Textbook of Organ Transplantation

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SECTION 1

History/Introduction

Introduction

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To understand organ transplantation is to understand all of modern medicine, and to practice organ transplantation is to comprehend the fundamental challenges of advanced healthcare delivery in general.

Although these introductory statements are biased by a lifetime of study and admiration of this field, they are not wholly derived of hyperbole. Indeed, when considered objectively, there are few serious students of medicine that would argue the principles they espouse.

Consider the most fundamental diagnosis of medicine: alive or dead. The objective determination of death has been conceived in large part in pursuit of ethical grounds for organ donation; the concepts of brain death are unarguably intertwined with clinical organ donation, and the transplant clinician must understand this most critical of states as much as anyone in any field. Similarly, beyond knowing what constitutes a living individual, the transplant professional is continuously defining where the individual resides by objectively demonstrating that most of *what* we are exists as parts in support of *who* we are. From a utilitarian standpoint, the vast majority of a person is replaceable! This concept is experienced in transplantation as a matter of fact, and the transplant professional's proximity to it provides a unique vantage point relative to other specialties. At a more granular level, the transplanter (a term used to avoid distinguishing between scientists, surgeons, non-surgeons, and the numerous combinations thereof ubiquitous in the field of transplantation) uses biological definitions of individuality, namely molecular histocompatibility, as a daily tool of the trade. This fundamental biological characteristic has arisen through the study of transplantation and is as central to transplantation as brain death is to organ donation. From these high level examples, it is clear that the practicing transplanter more regularly deals in rather heady biological concepts than many other professions. That same characteristic applies to the most difficult of ethical concepts, as transplanters, more than any other specialists, must grapple daily with very practical applications of beneficence, justice, and utility.

Beyond the high-level concepts discussed above, the delivery of transplantation has emerged in such a way as to solidify its location in the core of the day-to-day details of medical practice. The nature of transplantation has always been to treat those with end stage illness, and while the etiology of the disease in question may be organ system specific, the consequences of any organ disease requiring transplantation become systemic in end stage. As such, transplanters have always had to treat the entire patient simply as a matter of practicality. The patient in fulminant hepatic failure not infrequently has critical brain, cardiopulmonary and renal dysfunction, as well. While these resolve when the underlying liver failure is reversed (in a way so dramatic as to have been unimaginable before the advent of liver transplantation), the transplanter must understand these systems exceptionally well just to get the patient to the operation; he or she cannot be overly focused on an organ of choice. Similarly, given the complexity of post-transplant immunomodulatory management, the transplanter's need to maintain a role in primary care and rehabilitation eclipses that of most subspecialties. As the need to stay involved for immune management has combined with improving life spans of transplant recipients, the understanding of long-term health maintenance required of the transplanter far exceeds that required of any other subspecialist. The transplant surgeon knows far more about medical management of diabetes and hypertension than the general surgeon, and the transplant physician understands surgical complications and wound healing much better than their non-transplant counterparts. Additionally, patients with transplants get the same diseases as patients without transplants, and thus, any illness arising in a transplant recipient will (or at least should) involve a transplanter at some level. A reciprocal effect of the growth and success of transplantation has impelled transplantation to be a core aspect of almost all subspecialties. One cannot consider himself or herself a fully trained cardiologist, endocrinologist, gastroenterologist, intensivist, hepatologist, nephrologist, pediatrician, pulmonologist, or surgeon without at least rudimentary knowledge of transplantation.

Transplant pharmacology similarly has driven pharmacology in general. The concept of therapeutic immune suppression derives from transplant applications and the first clinically approved monoclonal antibody (muromonab) and engineered biologic (daclizumab) were introduced in transplantation. As transplant pharmacology is with rare exception, poly-pharmacy, the role of the clinical pharmacist has been a long-standing feature of transplant care delivery, a model that many other areas have since adopted.

The means by which transplantation has been practiced is turning out to be prophetic for healthcare's ongoing evolution. Transplantation has always been a multidisciplinary craft, as evidenced by the inclusive nature of the depiction of the first successful transplant (see Figure 1.5, Chapter 1). From the start of the field, surgeon and non-surgeon physicians, nurses, allied health professionals, and basic scientists have mingled with a symbiotic fluidity that has typically exceeded that in other fields of medicine. Transplantation has not *become* a team sport, it *always has been* a team

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sport, and as such has stood as a tested example of the way forward for healthcare delivery. At a higher hierarchical level, transplantation has long ago adopted means of healthcare delivery that are just now emerging as vogue: elements of accountable care networks, capitated payment plans, pay for performance agreements, public/private partnerships, and rationing algorithms have been in place for transplantation for decades, and again, other fields of medicine can look to transplantation as a source for data on how these health services models are likely to perform.

In a similar vein, transplantation has led the way in terms of keeping track of outcomes on a local and national level, using these data to provide objective means of charting the future. The limited availability of donors and the life-or-death nature of the diseases treated, no doubt, have stimulated the desire to measure and improve the field. While other fields may have differences in the degree of these basic restraints (scarcity and severity) at some level they are applicable, and the model of transplant clinical databases has much to teach other areas of medicine. Transplantation has been aggressive in reporting its complications, and this has led to fundamental revelations in the biology of cancer, infectious diseases, metabolic syndrome, and numerous conditions. Basic physiology has also derived incalculably from the natural experiment of organ replacement.

With all this said, the breadth and depth of content implied by a textbook aiming to cover organ transplantation as a whole might be considered daunting. However, it is no more so than the challenges routinely faced in our field: replacing one person's heart with that of another, operating within the Venn diagrams of allo- and protective immunity, ethically allocating scarce donor organs, paying for it all . . .

Thus, this textbook has been created with the opening sweeping statements at its heart. It is not meant to cover everything to its maximal depth (there are texts dedicated exclusively to each of most of the topics covered in this text), but rather is designed to provide a reasonable introduction to most of what transplanter's do. It is arranged with topics presented in an order that a clinician might encounter them. Basic concepts are presented first, moving from requisite historical and general scientific concepts to transplant-specific biology and the fundamental principles of alloimmunity and immune modulation. Beginning, as do all transplants, with donor considerations, the clinical sections of the text then proceed through pre-operative, operative, and post-operative topics. Each of these sections contain chapters specific to the major organ systems presented in order of clinical volume: kidney, liver, heart, lung, pancreas and islet, intestinal and multivisceral, and in selected areas, vascularized composite transplantation. Within the pre-operative section, specific chapters related to the complex management of critically ill patients with end stage organ disease are presented, including intensive care and artificial life support devices. A technique for each major operation is presented in the operative section, acknowledging that there are numerous ways to do everything. The goal is to provide the fundamental starting point for the surgeon, and a reasonable idea of the scope of a procedure for the non-surgeon. The postoperative section includes organ specific post-transplant management, including acute and chronic management issues, as well as substantial attention to immune management and its consequences, particularly infectious and malignant complications. Rejection, being a central diagnosis driving much of a patient's post-operative care, is presented in two ways: from its organ-specific histopathological definition, and from an organ-specific clinical management vantage. The postoperative section ends with current accountings of long-term outcomes for each transplanted organ type.

We have chosen to provide a specific section dedicated to pediatric transplantation. While much of the biology and conduct mirrors that of adult transplantation, there are numerous areas, particularly related to indications, technical nuances and complications that clearly are unique to children. Indeed, the pediatrician will find the pediatric section to be a very satisfactory primer for transplantation in children in and of itself, complete with its own introductory comments.

As discussed above, the infrastructure and organizational requirements, ethical principles, and administrative guidelines for transplantation are exceptional examples of similar issues now facing the healthcare community at large. As such, the Textbook closes with sections dealing with these issues, both to provide the student of transplantation with transplant-specific knowledge of "how things get done", and to provide the general student of an example of how they might be done in other fields.

The overall goal of this textbook is to bring together a reasonable representation of modern transplantation in all its complexity into a single place. It is anticipated that there are several settings in which such a reference work will be useful. For the graduate or medical student, resident, or allied health trainee, this will serve as a centralized touchstone to help them orient their thoughts and prepare for rotations in transplantation. For non-transplant professions whose practice involves the care of patients awaiting or following a transplant, such as cardiologists, endocrinologists, gastroenterologists, hepatologists, nephrologists and pulmonologists, this will provide sufficient information to help in following patients and understanding when referral is prudent. Indeed, there are two chapters specifically directed toward the concerns of the community physician. For the established transplanter, the knowledge here will be useful when considering aspects of transplantation not typically encountered: for example information about heart transplantation for a kidney transplanter; clinical information for the basic scientist (or vice versa); or background information about a topic to aid in the interpretation of an article in the primary literature. Finally, for the transplant unit or hospital administrator, the content herein will provide substantial subject matter information helpful in organizing and staffing transplant cost centers, and understanding the contribution margins and risks derived from clinical transplant practices.

This first edition has emerged slowly, hindered somewhat by the substantial inertia of its scope. At some point, completeness has given way to practicality, and it is hoped that this same property will aid in its continuation–it is not, and may never be, "done". As the Textbook will be published in an electronic format, it will allow for continuous updates, and it is planned that approximately 10% of the content will be updated annually, making this a perpetually current resource. It will not replace the primary literature, but will be a constant source of information on which to base readings from the primary literature. Future enhancements linking the Textbook directly to the primary literature, particularly journals published by Wiley-Blackwell, are underway, and should provide a new paradigm for textbook utility.

CHAPTER 1

A Brief History of Clinical Organ Transplantation

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Introduction

Transplantation is one of the most visible and influential medical accomplishments of the twentieth century. Arising from technical advances and insights of the early twentieth century, the concept that transplantation was technically feasible gave rise to earnest investigations into its physiological barriers by a small number of visionary investigators driven by recognized clinical problems. From their descriptions of the genetic and immunological basis for graft rejection and the pioneering work of the first transplant surgeons, the clinical practice of transplantation rapidly became interwoven into the fabric of clinical care, not only from a practical standpoint but also as an example of the immense possibilities and challenges inherent in advanced medical practice. Multiple concepts now taken for granted in modern medical practice, such as immunosuppression, monoclonal antibody use, and indeed the concept of death as a definable state, have their origins in transplantation. Numerous modern ethical quandaries also trace their roots to problems introduced by transplantation.

The history of transplantation has been the subject of numerous books, and while its in-depth treatment is not practical in this text, it is important to make evident the major contributions that brought the field to where it stands currently: a prime example of the power of science and medicine combined for a common good. This chapter will provide the reader with a reasonable orientation to the history of the field, referring to more definitive works when necessary and to other chapters in this text. In doing so it will serve as an important introduction to the subject of organ transplantation in general. Throughout this textbook, more specific historical references will be introduced in individual chapters.

The concept of transplant surgery

The idea of organ transplantation is a surgical concept based on an understanding of anatomy and the physiological requirements of an organ to stay viable and fulfil its biological role. Its origins relate to intuitive concepts of like replacing like that date back hundreds of years, including the oft cited Catholic miracle of Cosmos and Damian. However, its serious pursuit arose independently from work conducted in a number of European countries in the early twentieth century, the products of which led to the kidney as the chief candidate for speculations on organ transplantation. Kidney failure was a relatively common fatal condition, the physiological role of the kidney was understood and the kidneys were well defined anatomically. Importantly, it was recognized that one well-functioning organ was sufficient to maintain an individual in good health for a normal lifespan. The kidney's essential anatomical connections, namely a single artery and vein and a urinary drainage vessel, were apparently sufficient for it to perform its physiological role. Nerves, lymphatic and fascial connections, although important, did not seem to be essential. At the beginning of the twentieth century, Alexis Carrel (Figure 1.1) devised a method of joining blood vessels together surgically [1], a technique that became the subject of his 1912 Nobel Prize in Physiology or Medicine. Carrel himself used this technique to show that a kidney could be removed and transplanted and would function after restoration of the arterial in-flow and venous out-flow, provided these surgical procedures were undertaken expeditiously, since a prolonged period without a blood circulation led to irreversible damage from ischaemia.

In cats and dogs Carrel and Guthrie proved the technical feasibility of the operation and also observed that moving a kidney from an animal to another site in the same animal could result in long-term survival of the kidney and the animal after removal of the opposite kidney [2]. However, transplanting a kidney from one individual to another, after a short period of satisfactory kidney function, was soon doomed to failure. Carrel recorded this observation, but at that time there was no explanation for the fairly rapid failure of what are now called renal allografts [3]. Priority for the transplantation of a kidney in a human patient has been claimed and disputed by a number of surgeons and their historians. Transplants to the brachial or femoral vessels of human kidneys or animal kidneys to humans were described. The early claims and a critical discussion of these unsuccessful experiments can be found in Dr. Francis Moore's book *Transplant* [4]. None of them functioned for a long period of time and with hindsight the procedures were premature in relation to the knowledge that was gradually accumulating from Carrel and Guthrie's experiments.

Transplant biology

In the 1940s and 50s, Morten Simonsen in Denmark and William Dempster in London independently described experiments similar in nature to those of Carrel and Guthrie and long-term, life-sustaining function of autografts was confirmed. However, experimental allografts were shown to fail universally and a second kidney transplanted from the same donor to the same recipient was destroyed almost instantaneously. These observations were the culmination of many years of painstaking research by Simonsen and Dempster, who also reported on the use of cortisone in renal

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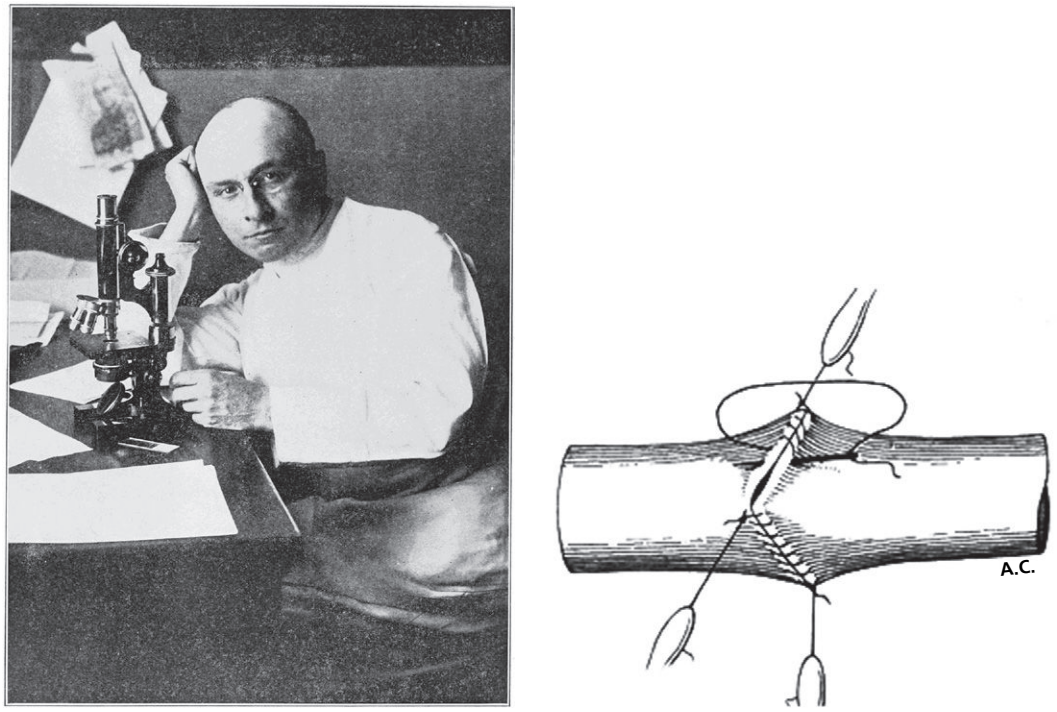


(A) (B)

Figure 1.1. (A) Alexis Carrel, widely considered the father of surgical transplantation for his development of (B) the techniques for vascular anastomoses, an accomplishment recognized by the 1912 Nobel Prize in Physiology or Medicine.

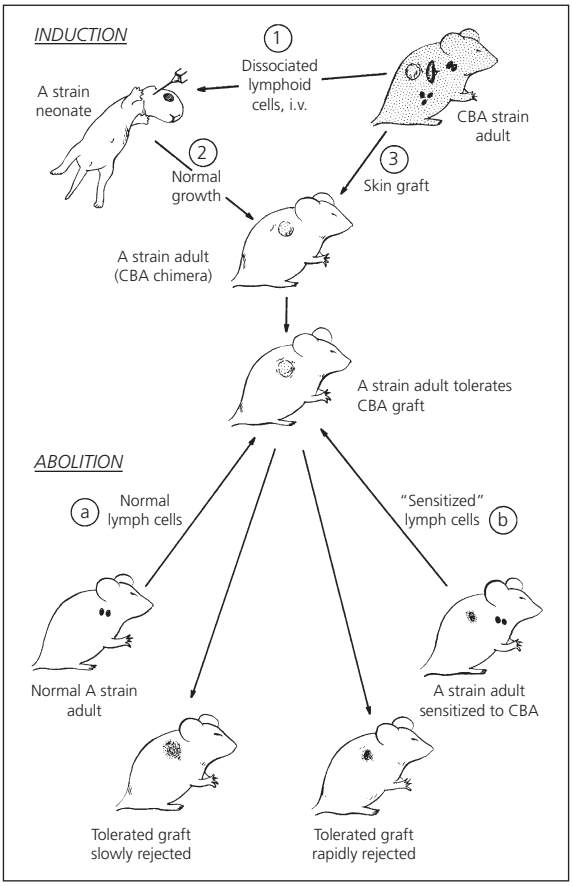
allograft recipients. Both workers described histological changes that occurred in renal allografts. The renal cortex became infiltrated with neutrophils, eosinophils, macrophages, lymphocytes and pyronin-positive plasma cells, and these inflammatory changes were accompanied by necrosis of nephrons. Both Simonsen and Dempster felt that it was likely that the cells originated from the grafted organ and were in fact a graft-against-host reaction [5,6]. Subsequently, using a newly developed isotope labelling technique it was shown by Porter and Calne that most of the infiltrated cells came from the recipient [7].

At the time that these experiments were being performed by Simonsen and Dempster in canine renal allografts, Peter Medawar (Figure 1.2) and his colleagues had studied the biology of rejection of skin grafts in rabbits and mice. The first set of grafts became infiltrated with leukocytes after a few days, the graft becoming necrotic after 7–10 days. A second set of grafts from the same donor were usually destroyed immediately and never achieved a capillary circulation. They were called 'white grafts'. The immune nature of skin graft rejection was proved beyond doubt by Medawar's group [8], and from their experiments it seemed likely that kidney grafts between identical twins would behave like skin grafts between identical twins and, provided the surgery was satisfactory, would be accepted without any immune reaction. From this scientifically established basis reciprocal skin grafting should differentiate identical from non-identical cattle twins, and Medawar and his colleagues were confident that this would be straightforward. However, they were astonished, and presumably initially disappointed, when the non-identical cattle twins accepted skin grafts in the same way that grafts were accepted between identical cattle twins [9]. They became aware of studies of non-identical cattle twins by Ray Owen who had observed that non-identical cattle twins frequently had blood groups of more than one type, circulating in apparently healthy animals [10]. This would be equivalent to a human having some red cells Group A and some Group B, which was know not to occur because of natural antibodies. Owen described the unusual status of their red cells blood groups as 'chimeric' from the fanciful resemblance to the mythical ancient Greek animal with organs derived from different species (Figure 1.3).

Medawar and his colleagues, Billingham and Brent, then set about performing a series of carefully controlled experiments between inbred strains of mice. The individuals of each strain after many sibling matings would be regarded as identical or 'isologous' individuals, so skin grafts were accepted between members of each inbred strain. However, grafts from a given strain transplanted into individuals of a different strain would be rejected, by what was later called the 'allograft' reaction.

Based on these observations in the cattle twins, Medawar's group found that injecting cells from one inbred strain into the foetus of another strain, although a formidable technical achievement, resulted in some survivors, which were then rendered 'tolerant' to grafts from the strain that donated the tissue (Figure 1.2B) [11]. They later found that this tolerance could also be reproduced with neonatal animals, where the technical challenges were still grave but less demanding than in the foetus. This discovery of 'specific immunological tolerance' was awarded the Nobel Prize in Medicine or Physiology and was a fundamental advance in immunology and also raised the practical question of whether the plasticity of the developing foetal immunological system could be reproduced in an adult, at least temporarily while a graft was performed, although it would be important that normal immunity would later be restored. Another factor of importance observed by Medawar's group was that if lymphocytes were included in the donor inoculum they

(A)



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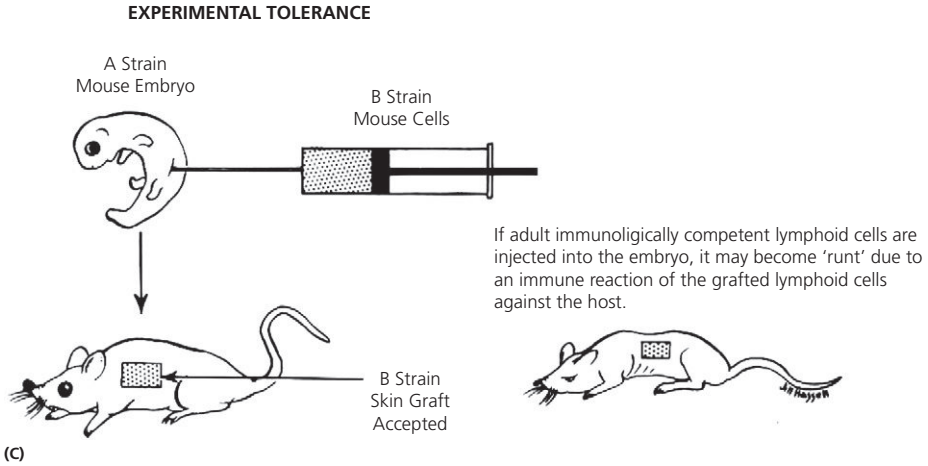

(C)

Figure 1.2. (A) Sir Peter Medawar, who won the 1960 Nobel Prize in Physiology or Medicine for (B) his studies into the biology of acquired immunological tolerance [11]. (C) The description of 'runt disease', a primary demonstration of the concept of graft-versus-host disease.

could react against the recipient, causing a wasting illness which they called 'runt disease' [12]. This was an example of a graft-versus-host reaction, which was subsequently recognized to be extremely important in bone marrow transplantation, and also relevant in some organ transplants, particularly the liver where many lymphocytes are transplanted together with the donor liver.

In the early 1950s, David Hume performed a series of carefully observed clinical kidney grafts, joining the renal artery and vein to the femoral vessels of the recipient, bringing the ureter through the skin [13]. Despite no serious attempt to impair the immunity of the recipients, some of the grafts functioned and one continued to produce urine for nearly 5 months. When it failed the kidney was found to have both cellular infiltration and severe arteriolar narrowing typical of what later was called 'chronic allograft rejection'. The investigators wondered if these somewhat less severe rejections in human kidneys than those observed in animal experiments was

Figure 1.3. The chimera, a creature from Greek mythology referenced in Homer's *Iliad*, which is amalgam of a lion, snake and goat and has become a recognized symbol of transplantation. Apulian plate, 350BC, Musee Louve.

due to the fact that the patients all suffered from uraemia, which was both generally debilitating but also possibly impaired the normal activity of the immune system.

Concurrently, experimental studies were also performed at the Peter Bent Brigham Hospital in Boston by the departments of surgery under Professor Francis Moore and medicine under Professor George Thorn. The surgical experimental lab. was headed by Joseph Murray, a plastic surgeon who had a background in very intricate surgical correction of dreadful facial deformities (Figure 1.4). He brought his skills to the laboratory and developed an improved surgical technique of kidney transplantation in dogs, in which the renal artery and vein were joined to the iliac vessels and the ureter implanted into the bladder, avoiding the discomfort of a urinary fistula with the concomitant danger of ascending infection. Professor Rene Kuss pioneered the technique of pelvic renal transplantation clinically in Paris in 1953 when a kidney graft from a mother to son, implanted using the pelvic site approach, functioned for 23 days [14]. This was an important advance in providing a surgically acceptable procedure.

Early clinical implementation

In 1954, a patient with renal failure was referred by his doctor to Dr. Merrill, a nephrologist in Dr. Thorn's department, with an accompanying note saying that 'you may be interested to know that this patient is one of identical twins and this could be relevant to your management', raising the possibility of a kidney transplant from the patient's identical twin. After careful evaluation of the ethical aspects and also a demonstration that the twins were in fact identical by accepting reciprocal skin grafts, the first identical twin transplant was performed by Dr. Murray and his colleagues at the Peter Bent Brigham Hospital and was a spectacular success [15] (Figure 1.5). It showed that as in animal experiments but also in man, if immunological rejection could be avoided, the surgery could produce an excellent long-term therapeutic result for the recipient and also the unilateral nephrectomy in the donor would not lead to long-term harm. For this and his subsequent work, Joseph Murray was awarded the Nobel Prize in Physiology or Medicine in 1990. Incidentally, the first twin donor survived for more than 50 years in good health, and served as the leading edge of follow-up regarding the safety of living donation.

Figure 1.4. Joseph Murray, shown in front of a depiction of his landmark performance of the first successful kidney transplant. Dr. Murray won the 1990 Nobel Prize for Physiology or Medicine. Photograph reproduced from *American Journal of Transplantation*, January 2013, with permission from John Wiley & Sons.

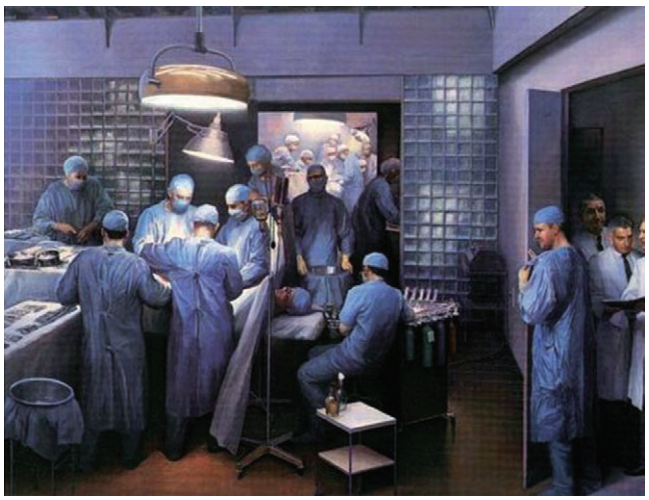

Figure 1.5. *The First Successful Kidney Transplantation*, by Joel Babb, 1996. Harvard Medical Library in the Francis A. Countway Library of Medicine.

In certain circumstances the original autoimmune cause of renal failure in the recipient recurred in the transplanted kidney, leading to its demise despite (or perhaps because of) being derived from an

identical twin [16], and this outcome impelled the search for means of impairing host immunity. In studies on bone marrow transplantation it had been shown that the immune system of rodents could be eliminated by a lethal dose of total body X irradiation and animals could be rescued by injection of autologous bone marrow from the same strain. The bone marrow cells given intravenously homed to the bone marrow of the recipient and populated not only the blood-forming elements but also the lymphoid cells in the lymph nodes and spleen, where they could restore immunity [17]. If the bone marrow was not autologous there was danger that the transplant would fail or if partially accepted, could result in a fatal graft-against-host reaction. Later developments in bone marrow transplantation became possible using less-severe non-ablative conditioning of the recipient than the original total body irradiation and has proved to be therapeutically successful, and even the graft-against-host reaction has been harnessed as a graft against leukaemia [18,19].

The success of experimental and clinical bone marrow transplantation following total body irradiation led to hopes that similar conditioning might be effective in recipients of kidney grafts. There was one encouraging result in the dog [20] but unfortunately with the exception of two kidney grafts between non-identical twins, one at the Peter Bent Brigham Hospital and the other in Paris, all the attempts at total body irradiation failed with disastrous results, rejection not being controlled and overwhelming sepsis becoming a common terminal event [21,22]. To reduce the toxicity of total body irradiation, repeated small doses of irradiation to the spleen and lymphoid organs was developed by Slavin and Strober in Stanford [23], a technique that continues to be investigated in the pursuit of clinical tolerance (see Chapter 76).

In the late 1950s it was clear that an alternative superior method would be needed to condition the recipients of kidney grafts more safely, and an important pointer was the demonstration that the antileukaemia thiopurine drug, 6-mercaptopurine, would prevent rabbits challenged with foreign protein antigens from producing antibodies. This effect persisted after the 6-mercaptopurine treatment had been stopped and was described by the authors, Schwartz and Dameshek in 1959, as 'drug-induced immunological tolerance' [24]. 6-mercaptopurine was then investigated in London and Richmond, Virginia as an immunosuppressant in dogs with renal allografts, resulting in prolonged allograft survival but not tolerance [25,26]. However, the effect was superior to total body X irradiation. There was a high incidence of infections caused by the severe impairment of immune reactions. Hitchings and Elion, who had synthesized 6-mercaptopurine, provided a number of experimental compounds that they felt were worth investigating as possibly having better therapeutic indices than 6-mercaptopurine. One of these agents, azathioprine, was found experimentally to be slightly superior to 6-mercaptopurine and became an anchor drug for a landmark series of animal experiments and subsequently for the next phase of clinical immunosuppression, which was commenced at the Peter Bent Brigham Hospital [27]. Initial clinical results were disappointing and it was not until corticosteroids were added to the immunosuppressive regimen that some successes were obtained in allografts between unrelated individuals [28]. Azathioprine remains indicated for transplant immunosuppression and is described in more detail in Chapter 98.

Steroids had previously been investigated by Medawar and Dempster, but alone were not very effective. However, when combined with azathioprine, better results were obtained. Tom Starzl carefully documented this in Denver, in recipients of renal allografts. Goodwin had already shown that a bolus of steroids could restore function, at least temporarily, to a kidney allograft that was undergoing acute rejection [29,30].

With day-to-day monitoring of patients and adjustment of drug doses to try and find a level that would prevent rejection without severe toxicity, some 50% of renal allografts were functioning at a year, but failures were a severe burden for the patients, their relatives, and the surgeons involved. Nevertheless, during this phase techniques of experimental heart transplantation were developed by Lower and Shumway in Stanford [31]. Additionally, Starzl in Denver and Francis Moore at the Peter Bent Brigham Hospital in Boston each developed successful surgical techniques for transplanting the liver [32,33]. Experimentally head and limb transplants were performed by Demikov in Moscow, but at that time these were little more than a surgical curiosity rather than a serious move forward towards clinical application [34].

It became apparent that transplants between close blood relatives who were not twins had better results than between unrelated donors. The basis for variation was correlated with tissue groups, which were gradually becoming clarified by Dausset, Payne, Van Rood and Terasaki [35–38]. These human leukocyte antigens (HLA) were separate from blood groups, the typing of which also was required to avoid immediate graft failure due to preformed antibodies against red blood cells groups. The historical aspects of the development of HLA and its testing are covered in Chapter 3.

Starzl performed the first clinical liver transplants in 1963 [39]. However, the early failure of these grafts and the demise of the patients led to Starzl imposing a moratorium until 1967 when he recommenced a liver transplantation programme at the same time Barnard performed the first clinical heart transplant. Additional details on the first paediatric cardiac transplant can be found in Chapter 115 [40,41]. The media excitement towards the heart transplant was unprecedented in medical history and led to unfortunate consequences because all over the world cardiac surgeons attempted clinical cardiac transplantation 'to join the club'. This was extremely worrying because most of the surgeons had no background knowledge on the biology of transplantation and the difficulties of prescribing immunosuppressive therapies.

Using aziothioprine and steroids the results of cardiac and liver transplantation were poor, but a few patients did very well. Many other agents and procedures were investigated to try and improve immunosuppression. They included total drainage of lymph from the thoracic duct and ex vivo irradiation of the kidney and circulating blood. These procedures were unsuccessful, but the concept of raising antibodies against human lymphocytes proved to be more promising. This had first been investigated experimentally by Woodruff and colleagues in Edinburgh in organ grafts after a demonstration of effective antilymphocyte serum produced in rodents to prolong skin grafts [42]. The antilymphocyte antibodies were polyclonal, that is they reacted against many different epitopes that were generated when lymphocytes or thymocytes were injected into a different species, for example the horse and rabbit. These antibodies were difficult to purify and tended to vary in efficacy and toxicity from batch to batch, but some were very powerful and, as described in Chapter 98, refined rabbit antihuman thymocyte antibody is still used clinically in patients [43].

The modern era of immune management

In 1978, Borel, an immunologist working in Sandoz laboratories in Basel, Switzerland, described the immunosuppressive effects of a

fungal cyclic peptide, cyclosporine, which was being investigated as a possible antibiotic [44]. Its antibiotic properties were weak but its immunosuppressive activity, both in vitro and in vivo with skin grafts in mice was impressive. On the basis of Borel's observations we, and workers at Northwick Park, investigated cyclosporine in rats transplanted with heterotopic cardiac allografts and found cyclosporine to be extremely effective [45,46]. Our studies were extended to canine renal allografts and orthotopic cardiac allografts in pigs. In these species, and later in the rabbit, cyclosporine was shown to be a powerful immunosuppressant with few side-effects [47]. However, when it was first used in clinical renal transplants it was found to be remarkably nephrotoxic, a property that had not been suspected in the animal experiments [48]. The nephrotoxicity was particularly severe as the dosage used in the first clinical trials was based on the animal experiments and found later to be much too high. Cyclosporine proved to be a watershed in immunosuppression, with the 1-year functional survival of renal allografts increased from 50 to 80%. This early improvement changed the attitude of the medical profession towards organ transplantation, which previously had been extremely sceptical, and as a result there were only approximately 10 units worldwide seriously doing clinical organ transplants. After the introduction of cyclosporine there were, within a few years, more than 1000 centres and the anticipated shortage of organ donors suddenly became a major consideration, which has escalated ever since. The mechanism of action of cyclosporine is detailed in Chapter 17, along with descriptions of all medications specifically indicated for transplantation.

From Japan the streptomyces-derived compound, tacrolimus, was developed by the Fujisawa Company and shown by Ochiai to prolong experimental graft survival at a very low dosage [49]. After early experimental organ allografts in different species, tacrolimus was used in clinical organ transplantation and fully and exhaustively investigated by Starzl's group in Pittsburgh [50]. Tacrolimus has a similar nephrotoxic effect to cyclosporine but the hirsutism and gum-swelling, which were the common side-effects in cyclosporine-treated patients, did not occur in patients treated with tacrolimus.

The production of monoclonal antibodies by Kohler and Millstein in 1975 provided an agent with a single molecular target [51]. This was quickly exploited for use in transplantation, with the development of OKT3, a murine-origin monoclonal antibody specific for the CD3 determinant on T cells. This agent effected prompt, albeit transient, reductions in peripheral T-cell counts and was shown to reverse established acute rejection [52]. In 1986, OKT3 became the first monoclonal antibody approved for use in any human disease, and while its side-effect profile, clearance by a mouse-specific antibody response, and the emergence of other more tolerable therapies, eventually led to its withdrawal from the market, OKT3 served as a proof-of-concept for the promise of monoclonal antibody therapy in medicine in general. Similarly, the concept of humanization, that is genetic engineering of an antibody to make it largely composed of human structures to avoid a neutralizing antibody effect, was first launched in transplantation with the development of daclizumab [53]. As with OKT3, a completely new therapeutic approach first found its way into the clinic through transplantation. This drug also has been removed from modern practice, but others now find their way into our field through off-label use (see Chapter 99). Conceptually, import in this regard derives from the work of Waldmann and Winter who developed the lymphocyte-specific humanized monoclonal antibody Campath 1H or alemtuzumab, an extremely powerful antilymphocyte antibody, which eliminates lymphocytes almost completely from the circulation within a few hours [54]. It was developed for the treatment of chronic lymphatic leukaemia but was shown to be a valuable induction agent in clinical kidney transplant patients, allowing maintenance immunosuppression to be reduced to a significantly low level, which eliminates most of the toxic side-effects of maintenance drugs. This concept of powerful almost, or '*prope*', tolerance antilymphocyte induction therapy, followed by very low maintenance, does not generally result in tolerance, but is probably currently the best available treatment for recipients of organ grafts [55,56].

It has been known from the early experimental liver grafts in pigs and later in rodents that liver grafts were less severely rejected than other tissue and sometimes were accepted long-term as an 'operational' tolerance in animals not given any immunosuppression. The transplanted liver often underwent biochemical and histological rejection, which recovered spontaneously [57–59]. These observations were a background to 'experiments' performed by patients in Pittsburgh who were non-compliant in taking immunosuppression, without telling their doctors. Some of these patients with liver transplants have survived for more than 20 years without any immunosuppression and justifiably would be regarded as operationally tolerant. As discussed in Chapter 76, patients in whom immunosuppression was deliberately stopped or weaned, because of infection or as a policy to see if they were tolerant, produced variable results. Some patients accepted their grafts without deterioration indefinitely, but others developed rejection and had to return to immunosuppression. Even an extremely low dose of immunosuppression may be important in recipients of organ grafts; stopping a very low dose may lead to rapid rejection, which may be difficult to reverse [60,61].

A more biological approach to immunosuppression has been studied for some years based on successful murine experiments. Engagement of foreign tissue with the recipient immune system involves not only recognition and reaction to antigenic epitopes, but also a complicated secondary signal that binds the cells presenting the antigen to the immune reactive cells. The second signal has been studied in great detail and various methods of blocking the second, now costimulatory, signal have shown encouraging results experimentally and have now been investigated in the clinic [62]. This is described in more depth in Chapter 11.

Ethical challenges

In the past 50 years, organ transplantation has come a long way from a theoretical, fanciful concept to a fully established, practical therapy. There are still many biological obstacles to be investigated and overcome, particularly recurrent disease in the transplanted organ, the development of renal damage and diabetes associated with calcinurin-inhibiting drugs, and infection and malignancy, especially lymphoma and skin cancers. All these unsatisfactory features of organ transplants have gradually become understood and various methods of avoiding these side-effects and treating them have been developed. Indeed, an in depth understanding of these adverse conditions form the foundation of the knowledge required for the clinical practice of modern transplantation, and is approached to some degree in most chapters in this text. The success of organ grafts has raised ethical questions, which have emerged like 'a can of worms', totally unexpected by the pioneers of transplantation in the early days, but covered in numerous chapters in this textbook (e.g. Chapters 136–139). As the results of organ

transplants improve, so the demand increases, yet the donor supply does not increase *pari passu*. There are not sufficient deceased donor organ transplants in any country. Indeed, public opinion is often not in favour of deceased donation nor are some governments prepared to invest in the infrastructure necessary to educate the public or provide the medical facilities necessary for intensive care of potential donors. Spain has been the most successful nation in all areas with deceased organ donation of more than 30 cases per million but with improved treatment of head injuries and less road traffic accidents than previously, the 'donor pool' has become reduced [63,64]. This is detailed specifically in Chapter 53.

Living donors have been used extensively since the beginning of organ transplantation in identical twins. Usually the kidney transplantation can be performed ethically between related and unrelated family members and liver transplantation from parents to children. However, beyond these stipulated cases the ethics of organ donation have been strongly questioned (see Chapter 138). Recently, a child in China sold his kidney without his parents knowing to obtain cash to buy an iPod [65]. Many thousands of prisoners executed in China had their organs removed for transplantation. The transplant community in the West condemns this practice as unacceptable on a variety of grounds, including the use of organs to generate income from rich foreign 'organ tourists'. Pressure can be put on individuals to donate within a family and relations of the patient may feel forced to acquiesce or have a terrible guilt feeling if they refuse. Attitudes to donation between donor and recipient may change. A New York surgeon who had given a kidney to his wife, later divorced her and was awarded considerable damages in consideration of his previously considered altruistic gift [66]. Liver transplantation from a living adult donor to another adult has risks that are very difficult to explain to the prospective donor. Not only is there a high likelihood of prolonged morbidity, but there is a possibility that organ donation will cause the donor to lose his or her job and mortality as a result of the donor operation is high enough to be considered a significant risk. Five partial liver donors to adults have been reported to have developed liver failure themselves, four died and one was rescued by a transplant [67]. Rich patients may buy organs illegally. Seldom do the rich donate organs to poor recipients.

It is unlikely that the ethical difficulties will be easily overcome, but defining them is the first step. In the middle 1960s, attempts were made to transplant organs from animals to man. A variety of well-documented kidney transplants were reported from New Orleans, Denver, and Richmond, Virginia and there were also sporadic attempts at other organ xenografts. To date, none have been successful long-term apart from one kidney transplant from a chimpanzee to a patient in new Orleans, which functioned for 9 months, eventually succumbing to chronic rejection [68]. A great deal of effort has been put into studying xenograft reaction and defining the barriers, and this subject receives in-depth treatment in Chapter 12. However, despite intense and expensive experimentation, each barrier appears to be rather like an opaque hazard in a steeplechase where it is necessary to overcome the first barrier to see the next one and currently we do not know how many barriers there are or even if the race can be won.

Summary

In a brief period of less than 60 years, transplantation has emerged from its humble origins as a experimental curiosity to being an accepted means of life-saving therapy for most end-organ diseases. Its success has become increasingly expected, and its implementation increasingly seen as routine. In the foreseeable future, despite (or perhaps as a result of) exceptional advances in overcoming the surgical and immunological barriers to transplantation, the shortage of organ transplants will remain, and it is incumbent on the transplant community to explain honestly the situation to the public and their governments, and to face the unwelcome fact that not everybody who might benefit from a transplant will receive one; this is especially true in poor nations. As the readers makes their way through this comprehensive text, they will find vestiges of the historical landmarks outlined briefly in this introductory chapter. It is hoped that as one consumes the specifics of the expansive practice that is modern day organ transplantation, they will recognize the common thread that moves through it, from fundamental biology and discovery to similarly fundamental ethical concerns that define our humanity. In doing so, the student of transplantation will best appreciate the privilege and responsibility that this field imparts upon them.

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SECTION 2

Transplantation Biology

CHAPTER 2

An Overview of Physiologic Immunity

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Introduction

An understanding of one's response to a transplanted organ requires a fundamental understanding of the immune system in general, and its function in the context of physiological homeostasis. Indeed, it is important to recognize that transplant-directed allo- or xenoimmunity is mediated by processes that evolved with selective pressures completely unrelated to organ transplantation, and, thus, the components of a transplant-specific response derive from a system that has come into being to maintain one's integrity amongst numerous environmental pathogens and threats to individual integrity. Humans are exposed continually to diverse pathogens, including viruses, bacteria, fungi, and protozoa, as well as many multicellular parasites. If not prevented or counter-acted upon, infection by these pathogens results in illness and potentially death of the host. Moreover, daily physical activities can cause varied levels of trauma and associated tissue injury that must be managed satisfactory to maintain normal body function. To cope with these challenges, an intricate immune system has evolved, consisting of generalized barrier mechanisms, an array of highly responsive hematopoietic cells, and an arsenal of specialized effector molecules.

Although immunology, the study of the immune system, is generally considered a modern science [1], it has a long history, spanning ancient times to the present day, during which physicians and scientists have studied the role of immunity in health and disease. As early as 1 AD, the ancient Roman Celsus documented in *De Medicina* the heat, swelling, pain, and redness (*calor, tumor, dolor, and rubor)* that results from our body's inflammatory response to injury. In recent history, we have elucidated many of the cellular, and now molecular, processes that constitute the immune response. As our understanding of immunology has grown, so has our appreciation of the role of the immune system in the normal physiology and function of tissues and organs. Likewise, translational efforts have continued to harness an understanding of normal, pathologic, and therapeutic immune responses to improve the human condition. The study of immune responses to transplanted tissues and organs and how these can be controlled (transplant immunology) has been an area of strong academic interest and clinical success. In this chapter, we provide a brief general introductory framework of the cellular and molecular immunology necessary to comprehend subsequent, more specific and detailed chapters on the mechanisms of transplant-specific immunity (see Chapters 5, 6, and 7 for transplant-specific treatments of basic immune mechanisms). It will also establish the general vocabulary and knowledge base required to understand the references to immunology that are ubiquitous in the practice of transplantation, as will be evident throughout this text. Excellent contemporary immunology textbooks [1,2] elaborate core concepts of protective immune reactivity to foreign material and pathogens and the processes of adverse immune responses, termed immunopathology.

Generalized protective barriers and defense factors

Protective barrier tissues, such as the skin and the mucus-lined and/or ciliated epithelium of the respiratory tract serve as first lines of defense and prevent invasion by most pathogens (Figure 2.1A). Likewise, the low pH environment of the gastrointestinal system and genitourinary tract is inhospitable to most organisms. Sites susceptible to invasion, such as the conjunctiva of eye or the oral cavity, are protected by antibacterial enzymes, such as lysozyme, β-defensins, or phospholipase A. These barrier tissues also support a critical commensal bacterial flora, or microbiota, which prevents infection by "crowding out" pathogenic organisms and also play a critical role in shaping immune reactivity, especially in the gut mucosa [3].

Tissues and organs of the immune system

The lymphoid organs are categorized as primary and secondary lymphoid organs (Figure 2.1B). The primary lymphoid organs, specifically the bone marrow (BM) and thymus, are sites of lymphocyte development. The hematopoietic compartment of the human BM generates approximately 500 billion blood cells each day, and produces the diverse hematopoietic cells that support the body's innate and adaptive immune systems (Figure 2.1C). The BM and thymus constitute the primary lymphoid organs involved not only in the production but also the selection of lymphocytes, selection defined by which cells will most appropriately interact with the individual's cellular constituents both to maintain homeostasis and avoid autoimmunity. The thymus provides an inductive environment for the development of T cells from hematopoietic progenitors. Moreover, thymic stromal cells permit the selection of functional and self-tolerant T cells. Thus, the thymus is of

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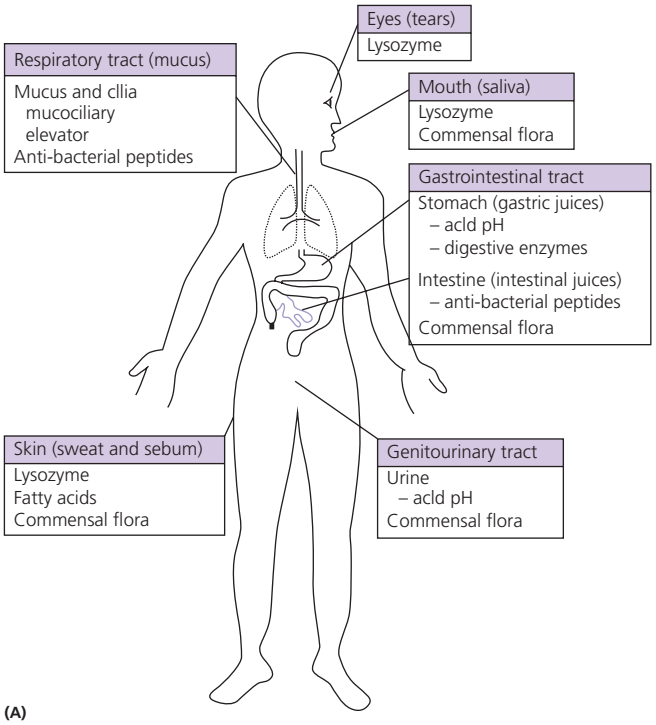

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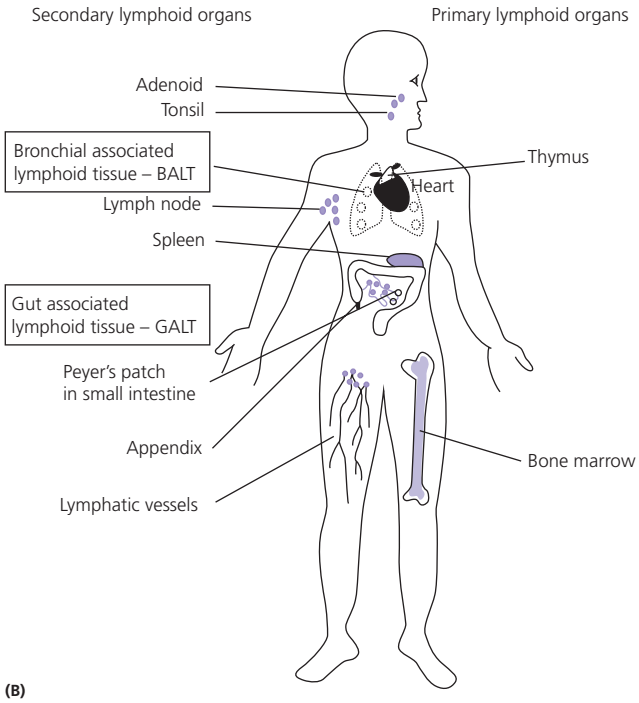

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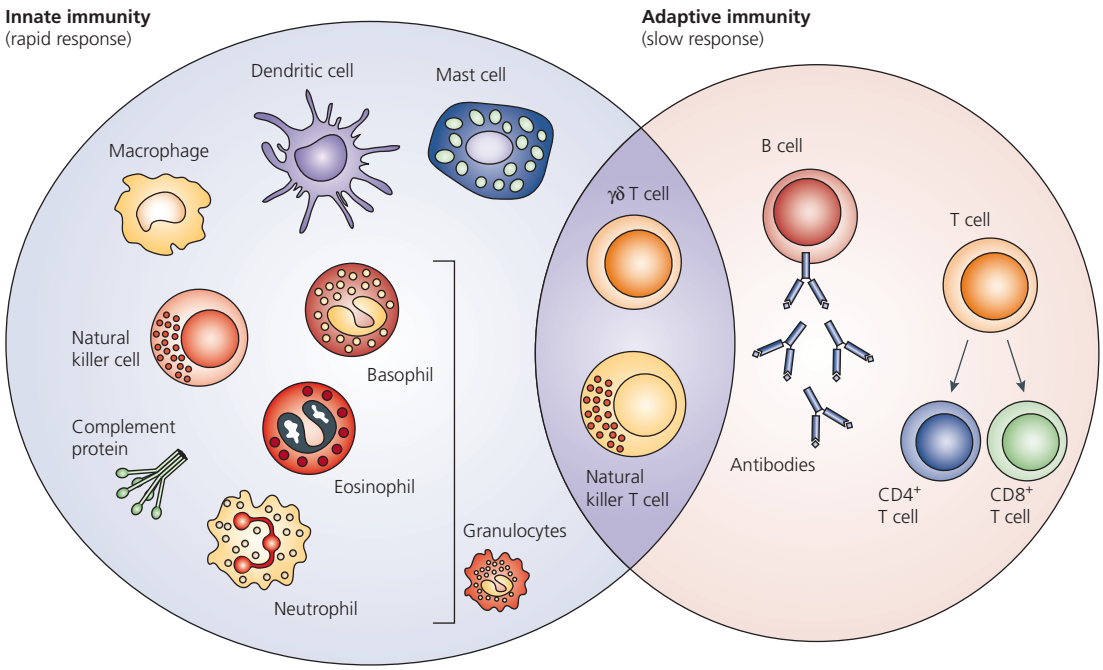

(C)

Figure 2.1. The components of the immune system. (A and B) Reprinted from Keogan MT, Wallace EM, and O'Leary P (2006), Concise Clinical Immunology for Healthcare Professionals. Figure 1.2.1 page 9, and Figure 1.3.2, page 16, with permission from Taylor and Francis Books [103]. (C) Reprinted by permission from Macmillan Publishers Ltd: Dranoff G, Cytokines in cancer pathogenesis and cancer therapy Nature Reviews Cancer 2004, 4: 11–22, copyright (2004) [104].

crucial importance in the maintenance of self-tolerance (central tolerance, see below).

Secondary lymphoid organs provide sites for collection of interstitial bodily fluid, or lymph, which is mainly blood plasma that may contain material derived from invading pathogens. Contained in this material are substances (or antigens; Ag) that elicit adaptive immune response. Likewise, secondary lymphoid organs, including the lymph nodes, spleen, and other regional lymphoid tissues, are a very important nexus for communication between innate and adaptive immune cells, and thus the site where most immune responses are initiated. The lymphatic system is connected by a series of vessels that act as part of the body's extracellular fluid recirculation system in which lymph is collected and transported to the lymph nodes, where it can be conveyed back into the blood stream.

Cells of the innate and adaptive immune system

Although the barriers and general protective substances described above are highly effective in preventing pathogens from infiltrating sites of exposure, specialized hematopoietic cells are exceptionally well equipped to counter pathogens that breach these defenses. These white blood cells, or "leukocytes," circulate in the blood, navigate the lymphoid system, and monitor/patrol/survey peripheral tissues, including the commonly transplanted organs (Figure 2.1C). Leukocytes are also critical in their support of wound healing and resolution of tissue damage.

Leukocytes are grouped in two integrated arms of the immune system: the innate and adaptive arms, based on their respective functional attributes and the uniqueness of the cell surface receptors they express. However, other important differences, described elsewhere in detail [4], exist between cells of the innate and adaptive immune systems. In brief, innate immune cells can detect pathogens and local tissue damage and respond immediately following ligation of a single activating signaling receptor. This contrasts with adaptive immune cells that require multiple permissive signals provided by other immune cells to become fully immunocompetent. Importantly, following their activation, adaptive immune cells undergo proliferation and division into clones with identical Ag recognition ability, thus amplifying the resultant immune response. Another important distinction is the ability of adaptive immune cells to persist in the body long term following their stimulation and division, and their ability to mount a "memory" response to a particular Ag (see below). Specifically, this allows a more rapid and intense response following repeated exposure to the same Ag. Innate immune cells have short life spans, and given their lack of clonal expansion, any secondary responses to an Ag will appear identical, in both time and amplitude, to a primary response. However, there is a strong inter-relationship between the cells of the innate and adaptive immune systems in regard to transplant immunity, and it is therefore difficult to describe their functions and immune responses in isolation from each other. Furthermore, evidence has accumulated that cellular "crosstalk" between innate and adaptive immune cells is important for the generation or regulation of immunity. Thus the concept of purely "innate" or "adaptive" immune responses may be more of a practical generalization than reality.

Innate immune cells

Thought to have evolved long ago as a host defense mechanism against various pathogens, cells of the innate immune system are recognized as the body's first line of defense against bacteria, fungi, yeasts, and parasites [5]. The innate immune system is comprised of inflammatory cells, often granulocytic and/or phagocytic cells, which are activated rapidly by pathogens following detection of pathogen-associated molecular patterns (PAMPS; [4,6]) and endogenous damage-associated molecular patterns (DAMPS; [7]) by pattern recognition receptors (PRR). In an important distinction from adaptive immune responses, innate cells are non-clonal, with all cells of a lineage identical in displaying the same, germline encoded receptors. Innate immune cells include leukocytes that contain granules consisting of preformed antimicrobial agents, enzymes, and other immunoregulatory material within their cytoplasm. These are classified, firstly, as granulocytes, which include neutrophils, eosinophils, basophils, and mast cells. A second group of innate immune cells, recognized for their capacity to take up and selectively process exogenous materials, includes monocytes, macrophages, and dendritic cells (DC), and are classified as "monocular phagocytes" [8,9]. A third group of innate cells with some attributes of adaptive immune cells are gamma-delta ($\gamma\delta$) T cells [10], which have ill-defined immune functions, and natural killer (NK) cells [11], which are important for their ability to kill virally infected cells or cancer cells without prior sensitization. Chapter 7 details transplant-specific mechanisms of innate immunity.

Granulocytes

In general, granulocytes respond to activation following perception of invading microbes or local inflammatory stimuli with a rapid discharge of the granulocytic and cytoplasmic immune mediators that are directly toxic to pathogens and promote local inflammation and immune responses.

Neutrophils

Neutrophils comprise a large proportion of circulating leukocytes and are of paramount importance in effective responses to microbial invasion and tissue injury [12]. Neutrophils express surface receptors and molecules that allow them to both sense and migrate to sites of infection and inflammation. Typically, the first immune cells to arrive at these sites, neutrophils phagocytose or ingest complement-opsonized (see below) microbes and damaged tissue. Neutrophils also can deploy antimicrobial reactive oxygen species (ROS) and cytotoxic substances contained within their granules, as well as augment local innate and adaptive immune responses through production of a diverse range of inflammatory mediators that influence both innate and adaptive immune cells. Neutrophils are also emerging as crucial facilitators of inflammation resolution and wound repair.

Mast cells, eosinophils, and basophils

Mast cells are rare, but long-lived cells present in peripheral tissues, especially innervated and vascularized regions [13]. Activation causes mast cells to release granules containing histamine, which increases vascular permeability and stimulates local nerves. Mast cells can also generate lipid-derived eicosanoid messengers that module the local environment and promote chemotaxis, that is the migration of other immune cells towards increasing concentrations of chemoattractant. Eosinophils and basophils are equally rare granulocytic cells that circulate in the blood and are implicated in allergic and antiparasitic responses supported by the secretion of histamine, heparin, cytokines, particularly IL-4, and chemotactic agents [14,15].

Natural killer (NK) cells

Natural killer (NK) cells are a lineage of cytotoxic innate immune cells that are especially adept at the removal of abnormal cells [16,17]. NK cells, and related NK T cells, are a heterogeneous collection of lymphocytes specialized for the elimination of pathogen-infected cells and abnormal cells. They survey the periphery with a diverse set of germ-line-encoded cell-surface receptors belonging to the immunoglobulin (Ig)-like receptor and C-type-lectin receptor families. Their ability to act as effector cells is regulated by these surface receptors, which act to inhibit or enhance NK cell cytotoxicity.

Mononuclear phagocytes

Phagocytosis, or actin-dependent, active uptake of comparatively large particles (>0.5 μm), is carried out by neutrophils and a subset of innate cells classified as mononuclear phagocytes, which includes monocytes, macrophages, and DC [8,9]. Monocytes, macrophages, and DC are, like neutrophils, considered "professional" phagocytes and perform the bulk of phagocytosis in the body [18]. Mononuclear phagocytes utilize phagocytosis not only for clearance of pathogens, but also employ this process to take up, process, and present Ag in a form recognized by adaptive immune cells [19].

Several additional, essential characteristics enable mononuclear phagocytes to move beyond a simple phagocytic effector role and to act as crucial immunological sentinels that coordinate the response of the innate and adaptive immune response. Monocytes, macrophages, and especially DC, are well equipped to sample the local environment for Ag that is captured, processed, and then presented in a form that enables T lymphocytes (T cells) of the adaptive immune system to recognize it and mount an appropriate response if necessary. As such, macrophages and DC are often described as professional Ag-presenting cells (APC). The events underlying Ag processing and presentation are described below in more detail.

Monocytes, macrophages, and DC, like other innate immune cells, express diverse, germ-line encoded environment-sampling receptors. These PRR, which enable the cells to detect PAMPs on exogenous pathogens [4] and DAMPS released by local tissue damage/cell death [20,21], include the well-studied Toll-like receptors (TLRs) [22], as well as cytokine receptors, C-type lectin receptors, NOD-like receptors, and RIG-I-like receptors [23]. Triggering of these receptors implements programs of activation and differentiation of mononuclear phagocytes that result in distinct, proinflammatory myeloid cell subsets that precisely orchestrate the subsequent adaptive immune response, especially that of T cells which recognize Ag displayed on these APC. In quiescent environments and in the absence of activating stimuli, mononuclear phagocytes are described as "immature" APC, and act in a tolerogenic fashion to suppress responses of T cells reactive to the presented Ag.

Dendritic cells (DC)

In general, DC are rare, ubiquitously distributed, migratory leukocytes, derived from $CD34^+$ hematopoietic stem cells [24–27]. They convey Ag from peripheral sites, such as skin or other non-lymphoid tissues, to T cells in secondary lymphoid organs. DC are extremely well equipped for Ag capture and for processing and presentation of Ag to rare T cells expressing specific receptors that recognize Ag peptides bound to major histocompatibility complex (MHC) molecules.

DC comprise multiple subsets (i.e. conventional migratory DC, lymphoid tissue-resident DC, inflammatory DC, and plasmacytoid DC) that differ in phenotype, location, and function [28,29]. Conventional migratory DC located in non-lymphoid organs, including epidermal Langerhans cells (LC) and DC in the intestinal mucosa, lung, or kidney, are characterized by the expression of CD11c, CD11b, or CD103 and are potent initiators of T-cell immunity [30–32]. Their common feature is an ability to migrate via afferent lymphatics from peripheral non-lymphoid tissues to draining lymph nodes (LN). In doing so, migratory DCs not only undergo maturation, but also fulfill the important role of transporting peripherally acquired Ag into the LN wherein they may pass Ag to resident DC (cross-presentation of Ag). In addition, recent studies propose that productive naïve T cell–migratory DC interactions may also occur in peripheral sites (non-lymphoid tissues), interactions that may further promote the quantity and quality of T-cell activation [33]. Lymphoid tissue-resident DC, such as $CD4^+$, $CD8^+$, or double negative DC, are found exclusively within lymphoid organs, where they differentiate from precursors that directly populate these tissues [34,35]. Plasmacytoid DC (pDC) differ substantially from conventional migratory and lymphoid resident DC, by being potent producers of key antiviral cytokines, such as type-I interferon (IFN) [36]. In addition to migratory, lymphoid-resident and pDC, inflammatory DC can develop from monocytes, both in the periphery and in secondary lymphoid organs under conditions of inflammation [37].

Critical immune regulatory functions of DC

In general, DC function primarily to convey Ag from peripheral sites, such as skin or other non-lymphoid tissues, for inspection by T cells in secondary lymphoid organs [24,38]. Importantly, DC then either promote or suppress the immune response of reactive T cells, depending on necessitating conditions in the body.

In the non-lymphoid tissues, which include the commonly transplanted organs (kidney, heart, liver, and pancreas) DC reside as "immature" APC [39–42]. When freshly isolated, they express few surface MHC molecules, which are highly polymorphic surface proteins generated from co-dominantly inherited genes. These molecules are critical to T-cell immune responses due to the strict requirement that Ag must be presented in the context of MHC for T-cell recognition and reactivity (see Chapter 5). Cells classified as APC, especially DC, take up Ag efficiently either by phagocytosis or receptor-mediated endocytosis, process the Ag in specialized intracellular compartments, and present Ag-derived peptides bound to MHC molecules on their cell surface to T lymphocytes [43] (Figure 2.2). MHC class II molecule complexes on APC interact with $CD4^+$ T cells, while MHC class I molecule complexes interact with $CD8^+$ T cells. The Ag-derived peptides presented by MHC class I molecules originate mostly from intracellular sources of pathogens and are degraded by the cytosolic proteasome, whereas peptides presented by MHC class II molecules are acquired from extracellular sources, and degraded in the lysosomal compartment [44,45]. An additional process bridging the two pathways, called cross-presentation, consists of either exogenous Ag presentation by MHC class I molecules to $CD8^+$ T cells, or by cytosolic (endogenous) proteins degraded through autophagy (a catabolic process involving degradation of a cell's own components through lysosomal machinery) and presented by MHC class II molecules to $CD4^+$ T cells [46,47]. Among APC, DC are considered the prototypic professional APC, due to their exquisite expression of T-cell costimulatory and intercellular adhesion molecules and their

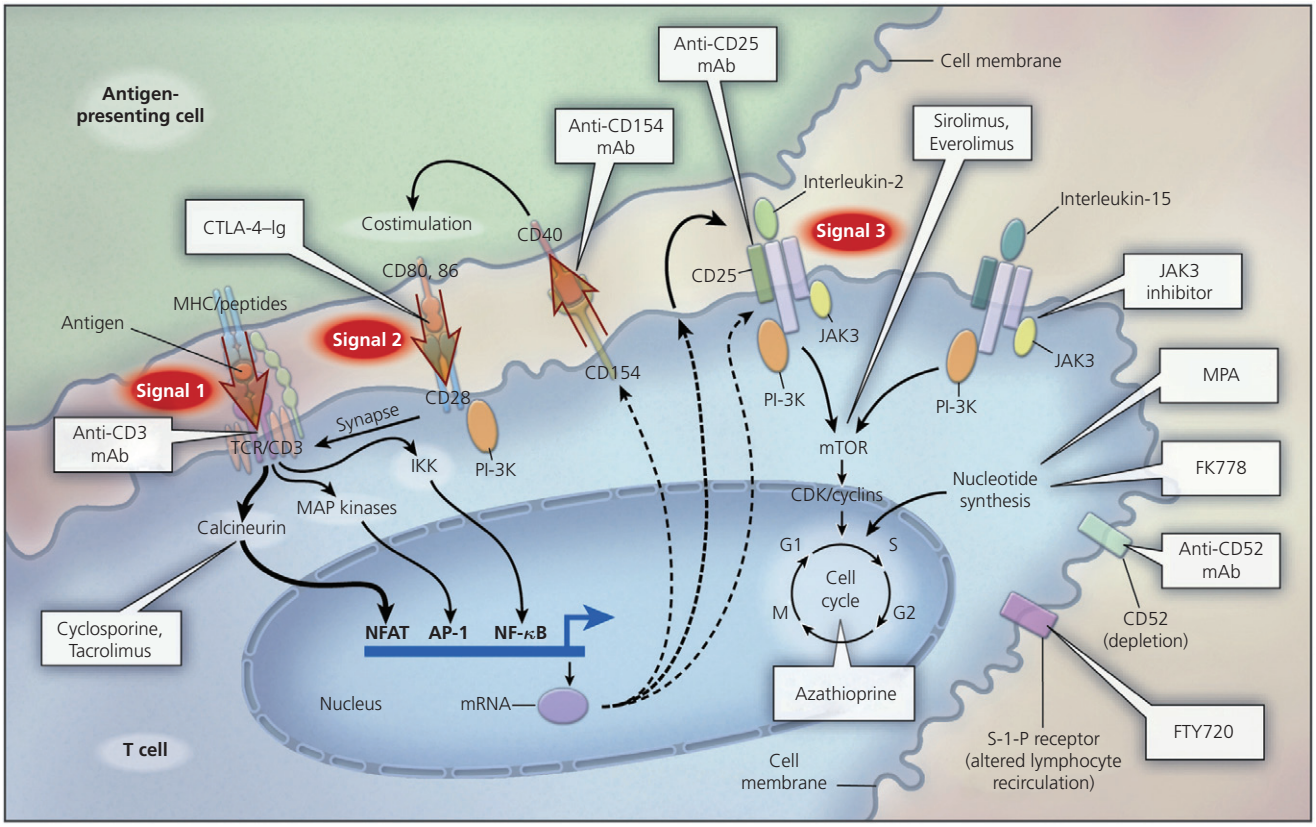

Figure 2.2. Antigen-presenting cell–T-cell interaction, depicting the three signals required for T-cell activation. From Halloran PF. Immunosuppressive Drugs for Kidney Transplantation. NEJM 2004 351: 2715–19. Copyright © 2004 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society [105].

ability to secrete proinflammatory cytokines, critical for effective naïve T-cell priming [43]. Macrophages and activated B lymphocytes can also present Ag via their MHC class II molecules, but are less efficient APC for priming naïve T cells. Immature DC, although poorly stimulatory, are sensitive to local changes perceived by environment-sampling receptors (i.e. TLR) which detect PAMPS or DAMPS, signifying local "danger." This triggers signaling pathways that direct DC "maturation," which consists of up-regulation of costimulatory molecules and the secretion of bioactive proinflammatory cytokines that facilitate immune responses.

Macrophages

Similar to DC, macrophages are highly plastic, monocyte-derived cells that acquire different molecular and functional phenotypes following integration of environmental and cellular cues [48]. They patrol tissues by sensing the presence of infectious agents or conditions of stress/injury [49]. This role is exerted through expression of the aforementioned germ-line-encoded environment-sampling PRR. Engagement of these receptors initiates a complex sequence of intracellular and extracellular modifications referred to as activation. Activated macrophages are capable of endocytosis and Ag processing and express MHC class II and costimulatory molecules to varying degrees, according to their activation state. Thus, like DC, activated macrophages are professional APC capable of effective Ag presentation to T cells. A key difference, however, is that although both cell types can present Ag to primed T cells, only DC can present Ag effectively to naïve T cells (see below) [25]. Thus, it is likely that during the response to a pathogen (or transplanted tissue), Ag presentation by DCs is essential to initiate a robust adaptive response whereas macrophages, which exist in greater numbers within inflamed tissue, act at the interface between adaptive and innate responses by integrating signals from both primed T cells and the inflammatory environment.

In addition to their response to PAMPs and DAMPs, macrophages reach different functional states based on their interaction with fully differentiated effector T cells and exposure to specific cytokine milieu. Macrophages were described initially as either classically or alternatively activated, with these phenotypes referred to as M1 and M2, respectively [50]. M1 macrophages are instrumental in immune responses against intracellular microbes and tumors [51]. M2 macrophages are more heterogeneous, but generally play a role in killing and encapsulation of extracellular parasites, resolving type-1 inflammation, and promoting tissue repair and remodeling. M2 macrophages also play a role in immune regulation and promote tumor progression. M1 and M2 macrophages are not only distinct in function, but also express different receptors and enzymes required for their activities. M1 macrophages express high levels of inflammatory cytokines (IL-12, IL-23,TNF, IL-1β, and IL-6) and chemokines (CXCL9, CXCL10, CXCL11, CCL2, CCL3, CCL4, CCL5, and CXCL2), as well as enzymes involved in the generation of ROS and nitric oxide (NO; [51]). M2 macrophages express lower levels of inflammatory mediators, but high levels of IL-10, scavenger, mannose, and galactose receptors. Importantly, in

mice, M2 express the enzyme arginase-1 that inhibits the NO generation pathway (through inducible NO synthase (iNOS)) to generate ornithine and polyamines which are instrumental in tissue repair and fibrosis.

Although this M1/M2 dichotomy simplifies understanding of the function of macrophages as key components of the immune system, more recent investigations clearly indicate that macrophages cannot be rigidly categorized, as they can express many shades of the inflammatory spectrum determined by tissue, stimulus, and phase of inflammation. Additionally, new functions have been attributed recently to macrophages isolated at different phases of the immune response and terms such as "resolution macrophages" and "regulatory macrophages" have been proposed, further emphasizing the complexity and breadth of activity of these cells [52].

Adaptive immune cells

Adaptive immune cells, consisting of T and B cells, express unique variants of prototypic Ag receptors on their surface, described respectively as T cell and B cell receptors (TCR and BCR). The Ag specificity-determining ("variable") regions of T and B cell Ag receptors are generated via gene rearrangement by site-specific recombination machinery encoded by the recombinase activating genes 1 and 2 (*RAG1* and *RAG2*) [53]. The stochastic process generating the Ag-detecting region of the TCR and BCR instills adaptive immune cells with the potential to recognize and mount specific immune responses to any Ag. In addition to Ag from pathogens, these Ags may include self Ag, and thus immature T and B cells must undergo a selective maturation process to eliminate self-reactive and potentially autoreactive T cells. BM-derived T-cell precursors migrate to the thymus, where those expressing functional, but weakly autoreactive, TCR are selected for release to the periphery. B cells, following their generation in the BM, remain there and progress to mature B cells with the help of BM stromal cells. More details on the selective processes that T and B cells undergo are provided in the section on immunological tolerance (see below). Relationships between these cells and transplant immunity can be found in Chapters 5 and 6.

T-cell immunobiology

In addition to their identification by expression of TCR, T cells are easily identified by surface expression of CD3, a crucial signaling component of the TCR [54] (Figure 2.2). T cells are divided into two major and functionally distinct subsets, based on surface expression of CD4 or CD8. CD4 and CD8 act as co-receptors to the TCR and are critical for Ag recognition. Likewise, expression of CD4 and CD8 signifies the distinct functional roles of two critical T subsets in adaptive immune responses. In general, $CD4^+$ and $CD8^+$ T lymphocytes display largely non-overlapping functions and co-operate in generating protective immunity in the host.

T-cell recognition of Ag in MHC via the TCR

Either the CD4 or CD8 molecule on T cells, in combination with the CD3 molecule and the α/β and γ/δ chains of the TCR heterodimer, form the survey and signaling machinery of T cells. This assembly of T-cell surface proteins, CD4 or CD8/CD3/TCR is referred to as the TCR complex [55]. As alluded to above, T cells scan peripheral tissue and other cells for potential pathogens/foreign Ag through their unique TCR complexes. They receive signals in the form of information delivered by MHC molecules on the surface of other cells of the body, in particular APCs. In the case of $CD8^+$ T cells, antigenic peptide is viewed in the context of MHC class I, whereas $CD4^+$ T cells detect Ag on MHC class II. In both cases, interactions between the TCR and MHC are stabilized by the T-cell co-receptors (CD4 or CD8) which interact with the MHC molecules. The interaction of a TCR with a MHC molecule displaying its specific or cognate peptide Ag, triggers signal transduction pathways, leading to phosphorylation of key signaling molecules, including zeta-chain-associated protein kinase 70 (ZAP70)/CD3ζ, spleen tyrosine kinase (Syk), extracellular signal-regulated kinase (ERK1/2,) phosphoinositide-3-kinase/serine/threonine kinase Akt (PI3K/AKT), and nuclear factor of activated cells (NFAT) pathways, followed by downstream expression of master transcription factors, chromatin remodeling and cytoskeleton reorganization [56,57]. As a consequence of meaningful TCR-induced signaling, effector functions, such as cytokine secretion and cytolytic responses against target cells, can be executed.

$CD8^+$ T cells

Activated $CD8^+$ T cells, also referred to as cytotoxic T cells (Tc), are considered to be primary effectors in the removal of cells infected with intracellular pathogens. Tc release several cytokines, small signaling molecules that influence immune and other cells (see Cytokines Section below), and cytotoxic molecules that destroy target cells. These cytotoxic substances include granzymes, serine protease that induce target cell death by activating proapoptotic pathways, and perforin, which supports granzyme introduction into target cells leading to osmotic cell death [58]. In addition, activated $CD8^+$ T cells express FasL(CD95L), which can initiate apoptotic death of target cells expressing Fas (CD95) on their surface [59].

$CD4^+$ T cells

$CD4^+$ T cells, also known as T helper cells (Th), are of critical importance in secondary lymphoid tissues during the initial phase (priming) of the immune response. Thus they provide specific instructions to effector and memory $CD8^+$ T cells, B cells, or other immune cells (including APC), via cytokine release and expression of immunomodulatory (costimulatory) surface molecules. Moreover, in peripheral tissues, $CD4^+$ T cells may release chemokines that attract other immune cells to amplify the immune response or exert direct effector functions. Effector Th cells are crucial regulators of both adaptive T- and B-cell responses, as well as coordinators of the innate immune response. Th cell cytokine production profiles, surface molecules, and specific transcription factors are often used to categorize Th cells into distinct functional subsets (Figure 2.3) (described more completely below in Section The Cytokine Milieu and Th Cell Polarization; reviewed in [60]).

Need for APC costimulation for T-cell activation

Costimulatory molecule engagement during naïve T activation is critical for T-cell survival, proliferation, and polarization (Figure 2.2) (see Chapter 5). Upon recognition of CD28 (the costimulatory molecule expressed constitutively by naïve T cells), by its ligands CD80 and CD86 expressed on DC, naïve T cells up-regulate the high-affinity IL-2 receptor α chain (CD25) and CD40L (CD154), express antiapoptotic Bcl-xL molecules, and activate the mechanistic target of rapamycin (mTOR, previously known as the mammalian target of rapamycin) pathway, thus initiating T-cell proliferation [61]. In addition, ligation of CD40 on DC by inducible CD40L on activated T cells further licenses DC activation (up-regulation of

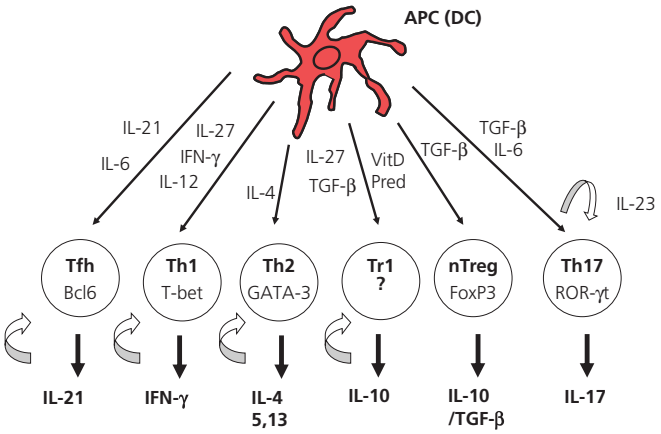

Figure 2.3. Polarization of naïve T cells into distinct helper T-cell subsets. T-cell polarization fate is determined by DC (APC) instructions conveyed to T cells which, in addition to the type of Ag (signal 1) and the nature of costimulatory factors (signal 2), provide cytokine instructions (signal 3). These signals are reinforced by cytokine release from Th cells themselves (feed-back positive loops); the cytokines instructions and master transcription factors are shown for each T helper subset.

MHC and costimulatory molecules and secretion of inflammatory cytokines) and increases the strength of cognate T-cell signal recognition [62]. Alternatively, naïve T cells that come in contact with their cognate MHC Ag on an immature APC, in the absence of costimulation, become non-responsive or anergic to cells expressing this MHC Ag [63].

The cytokine milieu and Th-cell polarization

It is well accepted that the local cytokine milieu influences T-cell polarization and lineage differentiation. The signature signal transducer and activator of transcription (STAT) molecules, which sense the extracellular cytokine environment, thus dictate the expression of master transcription factors by T cells (Figure 2.3), their cytokine signature, and the type of pathogens controlled, while silencing other T-cell lineages with inappropriate cytokine expression.

Specifically, Th1 cells express the transcription factor T-bet, secrete IL-2, IFN-γ, and TNF and serve to control intracellular pathogens [64]. STAT4 and STAT1 are important signaling molecules which receive signals from IL-12 and IFN-γ to further induce T-bet, and maintain Th1 commitment, and to repress other transcription factors, such as GATA-3, ROR-γt, and Bcl-6, and thus suppress IL-4, IL-17, and IL-21 production, respectively. Th2 cells express the transcription factor GATA3, secrete IL-4, IL-5, IL-10, and IL-13 and control extracellular infections and helminth infestations [65]. STAT3 and STAT6 further promote Th2 cell generation and commitment from naïve T cells, while antagonizing Th1 and Th17 generation. Th17 express ROR-γt and IL-17, and protect against extracellular bacteria and fungi. IL-6 and IL-23 drive Th17 responses by activating STAT3 to induce ROR-γt [66]. T follicular cells (Tfh) secrete IL-21 and are critical for providing B-cell help, Ig class switching, and Ab production [67]. They express Bcl-6, the activity of which is regulated by STAT3, IL-6, and IL-21 [68]. Th22 and Th9 are other newly described T-cell subsets whose individual significance is still under debate [69,70].

T-cell subsets that inhibit other T cells' immune responses (both CD8 and CD4 responses) in an Ag-specific manner have been identified in humans and mice [71–74]. These cells are termed regulatory T cells (Treg). Naturally occurring Treg are distinguished by display of the α chain of the IL-2 receptor (IL-2R; CD25) and by the transcription factor, forkhead box p3 (Foxp3), see below under Immunologic Tolerance. A discussion of regulatory T cells in transplantation is found generally in Chapter 5 and specifically in Chapter 8.

T-cell plasticity

The various Th subsets, with their distinct immunologic functions, as discussed above, have been regarded historically as committed "lineages" because they display elements of stability and heredity [75,76]. However, recent reports refute this dogma and provide direct evidence of T-cell subset plasticity, implicating epigenetic modifications responsible for triggering complex transcriptome signatures that allow flexibility in T-cell phenotypes [77]. Thus, GATA3/Th2 cells for example could be reprogrammed to adopt a GATA3$^+$/T-bet$^+$ and IL-4/IFN-γ phenotype in a model of lymphocytic choriomeningitis virus (LCMV) infection [78]. RORγt/IL-17 cells can be converted to RORγt$^+$/T-bet$^+$ and IL-17$^+$/IFN-γ$^+$ producers in a model of autoimmunity [79]. Likewise, IL-21 and Bcl-6 expression appears not to be restricted solely to Tfh, because these cells can also secrete Th1,Th2, and Th17 cytokines [80]. The plasticity versus stability of these T-cell subsets remains to be further elucidated given increasing knowledge of the close relationship between transcription factors and the state of cell chromatin and gene expression, as reflected by downstream cell effector function.

B-cell immunobiology

In mammals, there is no single discrete organ of B-cell development that functions as a homolog of the thymus for T cells. Instead, B cells develop at multiple sites, including the liver, BM, and spleen. Although their immunological functions are quite distinct, there are many similarities between T and B cells, including development, receptor structure, tolerogenic mechanisms, and signaling. One of the most important of these is evident from the nomenclature of the gene family to which the TCR belongs, that is the Ig supergene family. Ig genes, expressed exclusively in B cells, are the prototype of a large number of immunologically important genes, including the TCR. Secreted Ig gene products are Abs, the basis for humoral immunity. Membrane-bound Ig gene products are BCR for Ag (BCR), the B cell "equivalent" of the TCR. The processes of B-cell development, selection, and elimination or inactivation of autoreactive cells are very similar to those for T cells and will not be elaborated upon further. There are, however, two important processes that are unique to B cells: isotype switching and affinity maturation or somatic hypermutation. The transplant-specific elements of these processes are highlighted in Chapter 6.

Isotype switching

There are five primary classes (isotypes) of Ig: IgM, IgD, IgG, IgE, and IgA, determined by which constant region is used by the heavy chain, that is the ξ, δ, γ, ε, or α segments, respectively. Mature naïve B cells express both IgM and IgD on their surface, the only instance in which B cells express more than one Ig isotype. Both IgM and IgD function as BCR for Ag. After activation, B cells undergo a process of isotype switching to either IgG, which has several subclasses (IgG1, IgG2a/c, IgG2b, IgG3 in mice and IgG1, IgG2, IgG3, and IgG4 in humans), IgE, or IgA as part of further differentiation and expansion.

Somatic hypermutation and affinity maturation

Following immunization with an Ag, B cells undergo somatic point mutations in their Ig genes. The purpose of somatic mutation is to refine the Ig repertoire further. The mutation process is essentially random; therefore, some mutated Ig genes have unchanged Ag affinity, while some have higher, and others have lower Ag affinity. Since B-cell expansion, maturation, and survival are dependent upon continued Ag stimulation, those B cells whose somatic mutations have led to high-affinity Ig genes will have a selective survival advantage. The result is affinity maturation during the immune response.

B-cell activation

T-independent Ags

In general, B-cell responses to Ag require T-cell help (see below). However, a few types of Ag (i.e. those containing repetitive epitopes) are highly efficient at cross-linking membrane Ig (the B-cell receptor for Ag). These Ags, most commonly bacterial capsular polysaccharides, deliver such potent signals to the B cells that, unlike most protein Ags, T cells are not required. Therefore, these Ags are termed T-independent Ags.

Cognate interactions provide costimulatory signals

Most B cell Ags are probably unable to cross-link Ig extensively and, therefore, activate B cells by themselves. They depend on a process known as T-cell : B-cell cognate help. In this instance, the Ag binds to the B-cell surface Ig, is internalized, and is presented to $CD4^+$ T cells on B-cell MHC class II molecules. The activated T-cell : B-cell contact initiated in this way also allows the B-cell surface receptor CD40 to bind to its ligand on the T cell (CD40L, CD154). Signals through CD40 then act in concert with T-cell-derived cytokines to activate the B cell.

Ab secretion

Following appropriate activation and signaling, previously naïve B cells secrete IgM and proceed in one of two directions: development into memory cells or differentiation into Ab-secreting plasma cells. The latter fate is accompanied frequently by somatic mutation and isotype switching to IgA, IgG, or IgE, although some plasma cells do not class switch and secrete IgM. This process occurs over a period of 2 to 3 weeks, so that the initial Ab response in a naïve animal is IgM, with subsequent maturation to IgG and IgA. Abs can function in several different ways, varying by isotype. These mechanisms include fixation of complement (see below), opsonization or phagocytosis by fragment crystallizable receptor $(FcR)^+$ cells, opsonization for lysis by cells capable of Ab-mediated cellular cytotoxicity (whereby innate effector cells kill cells coated with Abs when activated through a receptor for the Ab), and induction of eosinophil degranulation.

The complement system

The complement system (Figure 2.4) is a complex system of proteolytic enzymes, regulatory proteins, and lytic proteins directed at

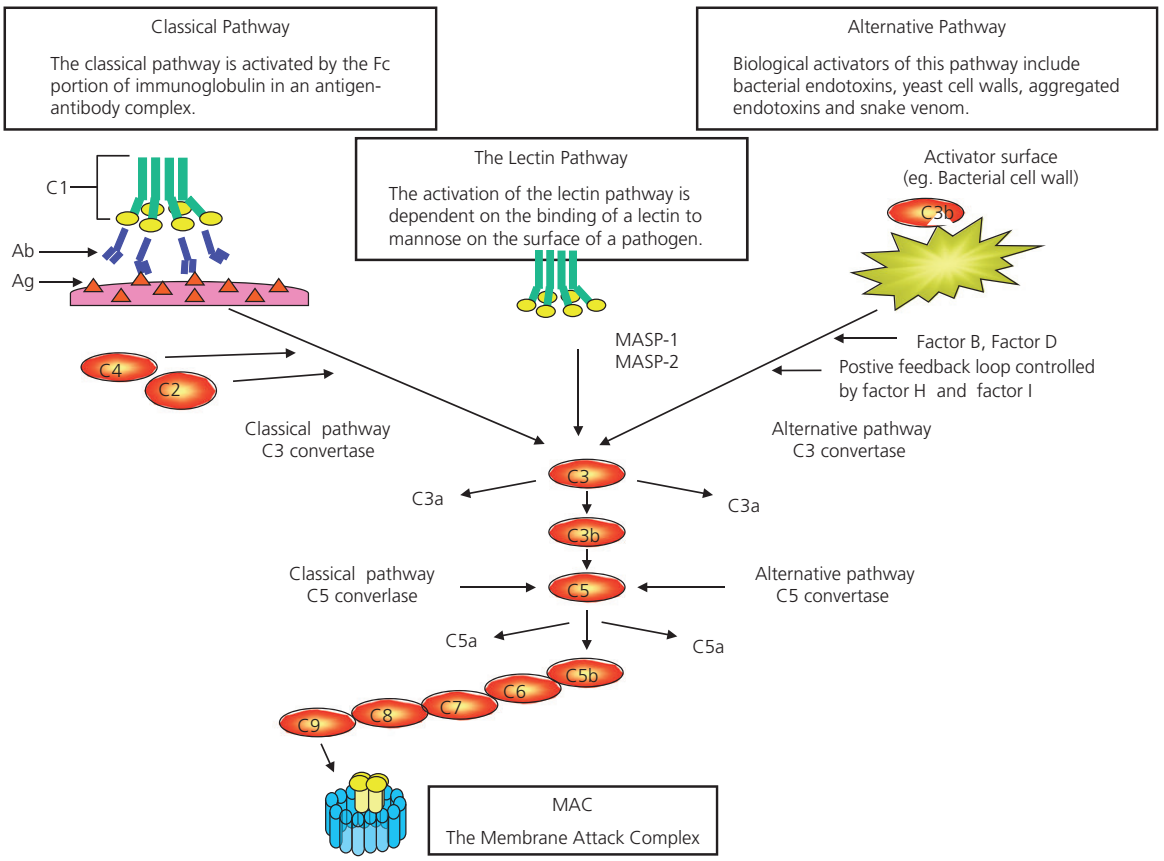

Figure 2.4. Pathways of complement activation. Complement activation is mediated by three pathways: the classic pathway, which is initiated by antigen–antibody complexes, the alternative pathway, and the lectin pathway. All pathways converge on the activation of C3 convertase, and end with the formation of membrane attach complex (MAC), which mediates cell lysis by breaking down cell membrane.

target cells [81,82]. It can be divided into three components, leading to the activation of C3, which is critical for opsonization of bacterial Ags. The split fragment C3b activates a membrane attack complex (MAC) (C5, C6, C7, C8, C9) which causes membrane damage, leading to osmotic lysis of target cells [83].

The three activation pathways are named the "classic," the "lectin binding", and the "alternative" pathways. The classic pathway of complement activation involves binding of the first component of complement (C1), to aggregated IgM or IgG Abs in the form of an Ag–Ab complex. Activated C1 is a proteolytic enzyme; the C1 esterase is capable of cleaving the next two components of the classic pathway (C4 and C2) into several fragments. The complex of C4b with C2a is a serine protease capable of cleaving C3, releasing C3a and C3b. The lectin pathway uses mannose-binding lectin (MBL)-associated serine proteases (MASP) to cleave C4b with C2a that, in turn, splits C3 into C3a and C3b. C3 can also be cleaved by a convertase that develops from the alternative pathway, which can be initiated not only by Ag–Ab complexes but also by bacteria, LPS, and many other activating surfaces. In this pathway, factor D is capable of hydrolyzing another factor, factor B, only after the latter has been bound by the C3b fragment of C3. This leads to the C3bBb complex, which is an alternative pathway C3 convertase. C3b is recognized by receptors on various cell types, including macrophages and B cells. Binding of C3b to Ab-coated bacteria is an essential step for the phagocytosis of these agents by macrophages bearing receptors for C3b. On the other hand, C3b is critical for the engagement of the terminal components of complement (C5, C9) to form the MAC, which causes cell lysis. The C5b–C9 is responsible for the complement lesion in membranes.

In addition to the role of the complement system in opsonization and as a lytic material, several of the complement fragments (C3a, C5a) formed during activation are potent mediators of inflammation. C3a binds to receptors on mast cells and basophils and induces the release of histamine and mediators of anaphylaxis. C5a is also a chemotactic attractant for neutrophils and monocytes [84]. Further information about the role of complement in transplant rejection can be found in recent reviews [85,86] (see Chapter 7).

Cytokines

Cytokines are peptides, produced by all nucleated cells, that exert pleiotropic regulatory effects on hematopoietic and other cells that participate in host defense and repair. They include lymphocyte-derived and monocyte-derived factors, hematopoietic growth factors (colony-stimulating factors; CSF), connective tissue growth factors, and chemotactic factors (chemokines). Extensive details of cytokine biology and their applications can be found in [87]. Most cytokines act locally, over short distances, as autocrine or paracrine intercellular signals, and, with few exceptions, reach the circulation and initiate systemic responses only occasionally. Cytokines generally are not produced constitutively, but are generated in response to danger signals to offset challenges to host integrity. Their functions maintain homeostasis by regulating innate host defenses and the immune system, through damage control, and by promotion of tissue repair. Cytokines are very potent and active at pM to nM concentrations. Their characteristics are summarized in Table 2.1.

Whereas many cytokines are now termed interleukins (IL) (e.g. IL-1 was formerly monocyte-derived lymphocyte-activating factor), others retain their older names (e.g. IFN-α/β, TNF, lymphotoxins (LT-α and LT-β), transforming growth factor (TGF)-β, leukocyte inhibitory factor (LIF), most CSFs and many others). The "older" names suggest only one (usually the first recognized) function of these pleiotropic agents. The "IL" terminology accommodates broader roles and, as the number of ILs has increased, now up to 35, there has been explosive interest in the molecular biology and function of these mediators of inflammation and immunity. Structural features shared by certain cytokines has allowed their grouping into "families" (Table 2.2), such as the TNF family of cytokines and the IL-1 and TGF-β families. Numerical designations have been approved for the four subfamilies of chemotactic cytokines (chemokines) and chemokine receptors (see Table 2.1) consisting of the letters CXC, C, CX3C, and CC, plus a consecutive number.

Table 2.1. Characteristic features of cytokines

- Most are simple polypeptides or glycoproteins ≲30 kDa
- Constitutive production is low or absent; production is regulated by inducing stimuli at the level of transcription or translation
- Cytokine production is transient and typical action is autocrine or paracrine, not endocrine
- Cytokines bind to specific, high-affinity cell surface receptors (Kd of 10^{-9}–10^{-12} M)
- Most cytokine actions result from an altered pattern of gene expression in target cells. Cytokine actions lead to increased (or decreased) cell proliferation, changes in differentiation state, and/or a change in function(s)
- While individual cytokine actions can be quite broad and diverse, at least some action(s) of each cytokine is (are) targeted at hematopoietic cells

Table 2.2. Shared structural features of cytokines permit their grouping into families

Family	Representative members
IL-2/IL-4	IL-2
	IL-4
	IL-5
	GM-CSF
IL-6/IL-12	IL-6
	IL-12*
Interferons-α/β	IFN-α (many subtypes)
	IFN-β
	IFN-ω
	IFN-τ
Tumor necrosis factors	TNF-α
	LT-α (TNF-β)
	LT-β
	Fas ligand
	CD40 ligand
	TRAIL
IL-10	IL-10
	IL-19
	IL-20
	IL-22
IL-17	IL-17
	IL-25
IL-1	IL-1α
	IL-1β
	IL-1R antagonist
	IL-18
TGF-β	TGF-β
	Bone morphogenetic proteins
	Inhibins
	Activins
Chemokines	CXC subfamily (CXCL1-16)
	CC subfamily (CCL1-28)
	C subfamily (CL1/lymphotactin)
	CX3C subfamily (CX3CL1/fractalkine)

*IL-12 is a heterodimer in which one subunit is structurally-related to IL-6, and the other subunit shows partial homology to the extracellular domain of the IL-6R (α chain).
TRAIL, TNFR apoptosis-inducing ligand; LT, lymphotoxin.

Structural features and categories (families) of cytokines

Some families include proteins whose primary sequences show a high degree of homology, for example all IFN-α/β family members show at least 30% homology in their amino acid sequences. In other instances, the structural relationship is more distant and based mainly on a common proposed tertiary structure and spatial organization. An example of the latter type of structural relationship is the IL-2/IL-4 family. Members of this family contain four α-helical regions in a spatially similar arrangement. Despite the identification of many structurally distinct cytokines, they can be organized into groups with functional similarities based on shared receptor (R) usage. This applies to IL-1α and IL-1β, as well as to TNF and LT, which use shared and unshared receptors. Receptor chains are shared by cytokine groups. For example, the IL-2γR chain is shared by IL-2, 4, 7, 9, 15, and 21. The gp130 chain of the IL-6R is shared by IL-6, IL-11, LIF, and other cytokines. TNF family members share receptors with homology to the TNFR. These include TNF, LTα, LTβ complex, Fas ligand, CD70, CD40L, CD30L, nerve growth factor, and more. A receptor β chain is shared by IL-3, IL-5, and granulocyte–macrophage (GM)-CSF. Homologous G-protein-coupled receptors are used by IL-8 and chemokine family members. Receptors for IFN-α, β, ω, γ, and IL-10 also exhibit homology, as do those for the TGF-β family. Because IL-12 and IL-18 have overlapping activities, their receptors may also be related.

The importance of cytokines

Cytokines are the principal orchestrators of defense processes and, as such, play key roles in host responses to exogenous and endogenous insults, repair, and restoration of homeostasis. They mediate host responses to tissue injury, invading organisms, tumors, and to cell and tissue transplants. Importantly, microbial pathogens can produce molecular variants of cytokines (e.g. viral IL-10), their receptors, and chemokine antagonists which subvert host inflammatory and immune defenses. Deletion of these products reduces the pathogenicity of these viruses. The development of highly sensitive methods of detection has revealed the presence of detectable, but low levels of cytokines associated with a variety of binding proteins in the serum. This probably reflects the production of cytokines in response to the many non-pathogenic stimulants present in the normal environment. Detection and measurement of cytokines in disease states may provide useful diagnostic tools. Moreover, the therapeutic administration of pharmacological doses of cytokines is employed in a wide variety of infectious diseases, autoimmune disorders, and malignancies. This has also prompted the evaluation of cytokine antagonists for their anti-inflammatory properties (e.g. TNFR).

Synergistic and antagonistic interactions

Actions of cytokines are contextual and can be influenced profoundly by the milieu in which they function and, in particular, by the presence or absence of other biologically active factors (i.e. other cytokines, hormones, growth factors, prostaglandins, microbial products, etc.). Under normal conditions a cell rarely, if ever, encounters only one cytokine at a time. Thus, a cell is more likely to be exposed to multiple cytokines and other biologically active factors, resulting in biological actions that reflect various synergistic and antagonistic interactions of various factors present. Table 2.3 summarizes typical features of cytokine actions.

Table 2.3. Characteristic cytokine actions

Action	Features
Pleiotropy	Multiple target cells and actions
Redundancy	Distinct cytokines have similar actions
Synergism/ antagonism	Exposure of cells concurrently to >1 cytokine may lead to qualitatively different responses (e.g. synergy between IFN-γ and TNF-α)
Cytokine cascade	A cytokine may increase (or decrease) production of another cytokine
Receptor transmodulation	A cytokine may increase (or decrease) the expression of receptors for another cytokine or growth factor
Receptor trans-signaling	A cytokine may increase (or decrease) signaling by receptors for other cytokines or growth factors

Stimulatory and inhibitory properties of cytokines

An important characteristic of cytokines is their ability to stimulate or inhibit the production of other cytokines. Consequently, many cytokine actions are indirect, that is they are due to an increase or decrease in production of other cytokines, resulting in an altered biological response. An example of such an indirect action is that the mitogenic action of IL-1 in mouse thymocytes involves stimulation of IL-2 production, and that IL-2 is the actual effector molecule responsible for stimulation of thymocyte proliferation. The stimulatory effect of IL-1 on IL-2 production and the role of this cytokine interaction in T-cell proliferation typify the actions of many other cytokines. Another example is the stimulation of IFN-γ production by IL-12. IL-12 that is produced by activated APC induces IFN-γ production in T cells and NK cells and is a major regulator of IFN-γ production. IL-25 stimulates IL-4, IL-5, and IL-13 production in T cells.

Although less numerous than reports of their stimulatory interactions, cytokines can also inhibit cytokine production. Thus IL-10 inhibits cytokine production by Th1 cells and by monocytes/macrophages/DC. Many of the anti-inflammatory and immunosuppressive actions of TGF-β are also due to its ability to suppress cytokine production in T cells and APC. A good example of stimulatory and inhibitory interactions involving multiple sets of cytokines is the development of polarized Th1 and Th2 responses. Thus Th1 cells secrete IL-2 and IFN-γ and are efficient in activating cellular immune responses that enhance the elimination of intracellular pathogens. By contrast, Th2 cells secrete IL-4, IL-5, IL-10, and IL-13, which promote Ab responses, especially IgE production, leading to allergy. A major player in these processes is IL-12, which promotes IFN-γ production and Th1 development.

Redundancy and pleiotropy

Extensive redundancy and pleiotropy in cytokine actions can make it difficult to predict the uniqueness and necessity for individual cytokine actions in vivo. Determining how cytokines function in the intact host has been aided greatly by the development of the technique of targeted gene disruption. However, a great deal still remains to be learned about the complex interactions that eventually determine the outcome of cytokine actions in the complex in vivo environment.

Immunological tolerance

T and B cells are highly effective in defending the host from invasion and colonization by pathogens; however, they can also attack the host. This risk arises during ontogeny, from the mechanism

used to generate T- and B-cell Ag receptors (TCR, BCR). As described above, these receptors are generated by joining V, D, and J segments at the TCR and Ig loci. The emerging repertoires contain unpredictably diverse V segments and include receptors capable of binding to Ags from self-proteins. Multiple checkpoints are needed to prevent either the generation or functional activity of autoreactive lymphocytes [88]. Furthermore, as lymphocytes are generated continuously throughout life, there is a life-long need for these controls.

Although tolerance-promoting mechanisms are tightly integrated and often act at the same time, they can be categorized in two major groups: (1) checkpoints that control the development of lymphocytes—commonly referred to as "central tolerance," and (2) checkpoints that influence the activity of fully developed lymphocytes, or "peripheral tolerance." Understanding the mechanisms of tolerance is key to understanding how to treat and monitor transplant recipients, and a thorough discussion of these mechanisms as they relate to transplantation tolerance is found in Chapter 11. This is particularly important when considering that some immunosuppressive drugs, while blocking lymphocyte activation, also inhibit intrinsic programs of tolerance induction. Thus, careful consideration must be afforded to their optimal application.

T-cell central tolerance

During intrathymic T-cell development, the spontaneous rearrangement of TCR chain segments is halted only when the newly formed TCR binds to an intrathymic ligand [89]. Stronger and/or longer positively selecting signals generated as a result of co-ligation of the TCR and the CD4 co-receptor results in instruction of CD4 helper lineage fate, while weaker and/or shorter signals initiated by the TCR and the CD8 co-receptor result in CD8 cytotoxic lineage fate determination. Very strong signaling, however, results in apoptosis of the engaged thymocytes—commonly referred to as "negative selection." Deletion of thymocytes is based on the Ags present in the thymic medulla, raising the issue of how tolerance to tissue-specific Ags occurs. Presentation of peripheral tissue-specific Ags within the thymus is made possible either by immigration of peripheral APC (DC and B cells), in addition to promiscuous Ag expression by thymic epithelial cells facilitated by the recently discovered transcription factor Aire (autoimmune regulatory) expressed in the thymic medulla.

Not all thymocytes that recognize self-Ag with high avidity are deleted. Engagement of TCR ligands with high affinity can induce the generation of Treg that play an essential role in maintenance of self-tolerance at the level of lymphocyte activation (see below). This is one point where the distinction between central and peripheral tolerance becomes indistinct, complicating our understanding of the contribution of each individual mechanism.

T-cell peripheral tolerance

Autoreactive T cells can escape negative selection when their TCR is of sufficiently low avidity for self-proteins, or when they bear TCR with high avidity for tissue-restricted Ags that are not expressed in sufficient amounts in the thymus. For these T cells, immune tolerance must rely on peripheral mechanisms [90].

Ignorance

One barrier to self MHC complex recognition is the physical separation of potentially autoreactive T cells from the parenchymal cells that express the target Ag. Naïve T cells circulate from blood to secondary lymphoid organs, to efferent lymph, and then back again to the blood. Thus, naïve T cells are excluded from non-lymphoid peripheral tissues, where the likelihood of coming in contact with a tissue-resident cell expressing a high density of a tissue restricted Ag is higher. These specific trafficking patterns of naïve T cells promote a state of "ignorance" (unawareness) to tissue-specific Ag.

Deletion and anergy

As indicated earlier, activation of T cells requires the integration of signals generated through the TCR and costimulatory molecules engaged in the interaction with mature APC. This requirement enacts an important checkpoint for the maintenance of tolerance [91,92]. When a T cell recognizes a target Ag in the absence of costimulation, the engaged T cell initiates an abortive activation process that results in the deletion of most of the daughter cells. This takes place through activation of a suicide program incorporated in the T-cell differentiation mechanism, which is commonly referred to as apoptosis. Interestingly, not all reactive cells that encounter their Ag in the absence of costimulation are eliminated. During abortive T-cell activation, the remaining cells enter a state of functional inactivity defined as "anergy." Similarly to apoptosis, anergy is a program enforced through a delicate balance between activation and inhibition of specific genes. The main characteristic of anergic cells is their inability to respond to subsequent engagement of the TCR with the same Ag, even when this new encounter occurs in the presence of costimulation. Although the advantage of the apoptotic program is evident, the benefit of maintaining circulating anergic cells is still under investigation. It is believed to provide two main advantages; first, it guarantees the existence of cells able to recognize a different Ag (pathogenic) with sufficient affinity to allow reversal of the anergic state, thus contributing to the maintenance of a wide repertoire of patrolling cells. At the same time, the anergic cells could join the pool of regulatory cells that control the activation of other autoreactive lymphocytes, as indicated below.

Regulatory T cells

As mentioned briefly in the previous sections, T lymphocyte development is characterized not only by the selection of potential effector cells that will attack their target, but also by T cells with the ability to regulate the activation of the immune system, so-called Treg (also see Chapter 8) [93]. First recognized as $CD25^{+}CD4^{+}$ T cells, and later shown to specifically express the transcription factor Foxp3, these cells are naturally present as a functionally distinct T-cell subpopulation, and their deficiency produces autoimmune disease. Evidence is now accumulating that Treg buffer almost every adaptive immune response, both physiological or pathological. The thymus of an adult animal produces natural Treg continually. Their selection appears to be induced on high-affinity, self-reactive TCRs, although more understanding is needed. Once in the circulation, these cells are programmed to control immune reactivity in every circumstance where self-Ags are involved. This is achieved through multiple mechanisms [94]. These range from the ability of Treg to interact with APC and reduce their presenting and T-cell stimulatory function, to active inhibition of the activation and proliferation of naïve T cells, as well as control of the damaging function of fully differentiated T effector cells. Several mechanisms of suppression may operate synergistically, and in a complementary manner.

The involvement of Treg in almost every immune response is more easily understood when it is considered that low-level

autoimmunity may occur frequently as a result of tissue damage due to microbial infection and consequent activation of DC that present self-Ag to self-reactive T cells. Treg are automatically recruited to sites of inflammation, activated, and able to suppress autoreactive T cells and thus prevent the progression of autoimmunity to chronic autoimmune disease.

In addition to thymic production of natural Treg, naïve T cells in the periphery can be induced to upregulate Foxp3 expression and consequently acquire Treg functions. There is accumulating evidence that such induced Treg are present at least in the intestine. Although it remains uncertain to what extent such induced Foxp3$^+$ Treg contribute to systemic self tolerance, it is reasonable to speculate that they play complementary roles and that deficiency of both natural and induced Foxp3$^+$ Treg is responsible for severe autoimmune diseases.

Another mechanism contributing to the decision between tolerance and immunity is the capacity of B cells to act as regulators rather than effectors [95,96]. Although the first description of regulatory B cells (Bregs) dates back many years, they have recently re-emerged in the form of a population of IL-10-secreting B cells in mice, with a comparable population in humans that exhibit the capacity to control both T-cell- and B-cell-mediated responses.

B-cell central tolerance

B cells belong to three distinct lineages: B1a, B1b, and B2 cells. B1 cells respond in a T-cell-independent manner, and seem essential for the production of Abs against some pathogenic bacteria and parasites that require an innate-like response. Conversely, B2 cells are activated through T-cell licensing in germinal centers and are required for adaptive immunity. These complementary activities underscore the need for multiple mechanisms acting at different stages of B-cell development and activation to control the possible production of self-reactive Abs [89,97].

It is estimated that more than half of all newly generated BCRs are capable of binding self-Ags. However, not all self-Ag-binding BCR are necessarily detrimental to the organism. For example, a certain proportion of BCR may bind self Ags with too low an affinity to trigger an autoimmune response, while they may bind strongly enough to Ags derived from invading pathogens to exert a protective host defense effect. This further underscores the complexity of the process of purging or controlling specifically those autoreactive B cells that are harmful.

Mature B1 and B2 cells emerge only after passing through checkpoints, several of which enforce self-tolerance through assessment of Ag avidity (affinity and density) and the signaling threshold of the BCR. During the formation of a pre-B cell in the BM (or fetal liver), a significant number of cells are blocked in their development. It is still unclear how much of this negative selection of pre-BCRs is mediated by binding of self Ag and how much is due to lack of formation of a functional receptor. Unfortunately, almost nothing is known about the self-Ag-presenting modes that establish central B-cell tolerance. In human B-cell development, some, but not all, autoreactive cells are lost during the transition from pre-B to immature B cells, defining an additional checkpoint for self-reactivity that needs to be better elucidated. Less controversy exists for immature B cells (confirmed in both rodent and human systems). At this stage, autoreactive BCRs can be purged by "receptor editing," a process through which Ag binding induces continued rearrangement of Ig gene segments. This process results in a change in the specificity of the autoreactive population, as it is also paired with the clonal deletion of B cells that retain their self-reactivity.

B-cell peripheral tolerance

The existence of checkpoints during B-cell development in the BM allows approximately 10% of all newly generated immature B cells to emerge from the BM as transitional (T1 then T2) cells. Although fairly efficient, these mechanisms do not remove all potentially autoreactive B cells, as these transitional cells migrate to the spleen, where they may encounter peripheral self-Ags absent in the BM. High-avidity interactions with these Ags lead to rapid deletion of B cells at the T1 stage. By contrast, low or very low avidity interactions result in the induction of anergy. This state is characterized by desensitization of BCR signaling, resulting in decreased responsiveness, failure of Ag-presenting function and Ab-producing capabilities, and an inability to compete for limiting amounts of B-cell survival factors. Furthermore, the remaining transitional B cells that interact with moderate intensity to either self or foreign Ags relocate, similarly to mature naïve B cells, to the T-cell zone in search of help. In the absence of T-cell help (or alternative costimulatory signals), these stimulated transitional B cells die within 2–3 days, irrespective of their specificity.

Differently from T cells, B cells, once mature, can further expand their BCR repertoire of specificity through somatic hypermutation of Ig variable region genes within germinal centers following Ag stimulation. This leads inevitably to the appearance of cells expressing antiself receptors and requires additional control mechanisms. The environment of germinal centers plays a pivotal role in preventing the development of a self-reacting repertoire among mature naïve B cells. This represents a microenvironment in which hypermutating B cells are positively selected on the basis of affinity, but negatively selected against self-reactivity due to local competition for Ag presented on follicular DC and access to help provided at this site by Tfh cells. Similarly to transitional B cells, germinal center B cells are deleted following Ag exposure if they do not receive survival signals from Tfh cells.

Immunological memory

When the immune system is challenged a second or subsequent time by the same foreign material, the response is generally characterized by faster kinetics of lymphocyte activation (both of T and B cells) and elimination of the foreign material, a phenomenon referred to as immunological memory [98].

T-cell memory

As described earlier, a productive interaction between a T cell and an APC triggers the former to divide and become an effector cell. Under physiological conditions where these effectors (a blend of CD4 T subsets and CD8 cytotoxic T cells) execute a rapid clearance of the triggering Ags, the number of effector cells peaks about a week into the response. About 90% of these effector cells then die during a 1- to 2-week-long contraction phase, which leaves a residual population of long-lived memory T cells. These memory cells are phenotypically and functionally distinct from naïve T cells, the main characteristic being a lower activation threshold that allows them to respond rapidly upon restimulation [99]. There is recent recognition that the distinction between naïve and memory status has marked implications with regard to a cells capacity for alloreactivity, and this is covered in depth in Chapter 9. Memory cells are heterogenous however, and are thought to exist in at least two classes [100]: effector memory T cells (Tem cells) and central memory T cells (Tcm cells). Tem cells express homing receptors that facilitate migration to non-lymphoid sites of inflammation and

produce a variety of microbicidal cytokines within several hours of TCR stimulation. Conversely, Tcm cells do not produce prototypic "effector" cytokines immediately after stimulation through the TCR, although they do secrete IL-2 and proliferate extensively and acquire effector cytokine production later. Additionally, these cells express receptors (e.g. CD62L and CCR7) that promote migration to lymph nodes and mucosal lymphoid organs. It has therefore been postulated that Tcm cells circulate to these locations and probably therein undergo secondary responses representing a source for new Tem cells.

These characteristics indicate that the generation of Tem and Tcm cells confers a qualitatively and quantitatively distinct immune response upon successive (but interrupted) exposures to Ag. This leads to an improved secondary immune response compared to the primary response, one that is greater in magnitude, faster, more sensitive to low doses of Ag, and more effective in the diversity or complexity of secondary effectors.

B-cell memory

As discussed above, B-cell activation comprises a process of selection in the germinal centers (influenced by interactions with APC and by the environment defined by activated T cells) that results in "affinity maturation" of the responding cells. After this process, some B cells differentiate into Ab-secreting plasma cells, while others become memory B cells (Bmem) [101,102]. These Bmem stop dividing and enter a quiescent state that allows them to persist for a long time, the entire life of an individual in some instances. Similarly to the memory response of T cells, the existence of Bmem confers different characteristics on a secondary humoral response. This response develops in a shorter time (approx 3–5 versus 7–10 days in a primary response), is typified by a greater amount of Ab production and lasts for a longer period. Additionally, the secondary response is characterized by secretion of Ab with higher affinity for the Ag (due to the initial affinity maturation process) and by the presence of isotypes other than IgM (mainly IgG, IgA, and IgE) that are better suited to respond rapidly to the multitude of Ags associated with a pathogen previously encountered and to serve different anatomical sites.

Summary

Immunology in general forms the basis for understanding how one exists as an individual within a sea of pathogens and threats to one's homeostatic integrity. While this is fundamental to the maintenance of health, it runs counter to the practice of organ sharing, and, as such, is integral to the requirements for immune modulation that result in the successes and challenges of modern transplantation. Throughout this text, the reader should recognize that the numerous interconnected elements of physiologic immunity that require constraint to allow for allograft survival, evolved first as important means of self-preservation. As such, the transplant researcher and clinician should be cognizant of the consequences of excessive suppression of immune function, and strive to work within the confines and elegance of the immune system.

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CHAPTER 3

Transplant Antigens: A Brief History of HLA

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Introduction

One of the most significant discoveries relevant to the practice of organ transplantation was the elucidation of the major histocompatibility complex (MHC), known in humans as HLA and mapped to chromosome 6. Histocompatibility typing, essentially defining the content of the MHC for a donor and recipient pair, has been an established practice in transplantation since its methodology was established, and alloimmune responses are now accepted to be largely responsive to polymorphisms mapped to this region. Indeed, the development of alloantibodies toward HLA polymorphisms is a fundamental metric used to establish organ allocation, and is increasingly recognized as an indicator of ongoing alloimmune destruction of a transplanted organ. This chapter describes the major events culminating in the modern understanding of HLA, and will be useful to the reader in orienting to the concepts of transplant biology in general. It specifically serves as a companion to the broader history of clinical transplantation found in Chapter 1, and to the specific chapters on modern histocompatibility lab and alloantibody testing in Chapters 36 and 89, respectively.

Early investigations

The advent of clinical kidney transplantation in the late 1950s drove the development of knowledge of HLA. When Joseph Murray and David Hume boldly transplanted kidneys in humans [1,2], relatively little scientific support existed for such human experimentation. Skin grafts with animals [3] and kidney transplants in dogs [4] had survived only a matter of weeks. Studies in mice by Peter Gorer and George Snell [5] had shown that a histocompatibility system existed, and that it controlled whether tissues could be grafted from one animal to another. It was called the "H-2 system," and there was general agreement that a similar system probably existed in humans. The challenge then was to identify this human histocompatibility system.

After discovery of the ABO system [6], how antigens of the red cell could be detected was well known. Researchers then turned to white cells as the best avenue for isolating the factors of histocompatibility in humans. Because transfusion reactions in patients who had been multiply transfused suggested that antibodies were involved, Peter Miesher [7] and others used techniques borrowed from work with red cells—mainly agglutination—to begin studies on leukocyte antigens. Jean Dausset, working at a blood bank in France, found antibodies in three patients that reacted similarly. The discovery led him to postulate the first leukocyte antigen group, which he called MAC [8]. Johannes van Rood, also working at a blood bank, but in Holland, studied leukoagglutinating antibodies in pregnant women, searching for specificities [9]. From the outset, the problem was the unreliability of the agglutination test because of difficulties in handling granulocytes. To resolve the variability problem, Van Rood came up with the idea of using computers to sort out complex reactions, resulting in the identification of the 4a and 4b specificities [10]. At about the same time, Rose Payne at Stanford—also working on leukoagglutinating antibodies in pregnant women [11]—collaborated with Walter and Julia Bodmer to find two antigenic groups, which they called LA1 and LA2 [12].

Recognizing that we researchers needed to agree on methods used for tissue typing, the first histocompatibility workshop was organized at Duke University by Bernard Amos in 1964 to compare methods [13]. Five labs used the leukoagglutination test, and one or two labs demonstrated tests by mixed agglutination, complement fixation, indirect antiglobulin consumption, mixed lymphocyte culture, and the microlymphocyte cytotoxicity test [14]. In the second workshop (1965), hosted by Van Rood in Holland, ten labs used leukoagglutination, and only Ruggero Ceppellini and our lab used the microcytotoxicity test (Figure 3.1) [15]. Most important, we were now able to cross-compare results of tissue types identified in the different labs because we all typed the same panel of blood donors. We began to see that we had, among us, identified ten types [15]. Work continued furiously in the various labs, and 2 years later, in 1967, the third histocompatibility workshop was organized in Torino by Ceppellini, with 16 labs participating [16]. We all typed 11 families, making it possible to see for the first time that a *single* system or genetic locus was producing all these reactions.

After the workshop, a WHO conference was held to decide on nomenclature [17]. Amos summarized the wrangles in the committee [18] over whether to call the system "Hu," as Dausset had called his, or "LA," as Payne and the Bodmers had called theirs; and as noted by Amos [18], I suggested "HLA" as a compromise, though at the same time, as part of the compromise, the first antigen of Dausset came to be called HLA 2 and Payne and Bodmer's LA 1 became HLA 1. The association of this nomenclature with the term "human leukocyte antigens" was eventually applied post hoc, but not part of the actual naming convention as it originated.

With 13 antigens identified and now placed in one locus, the principal basis of the HLA system was established in 1967, a bare 3 years after the first workshop. Flemming Kissmeyer-Nielsen provided evidence that there were at least two parts to the system: the A and B loci [19]. At the fourth workshop, at UCLA in 1970,

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

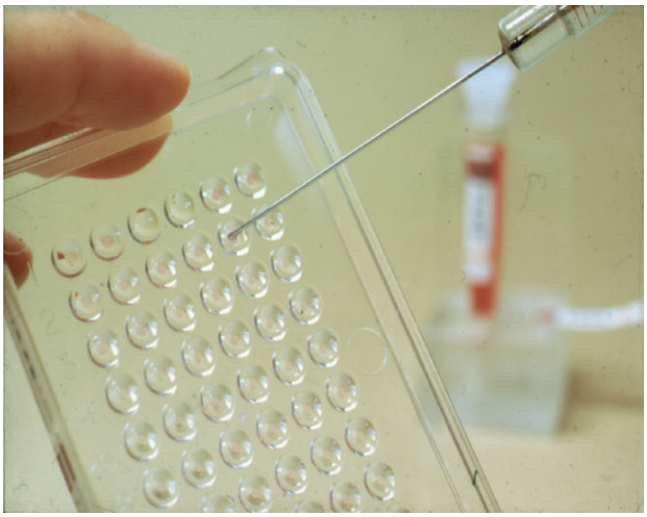

Figure 3.1. The microlymphocyte cytotoxicity test was introduced in 1964. It utilizes 0.0001 mL of antisera, 0.001 mL of lymphocytes, and 0.005 mL of rabbit complement. The drop of oil in each well prevents evaporation and allows the addition of successive reagents into the well without the necessity of removing and adding a cap, because the oil always goes to the top. (From the author's personal collection.)

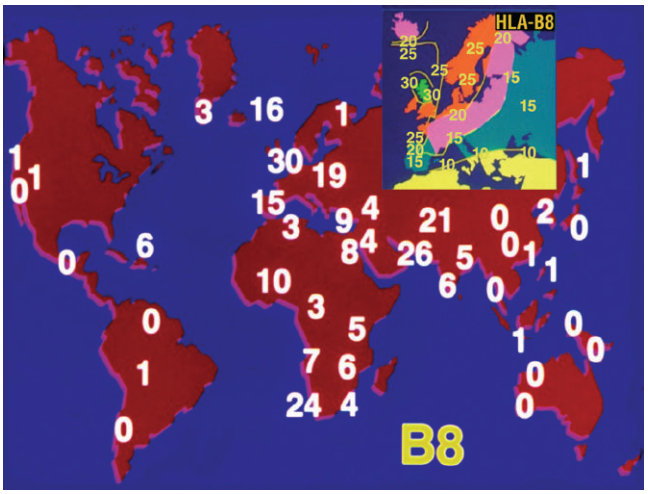

Figure 3.2. The worldwide distribution of HLA B8 antigen. The numbers indicate the frequency within each population. B8 can be seen to be a Caucasian antigen, rarely present in Asia and in American Indians. Even among Caucasians, as shown in the insert, the frequency of B8 shows a gradient in Europe from north to south. (From the author's personal collection.)

the microcytotoxicity test [20] was adopted as the international standard. This method was used at all subsequent workshops. In 1970, 116 sera were exchanged between 15 labs, and, thanks to the microcytotoxicity test, as many as 298 families could be tested. We ended up with 27 specificities [21].

It should be noted that during this time, Baruj Benacerraf was conducting experiments involving the immune response of outbred animals immunized with antigens with restricted heterogeneity, such as hapten conjugates of poly-L-lysine. His observation that the immune response varied and could be mapped to a genetic locus of what he called immune response genes were consistent with the concepts of the MHC.

A major application of HLA for anthropological studies was inaugurated by Dausset at the fifth workshop, in Evian (1975). Typers were assigned various geographic areas, and a world map charting the frequencies of HLA specificities was compiled [22]. Major racial groups could be identified, and racially associated specificities tracked in various national groups. For example, B8, which appears in Caucasians, was shown to vary from 30% in Scots, to 20% in French, 10% in Italians, and 5% in the Caucasians of Middle Eastern countries (Figure 3.2).

This great diversity in human populations led to the use of HLA in paternity testing: the father could almost certainly be identified because the inherited paternal chromosome would be unique [23]. A small tissue-typing triumph occurred when it demonstrated that two non-identical twins had different fathers [24].

HLA C locus antigens were identified at the sixth workshop, in Aarhus [25], and DR locus antigens at the seventh, in Oxford [26]. By the time the eighth workshop, in Los Angeles, was over, 78 specificities had been defined [27]. Since that time, seven more international workshops have been held—one every 2 to 3 years—to further define HLA specificities. The major change during that time was the introduction of DNA technology, which has led to the identification of more than 5000 HLA specificities.

When, at the UCLA tissue-typing lab., we discovered how to keep lymphocytes alive for as long as 2 weeks, we started the International Cell Exchange (1974) [28]. Four cells were sent every month to international laboratories to test blind and report their typing. This aided in worldwide standardization of HLA typing. The monthly cell exchange continues to this day, 38 years later.

In 1980, the Nobel Prize in Physiology or Medicine was awarded to Jean Dausset, George Snell, and Baruj Benacerraf "for their discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions" (Figure 3.3). Ten recollections of the pioneers of HLA appear in *History of HLA*, edited by Terasaki, and published by the UCLA in 1990.

HLA and disease

Patients with almost every known disease have been HLA typed to see if particular specificities are associated with the disease. There was a frenzy of activity in the 1970s, and, by the time we wrote a summary book in 1985, there had been more than 4000 papers published on more than 540 diseases [29]. From this large body of work, little remains of clinical value: only testing for B27 to diagnose ankylosing spondylitis [30,31] is widely used. Narcolepsy and DR 52, DQ1 are as highly associated [32], or perhaps more so, but this association does not seem to be used as much clinically. Autoimmune disease is clearly associated with DR3 and DQ2 [29] but the association is not high enough to be clinically useful. Proof of the idea that HLA is linked to an immune-response gene—and therefore linked to many diseases—has not materialized. It had been proposed that the HLA molecule and the molecule of various infectious diseases contain similar amino acid sequences, but such mimicry of HLA with pathogens has not been clearly shown for any disease. HLA was associated with adverse reactions to abacavir [33], a finding that suggests a different area of HLA application. But, overall, the initial high hopes that HLA

(A) (B) (C)

Figure 3.3. Recipients of the Nobel Prize in Physiology or Medicine in 1980: (A) Baruj Benacerraf, (B) Jean Dausset, and (C) George Snell, "for their discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions." (From the Nobel Foundation: *The Nobel Prize in Physiology or Medicine 1980*. Nobelprize.org. 3 Apr 2013. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1980/)

might be strongly associated with many diseases have been disappointed.

HLA and transplantation

One area of medicine in which HLA was shown to be of great importance was in transplantation. Again at UCLA, we realized that a meaningful compilation of transplant outcomes—to gauge the effect of matching donor–recipient tissue types—could only be accomplished by testing patients from distant transplant centers because only a few transplants were then being performed at each center. Accordingly, from 1964 on, we started a system of mailing blood samples [34] so that transplants as far away as Paris could be analyzed [35]. This was possible because of our use of lymphocytes as targets—lymphocytes being able to survive for several days. This program let us document the effectiveness of tissue typing before other labs. We tested blood from donors and recipients from eight different centers, and, in 1968, first showed that kidney transplants from HLA-identical sibling donors have the highest graft survival [36].

Then we discovered a great anomaly. As we accumulated more and more cases, it became evident that mismatched transplants were doing well, contrary to everyone's expectation. I saw no alternative but to announce this finding at The Hague International Transplant conference in September 1970 [37]. This brought down a firestorm on our heads because, on "scientific" grounds, this could not be correct. Patients doing well *must* have been well matched. The NIH, which was funding our work at that point, put together a large committee to make a site visit within 3 months of the Hague meeting. An account of this is given in Thomas Starzl's book *The Puzzle People* [38]. Our NIH contract was cancelled in 1971, 6 months after the Hague meeting. In addition, Dausset called an urgent meeting of all tissue typers for January, in Paris, to gainsay our Hague statement. Results for the labs were pooled in hope that, collectively, they would show that we were wrong. The analysis was never published—presumably because the data did not support the sought-after refutation. Instead, chapters by the more prominent tissue typers were published in *Transplant Proceedings* volume 3, 1971, with each author suggesting that *something* was wrong with our analysis as emphasized by the lead editorial by Dausset and Rapaport [39]. On the left hand side of Figure 3.4 is the slide I showed at The Hague that caused so much trouble. On the x-axis is the clinical rank of the patients, with A being the best; F = failure and N = non-immunological failure. On the y-axis is the degree of HLA matching, with D being the worst match. As can be noted, many mismatched patients had a good clinical result. The right hand slide in Figure 3.4 shows the results of transplants performed between 1987 and 1995 from the United Network for Organ Sharing (UNOS) Kidney Transplant Registry. The same type of trend is shown, with mismatched patients doing well, and matched patients doing well, but with relatively small numbers.

The tissue-typing community's upset and turmoil seem understandable. It was "obvious" to everyone with a scientific background that HLA mismatching should result in early failures. Just as, earlier, immunologists who could not obtain more than a few weeks survival of animal skin grafts were shocked that Hume and Murray transplanted kidneys in humans, the same basic scientists concluded that it should be impossible to have mismatched transplants doing well. As noted, the HLA system's extreme polymorphism meant that almost all patients were different from their unrelated deceased donors. But Starzl had no inhibitions about flouting basic "scientific" theory. When he saw that mismatched patients were having good results, he decided that HLA matching should not hold him back from doing transplants. Indeed, he has stated that his major effort in liver transplants was made possible only when he decided to ignore HLA typing. He was the first surgeon to take this path; but others soon followed, and HLA typing started to get a bad name.

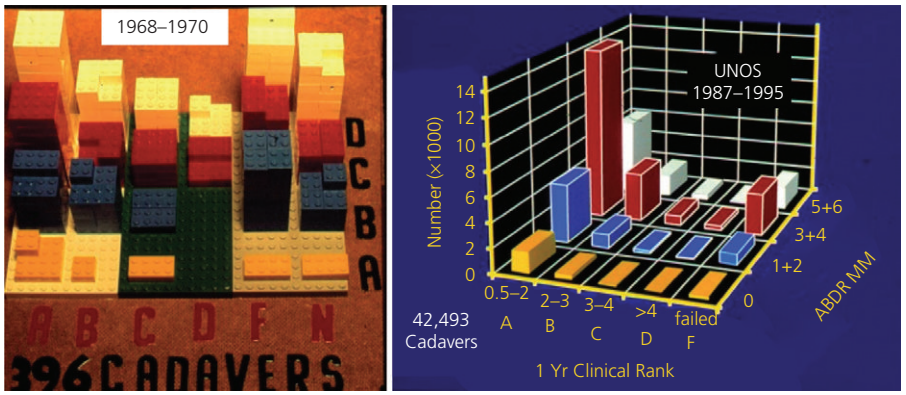

Figure 3.4. The correlation between clinical rank in the x-axis with the match grade in the y-axis is shown here for two periods. The left-hand figure was presented at The Hague International Transplant meeting in 1970. It shows the results for kidneys transplanted between 1968 and 1970. Note that although many badly mismatched transplants (shown in white) did poorly, many mismatched grafts had good results as shown by a clinical rank of A. This same tendency of mismatched transplants doing well is noted in transplants performed 17 years later as shown in the right hand graph. There were very few well-matched grafts in both eras, and they tended to do well. (From the author's personal collection.)

In the meantime, bone marrow transplants began, with the first successful operations relying on HLA typing to confirm the tissue match between the patients and their HLA-identical donors [40]. Donnall Thomas started his program that would encompass large numbers of bone marrow transplants, all based on HLA typing, which, for bone marrow transplants, was essential. But finding donors, other than siblings, was a major problem because the odds were against any given unrelated donor being a close enough match to the patient. The marrow donor program, started in 1987 with help from the Navy, was made into a national program to type the HLA of unrelated volunteer donors and create a registry to find HLA-matched donors for patients. The task becomes herculean as we increased the known number of HLA alleles, currently over 5000 types. Today, there are more than 9.5 million volunteer donors in the National Marrow Donor Program and 16 million donors registered worldwide.

The role of HLA typing for solid organs has been a completely different story. Despite the incipient "bad name" of HLA matching, when mismatches were made to work—thanks to advances in immunosuppression—clinicians recognized that perfect matches worked best [36]. When we found that mismatched patients were doing well, we started a kidney registry in 1971 to see if factors that influence outcomes could be identified. As a concomitant of that purpose, the UCLA registry became one of the most important resources for tracking the effect of HLA matching [41]. To overcome the obvious difficulty in obtaining good matches, we devised a simple cold storage method to send kidneys long distances to matched recipients [42]. This zero-mismatch program was supported by UNOS, and in 1988 the first series of 88 patients who received zero-mismatched kidneys was reported and further extended [43]. Since then, more than 21 000 kidneys have been shipped nationally to zero-mismatched patients using the UNOS system, and in all analyses of the effect of HLA matching, patients with well-matched kidneys have always survived at the highest rate, [44] while those with the largest number of mismatched antigens have generally fared the worst. Nevertheless, as a class, mismatched kidneys have done almost as well as the zero mismatched. Similarly, numerous analyses done of US data show that the difference between the grades of mismatch has not exceeded a few percentage points. This has impelled UNOS to discontinue allocating priority points for A locus matches in 1995, and B locus matches in 2003. The policy changes have not affected the overall outcome of transplants [45].

Currently, then, HLA matching is essential for bone marrow transplants, but not practical for organ transplants. Only by having a national registry and shipping kidneys to distant places has it been possible to obtain well-matched kidney transplants in about 14% of deceased-donor grafts in the USA.

HLA antibodies

HLA antibodies took a trajectory different from that of HLA matching. As noted earlier, only with antibodies was it possible to define the HLA system. However, aside from being used as reagents, antibodies have increased in importance in assessing the result of transplants. Antibodies in the recipient directed against the donor's antigens were found to cause hyperacute rejection of kidneys [46]. Moreover, antibodies present in patients before transplantation could be used to define a state of presensitization, with a higher risk of early failure [47]. It was important to determine the specificity of the antibodies so that donors having those specificities could be avoided. The method used for over 30 years was to test the serum with a panel of pretyped cells. From the reactions, it was possible, with the aid of computers, to determine the specificity of the antibodies. The problem was that because each cell has A, B, C, and DR antigens, it was difficult to determine which of the antigens had reacted to the antibody—resulting in inaccurate assignments. Only with the advent of recombinant cells has it been possible to coat beads with a single antigen so that each specificity can be detected accurately [48]. These techniques are detailed in Chapter 89.

In the past decade, HLA antibodies have zoomed in importance to transplantation because they are postulated as the *cause* of graft rejection [49]. Some of the evidence amassed leading to this conclusion is: almost all patients with a rejected kidney were shown to have antibodies [50]; antibodies could be eluted from kidneys that were rejected [51]; patients who developed antibodies after transplantation were shown to have a higher rate of failure than those without antibodies [52]—and this was true for transplants of kidneys (Figure 3.5) [53–56], hearts [57–59], lungs [60,61], livers

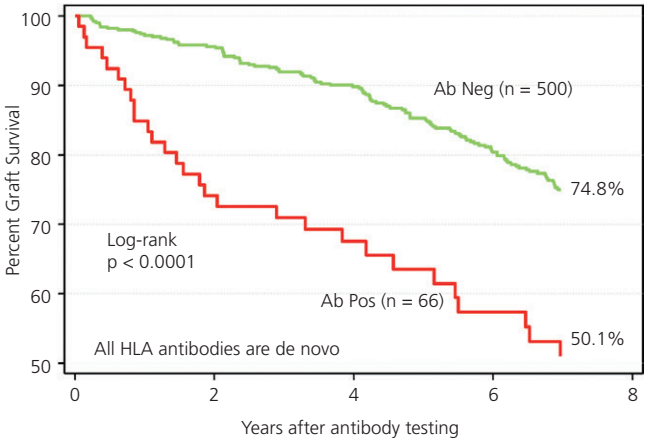

Figure 3.5. In 2002, as part of the International Histocompatibility Workshop, kidney transplant patients (n = 566) with well-functioning kidneys were tested once, and classified as negative or positive for HLA antibodies. After 8 years' observation, it can be seen that those who were positive on a single test have continued to lose their grafts compared with patients who were negative. (Adapted from the author's personal collection.)

[62], and pancreas [63,64]. Most important, patients with rejected kidneys could be shown to have antibodies detectable *before* rejection of the kidney. All these factors fit the nine criteria, developed by Bradford Hill and Richard Doll, that allowed a logical progression from association to causality [65]. We therefore concluded that HLA antibodies are causally related to rejection of grafts [66]. Recently accumulated evidence shows that following acute antibody-mediated rejection, antibody reduction led to superior survival compared with that of patients in whom antibody intensity was not reduced [67–69]. This finding was in acute rejection patients who all had histologic reversal of their rejection. Additionally, removing antibodies in kidney and lung transplant patients prior to evidence of allograft dysfunction was also shown to enhance allograft function and improve survival [70]. Improving function by removal of antibodies constitutes final proof that HLA antibodies cause graft rejection, and likely account for the majority of allograft loss.

Summary

HLA, the major histocompatibility locus in man, has had an intriguing history from the time it was first given its name in 1967. In the ensuing 44 years, HLA has emerged as the most polymorphic system known to man, with over 5000 types identified so far. This variety has made bone marrow transplants from unrelated donors extremely difficult. With most organs—such as hearts, livers, and lungs—it was impossible to obtain matches from unrelated deceased donors for transplant recipients. So transplant surgeons were forced to ignore HLA matching, relying instead on ever improving immunosuppression. Even in kidney transplants, use of healthy living unrelated donors has shown that the HLA system can largely be disregarded. However, it is becoming increasingly clear that, although mismatches can be initially ignored, antibodies are eventually formed against the mismatches in a number of patients. The donor-specific antibodies then react against the organs and cause graft loss. Recent findings show that removal of these antibodies results in longer graft survival, the final and persuasive indication that the antibodies had been causing graft loss. The HLA system is thus critical for transplantation, though in ways not initially envisioned.

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CHAPTER 4

Transplant Antigen Biology

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Introduction

Transplantation of organs or tissues from one individual to another is complicated by the presence of antigens that differ between the donor and recipient and provoke, or are targeted by, a destructive immune response. These transplantation or histocompatibility antigens are of varying types, complexity, and influence on the transplant process. The major blood group antigens, A and B, for example, which react with natural antibodies found in individuals negative for these antigens and cause immediate reactions, are limited in scope and therefore easily avoided through donor and recipient matching. The major histocompatibility complex antigens, however, are more challenging barriers to transplantation. Incompatibilities at the human leukocyte antigens (HLA) cannot be avoided, except between siblings that inherit the same set of HLA antigens from the parents. The extremely polymorphic HLA antigens themselves play key roles in normal immune responses and both their similarities and differences between individuals allow for alloimmune responses generated as a result of both direct and indirect antigen presentation. As a result, the immune response to allogeneic exposure may be many times more vigorous than the immune response to nominal antigens and transplantation requires lifelong manipulation of the recipient's immune system to prevent graft destruction. In this chapter, we describe the biology, genetics, structure, and function of the major human transplant antigens, HLA. We will also describe those non-HLA antigens thought to play a role in alloimmunity. This chapter complements Chapters 36 and 89, which detail the methods for detecting HLA differences and HLA-specific antibodies, respectively. As an understanding of HLA is paramount to an understanding of alloimmunity in general, the history of our understanding of HLA recounts much of the history of transplantation, and is presented in Chapter 3.

Genetics of the major histocompatibility complex (MHC)

The MHC in humans is an ~5 megabase region on the short arm of chromosome 6 [1,2]. The human MHC comprises 224 gene loci and is distinguished from other regions of the human genome by its high gene density. Nearly 57% of the MHC genes (128 genes) are predicted to be expressed and 40% of the expressed genes have known immune function. Figure 4.1 shows a schematic map of the major genes of the human MHC. These are traditionally grouped into three distinct non-overlapping regions, designated from the centromere to the telomere as the class II, III, and I regions, respectively.

MHC class I

This cluster comprises three sets of genes that encode proteins with similar structure but different properties. The **classical class I genes** (or class Ia) include three highly polymorphic genes (*HLA-A*, *HLA-B*, and *HLA-C*), which encode the membrane-spanning α chains (heavy chains) of HLA class I molecules. The α chains are expressed as heterodimers along with a non-covalently bound light chain, β_2-microglobulin (β_2m) which is produced by a non-polymorphic, non-MHC gene located on chromosome 15. All three class Ia molecules are normally expressed on the surface of all nucleated cells.

The **non-classical class I genes** (or class Ib) include three genes that encode a functional molecule (*HLA-E*, *HLA-F*, and *HLA-G*) and 12 pseudogenes that do not express a protein belonging to this category. The class Ib molecules display a high degree of similarity with class Ia products in sequence and structural association with β2m, but differ in two attributes: first, the expression of the class Ib molecules is restricted to certain cells types; and second, the class Ib molecules show limited polymorphism.

The **class I-like genes** (or class Ic) include seven MHC class I-related chain (MIC) genes, of which only two (*MICA* and *MICB*) encode functional molecules and the remainder are pseudogenes. The MIC proteins are structurally related to the HLA class I molecules, but fail to form trimolecular complexes because they do not associate with β2m and also do not bind peptides for presentation to T cells. The MIC gene expression is restricted mainly to fibroblasts and epithelial cells, and induced by stress in other cell types. In contrast to class Ib genes, both *MICA* and *MICB* genes are highly polymorphic.

The HLA class I genes comprise eight exons, each encoding a distinct domain: exon-1 (leader peptide), exon-2 (α1 domain), exon-3 (α2 domain), exon-4 (α3 domain), exon-5 (transmembrane region), and exons 6–8 (short cytoplasmic tail).

MHC class II

The class II cluster comprises genes that encode for the classical (HLA-DR, HLA-DQ, and HLA-DP) and non-classical (HLA-DM and HLA-DO) class II molecules. The class II molecules are heterodimers of α and β chains and each chain is encoded by a different gene in the MHC region (α-chain by gene-A and β-chain by gene-B) (Figures 4.1 and 4.2). The HLA class II genes comprise

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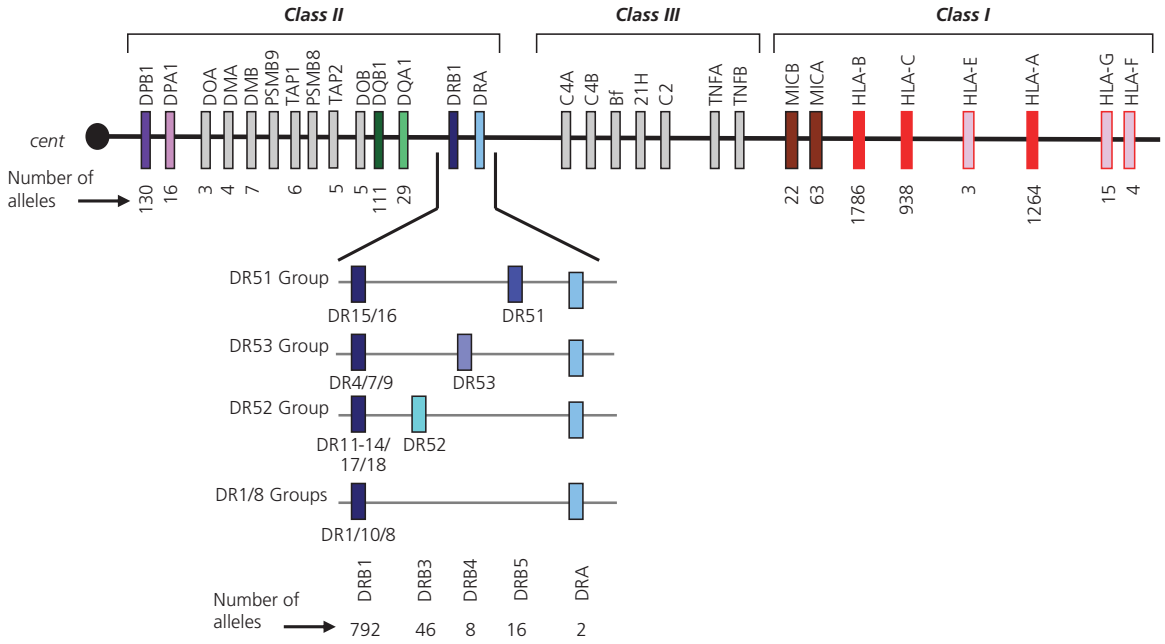

Figure 4.1. Genomic map of the human major histocompatibility complex (MHC). Schematic map of the human MHC on chromosome 6 comprising three non-overlapping regions: class I, class II, and class II. The centromere (circle) and major MHC genes (boxes) are indicated in order, but not drawn to scale. The haplotypic differences in DRB gene content in the class II DR region are indicated. The serological specificities encoded by each functional DRB gene are provided beneath the appropriate locus. The number of distinct alleles are indicated under each locus (source: IMGT/HLA database, Release 3.6, October 2011; http://www.ebi.ac.uk/imgt/hla/stats.html). The non-functional HLA gene fragments (pseudogenes) dispersed across the class I (*HLA-H*, *-J*, *-K*, *-L*, *-N*, *-P*, *-S*, *-T*, *-U*, *-V*, *-W*, *-X*, *-Y*, *MICC*, *MICD*, and *MICE*) and class II (*HLA-Z*, *DRB2*, *DRB6*, *DRB7*, *DRB8*, *DRB9*, *DQA2*, *DQB2*, *DQB3*, *DPA2*, *DPA3*, and *DPB2*) regions are not indicated.

seven exons, each encoding a distinct domain: exon-1 (leader peptide), exon-2 (α1 or β1 domain), exon-3 (α2 or β2 domain), exon-4 (transmembrane region), and exons 5–7 (short cytoplasmic tail). Unlike the classical class II genes, the non-classical class II gene products are not expressed on the cell surface, but are involved in peptide loading onto classical class II molecules.

The HLA-DR region contains a single monomorphic DRA gene and multiple polymorphic DRB genes. Nine distinct DRB genes have been identified, of which four (*DRB1*, *DRB3*, *DRB4*, and *DRB5*) are functional and polymorphic and encode the β chain of a specific class II molecule, and five are non-functional pseudogenes (*DRB2*, *DRB6*, *DRB7*, *DRB8*, and *DRB9*). The number and type of DRB genes are variable by haplotype, with five distinct gene-content haplotypes: the DR1-group, the DR8-group, the DR51 group, the DR52 group, and the DR53 group (Figure 4.1). All five haplotypes carry a *DRB1* gene, but the DR51, DR52, and DR53 group haplotypes contain an additional functional DRB gene (*DRB5*, *DRB3*, or *DRB4*, respectively), and therefore the DR51, DR52, and DR53 group haplotypes express two DR molecules—one from the *DRB1* gene (β-chain) and the DRA gene (α-chain) and another from the *DRB5*, *DRB3*, or *DRB4* gene (β-chain) and DRA gene (α-chain) (Figure 4.2).

The DQ region contains five genes (*DQA1*, *DQA2*, *DQB1*, *DQB2*, and *DQB3*) of which only *DQA1* and *DQB1* encode functional proteins, α and β chains, respectively, which together form the DQ heterodimer at the cell surface (Figure 4.2). In contrast to the DR genes, both *DQA1* and *DQB1* genes are polymorphic and consequently additional polymorphism is generated by different "cis" and "trans" chain combinations, creating four different DQ heterodimers. The DP region contains two DPA genes (*DPA1* and *DPA2*) and two DPB genes (*DPB1* and *DPB2*) of which only *DPA1* and *DPB1* are functional. As with the DQ genes, both *DPA1* and *DPB1* genes are polymorphic and thus may produce four different DP heterodimers by different "cis" and "trans" combinations.

The MHC class II regions include several non-HLA genes whose products are involved in antigen processing and transportation, such as genes of proteasome-related sequences (*PSMB8* and *PSMB9*) and transporter antigen proteins (*TAP1* and *TAP2*). The *PSMB8* and *PSMB9* are the IFN-γ induced subunits of the class I associated proteasomes that produce the antigenic peptides required by class I proteins for presentation to CTLs. The *TAP1* and *TAP2* are members of the ATP binding cassette gene superfamily involved in peptide transport from the cytoplasm to the endoplasmic reticulum.

MHC class III

The class III cluster comprises genes that have no structural or functional correlation with HLA genes. A large number of class III region genes encode molecules participating in antigen processing and presentation, the complement system, and in the inflammatory process. The class III region also includes several genes with no identified immune function.

Nature of HLA polymorphism

Most polymorphisms in HLA gene coding regions correspond to amino acid positions that interact with antigenic peptides or the T-cell receptor [3]. HLA class I polymorphisms are predominant in the first 180 amino acids of the heavy chain and the class II polymorphisms are found in the first 90–95 amino acids of the α

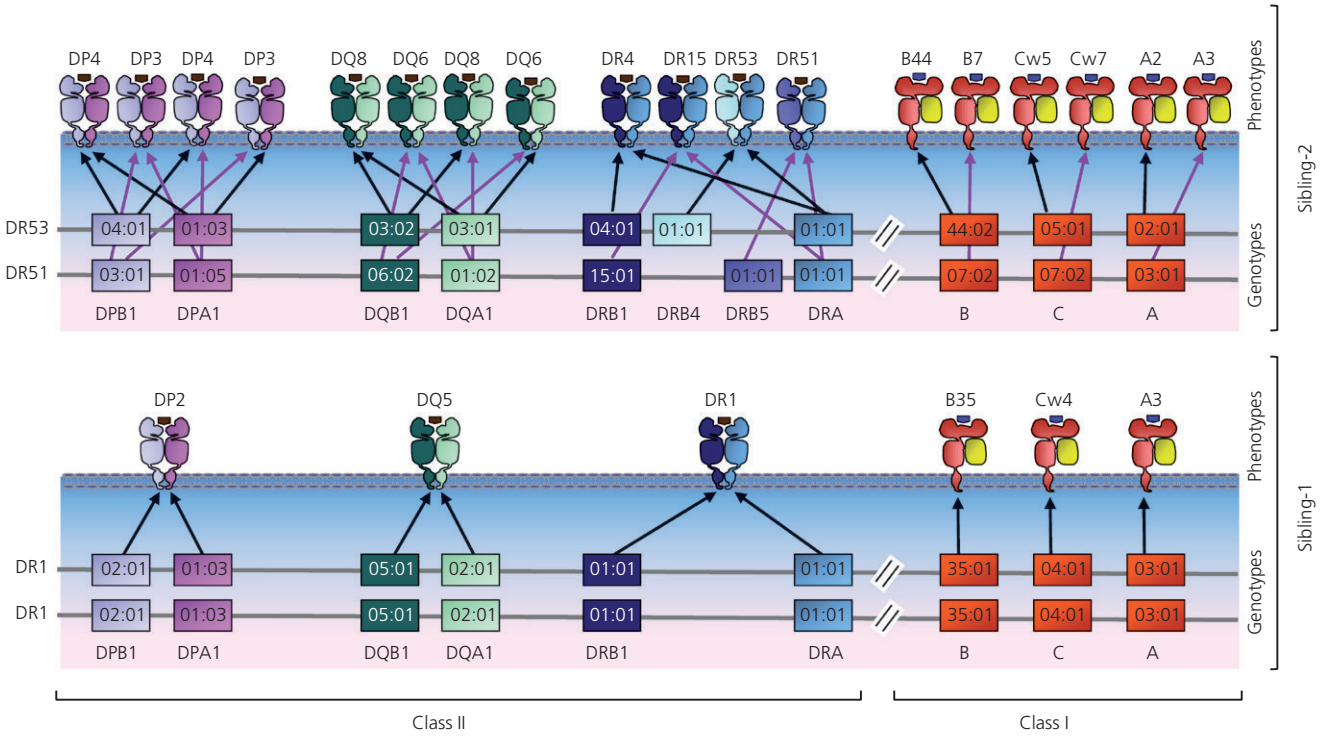

Figure 4.2. The number and type of HLA molecules can differ substantially even between two siblings. Distinct HLA genotype and phenotype constellations of two siblings within a same family are shown. Sibling-1 carries two copies of DR1-group haplotypes (one from the mother and another from the father) that are identical in all classical HLA genes (HLA-A*03:01, B*35:01, C*04:01, DRB1*01:01, DRA*01:01, DQB1*05:01, DQA1*02:01, DPB1*02:01, and DPA1*01:03), and therefore express a single HLA type at each locus (A1, B27, Cw7, DR1, DQ5, and DP4) for a total of six distinct HLA molecules. In contrast, sibling-2 carries the DR53 and DR51 haplotypes that display different alleles at each locus (A2, A3, B7, B44, Cw5, Cw7, DR4, DR15, DR51, DR53, DQ6, DQ8, DP3, and DP4) for a total of 18 different HLA molecules.

and/or β chains [4]. The exons or segments of the gene that encode these polymorphic residues are exons 2 and 3 for class I, and exon 2 for class II genes. DNA-based HLA typing methods target these highly polymorphic regions [5]. Comparison of HLA alleles reveals a characteristic pattern of sequence differences that are the result of recombination events between alleles of the same locus, which is presumably driven by positive Darwinian selection to promote diversity in the repertoire of HLA-binding antigenic peptides [6].

Every allele is a pastiche of sequence motifs shared with other alleles and thus most substitutions are shared by two or more HLA alleles of the same locus (Figure 4.3). The patchwork pattern of sequence polymorphism is probably generated by segmental exchanges through recombination and gene conversion [7]. The shared patchwork pattern of polymorphism complicates HLA typing as well as HLA antibody identification. Because of the extensive sharing of two distinguishing sequences of a given locus with other alleles of the same locus, it is often impossible to assign the actual allele pair unambiguously using current technologies. Additional tiered tests are performed to exclude some allele combinations. A current list of all allele combinations that provide ambiguous results is found at the IMGT/HLA database (<http://www.ebi.ac.uk/imgt/hla/ambig.html>), which provides a database for officially recognized HLA sequences.

Human diversity in HLA repertoire

Being diploid, humans have two copies of the MHC gene complex, one of paternal origin and the other of maternal origin. Because HLA genes are highly polymorphic, alleles of each HLA gene within an individual are almost always different (heterozygous) [8]. Because both HLA alleles are expressed at comparable levels on the cell surface (co-dominant expression), humans can differ in the number and type of HLA molecules expressed on their cell surface. For instance, an individual carrying two copies of DR1-group haplotypes (one from mother and another from father) that are identical in all classical HLA genes (*HLA-A*, *B*, *C*, *DRB1*, *DRA*, *DQB1*, *DQA1*, *DPB1*, and *DPA1*) can express a single HLA type from each of these loci (e.g. A1, B27, Cw7, DR1, DQ5, and DP4—in total six distinct HLA molecules) (Figure 4.2, sibling-1). In contrast, a sibling carrying the DR53 and DR51 haplotypes who displays different alleles at all HLA loci can express two HLA-A molecules, two HLA-B molecules, two HLA-Cw molecules, four HLA-DR molecules, four HLA-DQ molecules, and four HLA-DP molecules (18 different HLA molecules) (Figure 4.2, sibling-2). As a consequence of simple Mendelian inheritance, such an extreme diversity described in these two siblings can occur at the rate of 25% if their parents carry the following haplotypic constellations: mother with DR51 and DR1; father with DR53 and DR1.

Some HLA genes have well over 1000 alleles and their haplotype combinations produce extensive diversity in populations (Figure 4.1) [9]. The polymorphism is so high that in a mixed population (non-endogamic), no two individuals have exactly the same set of MHC genes and molecules. Such a high degree of polymorphism is important both within an individual and between individuals. Within an individual, inheritance of duplicate (homozygous HLA alleles) may result in a disadvantage against chronic viral infection

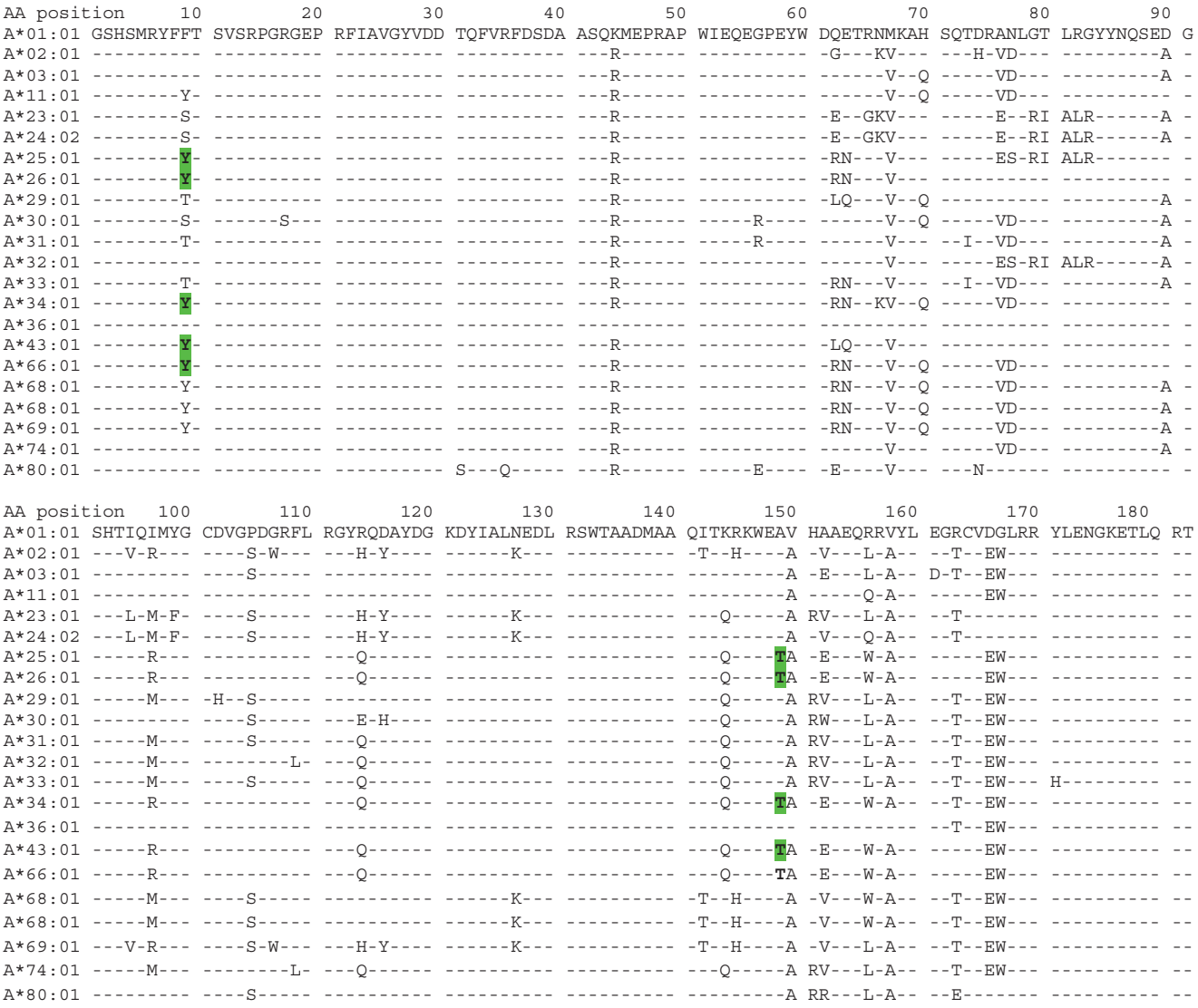

Figure 4.3. Nature of HLA sequence polymorphism. Amino acid sequences of α1 (top panel) and α2 (bottom panel) domains of common HLA-A allotypes representing major antigen groups. HLA-A*01:01 is the reference sequence listed at the top, and amino acid positions of other alleles identical to the reference are displayed as hyphens (-). Amino acid differences from the reference sequence are indicated by standard single letter codes. The majority of the substitutions are shared by more than one sequence. The green shadows identify amino acids that constitute epitope(s) shared by multiple allotypes HLA-A25, A26, A34, A43, and A66.

due to a diminished repertoire of viral peptides available for presentation to T cells. The hypothesis of heterozygote advantage (over-dominant selection) proposes that individuals heterozygous at HLA loci are able to present a wider range of antigenic peptides from a pathogen to the immune system than homozygotes, enhancing immune surveillance [10]. Maximum HLA heterozygosity of class I loci (A, B, and C) delayed acquired immunodeficiency syndrome (AIDS) onset among patients infected with human immunodeficiency virus-type 1 (HIV-1), whereas individuals who were homozygous for one or more loci progressed rapidly to AIDS and death [11]. Over-dominant selection would therefore be expected to favor individuals with the most divergent range of peptide binding specificities.

At the population level, HLA polymorphism provides a mechanism to limit the spread of epidemic pathogens, which might otherwise propagate unchecked throughout a host population. The presence of many different alleles in a population ensures there will always be at least a few individuals with a specific HLA molecule (rare or new alleles) able to load appropriate peptides to develop an adequate immune response to overcome the pathogen, so that the population survives the pandemic. The principle of rare allele advantage (frequency-dependent selection) is that an individual with a rare (e.g. new) MHC allele might respond better to a new pathogen variant, thereby conferring an advantage on that individual in a population in which the pathogen is prevalent [12]. Such frequency-dependent selection may have caused the increased frequency of HLA-B*53 in the Gambian population exposed to malaria. Similarly, HLA-B54 is found almost exclusively in Japanese and nearby Asian countries, and HLA-A74 is common among Blacks, but is very rare in other populations. Therefore, different

subsets of HLA alleles appear to have been selected over time in a given population and the total number of alleles maintained varies across human populations [13,14]. Furthermore, populations such as Native Americans may have experienced bottlenecks or founding events resulting in a more limited diversity of HLA alleles [15]. Linkage disequilibrium is a phenomenon where alleles at adjacent HLA loci are inherited together more often than would be expected by chance. For example, if HLA-A1 and HLA-B8 occur at gene frequencies of 16% and 10%, respectively, in a population, the probability of finding them together should be 1.6%. However, the actual occurrence of HLA-A1–B8 combination is significantly above the predicted incidence (about 8%). Positive selection is thought to be operating on the haplotype and that the linked loci confer a selective advantage for the host [16].

HLA nomenclature

The HLA nomenclature can be daunting as the change from serological typing (which defined antigens) to DNA typing (which defines alleles) resulted in some conflicts [9]. The HLA antigens were named sequentially as they were identified by antisera. Often specificities could be refined as antibodies "split" antigen specificities into two or more new specificities, often identifying alleles or allele groups within a family of related antigens. The more common HLA antigens have lower numbers, A2 or B7, and those that came later or were less common had higher numbers. HLA-B40, for example, was subsequently split into two groups renamed B60 and B61. The standard DNA nomenclature built on this basis and includes four fields (for example, HLA-A*02:01:01:01) delineated by a colon separating antigen, allele group, and designations for silent substitutions (those which don't result in a change in the protein) and differences in non-coding regions [9]. In the clinical setting, HLA typing for hematopoietic stem cell transplants is reported at the allele level (HLA-A*02:01) and HLA typing for solid organ transplants is reported at the antigen level (HLA-A2). The latter usually is the first two digits after the asterisk, but because the DNA nomenclature uses allele groups that are related by nucleotide sequence similarities, there are a few exceptions [17]: B*15:01 corresponds to the B62 antigen, B*15:02 to B75, B*40:01 to B60, and B*40:02 to B61. Similarly for HLA class II alleles, DRB1*03:01 corresponds to the DR17 antigen, DRB1*03:02 to DR18, DQB1*03:01 to DQ7, DQB1*03:02 to DQ8, and DQB1*03:03 to DQ9). In each case, the alleles correspond to splits of the parent antigen.

HLA epitopes and the structural basis of cross-reactivity

The localized and sometimes overlapping nature of HLA polymorphisms (shown in Figure 4.3) results in molecules with an array of alloantigenic structures, which range from those uniquely associated with a single allele to those shared by related HLA molecules. Immunization to a single HLA alloantigen (by pregnancy, transfusions, or allografts) can result in antibody production against a series of HLA antigens that share sequence motifs (termed epitopes) with the immunizing antigen. Antibodies adsorbed and eluted from purified HLA antigens attached to microspheres show reactivity patterns with HLA molecules that correlate with specific shared amino acids that map to regions of the HLA molecular structures and likely affect the antibody-binding site [18]. These antigens belong to cross-reactive groups (CREGs) as defined by specific antibodies. For instance, immunization to the HLA-A25 antigen can result in antibodies against HLA-A25, A26, A34, A43, and A66 that share amino acids at position 9 (tyrosine) and 149 (threonine), which are not present at these positions in other HLA antigens.

Structure and function of HLA molecules

The HLA class I and class II molecules differ in their structure, the cells on which they are expressed, the source of antigens they process, and the functionally distinct subsets of T cells with which they interact.

HLA class I molecular structure

The HLA class I molecules (A, B, and C) are heterodimeric transmembrane proteins that consist of a 32 kD α chain that is non-covalently associated with a 12 kD β_2-microglobulin (β_2m) light chain (Figure 4.4). The α chain is comprised of three segments α_1, α_2, and α_3, each of about 90 amino acids in length. X-ray crystallography studies revealed that the α_1 and α_2 domains fold together into a single structure consisting of two segmented α_1 helices lying on an eight-stranded β pleated sheet [19]. The folding of the α_1 and α_2 domains forms a cleft that binds self and non-self peptides. The peptide binding cleft of the HLA class I molecule is about 25 Å

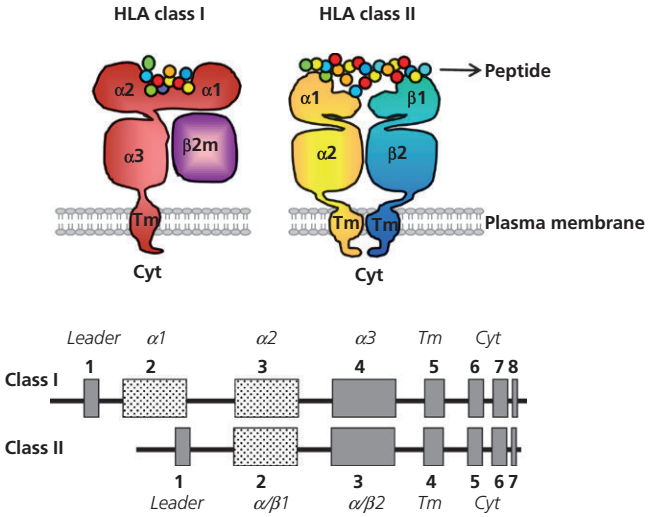

Figure 4.4. Structural organization of HLA proteins (top panel) and genes (bottom panel). The HLA class I molecule is a heterodimer of a membrane-spanning heavy α chain (encoded by *HLA-A*, *-B*, or *-C* gene) bound non-covalently to a non-MHC gene (located in chromosome 15) encoded light β2-microglobulin (β2m) chain, which does not span the membrane. The α chain folds into three domains: α1, α2, and α3. The α1 and α2 domains form an antigen-binding groove. The HLA class II molecule is composed of two transmembrane glycoprotein chains, α (encoded by *DRA*, *DQA1*, or *DPA1*) and β (encoded by *DRB1*, *DQB1,* or *DPB1*). Each chain has two domains, and the two chains together form a compact four-domain structure similar to that of HLA class I molecule. The α1 and β1 domains of class II molecules form a peptide-binding cleft. The class I genes comprise eight exons (shown in boxes) while the class II genes comprise seven exons. The protein domain encoded by each exon is shown in italics. Exons 2 and 3 of class I and exon 2 of class II genes (speckled boxes) encode the polymorphic antigen binding domains. Reproduced from Grody WW, Nakamura RM, Kiechle FL, Strom C (2010) *Molecular Diagnostics: Techniques and Applications for the Clinical Laboratory*, with permission from Elsevier. Copyright ©2010, Elsevier [115].

(Angstroms) long and accommodates small peptides of 8–11 amino acids in length.

The α_3 domain of class I binds non-covalently to β_2m, which is necessary for the correct folding of the molecule on the surface of the cell. The α_3 domain also contains a highly conserved α3 immunoglobulin-like domain that binds to the CD8 molecule on T cells [20]. The carboxyl terminal end of the molecule contains a hydrophobic transmembrane region consisting of 41 amino acid residues and a short cytoplasmic tail comprised of 27 residues. The stable expression of the class I molecule requires the assembly of a trimolecular complex formed by the class I α chain, β_2m and bound self or non-self peptide.

HLA class II molecular structure

The HLA class II molecules DR, DQ, and DP are heterodimers composed of two transmembrane glycoprotein chains comprising an α chain of 32–34 kD and a 29–32 kD β chain (Figure 4.4). The α_2 and β_2 domains fold to form a peptide binding cleft [21]. The polymorphic residues are primarily located in the α_1 and β_1 domains and participate in peptide binding and interactions with T-cell receptors. The ends of the class II peptide-binding groove are open and therefore can accommodate larger peptides of 30 residues or more in length [22,23]. One loop in the α_2 domain of class II serves as the docking site for the CD4 molecule. The carboxyl terminal ends of the α_2 and β_2 domains consist of a short hydrophobic transmembrane region, which anchors the molecule into the plasma membrane, followed by a short hydrophilic cytoplasmic tail.

Structural basis of peptide binding to HLA class I and class II molecules

Peptide binding to HLA class I and II molecules is guided by interactions between specific amino acid residues in the peptides and complementary amino acid residues that form the β-strands of the bottom of the cleft and the α-helices on the sides of the cleft. The polymorphisms that are found primarily in the α_1 and α_2 domains of the class I molecule and the α_1 and β_1 domains of class II molecules form the peptide-binding pockets. The polymorphic amino acid side chains alter the electrostatic surface charge, hydrophobicity, size, and shape of the HLA antigen pockets and govern whether a particular peptide will bind to the cleft [24,25]. The residues of the peptide that fit into these pockets are called anchor residues. The extensive polymorphisms alter the peptide binding characteristics of each HLA class I and class II allele so that each allele binds a unique set of peptides. The length of the peptides bound to class I molecules are generally eight to ten amino acids in length. The amino- and carboxyl-ends of the peptide are buried into the cleft and bind to conserved heavy chain residues via a series of hydrogen bonds. The peptides bound to class II molecules are longer (10–30 amino acids) and attach in an extended conformation with the ends of the peptide hanging over the edges of the cleft [21]. Whether a peptide will bind or not to a given class I allele is governed by the amino acid side chains that form the anchor residues of the antigen binding cleft. Therefore, the set of peptides that can be bound by a specific HLA class I allele often share a common "sequence motif." There are several websites that contain useful information on the peptide binding motifs and algorithms, which enable the prediction of specific peptides to bind HLA molecules including: http://www.ashi-hla.org/, http://www.ebi.ac.uk/imgt/hla/, http://www-bimas.cit.nih.gov/molbio/hla_bind/index.shtml and http://www.syfpeithi.de/.

Antigen processing and presentation

Antigen processing and presentation is the manner by which antigen-presenting cells express antigen on their cell surface in a form recognizable by lymphocytes. The cellular processes and surface molecules facilitating antigen presentation in general are discussed in more depth in Chapter 2. Antigen processing includes protein fragmentation (proteolysis), association of the fragments with HLA antigens, and expression of the peptide-HLA complex at the cell surface where they can be recognized by the T-cell receptor (TCR) on a T cell. HLA class I and class II molecules process and present antigen in a fundamentally distinct manner. HLA class I molecules present foreign and self-peptides that are generated in the cytosol of the cell (Figure 4.5) [26] whereas HLA class II molecules present protein antigens primarily, but not exclusively, from extracellular sources (Figure 4.6) [27–29].

The major source of peptides for HLA class I presentation are derived from processing of endogenous proteins into peptides by the proteasome [30] (Figure 4.5). The proteasome is a large multiprotein enzyme complex found in the cytoplasm of cells [31]. The proteasome is comprised of 14 subunits with broad proteolytic activity and functions to degrade cytoplasmic proteins into peptides. Protein targeting to the proteasome for degradation requires that the protein is first covalently linked to a small protein called ubiquitin [32]. The protein is then unfolded, the ubiquitin is removed, and the protein is degraded in the proteasome to a wide range of peptides. Interestingly, two of the proteasome subunits, PSMB9 and PSMB8, are encoded within the class II region and their activity is specifically turned on by proinflammatory cytokines such as INF-γ [33]. Thus, immune cells activated by INF-γ express variant proteasomes called "immunoproteasomes" which are characterized by unique catalytic subunits. The immunoproteasome has an enhanced catalytic activity compared to standard proteasomes and recent studies suggest that this enhanced activity of the immunoproteasome prevents the accumulation of degradation substrates that would otherwise accumulate during inflammation [34].

The loading of peptides on HLA class I molecules is a highly coordinated process involving several chaperone proteins that are present in the endoplasmic reticulum (ER) [35,36] (Figure 4.5). Following the synthesis of the heavy chain of HLA class I, it is targeted to the ER where it binds to the membrane-associated ER chaperones calnexin and Grp78. These chaperones are involved in the insertion of the class I polypeptide into the lumenal compartment of the ER and initiate heavy chain folding and intrachain disulfide bond formation. Upon dissociation from calnexin, the class I heavy chain associates with β_2m and binds to the ortholog chaperone calreticulin and is incorporated into the peptide-loading complex. The peptide-loading complex orchestrates the loading of an optimal peptide onto MHC class I molecules. The peptide-loading complex contains the two subunits of the transporter associated with antigen processing (TAP1 and TAP2), transmembrane glycoprotein tapasin, the soluble ER chaperone calreticulin and the soluble thiol oxidoreductase ERp57 [35]. The peptides are translocated into the endoplasmic reticulum via TAP1 and TAP2 molecules. The TAP genes are encoded within the class II region of the MHC, linked to the *PSMB9* and *PSMB8* genes [37]. TAP associates with peptides on the cytoplasmic side of the ER membrane and with class I molecules on the lumenal surface and delivers peptides directly to the class I molecules. In the ER, the peptides can be trimmed (if required) to the required length of 8-10 amino acids for binding to class I molecules by ERAAP (or ERAP1), a specialized ER-associated aminopepsidase that recognizes the peptide

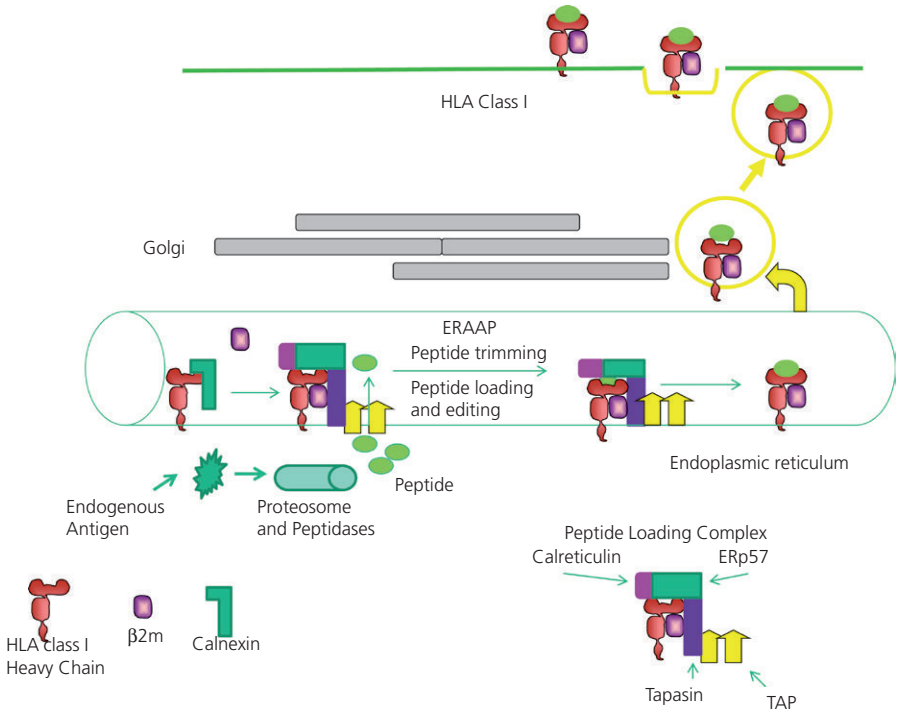

Figure 4.5. Schematic representation of MHC class I molecule peptide processing and presentation. MHC class I heavy chains assemble with β2m and are recruited to the peptide-loading complex in the endoplasmic reticulum. MHC class I molecules initially associate with the chaperone calnexin, which triggers folding and disulfide bond formation. Upon dissociation from calnexin, the class I heavy chain associates with β_2m and binds to the chaperone calreticulin and is incorporated into the peptide-loading complex. The peptide-loading complex contains the two subunits of the transporter associated with antigen processing (TAP1 and TAP2), transmembrane glycoprotein tapasin, calreticulin, and ERp57. The peptides are translocated into the endoplasmic reticulum via TAP1 and TAP2 molecules. Tapasin facilitates peptide loading and ERAAP functions to trim peptides at their amino-terminal ends before and after binding to the MHC class I molecules. The peptide-MHC class I molecules are then exported to the cell surface.

carboxyl terminus and trims the amino terminus to generate peptides of 8-10 residues in length [38]. The TAP-associated glycoprotein called tapasin is also encoded in the MHC and functions to facilitate peptide loading of class I molecules in the ER [39]. Tapasin bridges HLA class I molecules to the TAP molecules in association with the chaperone calreticulum and the thiol oxidoreductase ERp57, which together form the peptide-loading complex [39,40]. Tapasin stabilizes the empty class I dimer, retaining it in the ER until peptide loading by the peptide-loading complex. HLA class I molecules can be tapasin-dependent or tapasin-independent for peptide loading [40].

The primary repertoire of peptides bound to class I includes a variety of proteins derived from proteasomal degradation of intracellular products of the cytosol, including HLA molecules themselves. However, there are specialized cells, particularly macrophages and dendritic cells (DC) that can capture extracellular antigens and process and present these antigens to CD8 T cells through a process called cross-presentation [41]. During cross-presentation, it is thought that elements of the ER are incorporated into phagosomes and endosomes. Cross-presentation is important in presenting peptides from viruses that do not infect the DC [42]. Cross-presentation is also involved in tolerance to self-proteins that are not synthesized in the DC.

HLA class II molecules present peptides that are generated in the endocytic compartment of the cell (Figure 4.6). Many of the peptides presented by class II molecules are derived from exogenous antigens, but they can also be derived from the cytoplasm of the cell through the process of autophagy [43–45]. Exogenous antigens are internalized and then transported to lysosomes and endosomes containing proteolytic enzymes including legumain, cathepsin L, and cathepsin S [35]. Class II molecules are assembled in the ER with a protein called invariant chain (Ii) or CD74. The invariant chain blocks peptides from being loaded on class II molecules in the ER and chaperones the newly synthesized class II molecules to MCII, a specialized endosomal compartment where peptide loading occurs [46]. In the acidified MCII compartment the invariant chain is degraded by cathepsin-mediated proteolysis [47] to generate the class II-associated invariant chain peptide or CLIP [48]. CLIP binds to the cleft of the class II molecule and inside the cleft is protected from degradation. CLIP needs to be removed in order to make the cleft accessible to peptides produced from extracellular proteins. HLA-DMA and HLA-DMB are encoded within the class II region and function to catalyze the dissociation of CLIP and promote peptide loading in the endocytic compartment [49,50]. DM functions to ensure efficient association of peptides and edits complexes such that the stable peptide binders to HLA class II are favored [51]. The activity of DM can be further modified by another HLA class II encoded protein called HLA-DO, which can act as a peptide editor by altering the pH optimum of DM-mediated peptide loading of MHC class II to more acidic conditions [52]. The peptides that bind to class II molecules are generated by proteases called cathepsins, which are present in the endosomal compartments. Interestingly, there are differences in the repertoire of cathepsins used by different APC, which can influence the nature of the

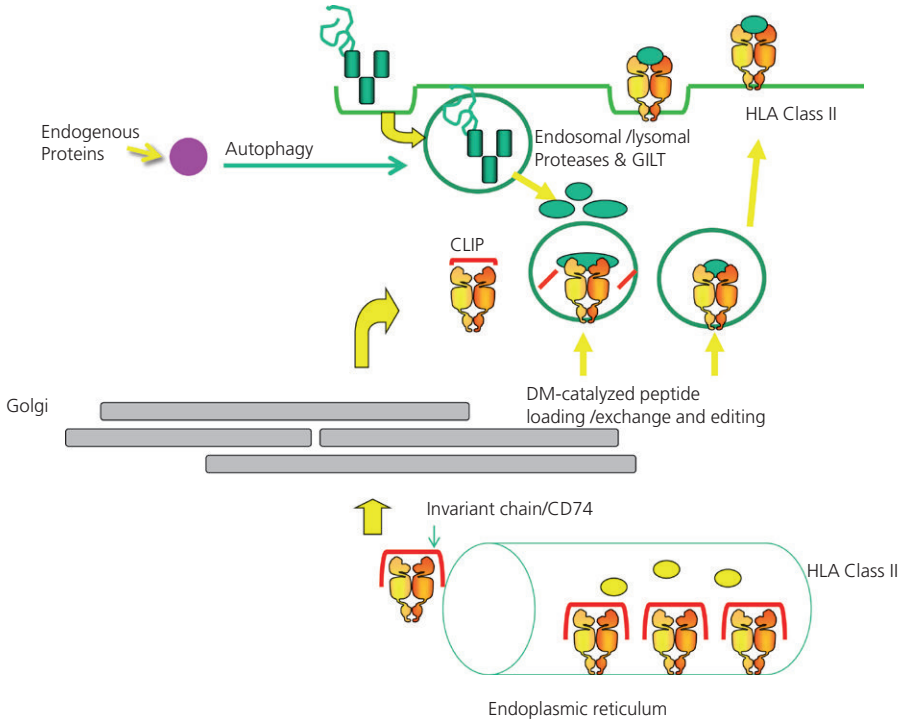

Figure 4.6. Schematic representation of MHC class II molecule peptide processing and presentation. MHC class II molecules assemble in the endoplasmic reticulum with invariant chain (Ii), which contains the endosomal targeting signal. In the endosomal compartments, Ii is cleaved leaving only the smaller fragment CLIP occupying the peptide binding cleft. Exogenous proteins are internalized into the endosomal pathway and their unfolding and fragmentation is catalyzed by the disulfide reductase GILT and lysosomal proteases. HLA-DM catalyzes the release of CLIP followed by the binding of peptide fragments and peptide exchange. HLA-DM edits the repertoire of peptide-MHC class II complexes that are transported to the cell surface.

peptides generated [53]. An additional lysosomal processing enzyme called gamma-interferon induced lysosomal thiol reductase or GILT has been identified for the presentation of some antigens with disulfide bonds [54]. Studies by the Cresswell lab. showed that GILT can also be important for the cross-presentation of a subset of viral antigens on MHC class I molecules [55].

Cytoplasmic and membrane cellular proteins may also enter the class II pathway of antigen presentation as a result of normal turnover of cellular proteins, a process referred to as autophagy. In this pathway, cytoplasmic proteins are trapped in ER-derived vesicles called autophagosomes, and these vesicles fuse with lysosomes and the cytoplasmic proteins are degraded and can be delivered to class II molecules as are peptides derived from exogenous antigens [56] (Figure 4.6). The processing and presentation of self-proteins is thought to be important in the process of central tolerance induction [57]. In addition to tolerance induction, studies show that some viral antigens can be intracellularly processed for cross-presentation by class II molecules [45], a mechanism which is likely to be important for protection against viral pathogens.

Differential expression of HLA class I and class II molecules

HLA class I and class II molecules have distinct distributions among cells, which reflect the different effector functions of the T cells that recognize them. HLA class I molecules present peptides from intracellular pathogens, commonly viruses, to $CD8^+$ cytotoxic T cells, which are specialized to kill any cell that they specifically recognize [58]. Class I molecules are constitutively expressed on all nucleated cells. The broad expression of class I molecule expression is linked to the function of class I molecules in presenting intracellular pathogens such as viruses. In contrast, HLA class II molecules present peptides from extracellular antigens to CD4 T cells, which in turn regulate the activation other effector cells of the immune system. Thus, HLA class II molecules are normally found on professional antigen-presenting cells, including B lymphocytes, dendritic cells, and macrophages. Cytokines, including INF-α, INF-β, and INF-γ and tumor necrosis factor a (TNF-α), increase the expression level of class I molecules. Class II molecule expression is augmented by INF-γ. Cytokines increase HLA class I and class II expression by increasing the rate of gene transcription. This effect is mediated by the binding of cytokine-activated transcription factors to regulatory DNA sequences in the promoter regions of the HLA class I and class II genes. The expression of HLA class II molecules is inducible on many cell types, including T cells, in response to cytokines. Transcription of class II genes is controlled by a master regulatory factor, which is known as the MHC-II transactivator, CIITA [59]. Differential activation of the three independent promoters that drive expression of the gene encoding the class II transactivator ultimately determines the exquisitely regulated pattern of MHC class II gene expression. In addition to increasing the transcription of the class I and class II genes and β2m, INF-γ also increases transcription of the proteasome and subunits of TAP.

HLA Class Ib molecules

HLA-E, F, G, and Hfe (HLA-H) molecules are non-classical class I molecules encoded within the MHC region [60]. These molecules

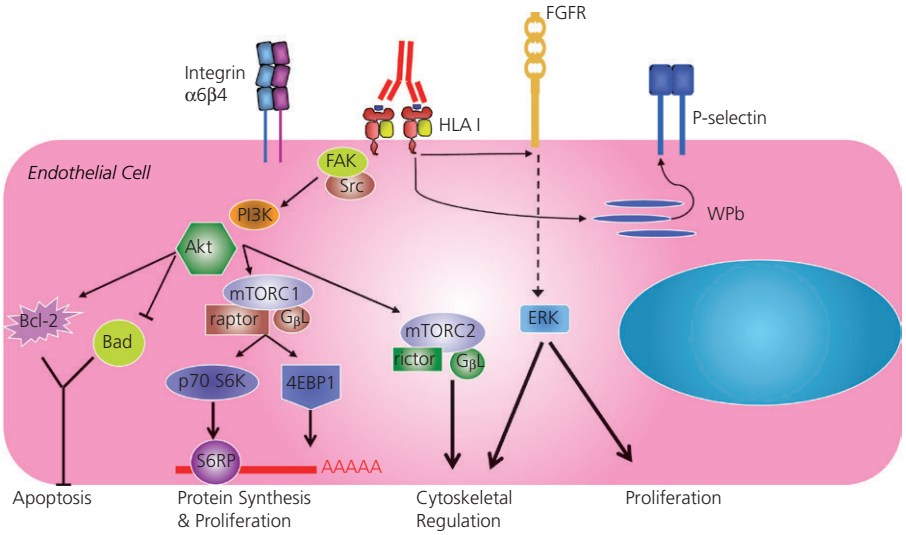

Figure 4.7. Cross-linking of HLA I by antibodies induces cell signaling pathways leading to cell growth, cytoskeletal modulation, and resistance to cell death. After ligation of HLA I on endothelium, cell surface expression of fibroblast growth factor receptor (FGFR) is rapidly increased, supporting increased cell sensitivity to growth factors and increased activation of ERK. Src and FAK are activated, resulting in activation of the PI3k/Akt axis. FAK is also an important regulator of the cytoskeleton, supporting focal adhesions. PI3K-dependent activation of Akt results in antiapoptotic and progrowth signals. Akt influences the apoptotic regulators, inhibiting the proapoptotic factor Bad and increasing levels of the antiapoptotic factors Bcl-2 and Bcl-xL. These actions result in cellular resistance to death from signals such as complement activation. Akt also targets the mammalian target of rapamycin (mTOR), which exists in two distinct complexes; mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTOR complex 1 triggers S6 kinase and S6RP activation, proteins that are essential during protein synthesis. In addition, mTORC1 regulates 4EBP-1 to increase translation of mRNA. In contrast, mTOR complex 2 acts on downstream cytoskeletal regulators to control the organization of the actin cytoskeleton. After HLA I cross-linking by antibodies, endothelial cells display dramatic stress fibers and focal adhesions, events that are dependent upon mTORC2. mTORC2 provides positive feedback to Akt. Finally, HLA I antibodies trigger rapid mobilization of endothelial cell vesicles containing von Willebrand Factor and P-selectin. This exocytosis leads to increased cell surface P-selectin, which initiates the leukocyte recruitment cascade.

associate with β2m and have a restricted polymorphism and bind a limited set of peptides. HLA-E is transcribed in most tissues. HLA-E has a limited polymorphism with only nine alleles (three protein variants). It has a binding cleft that holds a restricted set of peptides derived from the signal sequence of HLA-A, B, C, and G molecules. HLA-E binds to the CD94-NKG2A/C receptors on natural killer (NK) cells and modulate NK cell function [61].

HLA-G has a limited tissue expression and is primarily expressed in immune privileged sites including the trophoblast, thymus, and cornea [62]. In its soluble form, HLA-G is thought to inhibit maternal immunoreactivity to the fetus. HLA-G peptide presentation is limited to presentation of a nanomer peptide derived from some intracellular proteins. HLA-G interacts with leukocyte Ig-like inhibitory receptors (LIR-1 and LIR-2) and KIR2DL4 receptor. HLA-F is an intracellular protein rather than a membrane protein and its function is not well established.

HFE gene is a class I-like gene that associates with β2m for expression. HFE does not bind peptides but instead acts as a receptor binding site for the transferrin receptor to transport iron across the cell membrane. Individuals with mutations in the *HFE* gene (tyrosine 282) lack expression of *HFE* and cannot provide a negative feedback for iron absorption, which results in a disease called hemochromatosis. Hemochromatosis is a disease of iron overload and is associated with increased levels of iron in the serum and deposition in organs such as the liver.

HLA-mediated signal transduction

Cross-linking of HLA molecules induces signaling in lymphocytes, endothelial cells (EC), and smooth muscle cells (SMC). Cross-linking of HLA I or II with antibodies in B cells, T cells, or antigen-presenting cells results either in programmed cell death or activation and proliferation [63]. The pathways leading to death or proliferation in immune cells have common second messengers, including intracellular calcium, PLC, PKC, and Src family kinases [64–67]. Anti-MHC I antibodies also induce proliferation of human aortic EC [68–71] and airway epithelial cells [72,73], suggesting a process by which alloantibody may contribute to chronic allograft rejection and development of transplant vasculopathy (TV) [74].

Cross-linking of HLA class I molecules with antibody causes a molecular association between HLA I and the integrin subunit β4 and subsequent activation of intracellular signals leading to cell survival, proliferation, and migration (Figure 4.7) [75,76]. Upon antibody ligation of class I molecules, the tyrosine kinase Src is phosphorylated [77]. Src is a critical signal transducer, which regulates such cell functions as cytoskeletal changes, migration, mitogenesis, cell cycle progression, and cell survival. Next, focal adhesion kinase (FAK) phosphorylation permits complex formation with Src family kinases [71,77,78], allowing for maximal kinase activity. FAK then facilitates phosphorylation of paxillin, an adaptor protein involved in focal adhesion formation, which recruits other signaling molecules to specific compartments in the cell. One major functional consequence of FAK and paxillin activation is cytoskeletal rearrangement and formation or stabilization of focal adhesions [77,78], which are important in regulation of endothelial permeability as well as signal transduction [79]. HLA I ligation activates phosphatidylinositol 3 kinase (PI3K) and recruits 3-phosphoinositide dependent protein kinase (PDK1) and Akt, to the membrane. Akt contributes to cell proliferation by activating the mTOR pathway.

mTOR is present in two complexes in the cell. mTOR complex 1 (mTORC1) regulates ribosomal biogenesis and protein synthesis, and comprises mTOR, raptor, and GbetaL. mTOR complex 2 (mTORC2) includes mTOR, Sin1, GbetaL, and rictor, and participates in cytoskeletal regulation and feedback to Akt [80]. After HLA I ligation, mTOR is phosphorylated and forms signaling complexes that activate downstream targets, including S6Kinase, S6 ribosomal protein, and 4E-BP1. Additionally, RhoA is rapidly translocated to the cell membrane following HLA class I ligation in a manner that is associated with stress fiber formation and cytoskeleton reorganization [69]. These signaling cascades, which alter the cytoskeleton following HLA class I ligation, are linked to cell proliferation pathways, which involve mTOR activation [71,80]. In particular, mTORC2, which is composed of Rictor, Sin1, and GβL, has been shown to modulate the cytoskeleton through its stimulation of F-actin stress fibers, paxillin, RhoA, Rac1, Cdc42, and PKCα.

Stimulation of EC with a low dose of HLA I antibodies promotes cell survival and resistance to death [81–84]. HLA I cross-linking causes phosphorylation of the proapoptotic protein Bad, which results in its sequestration from the mitochondria by the 14-3-3 proteins (Figure 4.7) [85]. These Bcl family members suppress programmed cell death by regulating mitochondrial membrane permeability to prevent activation of caspases. Transplanted heart and renal allografts from patients with circulating DSA and mouse cardiac allografts undergoing AMR also had increased Bcl-2 expression, confirming these findings in vivo [84,86]. Other antiapoptotic genes, which protect from cell death, are up-regulated after HLA I ligation, including heme oxygenase 1 [83,87].

Further exploration of EC proliferation pathways revealed that following class I ligation, fibroblast growth factor receptor (FGFR) cell surface expression is up-regulated (Figure 4.7) [70,88]. Increased FGFR expression was associated with increased bFGF ligand binding and increased cell proliferation via the MAPK pathway. These data indicate that the up-regulation of FGFR and the resulting cell proliferation is an alternative pathway to the class I induced-FAK/actin-dependent cell proliferation pathway.

In addition to cell survival and proliferation signals, anti-HLA I antibodies also cause leukocyte recruitment through induction of adhesion molecules in the endothelium (Figure 4.7). Ligation of HLA with antibodies causes release of endothelial vesicles known as Weibel–Palade bodies and rapid presentation of P-selectin, which facilitates leukocytic cell adherence [89]. Study of chronic rejection in rat cardiac allografts showed a correlation between increased P-selectin expression in the intima and intimal thickening [90,91].

Killer cell immunoglobulin-like receptors (KIR)

In addition to the antigen presentation to cytotoxic T cells, the HLA class I molecules serve as ligands for NK receptors. The NK cells represent 5–25% of the mononuclear cell fraction of normal human peripheral blood. Historically, NK cells were considered to be components of innate immunity (see Chapter 7 of additional discussions of innate immunity) because their intrinsic spontaneous killing does not require any restriction by the target cell's HLA and the bound peptides. It is now clear that NK cells use a highly specific and complex target cell recognition receptor system arbitrated by the integration of signals triggered through a multitude of inhibitory and activating receptors of a wide array of conventional germline-encoded gene families [92].

KIRs are considered to be the key receptors involved in human NK cell development and function. Fourteen distinct KIR receptors have been identified, of which seven are inhibitory types and remainders are activating receptors. Upon engaging with distinct HLA class I ligands (HLA-A, B, and C), the inhibitory KIR receptors dampen NK cell reactivity. The activating KIR receptors are believed to stimulate NK cell reactivity when they sense unknown ligands expressed on the target cells, such as "induced-self" that are structurally related to HLA class I molecules (e.g. MICA and MICB), "altered-self" (HLA class I molecule loaded with foreign peptide), or pathogen encoded "non-self" (molecules associated with infection, tumor transformation, and allograft antigens). KIR loci are highly polymorphic and map to chromosome 19. KIR and HLA gene families segregate independently, yielding many individuals who express KIR receptors for which they lack HLA class I ligands, and vice versa, creating human diversity in the number and type of KIR-HLA gene combinations, some of which have been correlated with the susceptibility to certain diseases [93].

Because NK cells can spontaneously deliver effector function, it is critical that they do not attack surrounding healthy cells. To prevent such detrimental autoimmune responses, NK cells express at least one inhibitory receptor to the self-HLA class I molecule [94]. Interaction of inhibitory KIR receptors with cognate HLA class I ligands further sets the threshold of NK cell functional competence [95]. Abundant expression of HLA class I molecules on normal healthy cells provide ligands for a variety of inhibitory receptors of NK cells, and consequently healthy cells become tolerant to NK cell attack. Down-regulation of HLA class I expression due to certain viral infections, neoplastic transformations, or other forms of stress, relieves the inhibitory influence on NK cells, permitting NK cells to eliminate these unhealthy target cells, a phenomenon originally described as the "missing-self" hypothesis [96].

In an allogeneic transplantation setting, even within HLA compatible transplants (but not HLA-A,B,C identical transplants), the recipient NK cells expressing an inhibitory receptor can be activated when confronted with allograft cells lacking relevant HLA class I ligand for that inhibitory receptor [97]. A study by van Bergen and colleagues revealed that the absence of donor HLA class I ligands for the recipient inhibitory KIR receptors is associated with reduced long-term graft survival in HLA-A,B,DR compatible kidney transplantations [98,99]. Kunert et al. [100] found an association between a higher number of inhibitory receptors in the recipient's genotype and patients with stable renal function. Vampa et al. [101] found recipients carrying more activating KIR genes triggered more NK cytotoxicity against their donors than recipients carrying fewer activating KIR genes. These studies provide evidence that suppressing NK cell activity may be important to improve renal allograft survival.

Non-HLA transplant antigens

Although the immune response to HLA antigens plays the central role in allograft rejection, evidence shows that non-HLA antigens also contribute to the pathogenesis of acute and chronic rejection and decrease long-term graft survival of solid organ transplants [102]. While many of these non-HLA antigens remain poorly defined, the majority are autoantigens except for the MICA antigens [103,104] and are generally implicated by the presence of antibodies associated with graft dysfunction. The principal antigenic targets are expressed on cells of the allograft, including endothelium and epithelium. Antiendothelial cell antibodies (AECAs) represent a heterogeneous group of antibodies comprising both IgM and IgG subclasses and are directed against a variety

of antigenic determinants [105]. Other autoantigens, including vimentin and cardiac myosin (CM) in heart [106,107], collagen V (Col V) and K-α1 tubulin in lung [108,109], and agrin and angiotensin II receptor type I (AT1) in kidney transplantation [110,111], have been identified as immune targets with poor transplant outcomes. Pathogenesis of these autoantibodies in transplant recipients have been demonstrated in several ways: (1) they induce cellular damage in vitro; (2) they cause graft rejection when passively transferred in an animal model; and (3) their removal by plasmapheresis or immunosuppressants results in an improvement of clinical signs of disease.

It is easier to understand the pathogenesis of antibodies against extracellular antigens than antibodies directed against inaccessible intracellular antigens. However, during transplantation, tissue damage caused by cold ischemia reperfusion injury and inflammation caused by transplant surgery may activate antidonor alloimmunity via the direct and indirect allorecognition pathways, leading to the release of intracellular antigens from the injured cells. These alloantigens can be presented via the indirect recognition pathway to host T cells to generate autoreactive cellular and humoral immune responses. The indirect alloimmune response, once initiated, can spread to additional determinants within the primary target antigen, called intramolecular epitope spreading. It is likely that repeated stimulation of $CD4^+$ T cells with self-antigens surpasses the threshold of self-tolerance and leads to the development of autoantibody-inducing $CD4^+$ T cells. This post-transplant autoimmunity can contribute to graft failure. It has been demonstrated that administration of anti-MHC class I antibodies into the native lungs of mice triggered $CD4^+$ T-cell driven autoreactivity, production of IL-1β and TNF-α, and the development of antibodies to the self-antigens K-α1 tubulin and collagen V [112].

Anti-MICA antibodies

MICA is encoded by a highly polymorphic gene with at least 63 alleles located in the MHC class I region [113]. MICA antigens have a restricted tissue distribution and are expressed on epithelium, endothelium, keratinocytes, and fibroblasts. *MICA* genes contain a heat shock response element (HRE) promoter and their expression levels can be induced in response to cellular stress mediated by ischemia reperfusion injury, and cytokines such as IL-2, IL-4, and IL-15. MICA acts as a ligand for the activating NKG2D receptor of NK cells and subset of T cells. MICA neither binds β2m nor peptides.

Alloantibodies against MICA have been associated with acute and chronic vascular rejection of renal and heart transplants. In a large multicenter study, presensitization to MICA was associated with increased graft loss in renal recipients transplanted with donors who were well matched for HLA. MICA antibodies have been shown to mediate complement-dependent cytotoxicity against endothelial cells, suggesting these antibodies can cause complement-mediated damage of the allograft [114]. However, most studies reporting a role for MICA in transplantation have been indirect and circumstantial because the donor specificity of the antibodies was not ascertained.

Summary

The major histocompatibility complex (MHC) is a tightly linked group of genes present in all vertebrate species. The MHC encodes a variety of proteins associated with the immune system involved in antigen presentation, peptide transporter molecules TAP1 and 2, molecules that catalyze and regulate peptide exchange, complement components C2, C4, and factor B, cytokines TNF-α and TNF-β, heat shock proteins 70-1 and 2, 21-hydroxylase (involved in steroid metabolism), and other less well-defined products and regulatory elements. The HLA class I and class II cell surface antigens in humans that are the major transplantation antigens are encoded by the human MHC.

Some MHC genes are remarkably polymorphic. Humans have more than 10 000 HLA-A, -B, -C, -DR, -DQ, and DP alleles that have been identified, which encode at least 100 antigens that can be detected serologically. Diversity is generated by a number of interesting and novel genetic mechanisms and is likely selected by adaptations to pathogens and disease.

Each individual inherits a distinct constellation of MHC class I and class II molecules in a strict Mendelian fashion with a crossover frequency of about $<1\%$ between A and DQB1. The probability of siblings matched at the HLA-A, -B, -C, -DR, and -DQ loci is 25%.

The MHC molecules play a key role in immune responses by presenting peptides derived from protein antigens to T cells with specific receptors. Affinities for CD8 and CD4 cell surface molecules preferentially direct presentation of peptides from intracellular proteins to $CD8^+$ T cells and intracellular proteins to $CD4^+$ T cells by class I and class II MHC molecules, respectively. Intracellular protein peptides are loaded into the cleft of MHC class I molecules in the endoplasmic reticulum, whereas the cleft of MHC class II molecules is protected in the ER but is subsequently loaded with peptides from extracellular proteins in endocytoplasmic vesicles.

Allogeneic HLA molecules provoke both T- and B-cell immune responses. Antibodies may recognize a range of structural features (epitopes) that are unique, or shared by a few or many HLA antigens resulting in cross-reactivity.

Other non-HLA antigens may act as transplantation antigens as well, including cryptic antigens not normally expressed extracellularly and autoantigens, most of which probably stimulate the recipient immune system via direct or indirect presentation on HLA molecules. HLA molecules are also ligands for natural killer cells, which represent an emerging area of study for their role in clinical transplantation.

Acknowledgments

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CHAPTER 5

Cellular Mechanisms of Adaptive Immunity

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Introduction

Organs transplanted between genetically distinct individuals are rejected in a T-cell-dependent manner. The ability of host T cells to recognize antigens from transplanted organs within the same species is known as allorecognition and, in the absence of some degree of immune modification, results in recipient T-cell activation, proliferation, differentiation, acquisition of effector functions, migration to the allograft, and eventual destruction of the transplant. Innate immune cells are essential for initial presentation of alloantigen to naïve T cells in secondary lymphoid organs and, in the graft, can also be activated by effector T cells and be deleterious to the transplant via secretion of inflammatory factors. Additionally, activated T cells can help B cells produce alloantibodies, driving humoral immune mechanisms that can also damage the graft. In this chapter, we will review key features of allorecognition and T-cell-dependent allograft rejection. Details of humoral alloimmunity can be found in Chapter 6. This chapter will focus on mechanisms involved directly in T-cell-mediated graft destruction; more general discussions of physiologic cellular immunity can be found in Chapter 2. Specifics of the histological and clinical presentations of cell-mediated rejection are found in organ specific chapters on the histopathological syndromes (Section Histopathological Syndromes of Graft Rejection and Recurrent Disease) and clinical diagnosis and management (Section Clinical Allograft Rejection Syndromes: Diagnosis and Management) of graft rejection, respectively.

T-cell recognition of alloantigens: Direct, semidirect, and indirect presentation

T cells express a T-cell receptor (TCR) that can bind complexes formed by peptides positioned in the groove of MHC molecules, themselves expressed on the surface of antigen-presenting cells (APCs). T cells are educated in the thymus such that their TCRs recognize self-MHC complexes presenting peptides derived from endogenous self-proteins, with $CD8^+$ T cells recognizing MHC class I (HLA-A, B, and C in the human) and $CD4^+$ T cells engaging MHC class II (HLA-DR, DP, DQ). Detailed descriptions of MHC antigens can be found in Chapter 4. Thymocytes with TCRs that recognize self-peptide/MHC (pMHC) with high affinity are eliminated in a process called negative selection, which reduces the frequency of T cells capable of mediating autoimmunity, while thymocytes with sufficient affinity for self-pMHC to trigger survival pathways, but not enough to result in full activation, are positively selected. In contrast, thymocytes that cannot bind self-pMHC die by neglect. Thus, mature T cells that populate the body are "restricted" to recognition of self-MHC molecules. When these molecules present the normal array of endogenous self-peptides outside of the thymus, engaged T cells receive survival signals without being activated or proliferating [1]. Each of these T cells has the ability to be fully activated by a given "foreign" cognate peptide that may replace basal-state endogenous peptides on self-MHC molecules and may originate from an infectious organism, a somatically mutated protein, or an allogeneic cell. Presentation of an allogeneic peptide (derived from allogeneic MHC molecules or from polymorphic variations between donor and recipient non-MHC proteins termed **minor antigens**) in this context of self-MHC molecules is called **indirect presentation** and requires host APCs to pick up antigens from dying donor cells by phagocytosis, pinocytosis, or endocytosis (Figure 5.1).

Surprisingly, despite thymic selection producing T cells with TCRs restricted by self-MHC molecules, many T cells can also bind to allogeneic MHC molecules, presumably because there is no process by which alloreactive T cells would be eliminated in the thymus as allogeneic MHC molecules are not expressed in the thymus. In fact, whereas only approximately 1 in 10^6 T cells may recognize a "foreign" antigen on a given self-MHC allele, it is estimated that 1–10% of T cells can engage allogeneic MHC molecules directly [2]. This high precursor frequency had initially led scientists to hypothesize that T cells may bind allogeneic MHC molecules in a different manner from self-MHC molecules, perhaps contacting only MHC residues and thus being permissive to multiple peptides presented on each MHC allele. However, analysis of the few TCR/p/alloMHC crystal structures that have been solved (see below), suggests that the interactions are peptide-dependent [3]. The spectrum of receptor ligand interactions that make up one's alloimmune repertoire are considered further in Chapter 9. MHC molecules are highly polymorphic with hundreds of MHC alleles within the population, and each person being able to express up to six MHC class I and six MHC class II alleles (three of each on each chromosome 6; also see Chapter 4). Because each MHC allele can present a multitude of endogenous peptides, the

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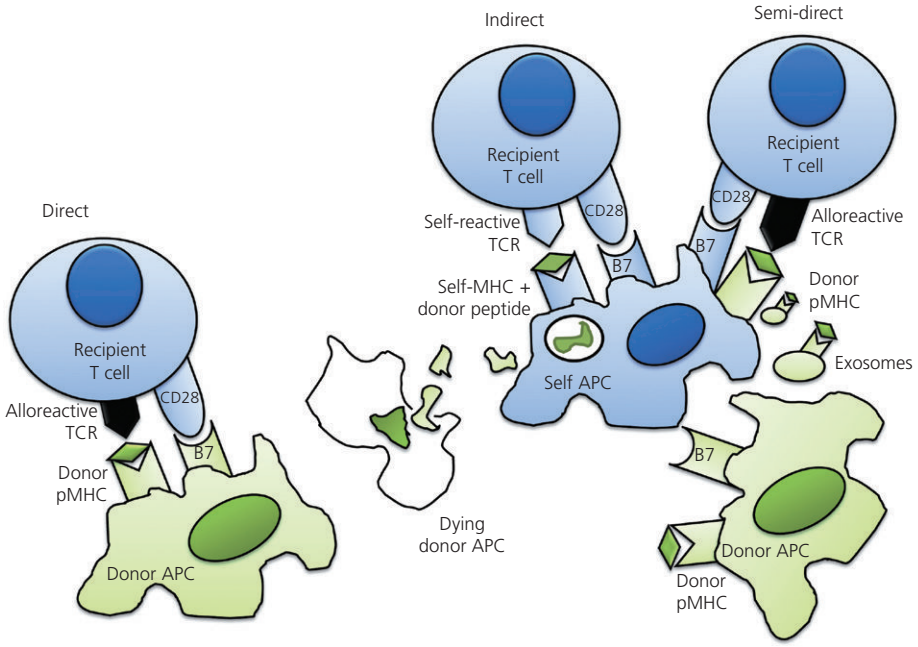

Figure 5.1. Types of allorecognition. T cells are selected in the thymus based on the restriction of their TCR to recognition of self-MHC molecules. However, a proportion of T cells can also recognize allogeneic MHC molecules/donor peptides either via cross-reactivity between allo- and self-MHC/peptide complexes or via distinct TCR/MHC docking sites. After transplantation, these T cells can recognize allogeneic MHC directly on the surface of donor APCs or parenchymal cells (direct recognition). In addition fragments of intact membranes containing donor MHC may fuse with membranes of recipient APCs either following cell–cell contact or cell–endosome contact, such that T cells can recognize intact donor MHC on recipient APCs (semidirect presentation). Finally, fragments of dying transplant cells can be picked up by recipient APCs and allogeneic peptides derived from polymorphic proteins distinct between donor and recipient can be presented by self-MHC molecules (indirect presentation). These mechanisms can all apply either to $CD4^+$ T cells recognizing donor or recipient MHC class II and $CD8^+$ T cells recognizing donor or recipient MHC class I.

combination of T cells that can each recognize a specific peptide with a given allogeneic MHC allele can add up to a very high number, possibly explaining the high frequency of T cells with direct alloreactivity.

Direct allorecognition by host T cells of donor peptide/donor MHC molecules can occur either on donor APCs contained in the transplanted organ (**direct presentation**), or following transfer of fragments of intact membranes from donor cells onto recipient APCs (**semidirect presentation**) [4] (Figure 5.1) (see Chapter 2). The latter may occur as a result of cell–cell contact between host and donor cells or via the transfer of donor exosomes that fuse with the plasma membrane of host APCs. Traditionally, MHC class I is described as predominantly presenting peptides derived from cytosolic proteins translocated to the endoplasmic reticulum by the transporter associated with antigen processing (TAP), and MHC class II presenting peptides from extracellular proteins delivered to endolysosomes by phagocytosis, pinocytosis, or endocytosis. However, it is now clear that extracellular proteins can be presented by MHC class I and cytosolic proteins by MHC class II. Delivery of intracellular cytoplasmic antigens to the endolysosomal pathway for class II presentation can occur from endosomes generated during autophagy [5,6] whereas extracellular phagosome cargo may seep out of the organelle into the cytoplasm by an incompletely understood mechanism and become available to the proteasome for processing, TAP translocation, and class I presentation [7]. These pathways further widen the possible peptide repertoires for both direct and indirect T-cell allorecognition.

Structural recognition of alloantigens by the TCR

The TCR comprises an α and a β chain that form three hypervariable complementarity-determining regions (CDRs) responsible for contacting amino acid residues on the peptide and the MHC molecule. Active investigation has been devoted to understanding how T cells raised to be restricted by self-MHC can recognize allogeneic MHC directly. A major obstacle to this research has been that the endogenous peptides naturally presented on MHC molecules are most often not known and could be derived from anywhere within the donor proteome. Only a handful of TCRs with both self-pMHC and allo-pMHC known ligands have been co-crystallized and directly compared [8]. Some structures have revealed molecular mimicry whereby allogeneic peptide/allo-MHC complexes mimic the conformation of foreign peptide/self-MHC although taking advantage of some TCR and pMHC plasticity (induced fit) to optimize interactions. Other structures have evidenced a distinct strategy of engagement by the TCR on self-MHC versus allo-MHC complexes, but in both instances including contacts on both the peptide and the MHC molecule itself. A report further demonstrates that alloreactivity is limited by the endogenous peptide repertoire, suggesting that allorecognition, like conventional recognition of foreign peptide on self-MHC, is critically peptide dependent and peptide specific [9]. It has also been suggested that T cells that express two TCRs of distinct specificities because of failed allelic exclusion of the α chain (1–8% of the T-cell repertoire) have a greater alloreactivity [10] (also see Chapter 9). As the rules

of allorecognition become better defined, it may become possible to predict cross-reactivity of host T cells on donor pMHC to help choose optimal donors with lower potential to activate recipient T cells.

T-cell costimulation and differentiation

In addition to requiring TCR engagement, full initial activation of naïve T cells (**T-cell priming**) can only occur when additional surface molecules termed costimulatory receptors are ligated simultaneously to the TCR [11]. Few costimulatory receptors are expressed on naïve T cells and CD28 is thought to be the principal one. CD28 is the receptor for CD80 (B7-1) and CD86 (B7-2) ligands expressed on APCs and further up-regulated during inflammatory conditions on APCs and some parenchymal cells. Blockade of CD28 during TCR binding has been shown to result in apoptosis or subsequent unresponsiveness of T cells to restimulation, a state termed **T-cell anergy**. These findings have led to the development of the reagents abatacept and belatacept that can bind CD80 and CD86 and prevent these ligands from engaging and activating CD28, therefore markedly reducing T-cell priming. Belatacept was approved in 2011 as an immunosuppressive drug for kidney transplantation. These agents prevent activation of alloreactive T cells after transplantation, hopefully leading to their apoptosis or anergy, without affecting T cells of other specificities whose TCR is not being engaged at the time of Belatacept treatment.

Following T-cell activation, T cells up-regulate many additional costimulatory receptors, including members of the immunoglobulin superfamily such as ICOS, from the TNF receptor family such as 4-1BB and OX40, and from the T-cell immunoglobulin mucin (TIM) family [12]. Activated T cells also up-regulate CD40L (CD154), a TNF family member that engages the TNFR family member CD40 on APCs and drives APCs to express higher levels of MHC, CD80, and CD86 and to produce cytokines. Many of the costimulatory molecules remain expressed after naïve T cells differentiate into effector cells and memory cells, such that it is more difficult to suppress previously activated than naïve T cells, as these receptors can lower the threshold of T-cell activation. Ligands for these costimulatory receptors are expressed on APCs and some of them can be up-regulated on parenchymal cells such as epithelial and endothelial cells.

In addition to expression of costimulatory receptors that can promote T-cell activation, stimulated T cells up-regulate members of the costimulatory family that inhibit or terminate T-cell activation and are sometimes called co-inhibitory receptors. These include CTLA-4 (binds to CD80 and CD86 with higher affinity than CD28), PD-1 (binds PDL1 and PDL2), BTLA (binds HVEM), LAG3 (binds MHC class II), and as yet unidentified receptors on T cells of newly described ligands on APCs that include B7-H4, B7S3, BTNL2, and VISTA. All these inhibitory receptors are members of the immunoglobulin superfamily, and their induction is necessary to restrain T-cell responses as animals deficient in these molecules have various degrees of lymphoproliferation and autoimmunity, which is lethal in the case of CTLA-4 deficiency. The ligands to these receptors are expressed on APCs, as well as on some parenchymal cells.

Upon stimulation, naïve T cells produce IL-2 and use it as an autocrine growth factor to proliferate. In addition, depending on the cytokines they encounter during their multiplication, they can differentiate into various types of effector T cells distinguished by the profile of cytokine production they acquire (Figure 5.2). $CD4^+$ T cells are often referred to as T helper cells (Th), whereas $CD8^+$ T cells are frequently called cytotoxic T cells (Tc) (see Chapter 2). Activated T cells exposed to IL-12 will differentiate into effector T cells that predominantly produce IFN-γ and are termed Th1/Tc1, whereas encounter with IL-4 will drive differentiation of Th2/Tc2

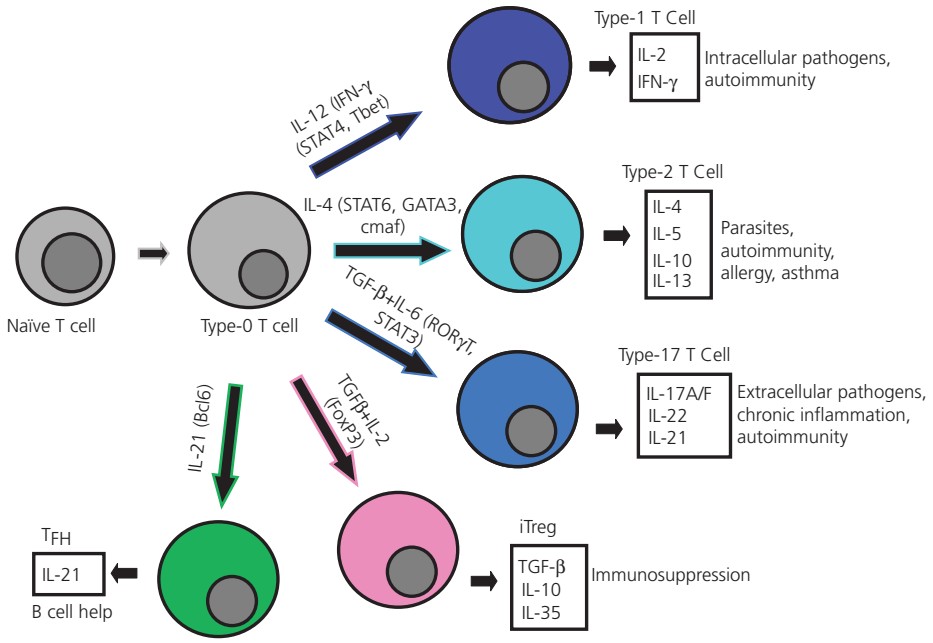

Figure 5.2. Pathways of T-cell differentiation. Upon activation, T cells can be driven toward different functional phenotypes, shown in this figure and based on varying exposures to cytokines and the actions of different transcription factors. In general, these take on prototypical patterns leading to the development of five major types of T cells: Th-1, Th-2, Th-17, induced T regulatory cells (iTreg), and T follicular helper cells (Tfh).

cells that produce IL-4, IL-5, IL-10, and IL-13, and exposure to TGF-β and IL-6 will result in differentiation of Th17/Tc17 cells that produce IL-17A (IL-17), IL-17F, and IL-22 [13,14]. These discrete cytokine profiles of effector T cells are each uniquely qualified to respond to specific pathogens, with Th1 cells playing a major role in the elimination of intracellular pathogens, Th2 cells fighting parasites, and Th17 cells being important against extracellular micro-organisms. Under some circumstances these cells can also become pathogenic, with Th1 and Th17 cells associated with autoimmunity and Th2 cells with asthma and allergies. In transplantation, acute allograft rejection has mostly been correlated with the presence of IFN-γ-producing Th1/Tc1 cells, although presence of IL-17 is becoming increasingly recognized in rejecting allografts and Th2 cells and Th2 cytokines also having been shown capable of driving rejection.

After proliferation and differentiation, T cells go through a contraction phase in which the majority undergo apoptosis or cell-cycle arrest as a means to terminate the immune response, with the remaining T cells acquiring memory characteristics. In transplantation, T cell-deficient mice transferred with memory T cells can reject allogeneic grafts with faster kinetics than those receiving naïve T cells [15]. Importantly, prior immune responses to microbes results in generation of pathogen-specific memory T cells some of which, like naïve T cells, can also be alloreactive [16]. These memory cells are less susceptible to conventional immunosuppressive therapies and can constitute an important barrier to allograft acceptance (see Chapter 9).

In addition to conventional $CD4^+$ and $CD8^+$ T cells, a subset of $CD4^+$ T cells has been shown to express the transcription factor Foxp3 and lack the ability to produce IL-2. Instead, these cells suppress the function of conventional T cells in vitro and in vivo and are now referred to as regulatory T cells (Tregs) (see Chapter 8)[17]. Tregs are essential to maintain T-cell homeostasis and prevent autoimmunity, as mice deficient in Tregs or mice in which Tregs are permanently eliminated after adulthood die rapidly of lymphoproliferation and autoimmunity. A congenital disease of loss of function mutations in the *Foxp3* gene is known in humans as IPEX syndrome and similarly leads to autoimmunity. Two types of $Foxp3^+$ Tregs have been described. The first one develops in the thymus, like conventional T cells, and is often called natural Tregs (nTregs). The second type develops from mature naïve T cells activated in the presence of TGF-β and IL-2 or retinoic acid in vitro or in vivo, and is referred to as induced or adaptive Tregs (iTregs). Tregs form about 5–10% of $CD4^+$ T cells in mice and humans. Both nTregs and iTregs can suppress immune responses and it has been shown that both are necessary, at least in mice, to maintain T-cell homeostasis [18]. In transplantation, it has been shown that iTregs can be induced following costimulation blockade protocols of tolerance induction [19], and both nTregs and iTregs can be visualized in accepted islet allografts [20]. However, the relative importance of each subset in controlling acute or chronic rejection remains to be established. Because nTregs and iTregs both express CD4, Foxp3, high CD25, and low levels of CD127, it remains challenging to distinguish them. It has been reported that nTregs express higher levels of the transcription factor Helios than iTregs [21], although this remains controversial, and that iTregs have specific sites within the *Foxp3* gene that remain partially methylated when compared to nTregs [22,23].

Because iTregs can be generated in vitro from PBMCs and expanded to very high numbers, clinical trials are exploring the safety and efficacy of transferring iTregs adoptively into transplant recipients. Initial trials in bone marrow transplantation have shown reduction of graft-versus-host disease (GVHD) and trials in solid organ transplantation are starting both in Europe and in the USA [24].

Innate immune signals that influence T-cell responses

Upon activation, APCs can secrete cytokines that help shape T-cell differentiation. Because the immune system evolved to fight infections, receptors that recognize molecular patterns present in microbes are critical in driving APC activation and bridging innate and adaptive immunity (see Chapter 2). Pathogen-associated molecular patterns (PAMPs) include endotoxin, flagellin, proteoglycans, unmethylated CpG motifs, double-stranded RNA, and many others [25]. These PAMPs can bind Toll-like receptors (TLRs) expressed on the plasma membrane and endosomal membranes of APCs, but also of T cells and some parenchymal cells [26]. Engagement of TLRs in APCs results in up-regulation of costimulatory ligands, thus facilitating their presentation of antigen to T cells, as well as production of cytokines such as type I IFN, IL-1, TNF, IL-6, and IL-12. These cytokines define the type of differentiation that the activated T cells will undergo, based on the appropriate response to the infecting organism, with, as mentioned above, IL-12 facilitating Th1 and IL-6 promoting Th17 differentiation. Importantly, molecular pattern-containing factors generated during sterile inflammation, such as that experienced by allografts during ischemia and reperfusion, can also activate some TLRs, mostly TLR2 and TLR4, either by ligating them directly or by sensitizing these TLRs to minimal concentrations of microbial ligands [27]. In the context of transplantation, several of these damage-associated molecular patterns (DAMPs) have been described that serve as adjuvants to augment alloresponses. HMGB1, heat shock protein (HSP), and hyaluronan have all been shown to potentiate alloreactivity [25]. In addition, haptoglobin, an acute phase protein, was reported to be up-regulated in transplanted tissues and haptoglobin-deficient skin displayed prolonged survival after transplantation in mice with more than half the animals retaining their grafts long term [28]. These data suggest that localized therapies to reduce TLR signaling by donor organs may be beneficial in transplantation.

Importantly, some TLRs are expressed in T cells themselves, including conventional T cells and Tregs. Engagement of TLRs in conventional T cells has been shown to promote T-cell activation and T-cell survival [29], while their engagement in Tregs can, depending on the circumstances, either enhance or prevent their suppression function perhaps by affecting epigenetic regulation of the *Foxp3* gene [30–36]. In transplantation, TLR engagement at the time of transplantation has been shown to prevent the induction of transplantation tolerance by costimulation-blockade therapies in mice and drive the differentiation of alloreactive T cells into Th1 and Th17 phenotypes, while inhibition of both IL-6 and IL-17 restored graft survival [37–39]. These data indicate that sensing of damage or infections by these innate receptors can shape the type of T-cell alloresponse to an allograft and promote T cell-dependent rejection by different effector mechanisms [40,41]. Therapeutic targeting of these inflammatory pathways is being considered to facilitate transplantation tolerance.

TLRs are not the only receptors that can sense damage and pathogens. Another type of membrane-bound receptor, C-type lectin receptors (CLRs), can engage fungal and bacterial PAMPs and trigger the production of inflammatory cytokines such as TNF and

IL-6. The other receptors described to date are all cytosolic and include NOD-like receptors (NLRs) that sense a variety of PAMPs, particulate matter, crystals, and other sterile inflammatory factors, to induce either IL-1 production downstream of the inflammasome or inflammatory cytokines, as well as RIG-I-like receptors (RLRs) that sense RNA, and receptors that sense DNA, both of which trigger type I IFN production (and IL-1 for some DNA-sensing receptors). The impact of these receptors on alloimmunity remains to be determined, but it is likely that dying donor cells will trigger several of these pathways thus enhancing alloreactivity.

Where are T cells primed after transplantation?

Donor parenchymal cells in a transplanted graft all express MHC class I and can therefore be recognized by host $CD8^+$ T cells with direct alloreactivity. Endothelial and some epithelial cells exposed to the stress of ischemia and reperfusion can up-regulate MHC class II and therefore be recognized by host $CD4^+$ T cells with direct alloreactivity. However, unlike professional APCs that also express MHC class II, such as activated dendritic cells, macrophages, and B cells, most parenchymal cells do not express costimulatory ligands and therefore cannot activate T cells, as T cells, especially in their naïve state, require engagement of costimulatory receptors such as CD28 at the time of TCR ligation to be able to mount a productive immune response. Therefore, most of the activation of T cells with direct alloreactivity is thought to be triggered initially by donor APCs carried by the graft and often termed passenger leukocytes. Studies performed by Lafferty and colleagues in the 1970s showed that elimination of passenger leukocytes from donor tissue by in vitro culture markedly reduced their immunogenicity [42]. Donor APCs are known to migrate into draining secondary lymphoid organs, spleen, and lymph nodes, patrolled by T cells in search of their cognate antigen. In addition, recipient APCs that pick up dying donor cells or membranes also migrate to secondary lymphoid organs to present alloantigen indirectly or semidirectly. However, as discussed previously, the precursor frequency of T cells recognizing alloantigen directly is higher than that of T cells with indirect allorecognition, such that direct activation of alloreactive T cells by donor APCs is believed to be the predominant pathway of T-cell activation initially after transplantation. As the half-life of donor APCs is short, the indirect pathway of allorecognition becomes predominant over time and may therefore play a role in later episodes of acute rejection and in chronic rejection. Evidence that secondary lymphoid organs are essential for alloreactive T-cell priming and graft rejection was demonstrated by using mice with genetic mutations resulting in lymph node agenesis (Aly/Aly mice) in which a surgical splenectomy was performed (see Chapter 14). Aly/Aly mice failed to reject allogeneic skin grafts whereas mice lacking both lymph nodes and spleen could not reject vascularized heart transplants [43,44]. Interestingly, lymph nodes also appear to be required for the induction of transplantation tolerance by costimulation blocking therapies [45], suggesting that T-cell activation can be aborted at the site of priming with long-lasting consequences.

Importantly, requirement for secondary lymphoid organs was only absolute for naïve T cells, as transfer of memory T cells into splenectomized Aly/Aly mice resulted in recall responses, propagation of alloreactive T cells, and transplant rejection independently of lymph nodes and spleen [46]. This property of memory cells enhances the challenge for their immunosuppression as they may be more difficult to inhibit before they can do damage if they can go directly to the graft.

Effector phase of the alloimmune response: T cells go to the graft

Once primed, naïve T cells proliferate, differentiate, and acquire effector functions. Their egress from lymph nodes is regulated by the sphingosine-1 phosphate (S1P) receptor and agonistic activity of S1P1 by the drug FTY720 has been shown experimentally to result in trapping of lymphocytes in thymus, lymph nodes, and Peyer's patches [47], although clinical trials with FTY720 at the doses tested have not revealed immunosuppressive efficacy. Once in circulation, effector T cells are thought to enter inflamed tissues in response to chemokine gradients, though graft infiltration and acute rejection can still occur when chemokine signaling is blocked, and the exact chemokines that recruit T cells to specific transplanted organs or the cells that produce them have not been fully uncovered [48]. Neutrophils that infiltrate allografts following ischemia reperfusion have been shown to promote recruitment of $CD8^+$ T cells into allografts and depletion of polymorphonuclear cells can synergize with costimulation blockade therapy to prevent rejection of cardiac allografts in mice [49], suggesting an avenue to reduce acute graft infiltration.

In considering how effector T cells can recognize alloantigen at the graft site and trigger transplant damage (Figure 5.3), one needs to consider separately $CD4^+$ and $CD8^+$ T cells with direct versus indirect allorecognition. $CD8^+$ T cells with direct allorecognition should be able to engage alloantigen on all nucleated transplant cells as they all express MHC class I. It has been shown that $CD8^+$ T cells can be activated by allogeneic endothelial cells and that cardiac allografts can be rejected when donor MHC class I is present only on non-hematopoietic cells in mice [50]. These results suggest that recognition of alloantigen in the vasculature is essential either for entry of direct $CD8^+$ T cells into the graft for subsequent killing of parenchymal cells, or that $CD8^+$ T cells can injure vascular endothelial cells resulting in downstream graft damage by nutrient deprivation or thromboembolic events.

Expression of MHC class II is more restricted than that of MHC class I, with only dendritic cells, macrophages, B cells, activated endothelial cells, and some epithelial cells expressing it. Therefore, $CD4^+$ T cells with direct allorecognition should have more limited access to alloantigen in the graft. Expression of donor MHC class II in cardiac allografts has been shown to be sufficient for CD4-mediated rejection in the absence of host MHC class II in mice [51]. Moreover, donor class II on hematopoietic cells was shown to be more important than on endothelial cells [52]. It is not exactly clear how engagement of donor hematopoietic cells in the graft can result in graft rejection by $CD4^+$ T cells, but it is thought that T cells may activate donor APCs to produce inflammatory factors that are toxic to parenchymal cells in the transplant. Once donor APCs have left the graft, interaction of direct $CD4^+$ T cells with class II-expressing parenchymal cells may result in their inactivation or death because of insufficient costimulation, or in a switch to a Th2 phenotype. Indeed, the frequency of direct pathway-reactive T cells is known to decrease over time after transplantation [4].

T cells with indirect allorecognition can also play a role in allograft rejection. For $CD8^+$ T cells with indirect allospecificity to be activated, they need to encounter host cells presenting peptides derived from donor MHC or other polymorphic proteins presumably acquired via phagocytosis of dying donor cells, with donor

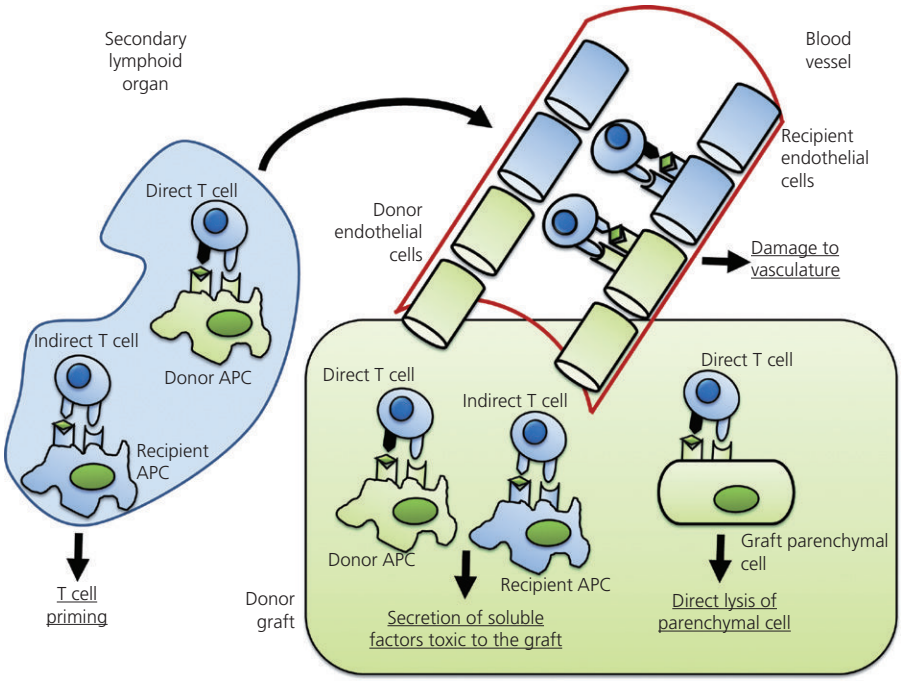

Figure 5.3. Mechanisms of T cell-mediated allograft rejection. T cells with direct and indirect alloreactivity can be primed in secondary lymphoid organs by donor and recipient APCs, respectively. Once activated these T cells proliferate, differentiate, acquire effector functions, and migrate via efferent lymphatics to the bloodstream. Upon reaching the transplant, alloreactive T cells may recognize recipient endothelial cells presenting allopeptides or donor endothelial cells in the graft and may mediate damage to the vasculature, therefore compromising the organ. Recognition of alloantigen on endothelial cells may also facilitate their recruitment/entry into the allograft. T cells that infiltrate the graft may recognize donor or recipient APCs presenting peptides. This may result in activation of both the T cell and the APC to secrete inflammatory factors that may damage the graft. Alternatively, T cells with direct alloreactivity may also recognize parenchymal cells and cause their death via Fas signaling or perforin and granzyme cytotoxicity. In some instances, $CD4^+$ T cells or $CD8^+$ T cells may be independently sufficient to reject a transplant; in other cases, activated $CD4^+$ T cells may help activated $CD8^+$ T cells develop their effector functions. In stable allografts, endothelial and parenchymal cells may not express costimulatory ligands anymore and their encounter may result in T-cell death or inactivation to perpetuate tolerance, rather than in T-cell activation and rejection episodes. Tregs with direct and indirect specificity may also be at play to inhibit alloresponses, both at the priming stage in secondary lymphoid organs and at the effector stage in the graft.

proteins escaping the endosome to enter the cytosolic TAP/endoplasmic reticulum/self-MHC class I pathway. Endothelial cells are capable of cross-presenting donor antigens in this manner [53]. Evidence for a pathogenic role of indirect $CD8^+$ cells in alloresponses has been revealed using TCR transgenic $CD8^+$ T cells specific for a male antigen presented by host MHC class I ($H\text{-}2^b$) and unable to recognize donor MHC ($H\text{-}2^k$). These T cells were shown to reject donor skin grafts with which they cannot interact, in an IFN-γ-dependent manner, and only if the vasculature expressed self-MHC molecules [54]. However, indirect $CD8^+$ T cells had no effect in a fully mismatched cardiac allograft model [55], thus questioning the impact of this pathway when direct recognition pathways are also operating.

The role of $CD4^+$ T cells with indirect recognition has been addressed similarly by using TCR transgenic T cells specific for a male antigen presented by host MHC class II. By using heart allografts from male mice with a disparate MHC alleles that cannot present the antigen to the transgenic $CD4^+$ T cells, it was found that host endothelial cells that progressively replace donor endothelial cells in the graft could activate the indirect $CD4^+$ T cells to induce graft vasculopathy and chronic rejection [56]. Overall, all these studies emphasize the important immunological function of endothelial cells, as APCs or as targets of effector T cells. Endothelial cells can also serve as targets for alloantibodies and innate immune cells, making them central players in acute and chronic rejection and deserving of special attention in future research [57]. B cells are also important for the priming of indirect $CD4^+$ T cells, as lack of MHC class II confined to the B cell compartment can result in reduced activation of alloreactive $CD4^+$ T cells and in turn diminished alloantibody production and prolonged cardiac allograft survival in mice [58].

The TCRs of Tregs can also have direct and indirect reactivity with alloantigen, but it has been shown that Tregs with indirect specificity have greater capacity to suppress alloresponses in vivo [59]. Interestingly, the inhibitory function of Tregs in alloresponses has been shown to depend on IFN-γ production by Tregs [60], an observation consistent with earlier reports demonstrating the resistance of mice deficient in IFN-γ-dependent signaling to transplantation tolerance [61]. Thus, IFN-γ may be a double-edged cytokine, important in the pathogenesis by Th1/Tc1 cells but also in the immunosuppressive function of Tregs. The predominant suppression of indirect alloresponses by Tregs has been confirmed in stable kidney transplant patients as depletion of Tregs in PBMCs resulted in enhanced IFN-γ production to HLA-DR-specific peptides [62]. The ability of Tregs to suppress indirect alloresponses positions them well to control chronic rejection, which may be more dependent on indirect presentation of alloantigen after passenger leukocytes have died. Indeed, the presence of a higher

number of Tregs in renal transplant patient biopsies containing inflamed fibrosis has been reported to correlate with better allograft survival [63]. Furthermore, Tregs with indirect specificity have been shown in rats to suppress in vivo production of alloantibodies [64] that are known to contribute to chronic rejection.

Another important consideration in the biology of Tregs following transplantation is whether they can suppress both T-cell priming in secondary lymphoid organs and effector T-cell responses in the allograft. Tregs have been shown to migrate from blood to inflamed islet allografts and back to draining lymph nodes, and to prevent migration of dendritic cells to secondary lymphoid organs [65]. Therefore, Tregs can reduce initial alloreactivity, T-cell priming and help the induction of tolerance. In addition, Tregs have been shown to accumulate in accepted allografts after costimulation blockade, and their sustained suppression at the graft site has recently been shown to be essential for infectious transplantation tolerance, a process by which new effector T cells become iTregs if their cognate antigen is expressed by APCs that can also present to existing Tregs [66,67]. These data indicate that Tregs also participate in the maintenance of tolerance.

Effector T cells: Th1, Th2, Th17, and cytotoxic T cells

Rejection of different organs has been shown to depend differently on T-cell subsets, at least in murine models (see Chapter 14). It is interesting to note that although the majority of the T-cell infiltrate in rejecting hearts is formed by $CD8^+$ T cells, cardiac allograft rejection can occur in the absence of $CD8^+$ but not of $CD4^+$ T cells, indicating that class II expression in the graft is sufficient for $CD4^+$ T cells to mediate rejection and suggesting alternative rejection mechanisms to cytolysis, as many $CD4^+$ T cells do not contain cytotoxic granules. Nevertheless, when the strength of the $CD4^+$ T-cell alloresponse is decreased such as in CD28-deficient mice, acute rejection becomes dependent on both $CD4^+$ and $CD8^+$ T cells, underscoring the participation of the latter T-cell subset, especially under conditions of immunosuppression [68].

Rejection of pancreatic islets has been reported to be dependent on either $CD4^+$ or $CD8^+$ T cells, depending on the strain combinations employed. Similarly, either $CD4^+$ or $CD8^+$ T cells are sufficient to drive skin and intestine allograft rejection. It may be that strong activation of $CD8^+$ T cells, as with Langerhans cells, obviates their requirement for $CD4^+$ T-cell help for their activation. However, $CD4^+$ T-cell help may be necessary for generation of alloreactive memory $CD8^+$ T cells [69].

The relative importance of distinct effector phenotypes, such as Th1, Th2, and Th17, in allograft rejection remains a subject of debate. IFN-γ production is most often associated with allograft rejection and T-bet, the signature transcription factor of Th1/Tc1 cells, can be detected in biopsies from kidney transplant recipients undergoing acute rejection [70]. However, mice deficient in IFN-γ are not impaired in allograft rejection but rather in allograft tolerance [61,71,72] and mice lacking STAT4, a transcription factor downstream of IL-12 without which Th1 differentiation is defective, do not display impaired allograft rejection [73,74]. Similarly, high serum concentrations of IFN-γ have been reported in patients without episodes of rejection [75]. It is important to note that IFN-γ and T-bet can be expressed by many cell types in addition to Th1/Tc1 cells, including NK cells, Tregs, and even dendritic cells, such that detection of this cytokine in mixed lymphocyte reactions does not equate Th1 alloreactivity.

Given their more recent discovery, Th17 cells have been under scrutiny in many pathological processes, with transplant rejection being no exception. IL-17 expression has been identified in many allografts undergoing acute or chronic rejection and IL-17 mRNA has been found in urinary sediments of kidney recipients [63,76–79]. Blockade of IL-17 was initially shown to prolong survival of rat cardiac allografts [80]. In mouse models, the pathogenic role of Th17/Tc17 cells has been evidenced principally in animals deficient in T-bet that become resistant to costimulation blockade-mediated induction of transplantation tolerance in a Tc17-dependent manner in one model or develop Th17-dependent accelerated vasculopathy in another [81,82]. In wild-type mice, signaling by TLR agonists at the time of transplantation was also shown to result in IL-6 production and promotion of IL-6/IL-17-dependent allograft rejection [38]. This is consistent with the demonstration that phagocytosis of infected apoptotic cells, which will trigger both TGF-β production from ingestion of apoptotic material and IL-6 production through stimulation of endogenous TLRs is a clinical situation in which Th17 cells may differentiate in vivo [83]. Additionally, exposure of self-antigens, such as collagen V or tubulin, following transplant injury has been reported to give rise to autoimmune Th17 cells with pathological consequences on allografts, both in animals and humans [84–88]. Importantly, some T cells can co-produce IFN-γ and IL-17 and may constitute a particularly pathogenic T-cell subset. It is important to note that differentiation into effector Th and Tc phenotypes may not be as terminal as initially thought and cells may retain pluripotency and plasticity to convert into other phenotypes depending on the environment [89], suggesting that it may be important to monitor T-cell function over time in transplant recipients to adjust immunosuppressive strategies to changing alloresponses.

Memory $CD8^+$ T cells are one of the first cells to enter allografts and initiate graft rejection [90], making this pathway important to suppress. The pathogenic role of effector and memory $CD8^+$ T cells in allograft rejection has been most often ascribed to their ability to produce IFN-γ and to lyse target cells via their perforin and granzyme-containing granules or via up-regulation of FasL (CD95L), which can kill by engaging the proapoptotic molecule Fas (CD95) on target cells [91,92]. In mice, FasL and perforin have been shown to play redundant, but obligatory, roles for acute rejection of pancreatic islets [93] with IFN-γ produced by the $CD8^+$ T cells themselves being also critical [94]. Perforin and granzyme are also important in chronic rejection, with knockout mice showing reduced apoptosis of endothelial cells and diminished subsequent allograft vasculopathy [95,96]. Importantly, granzyme B is also expressed in Tregs and plays a role in their suppressive function by allowing them to lyse $CD4^+$ T cells [97]. Consistent with this observation, expression of granzyme B in $Foxp3^+$ cells was necessary for the induction of skin allograft tolerance in mice [98], a reminder that effector pathways can have opposite consequences depending on the cells in which they are expressed.

Therapeutic implications

Because allograft rejection is absolutely T cell-dependent, immunosuppressive strategies have concentrated on preventing T-cell responses. The pathways targeted are covered in depth in Chapter 17. Most existing immunosuppressive agents such as calcineurin inhibitors or DNA synthesis inhibitors attempt to decrease T-cell activation and proliferation and do not induce transplantation tolerance. Experimental approaches to induce tolerance have aimed

either at deleting alloreactive T cells (using depleting antibodies, irradiation, or pruning strategies [99]), or at inducing their inactivation either intrinsically (through costimulation blockade or immunotherapy with tolerogenic APCs), or extrinsically (harnessing the suppressive power of Tregs, myeloid-derived suppressor cells, mesenchymal stem cells, or other cells) [99,100]. Interestingly, mTOR inhibition can promote iTreg differentiation while preventing differentiation of Th1, Th2, and Th17 cells [101], making rapamycin an attractive drug in transplantation, although it has also been shown to promote memory $CD8^+$ T-cell responses when used at lower doses [102,103]. Because deletion of T cells can paradoxically result in the proliferation of residual T cells to fill the space and their acquisition of memory characteristics with their inherent resistance to tolerance induction [104], a combination of partial deletion with induction of anergy and/or suppression of residual T cells may seem the more rational approach to the induction of tolerance.

Summary

The T cell and its biology remain at the core of transplant biology, and an understanding of the mechanisms involved in T-cell-mediated allorecognition remains fundamental to the practice of clinical transplantation. T-cell function precipitates essentially all of the clinical syndromes of acute cellular allograft rejection, and are central to most of the antibody-mediated forms of rejection, as well. Given the critical role of T cells in protective immunity, this biology also is relevant to an understanding of the infectious and malignant complications that continue to confound the long-term management of transplant recipients.

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CHAPTER 6

Humoral Mechanisms of Adaptive Immunity

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Introduction

Humoral mechanisms of immunology comprise fluid phase reactants, including antibodies, complement, coagulation factors, and cytokines. As part of the adaptive immune response, antibody has antigen-specific receptors; whereas complement and cytokines are antigen non-specific mediators of the innate immune system. All of these mediators can also engage cells of the innate immune system, notably platelets, granulocytes, monocyte/macrophages, and NK cells. These innate cellular and humoral mediators can greatly amplify and modify antibody-mediated injury. As a result, humoral mediators can cause a spectrum of allograft destruction, ranging from hyperacute through acute to chronic rejection. In this chapter we will first consider humoral mediators separately. Then we will discuss the interactions of antibody and mediators with recipient and donor cells that contribute to tissue injury in allografts. The chapter will focus on mechanisms involved directly in graft destruction; more general discussions of physiologic humoral immunity can be found in Chapter 2. Specifics of the histological and clinical presentations of antibody mediated rejection are found in organ specific chapters on the histopathological syndromes (Section Histopathological Syndromes of Graft Rejection and Recurrent Disease) and clinical diagnosis and management (Section Clinical Allograft Rejection Syndromes: Diagnosis and Management) of graft rejection, respectively.

Antibodies

The structure and function of antibodies is determined by multiple factors, all of which change as the immune response progresses. De novo antibody responses to allografts begin with low levels of IgM antibodies followed by IgG antibodies that increase in quantity and quality. Although IgM and IgG share fundamental elements of immunoglobulins, namely two antigen binding fragments (Fabs) joined to a constant fragment (crystallizable fragment or Fc), key structural differences generate distinct functions (Figure 6.1) (see Chapter 2). For example, the individual Fabs of IgM antibodies bind antigen with low affinity, but the combined binding avidity that results from ten identical Fabs in the pentameric structure of IgM is as much as 10^7 times higher. The pentameric structure of IgM also facilitates activation of complement by linking five Fcs together.

As the immune response progresses, antibodies switch from IgM to IgG, which undergo affinity maturation through a process of mutation and selection. This results in IgG antibodies with increasingly higher affinity Fabs. With time, the avidity of monomeric IgG alloantibodies exceeds avidity achieved by pentameric IgM. The switch to IgG brings several new dimensions to the immune response. The smaller (about 150 kD) monomeric IgG traverses capillary and venous barriers much more readily than the very large (900 kD) pentameric IgM, such that almost half of the IgG exists in the interstitial and lymphatic compartments. The evolution of subclasses of IgG has added functional diversity. Humans have four subclasses of IgG with different Fc structures that diversify function. IgG1 and 3 activate complement more effectively than IgG2, and IgG4 does not activate complement. Similarly, each subclass has different binding affinities for Fc receptors (FcRs) on leukocytes. The range of functions is further increased by multiple types of receptors for IgG Fc (FcγR) which are expressed by most populations of leukocytes [1]. In contrast, only one IgM-specific FcμR has been defined, which is expressed by T and B lymphocytes [2]. Leukocytes of both mice and humans express a family of FcγRs that have different affinities and that deliver stimulatory or inhibitory signals. Mice and humans both have one high-affinity FcγR (FcγRI) and multiple low to medium-affinity FcγRs that initiate activating signals, as well as one low to medium-affinity FcγR (FcγRIIB) which contains a negative regulatory immunoreceptor tyrosine based inhibitory motif (ITIM) in the cytoplasmic domain.

It is important to remember that antibodies are glycoproteins, and both the protein and carbohydrate portions of the molecule contribute to function. The protein structure determines the number and location of carbohydrate side chains. All IgG and IgM antibodies have glycosylation sites on the Fc, and the carbohydrate side chains can modify the interaction of Fc with complement and FcRs [3]. Moreover, a minority of antibodies has glycosylation sites in the Fab regions which can alter antigen binding significantly.

Understanding of immunoglobulin glycosylation has several important ramifications. First, variations in glycosylation that are known to occur in patients with differences in drug therapies, age, sex, or pregnancy [4–7] may alter the consequences of antibody responses to transplants. Second, Ravetch and colleagues [8] have modified the glycosylation of immunoglobulins to produce more immunomodulatory preparations of intravenous immunoglobulin (IVIg).

Numerous mechanisms have been developed to detect graft-specific alloantibodies. These are covered in Chapter 89.

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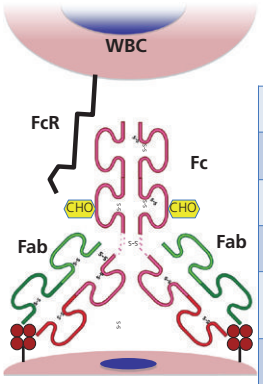

	IgM	IgG
Subtypes	None	1,2,3,4
C activation	+++	0 to +++
FcR	Limited	Multiple
Half-life	5 days	7 (G3) – 23 days
Function	Natural, early	Affinity Mature

Figure 6.1. Diagram of basic IgG antibody structure showing heavy chains (pink) and light chains (green), with constant regions (pale) and variable regions (dark). Papain cleaves IgG in the hinge region (checked) into three fragments: one Fc (fragment crystallizable) and two Fab (fragment antigen binding). The glycosylation site on the Fc is indicated by the yellow hexagon. The table compares important functions of IgM and IgG antibodies.

Antigens

Antigens are a critical part of the equation determining the results of antibody-mediated immunity. Antigenic variables that influence antibody function include structure and distribution. The high concentration of MHC class I antigens on all vascular endothelial cells provides an optimal target for antibodies to bind closely enough to activate complement and engage low as well as high-affinity FcRs on leukocytes. The expression of MHC class II antigens on endothelial cells is less uniform. In humans, for example, MHC class II antigens are constitutively expressed on all vascular endothelium in the heart, but confined to capillaries and veins in kidneys. However, the expression of MHC antigens is increased during ischemia–reperfusion and at sites of inflammation. An in depth discussion of MHC can be found in Chapter 4.

Like MHC antigens, the major blood group antigens A and B are expressed in high concentrations on endothelial cells. However, there are distinctions between MHC and blood group antigens [9]. The A and B antigens are terminal carbohydrate epitopes that can be linked to four different core saccharide chains attached to either lipids or proteins to form glycolipids or glycoproteins. The A and B epitopes have been shown to be covalently linked to various membrane glycoproteins as well as to soluble proteins such as von Willebrand factor (vWf) released by endothelial cells [10,11].

Other antigens that are still under investigation as potentially important targets for antibody-mediated rejection include MICA (major histocompatibility complex class I–related chain A), minor alloantigens, and autoantigens [12]. Unlike MHC antigens, these antigens are either expressed in low concentration, in intracellular locations, or as cryptic epitopes that are not exposed under normal conditions. Therefore, in quiescent conditions these antigens may not be optimal targets for antibodies. However, inflammation associated with transplantation (brain death of the donor, ischemia–reperfusion injury, and surgical trauma) or with subsequent rejection or infection can up-regulate the expression of these antigens to increase their susceptibility to antibody-mediated rejection. As an example of this interaction, it has been shown that antibody production to MICA is frequently a consequence of antibody responses to HLA [13].

Complement

When high concentrations of antibodies bind to allogeneic cells, the complete complement cascade can be activated by either the classical or lectin pathway depending upon the structure of the carbohydrate side chains on the Fc, which have variable terminal sugars (see Chapters 2 and 7). In contrast to most serum glycoproteins, only a minority of human IgG carbohydrates has a terminal sialic acid. Instead, the branches of these side chains frequently terminate with galactose residues that increase binding of C1q [14], the initiator of the classical pathway. Variation in antibody glycosylation is a well-known aspect of autoimmune diseases [15]. Antibodies that lack the terminal sialic acid and galactose (referred to as G0 antibodies) have exposed terminal *N*-acetyl glucosamine (GlcNAc) residues. Mannose binding lectin (MBL), the initiator of the lectin pathway, binds avidly to GlcNAc on G0 antibodies. G0 autoantibodies have been confirmed to activate complement through the lectin pathway in both humans and mice [14]. In mice, G0 IgG levels are higher in autoimmune-prone mice and increase with age and non-specific inflammatory stimuli [16]. In humans, the heterogeneity in terminal sugar residues is dependent on certain diseases, drug therapies, age, and sex [4–7].

Both C1 and MBL require two or more adjacent Fc regions of IgM or IgG for effective binding and activation. This permits the enzymatic subunits of bound C1 (namely C1r and C1s) or MBL (MBL-associated serine proteases; MASP) to start a series of enzymatic steps that can significantly amplify and diversify the immune response by producing increasing amounts of biologically active "split products" [17] (Figure 6.2). The details of the complement cascade are reviewed in Chapter 2. Briefly, C4 is first cleaved into two fragments—C4a and C4b. The small C4a fragment is a soluble mediator and the larger C4b contains a thioester group. This thioester can form a covalent bond with a hydroxyl or amino group on the closest protein or carbohydrate. This first enzymatic step can result in a significant amplification with about 25 molecules of C4b bound on or near the activating antibody [18]. More downstream, C4b serves as an attachment site for C2, which is then cleaved by C1s to generate C2a, which remains associated with C4b. The C4bC2a complex is the classical pathway C3 convertase that cleaves the central complement component, C3, into two fragments, C3a and C3b. The small C3a fragment is a fluid-phase vasoactive mediator that can bind to C3a receptors (C3aR) on nearby endothelial cells or mast cells. The larger fragment, C3b, is structurally homologous to C4b and contains a thioester group that can bind covalently. C3b has similar but more extensive functions compared to C4b. Like C4b, it serves as a ligand for cells with complement receptor 1 (CR1; CD35). Additionally. C3b can bind factor B of the alternative pathway of complement and engage the alternative pathway, which serves as an amplification loop that results in more C3 split products (Figure 6.3). As the result of the classical and amplification pathways, around 250 C3b fragments can be deposited on target cells for each C1 or MBL.

In combination with adjacent complement split products, C3b forms the classical and alternative C5 convertases (C4bC2aC3b and C3bBbC3b, respectively), which cleave C5 into C5a and C5b. It is at this point that the complement cascade intensifies its proinflammatory effects [19]. The small fragment, C5a, is quantitatively much

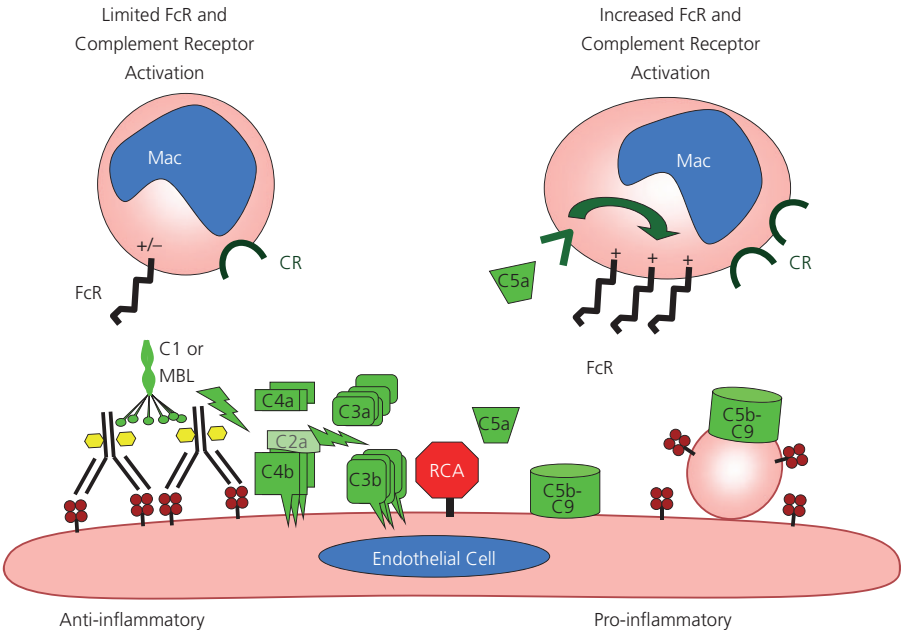

Figure 6.2. Diagram of increasingly proinflammatory effects of complement activation by antibody. Leukocytes with Fc receptors (e.g. neutrophils and macrophages) can be stimulated by antibodies bound to antigen. The strength of Fc receptor interactions with IgG is dependent on the carbohydrate side chains (yellow hexagons) on the antibodies. The carbohydrate side chains on IgG also interact with the first components of the classical and lectin complement pathways. At least two closely spaced IgG antibodies are required to initiate binding of one C1 or MBL molecule. C1 or MBL can enzymatically cleave many molecules of C4, which produce C4b that can bind covalently to cell membranes. C4b anchors the classical convertase (a complex of C4b and C2a) that cleaves large numbers of C3 producing C3b, which can bind covalently to tissues, and C3a, which is chemoattractant for neutrophils and macrophages. C3b joins C4bC2a in the formation of classical C5 convertase, which cleaves C5 into C5a and C5b. C5b initiates the formation of the membrane attack complex (MAC; C5b-C9). Both the C3 and C5 convertases are the target of several regulators of complement activation (RCA). As a result, endothelial lysis is not widespread in AMR, but sublytic amounts of MAC can stimulate endothelial cells to produce cytokines, express adhesion molecules, and shed microparticles containing the offending MAC. C5a is pivotal inflammatory mediator because it is an extremely potent chemoattractant for neutrophils and macrophages, and it also causes macrophages to up-regulate the stimulatory Fc RIII and down-regulate the inhibitory Fc RIIB for IgG. These receptors make macrophages more responsive to antibodies and complement split products. (Reproduced with permission from reference [17].)

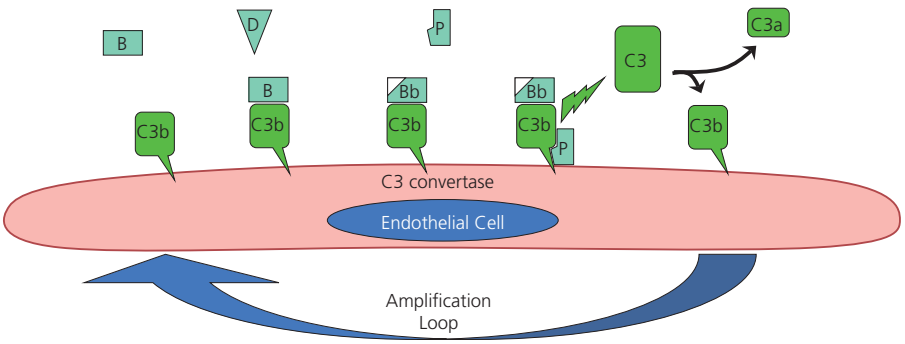

Figure 6.3. Once C3b is attached to a cell membrane, it can serve as the nidus for an amplification loop in which the alternative pathway generates more C3b. In this process, C3b acts as an anchor for factor B (B), which is then cleaved by factor D (D) to form the C3 convertase, C3bBb. Properdin (P) binds to this complex and stabilizes this enzyme. This stabilized enzyme complex has an increased half-life to cleave more C3 into C3b and creates a long-lived feedback loop.

more potent as a chemotactic agent than C3a and polarizes macrophages towards proinflammatory functions as described later. As shown in Figure 6.2, the larger fragment, C5b, initiates assembly of the terminal components of complement (C5b–C9) into a pore-forming structure, the membrane attack complex (MAC). As will be discussed below, MAC, even at sublytic concentrations, alters endothelial cell function.

Complement is a key component of the innate immune response to injury. Additional details regarding its role in this context are found in Chapter 7.

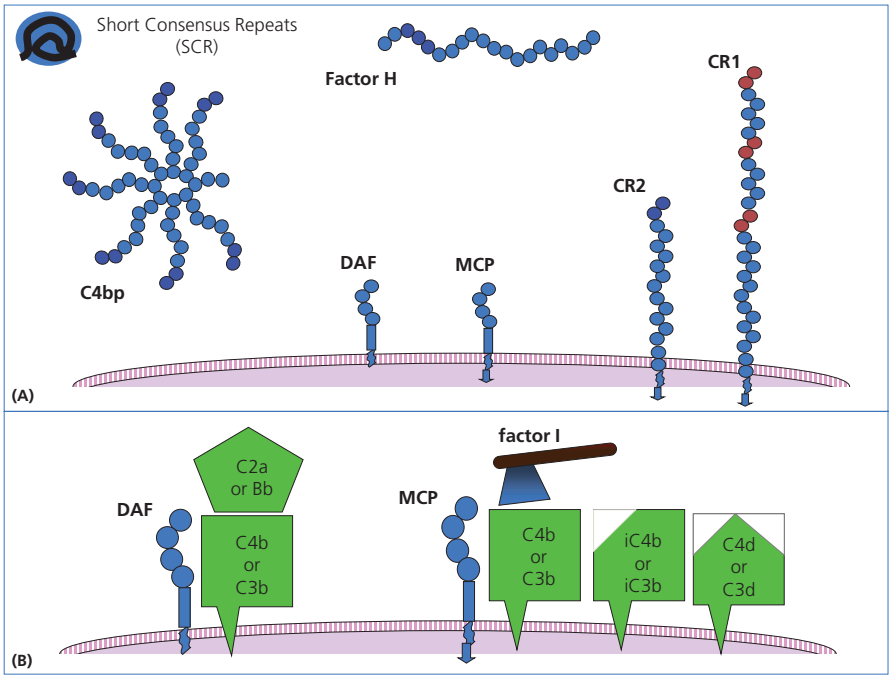

Figure 6.4. Diagram of the regulators of complement activation. (A) Structure of regulators that are composed of various numbers of highly conserved short consensus repeats. (B) Illustration of dissociation of convertases by decay accelerating factor (DAF) and co-factor function of membrane co-factor protein (MCP). DAF accelerates the dissociation of both the classical and alternative C3 convertases (C4bC2a and C3bBb). MCP serves as a co-factor for the enzymatic factor I to cleave C4b or C3b first to iC4b or iC3b, which are then further cleaved to C4d or C3d.

Complement regulation

Not surprisingly, this progression of potent effector molecules is highly regulated at almost every step. However, different regulatory molecules can modify the immune response in divergent directions. Regulation of complement is of increasing importance to transplantation both in understanding antibody-mediated rejection and as potential therapeutic interventions that modulate the complement system.

Regulation begins at the initial step of complement activation with C1 inhibitor, a serine protease inhibitor of the serpin superfamily. It provides a substrate to capture serine proteases, including C1r, C1s, and MASPs. This leaves the non-enzymatic C1q or MBL bound to antibody. In the setting of tissue injury, both C1q and MBL are critical for the clearance of apoptotic bodies by scavenger macrophages and the avoidance of autoimmunity [20,21]. It is possible that engagement of macrophages through C1q receptors also has beneficial effects in allografts. In fact, Bishop and colleagues have reported that allografts are rejected more rapidly by C1q-deficient mice [22].

As a serine protease inhibitor, C1 inhibitor has additional effects that are potentially salutary for transplants. These include inhibition of the kallikrein and coagulation systems [23]. More recent studies indicate that C1 inhibitor can bind to selectins on endothelial cells and interfere with leukocyte rolling [24].

The majority of complement regulators are focused on C4b and C3b. Many of these regulators are composed of various numbers of highly conserved short consensus repeats and are members of the regulators of complement activation (RCA) gene cluster, which is located on chromosome 1 (Figure 6.4A). In general, these regulators work through two mechanisms: dissociation of convertases and co-factor functions (Figure 6.4B). C4 binding protein (C4bp) and decay accelerating factor (DAF; CD55) accelerate the dissociation of C2a from C4b and decrease the half-life of this C3 convertase. In contrast, membrane co-factor protein (MCP; CD46) serves as a co-factor for the enzymatic factor I to cleave C4b or C3b first to iC4b or iC3b, which are then further cleaved to C4d or C3d. C4bp, factor H and complement receptor 1 (CR1; CD35) also function as co-factors for factor I (Figure 6.4A). The terminal complement components are regulated by protectin (CD59), which prevents the association of C9 with C5b-C8.

The balance among complement mediators and regulators is responsive to inflammation. IL-1, IL-6, IL-8, and TNF stimulate the liver to produce acute-phase proteins, among which are complement components and regulators. Hepatic production of MBL, C3, factor B, C5, C1 inhibitor, and C4bp are increased in response to inflammatory cytokines. At local sites of inflammation, both macrophages and certain parenchymal cells, notably epithelial cells, can produce complement components [25–27]. This is counterbalanced by the up-regulation of complement regulators on the surface of endothelial cells. In particular, DAF has been demonstrated to be up-regulated during antibody-mediated rejection in some renal and cardiac transplants [28,29].

The expression of regulators is sufficient to prevent lysis of most cells in allografts when moderate levels of complement are activated. However, high levels of antibodies can overwhelm the regulatory molecules and cause lysis of endothelial cells, which manifests as hyperacute rejection. Short of lysis, a range of outcomes is possible depending on the balance of split products that are produced.

Therapeutic intervention can moderate activation of complement at different steps. C5 has been recognized as a tipping point to intense inflammation. As a result, monoclonal antibodies that

block C5 cleavage, soluble C5a receptors, and antagonists of C5a receptors have been developed and are at various stages of clinical application [19]. The humanized anti-C5 antibody, eculizumab, has been demonstrated to inhibit antibody-mediated rejection in patients [30].

Human C1 inhibitor is now in clinical trials with renal transplant recipients. This approach is attractive because it inhibits the enzymatic components of both C1 and MBL but does not interfere with the apoptotic clearance mechanisms of C1q and MBL. The additional effects of C1 inhibitor on the kallikrein and coagulation systems [23] may also benefit transplant recipients.

Incompatibilities between humans and distant phylogenic species such as the pig significantly confound xenotransplantation. In depth discussions of complement regulation and preformed antibodies as they relate to xenotransplantation can be found in Chapter 12.

Cells

In addition to deposits of complement split products, the pathological changes observed in acute humoral rejection include endothelial cell activation and granulocyte or monocyte margination [31,32]. These responses are predictable from direct and indirect effects of antibodies on each of the cell types. Endothelial cells lining blood vessels of organ transplants are the first available target for antibodies. When antibodies cross-link MHC antigens on endothelial cells, von Willebrand factor and P-selectin are exocytosed within minutes from Weibel–Palade storage granules [33]. This results in platelet adhesion and activation, which extends the inflammation quantitatively and qualitatively because of the large numbers of platelets and the wide variety of mediators released by platelets [34,35]. Of particular relevance to transplants are the chemokines and ligands that engage neutrophils, macrophages, and T cells. These include IL-8, PF4 (CXCL4), RANTES (CCL5), P-selectin, and CD40L (CD154) that are stored in alpha granules of platelets. Activation of endothelial cells by antibodies is augmented by complement activation products beginning with the successively more potent anaphylatoxins (C4a, C3a, and C5a) and progressing to MAC. The anaphylatoxins increase vascular permeability by binding specific receptors on endothelial cells and mast cells [19]. Finally, MAC in sublytic amounts, and even incompletely assembled MAC (C5b-C8), cause calcium fluxes that activate endothelial cells [36–38]. This results in an immediate response of cell contraction and exocytosis of preformed adhesion molecules. In the presence of inflammatory cytokines such as IL-1α and TNF, endothelial cells activated by MAC synthesize chemokines for neutrophils and monocytes (IL-8 and MCP-1; CCL2), and up-regulate adhesion molecules (P-selectin, E-selectin, and ICAM-1) for leukocyte infiltration. Antibodies and MAC independently and together also induce endothelial cells to produce growth factors (PDGF and FGF) and to proliferate, responses that are of particular relevance to chronic rejection [39–41]. Unlike erythrocytes, nucleated cells react by rapidly shedding or invaginating membrane containing MAC [42,43], but this releases potentially antigenic microvesicles that can be acquired and presented to initiate or amplify adaptive immune responses.

Neutrophils and macrophages, which are characteristic participants in antibody-mediated rejection, both express multiple Fc and complement receptors. These receptors have interactive effects that are best illustrated by C5aR and FcR. In addition to being an extremely potent chemoattractant for neutrophils and macrophages, C5a also causes macrophages to up-regulate the stimulatory FcγRIII and down-regulate the inhibitory FcγRIIB for IgG [44], making macrophages more responsive to antibodies. Similarly, stimulation through FcR causes macrophages to produce more complement [45].

Although monocytes and macrophages are associated with rejection, they have a wide range of functions from proinflammatory to profibrotic to anti-inflammatory [46–48]. These subpopulations of macrophages have been most fully defined relative to T cells. In this context, proinflammatory (or M1) macrophages are stimulated by IFNγ and TNF from Th1 or NK cells to produce IL-12, IL-1, and IL-6 (also see Chapter 2). In contrast, profibrotic (or M2) macrophages arise in response to IL-4 from Th2 cells and characteristically produce TGFβ and connective tissue growth factor (CTGF). Finally, anti-inflammatory (or regulatory) macrophages respond to and produce IL-10.

Ischemic injury has been found to elicit a first wave of inflammatory macrophages followed by wound healing macrophages [48,49]. The macrophages that infiltrate allografts in response to different sets of humoral immune mediators have not been characterized. However, the most congruent analogy is that activation through C5aR and stimulatory FcR would polarize macrophages towards inflammation. At the other end of the spectrum would be macrophages stimulated by early complement components in the absence of terminal complement activation. As discussed in the complement section, C1q or MBL are known to mediate the removal of apoptotic blebs by macrophages without causing inflammation or fibrosis [50–54].

Types of humoral rejection

Concepts about the manifestations of humoral rejection were dominated by the original catastrophic experiences with hyperactue rejection. With increasingly sophisticated methods for detecting antibodies and probing biopsies, new concepts about more subtle effects of humoral immunity have evolved.

Hyperacute rejection

In hyperacute rejection, high levels of antibodies activate enough complement to exceed all of the regulatory barriers and significant lysis of vascular endothelial cells occurs. This disruption of vascular integrity results in hemorrhage and thrombosis. Copious amounts of complement split products also attract and activate numerous neutrophils that typify hyperacute rejection. A landmark publication in 1969 by Patel and Terasaki [55] described and validated a serological test for preformed antibodies. The history leading to this revolutionary realization is detailed in Chapter 3. This test demonstrated antibodies in about 30% of patients. Over a third of the patients with antibodies were cross-match positive for donor-specific antibodies (DSA). Hyperacute rejection occurred in 80% of the patients with DSA versus about 15% of the patients with antibodies to antigens not expressed on the transplant. Hyperacute rejection was very frequent (almost 41%) in patients who received second transplants. By 1974, Mel Williams reported, in a memorial symposium for David Hume, that implementation of the cross-match test had eliminated hyperacute rejection in a series of 24 patients with second transplants [56].

Acute rejection

The amount of complement activation depends on the titer and class of antibodies, and on the density of the target antigen.

Alloantibodies produced after transplantation by naïve or memory B cells do not immediately attain high titers. Low levels of antibodies result in less complement activation, which is better controlled by the many regulatory mechanisms.

When sufficient complement is activated that regulation of C3 convertase is overridden and significant amounts of C5 convertase are formed, then a complete picture of humoral rejection is found. This includes edema as the result of increased vascular permeability, deposition of C4 and C3 split products (C4d and C3d), and margination and infiltration of neutrophils and macrophages. Expert panels of pathologists have agreed upon these basic findings for antibody-mediated rejection in renal, cardiac, and pancreatic transplants [57–59]. A universal marker for monocytes and macrophages is usually employed to define the mononuclear infiltrates. This of course does not distinguish subtypes of macrophages.

When little complement is activated due to lower titers of antibodies, varying mixtures of non-complement activating antibodies, or antibodies to less densely expressed antigens, then the pathological findings are less obvious. Acute graft dysfunction has been described in the absence of C4d [60]. These cases have sometimes been attributed to complement-independent effects of antibody. It is also possible that these cases represent patients with low amounts of certain complement regulators. Low levels of circulating factor H, for example, allow the alternative C3 convertase to have a longer half-life, which would permit even small amounts of C4 activation to cause significant C3 and C5 production through the inadequately regulated amplification loop. The importance of normal factor H function in preventing excessive C3 activation has been demonstrated in animals treated with blocking antibodies to factor H and in humans with mutations that alter the functioning factor H [61]. The expression of membrane bound complement regulators could also be critical [62]. DAF, which is up-regulated in response to inflammatory cytokines, is expressed at lower levels in some cases of antibody-mediated rejection of both renal and cardiac transplants [28,29]. Again this would be expected to prolong the function of C3 convertase and result in more C3 activation.

Conversely, C4d has been found in some biopsies that do not show any other evidence of humoral rejection. It has been suggested that antibodies to donor-specific HLA in association with C4d deposits in the absence of inflammation and graft dysfunction represents subclinical rejection [63]. In non-human primate models, these findings can predate evidence of chronic rejection by months [64].

Chronic rejection

Clinically, humoral immune responses have been linked to chronic rejection in renal transplants [65]. Circulating DSA and C4d deposits in biopsies of renal transplants have been associated with eventual development of glomerulopathy. However, chronic glomerulopathy is much more prevalent than C4d deposition. Sis and colleagues have noted a high incidence of endothelial disturbance on a molecular level in the absence of demonstrable C4d deposits and this molecular signature of endothelial injury is correlated with chronic graft failure [66,67]. In part, this may reflect a selective process because high titers of antibodies that activate readily detectable amounts of complement would produce acute graft dysfunction. Lower titers of antibodies can cause complement-independent responses of endothelial cells and smooth muscle cells that can contribute to chronic vascular changes. These include up-regulation of fibroblast growth factor (FGF) receptors and increased proliferation [68]. Low levels of antibody and complement activation can also stimulate endothelial cells to endocytose or exocytose adulterated segments of their plasma membrane [42,43]. Experimental animal models have demonstrated that even large amounts of covalently bound complement split products (C4d and C3d) can be diminished to undetectable levels within 3 to 5 days after donor-specific antibodies are eliminated [69].

Accommodation and tolerance

Renal transplants incompatible for the major blood group antigens A and B have provided insights into two non-inflammatory outcomes of humoral immune responses: accommodation and tolerance. Antibodies to A and B antigens differ from antibodies to HLA in that "natural" hemagglutinin antibodies develop in the first year of life, presumably in response to cross-reactive carbohydrate antigens on bacteria that colonize the gut. Hyperacute or acute rejection can be avoided by depleting the natural antibodies in the recipient before and for the first weeks after transplantation. After this critical period, renewed production of antibodies does result in deposition of C4d in the peritubular capillaries, but often in the absence of margination of leukocytes or graft dysfunction [70,71]. The apparent resilience of grafts to antibodies and complement is termed accommodation. Several aspects of accommodation are instructive relative to humoral immune responses. The first relates to antibody titer. Antibodies to A and B blood group antigens can cause hyperacute rejection when the antibody titer is high and antibodies are not depleted before transplantation [72]. Recurrent antibody responses associated with accommodation usually develop gradually and attain only low titer. The development of hemagglutinins with titers >64 is associated with episodes of rejection [73]. Secondly, to allow grafts to accommodate, antibody depletion is maintained during the postoperative period when transplants experience non-specific inflammation due to ischemia–reperfusion and surgical trauma. Finally, the biochemical properties of the carbohydrate blood group antigens may be critical. The fact that the A and B antigens can be linked to soluble proteins released by endothelial cells such as vWf, as well as various membrane proteins in kidneys [10,11], may modify critical aspects of antibody distribution and complement activation. The typical markers of complement activation that are observed in accommodation, namely C4d and C3d, are end products of regulation by factor I. These split products do not necessarily indicate that the very proinflammatory C5 and MAC have been activated. Many activation products of the early complement components, notably C1q and iC3b, have anti-inflammatory functions. As noted in the section on complement, C1q is critical for the removal of apoptotic bodies without stimulating inflammation or antigen presentation. Similarly, when iC3b interacts with CR3 (CD11b/CD18) on antigen-presenting cells, production and secretion of the proinflammatory cytokines IL-1β, IL-6, IL-12, and TNF are decreased [74–76]. CR3 can also be up-regulated on T cells, and cross-linking this complement receptor inhibits T-cell proliferation and IL-2 release [77].

It is not clear whether tolerance to blood group antigens or HLA can be achieved through the activities of C1q or iC3b. Certainly, apparent cases of accommodation in the presence of antibodies to HLA are less stable than those for blood group-incompatible transplants. However, long-term tolerance to ABO-incompatible allografts can be achieved when ABO-incompatible hearts are successfully transplanted into infants within 6 months of birth. These transplants induce tolerance to the donor blood group as evidenced by the lack of B cells capable of producing antibodies to

donor blood group antigen [78]. The donor antigens remain expressed on the graft endothelium, but recipient B cells do not produce antibodies to donor blood group antigens, while antibodies do develop normally to non-donor blood group antigens. Over the long term, a minority of these patients have been reported to develop antibodies to donor blood group antigens [79], but these antibodies may recognize A and B epitopes attached to carbohydrate backbones expressed by erythrocytes used in hemagglutination assays and not the graft endothelium [80].

Summary

Humoral immunity encompasses a wide array of interdependent mediators, regulators, receptors, and cells that can create devastating pathological changes ranging from hyperacute to acute rejection. At the other end of the spectrum, humoral immunity may be associated with chronic changes or even down-modulate tissue injury. The appreciation of humoral mechanisms of graft destruction has improved markedly in recent years. Its future challenge will be to find means of controlling these processes so as to avoid their effects in a graft-specific manner.

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CHAPTER 7

Innate Immunity

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Introduction

Innate immunity is a key component of immunity in general, and, together with the adaptive immune system, it forms an intricate system that protects us from countless invading pathogens. The innate immune system also contributes to tissue homeostasis by eliminating cancerous and stressed cells. The innate response is carried out by innate immune cells, which include dendritic cells (DC), macrophages, monocytes, natural killer (NK) cells, neutrophils, mast cells, and eosinophils. In contrast to the cells of the adaptive immune system, namely T and B cells, innate immune cells do not possess somatically rearranged cell surface receptors that directly recognize foreign antigens. Instead, they express a plethora of pattern recognition receptors that engage conserved pathogen moieties, danger signals, and damaged cell products; they also express receptors for complement products, antibodies, and additional non-polymorphic activating and inhibitory receptors that constantly sense "self" or "missing self" to control cellular responses. These features allow innate immune cells to form the first line of defense and also influence the commencement, duration, and the character of adaptive immune responses.

Historically, transplant research primarily has focused on cells of the adaptive immune system, particularly the involvement of T cells in graft injury. This is rooted in the experimental observation that tissue and organ transplants often survive long term in the absence of T cells in allograft recipients. Recently, the contribution of innate immune cells to transplant outcomes (rejection or acceptance) is increasingly appreciated [1–3]. Innate immune cells can be activated by microbial products or by endogenous ligands (alarmins) from damaged grafts; some innate cells can respond directly to allografts. Such cells influence transplant outcomes through a variety of mechanisms, including the modification of the T-cell activation programs. In various settings, innate immune cells are closely involved in rejecting or protecting allotransplants, depending on the context and tolerizing protocols; they also contribute to a wide spectrum of graft injury. Therefore, innate immune cells are important contributors to alloimmunity in general, and, because of that, successful induction of transplant tolerance requires a better understanding and rational targeting of such cells following transplantation. In this chapter we review key cells and molecular pathways in the innate immune system, detail how the innate system responds to allotransplants, and summarize the challenges and opportunities in therapeutic targeting of the innate immune system in transplant settings. This chapter will complement Chapters 5 and 6, which deal with the cellular and humoral elements of the acquired immune response, respectively.

The innate immune system

The innate immune system consists of a variety of cell types, molecular sensors, cell surface activating and inhibitory receptors, and soluble mediators. The key elements in the system are discussed below in detail.

Dendritic cells

Dendritic cells (DC) are a rare cell type in the immune system, representing ~1% of total lymphoid cells; they are widely distributed in the body and are identified morphologically by having long dendrites projecting from the body of the cells (see Chapter 2). Dendritic cells are developed from bone-marrow precursors, and, based on anatomic locations, surface markers, and maturation status, they can be divided into various subsets [4,5]. DCs in the lymphoid tissues are called lymphoid tissue resident DCs and those in other sites are often termed interstitial DCs. Phenotypically, DCs express the β integrin CD11c, and, in combination with other surface markers, they can be further divided into **myeloid DCs** ($CD11c^+CD11b^+CD205^-$), **lymphoid DCs** ($CD11c^+CD11b^-CD205^+$), and **plasmacytoid DCs** ($CD11c^+B220^+PDCA^+$). Thus, DCs, though highly specialized, are extremely heterogeneous, and different DC subsets perform different functions in vivo. Earlier studies indicate that various DC subsets reside in different locations in the lymphoid tissues and exhibit striking difference in their expression of chemokines, chemokine receptors, and homing receptors. They also secrete different cytokines upon activation, which subsequently create different cytokine milieu for the differentiation of adaptive T cells. For example, plasmacytoid DCs produce copious amount of type I interferons whereas myeloid DCs secret high levels of IL-12 upon activation.

The primary function of DCs is to engulf, process, and present foreign antigens to cells of the adaptive immune system. However, DCs are very dynamic; their phenotypes and functions can be profoundly modulated by a variety of mechanisms. They express an incredibly complex array of toll-like receptors (see below) that allow them to respond to microbial products or endogenous tissue products collectively called "danger signals"; they are capable of

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producing and also responding to a plethora of inflammatory cytokines. These responses often result in further proliferation and maturation of DCs, as reflected by the heightened expression of MHC molecules and T-cell costimulatory molecules on the cell surface (e.g. CD80, CD86, CD40, and OX40 ligand etc.). During this process, DCs are often transformed into potent antigen-presenting cells (APCs) that are highly efficient in the activation of adaptive immune cells (see Chapter 6) Thus, depending on the presence or absence of "danger signals," DCs can remain in a state of resting cells or become fully activated, and there is evidence that resting and activated DCs may perform different functions in vivo. While activated DCs are potent triggers of immune activation, resting DCs are likely required for immune tolerance by engaging and supporting regulatory cells [6]. The demonstration that genetic ablation of DCs in naïve mice results in widespread autoimmune diseases suggests that DCs are essential not only for robust immunity but also for tolerance and immune homeostasis [7]. Thus, DCs provide an essential link between the innate and adaptive immune systems; they are also well positioned at the interface between immunity and immune tolerance.

Monocytes and macrophages

Monocytes and macrophages are mononuclear phagocytes with many special characteristics. Monocytes are developed from bone marrow myeloid precursor cells, reside in the blood, and, upon inflammatory triggering, they rapidly infiltrate inflammatory sites where they mature into macrophages [8]. In some organs, macrophages acquire additional tissue-specific features and become integral cellular component of the organ, such as Kupffer's cells in the liver and glia cells in the brain. In humans, monocytes constitute 5–10% of the peripheral circulating leukocytes in the blood. Although monocytes and macrophages are closely related, they can be distinguished by different surface markers. For example, monocytes are $CD11b^{high}$ and $F4/80^{-}$, and they express low levels of MHC class II and CD80, CD86, CD40 costimulatory molecules. In contrast, macrophages are $CD11b^{high}$ and $F4/80^{+}$, and also have high levels of surface MHC class II, CD80, CD86, and CD40 molecules. Besides differentiating to macrophages at inflammatory sites, monocytes also differentiate into myeloid DCs in situ in selected models. Additionally, $CD14^{+}CD16^{-}$ monocytes are highly efficient at phagocytosis and can produce high levels of inflammatory cytokines, whereas $CD14^{low}CD16^{+}$ monocytes express MHC class II molecules and are highly efficient at antigen presentation [8]. Furthermore, studies using intravital microscopy to directly examine blood monocytes in vivo revealed additional complexity. For instance, $Ly6C^{-}GR1^{-}$ monocytes patrol the vessel walls, and after extravasation differentiate into type II (M2) macrophages (see below), whereas $Ly6C^{+}GR1^{+}$ monocytes differentiate into dendritic cell-like cells or generate an type I macrophages (M1) [9].

Macrophages are a major cellular infiltrate at inflammatory sites; they primarily function as APCs and inflammatory cells [10]. Macrophages efficiently phagocytose foreign entities and then present the antigenic epitopes to T cells to initiate the adaptive immunity; they also produce, as well as respond to, a wide array of inflammatory cytokines, which further amplify their phagocytosis and APC functions. Similar to DCs, macrophages also consist of multiple phenotypic and functionally different subsets. The cytokine milieu in which macrophages are stimulated plays a decisive role in the polarization of macrophages. For instance, in an environment rich in IFN-γ, TNF-α, or IL-12, macrophages are polarized toward **type I macrophages** or M1 macrophages. M1 macrophages are highly inflammatory cells, facilitate Th1 induction, and exhibit cell-cidal activities, primarily through the production of reactive oxygen species and TNF [11]. On the other hand, **type II macrophages** or M2 macrophages are generated in an IL-4 rich milieu, and M2 macrophages exhibit immune modulatory function and promote wound healing [12,13]. Mechanistically, IL-4 stimulates arginase activity in macrophages, which converts arginine into ornithine, leading to enhanced collagen production and wound healing. M2 macrophages also produce the suppressive cytokines IL-10, TGF-β, and prostaglandin E2 which suppress T-cell activation. M1 and M2 macrophages may represent extreme ends of a rather wide spectrum of macrophage function, but they do highlight the plasticity of macrophages and the importance of environmental cues in determining macrophage functions [14].

Additionally, under certain conditions some macrophages can become potent inducers of $Foxp3^{+}$ Tregs, most likely via the expression of PD-L1, which indirectly contribute to immune regulation (covered in Chapter 8) [15,16], while others contribute to immune regulation and tolerance by differentiating into **myeloid-derived suppressor cells** (MDSC), a cell type with potent immunosuppressive properties [17]. Finally, there is emerging evidence that macrophages can be alloreactive and respond directly to allo [18] or xenoantigens [19], although the exact molecular basis for such alloreactivity remains to be defined.

Natural killer cells

Natural killer (NK) cells are a major cell type in the innate immune system and represent the third largest lymphocyte population in the blood (besides T cells and B cells). NK cells are well represented in the blood, spleen, lymph nodes, and other lymphoid tissues; they also reside in non-lymphoid sites in large numbers, especially in the liver and the lungs [20]. NK cells are present in cellular infiltrates at inflammatory sites where they closely interact with other innate and adaptive immune cells.

Mature NK cells exhibit remarkable cytolytic activities; they readily kill target cells without prior antigen priming, which contrasts sharply to adaptive T cells. Target cell killing is mediated primarily by the release of perforin and granzymes, which are tightly packed inside NK cells as large preformed granules, and target cell recognition by NK cells triggers rapid degranulation and target cell apoptosis. NK cells also produce copious amounts cytokines upon stimulation, and such cytokines include both proinflammatory (e.g. IFN-γ, TNF) and anti-inflammatory cytokines (e.g. IL-10, TGF-β), thus exerting diverse impacts on the nature of the immune response.

A key feature of NK cells is the multiplicity of cell surface receptors they express, and these receptors collectively control development, education, and effector functions of NK cells (Figure 7.1). Functionally, the NK receptors are divided into **activating receptors** and **inhibitory receptors** [21,22]. The activating receptors include natural cytotoxicity receptors or NCR (NKp46, NKp44, NKp30), c-type lectin-like Ly49 receptors in the mouse (e.g. Ly49H, Ly49D), and killer immunoglobulin-like receptors (KIR) and NKG2 family receptors (NKG2C, NKG2D) in humans. Certain NK subsets also express CD16 and CD27 which function as activating receptors. In mice, the inhibitory NK receptors belong to the Ly49 family (e.g. Ly49C, Ly49G, Ly49I), whereas human NK cells express inhibitory receptors of the KIR family, which include KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1, and KIR3DL2. In both humans and mice, NK cells also express the heterodimeric inhibitory receptor NKG2A-CD94. With few exceptions, most NK activating

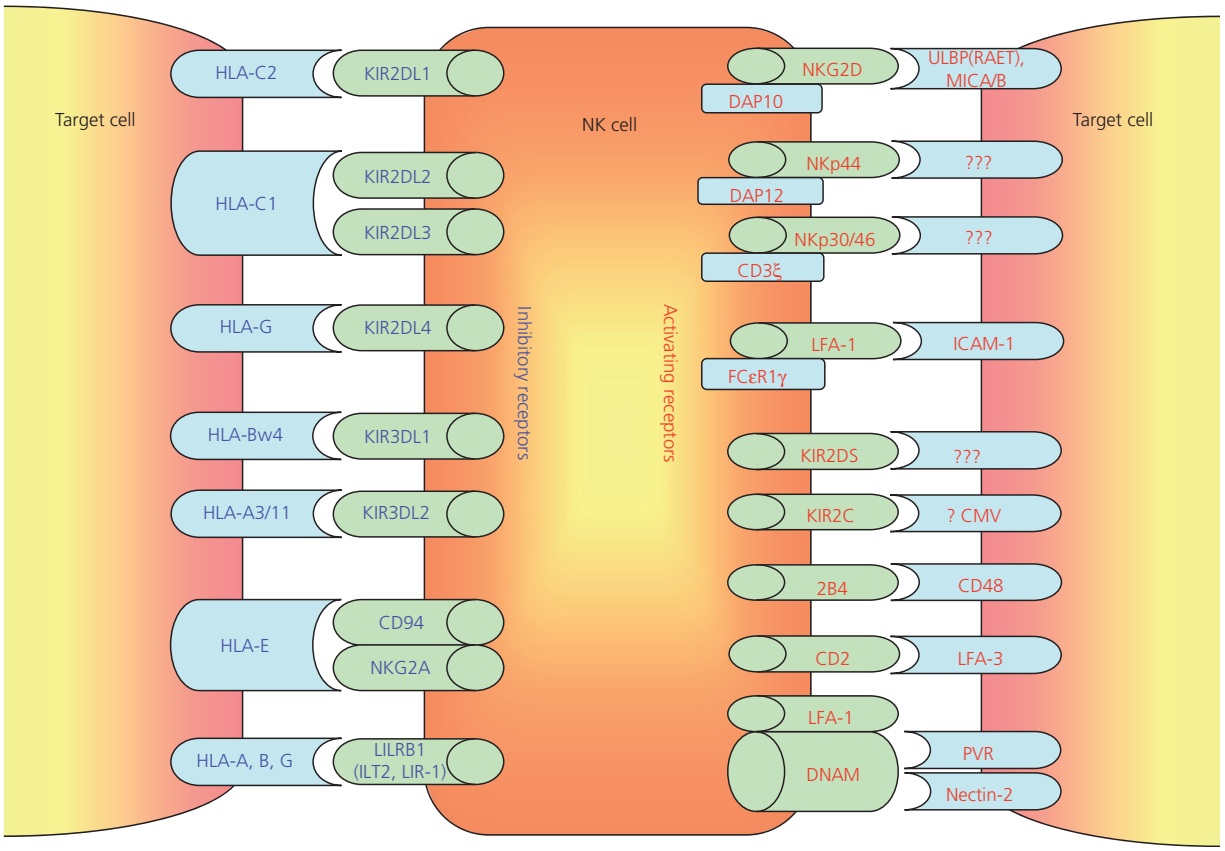

Figure 7.1. Human NK cells express multiple activating and inhibitory receptors. The NK receptors on human NK cells can be divided into activating receptors and inhibitory receptors. The ligands for the inhibitory receptors are self-MHC class I molecules, and those for the activating receptors include pathogens, some MHC molecules, and induced surface molecules on stressed or damaged cells. Signals from both activating and inhibitory receptors collectively control the function of NK cells.

receptors lack signaling motifs in their cytoplasmic domains. Instead, they recruit adaptor molecules DAP10, DAP12, CD3ς, or FcεRIγ to transduce activating signals. In contrast, the inhibitory NK receptors directly signal through the immunoreceptor-based tyrosine inhibitory motifs (ITIM) in their cytoplasmic tails. As can be envisioned, the ligands for such NK receptors are extremely diverse, which include pathogens, polymorphic MHC class I molecules or class I-related molecules, de novo induced molecules on damaged or stressed cells.

Much of the NK biology revolves around inhibitory receptors. As mentioned above, the ligands for inhibitory receptors are self-MHC class I molecules, and engagement of self-MHC class I by NK inhibitory receptors prevents self-destruction and ensures self-tolerance; such NK cells are also selected to undergo further maturation and become functionally competent killers against non-self-targets. This feature is developmentally acquired and often called "**NK education**" or "**NK licensing**" [23]. Most mature NK cells in the periphery express at least one inhibitory receptor, and therefore are fully licensed; the more inhibitory receptors individual NK cells express, the greater their killing potential toward target cells. Those that fail to express inhibitory receptors are rendered anergic or incompetent (**unlicensed NK cells**). Hence, upon encountering target cells that are missing or have down-regulated self-MHC class I molecules, NK cells will unleash their potent killing activities. This also applies in certain cases to MHC class I mismatched target cells in transplantation (both bone marrow and solid organ transplants) [24,25], and NK-mediated killing in this setting is called "**missing self-recognition**" [26]. However, in most cases, NK cells constantly survey the surroundings, integrate signals from both activating and inhibitory receptors, and then determine their actions. Thus, if multiple activating receptors are engaged, such activating receptors can override the inhibitory signals to initiate NK mediated killing of target cells. For instance, damaged and stressed cells often express multiple ligands for NK activating receptors in addition to self-MHC class I, and therefore are often killed by NK cells, a process also called "**induced self-recognition.**"

In essence, NK cells are controlled by the balance of inhibitory and activating signals, with the ultimate outcome determined by a hierarchy of signals. There is a predominance of inhibitory signals under physiological conditions. Under conditions of viral infection, NK cells that do not express inhibitory receptors (unlicensed NK cells) respond much better than those that express the inhibitory receptors. Also, NK licensing is a dynamic and continuous process in such a way that mature NK cells can be re-educated [27]. Additionally, different regulatory mechanisms may operate in the control of cytokine production by NK cells. The diversity and plasticity of NK cells suggest that their roles in immune responses are complex.

Evidence suggests that NK cells may have some features of adaptive immunity, specifically the acquisition of immunologic memory. Seminal studies in murine models showed that following activation, NK cells showed a similar pattern of expansion and contraction

as do $CD8^+$ T cells, and these NK cells persisted at increased frequencies and responded with increased magnitude and kinetics upon secondary rechallenge [28]. The implications of NK cell memory in transplantation are poorly understood.

Innate sensors

Innate immune cells respond vigorously to pathogens and tissue injuries, and this is mediated by a complex array of molecular sensors initially called **pattern recognition receptors or PRRs**. These sensor molecules recognize conserved pathogen-associated molecular patterns (PAMPs) derived from bacteria, mycobacteria, DNA and RNA viruses, fungi, and protozoans; they also recognize structures from damaged or stressed autologous cells known as damage-associated molecular patterns (DAMPs) or alarmins, and examples of these structures include heat shock proteins, peptidoglycan, high-mobility group box chromosomal protein 1 (HMGB1), heparin sulfate, glucose regulated protein, fibrinogen, hyaluronic acid, and nucleotide fragments (see Chapter 2). Engagement of these innate sensors by corresponding ligands elicits the activation of immune and inflammatory genes, and the products of which drive immune responses to eliminate invading pathogens or promote tissue repair [29].

There are three families of innate sensors indentified thus far and each family includes numerous members (Figure 7.2). **Toll-like receptors** or TLRs are by far the best-studied family [30]. Other two families include **NOD-like receptors** (NLRs) [31] and **RIG-like receptors** (RLRs) [32]. The TLR family consists of at least 13 members and, apart from TLR3, TLR7, TLR8, TLR9, which are intracellular receptors associated with endosomal membrane, the rest are transmembrane receptors consisting of an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain contains multiple leucine-rich repeat motifs (LRRs) that are involved in ligand binding; the intracellular domain signals through either the MyD88-dependent pathway, which activates the NF-κB transcription factor or the TRIF-dependent pathway, which stimulates type I interferons [33]. In contrast to the membrane-anchored TLRs, NLRs are free cytoplasmic proteins consisting of multiple functional domains involved in ligand binding, oligomerization, and signal transduction. There are 23 members in the NLR family, but NOD1, NOD2, NALP1-3, and Ipaf are the best studied ones. NOD1 and NOD2 activate NF-κB, with subsequent expression of potent inflammatory cytokines, including pro-IL-1β and pro-IL-18. NALP1-3, Ipaf form inflammasomes and activate caspase 1 and caspase 5, resulting in processing and production of active IL-1β and IL-18 [30]. RLRs are an interesting family of innate sensors; they are cytoplasmic proteins akin to NLRs. Similar to intracellular TLRs (TLR3, TLR7, TLR8, TLR9), RLRs stimulate NF-κB activation and type I interferon

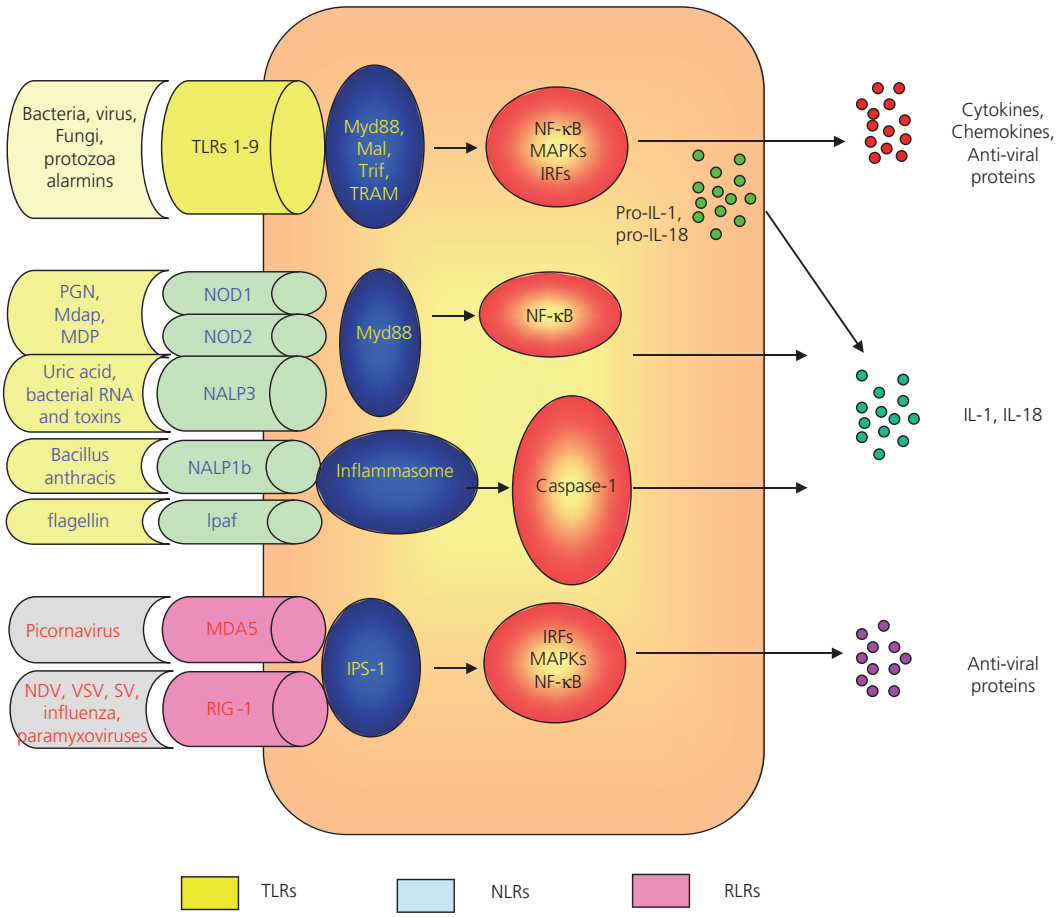

Figure 7.2. Molecular sensors expressed by innate immune cells. The innate sensors are broadly divided into three families, which include transmembrane receptors and intracellular ones. They respond to pathogen products and endogenous ligands from damaged cells, with certain degrees of specificities. Together, they provide an extraordinary early warning system to detect and respond to "danger signals."

production. However, RLRs sense viral nucleic acids in the cytoplasm whereas intracellular TLRs recognize viral products in the endosomal compartments [32]. In addition, RLRs are widely expressed in innate immune cells and rapidly up-regulated in response to type I interferons. Together, these families of sensor molecules provide an extraordinary early warning system against pathogens and tissue injuries.

TLRs, NLRs, and RLRs respond to pathogens with a certain degree of specificity. TLRs recognize extracellular and intracellular pathogens, and individual TLRs respond to different entities. NLRs sense intracellular bacteria-derived products while RLRs detect intracellular viral nucleic acids. These receptors also interact with one another to trigger optimal responses, as exemplified by the interplay of NOD1-2 and NALP1-3 in the generation of active IL-1 and IL-18. Stimulation of these receptors results in the activation of innate immune cells, production of potent proinflammatory cytokines, maturation of DCs, and initiation of adaptive immunity. Thus, the innate sensors play a critical role linking the innate and adaptive responses (see Chapter 5).

Complement

Complement is an integral part of the innate immune system and consists of numerous serum proteins (C1 to C9) (Figure 7.3). Serum complement is produced primarily in the liver and studies indicate that other tissues and cells also produce complement locally. Complement activation is tightly controlled by both positive and negative regulators [34]. Details of the three different pathways of complement activation are reviewed in Chapter 2.

The classical pathway of complement activation is a primary effector mechanism in antibody-mediated vascular injury. However, in other inflammatory responses including ischemia reperfusion injury, complement products can additionally function as a "danger signal" and aid in T-cell priming by acting as chemoattractants. Complement activation can be inhibited by multiple regulatory proteins. **Decay accelerating factor** (DAF, CD55) is a cell surface molecule that accelerates the decay of C3 convertases, thus preventing the amplification of complement cascade and formation of the downstream membrane attack complex [35]. Other inhibitory proteins include CD46 and CD59. Because of their regulatory properties, such inhibitory proteins have been targeted in preventing complement-mediated graft injury, including ischemia reperfusion injury, which is discussed below.

Aside from mediating cell lysis, chemoattraction, and innate cell activation, the role of complement has been markedly expanded recently to include costimulation of T-cell activation [36,37] and maturation of DCs [38]. T cells and DCs can produce complement components and also express receptors for C3a and C5a. Both cell types employ the complement pathway to optimally function. In this setting, complement produced in situ, rather than systemic complement, is critically involved [39]. Similarly, graft-derived complement has been shown to contribute graft injury following transplantation [40].

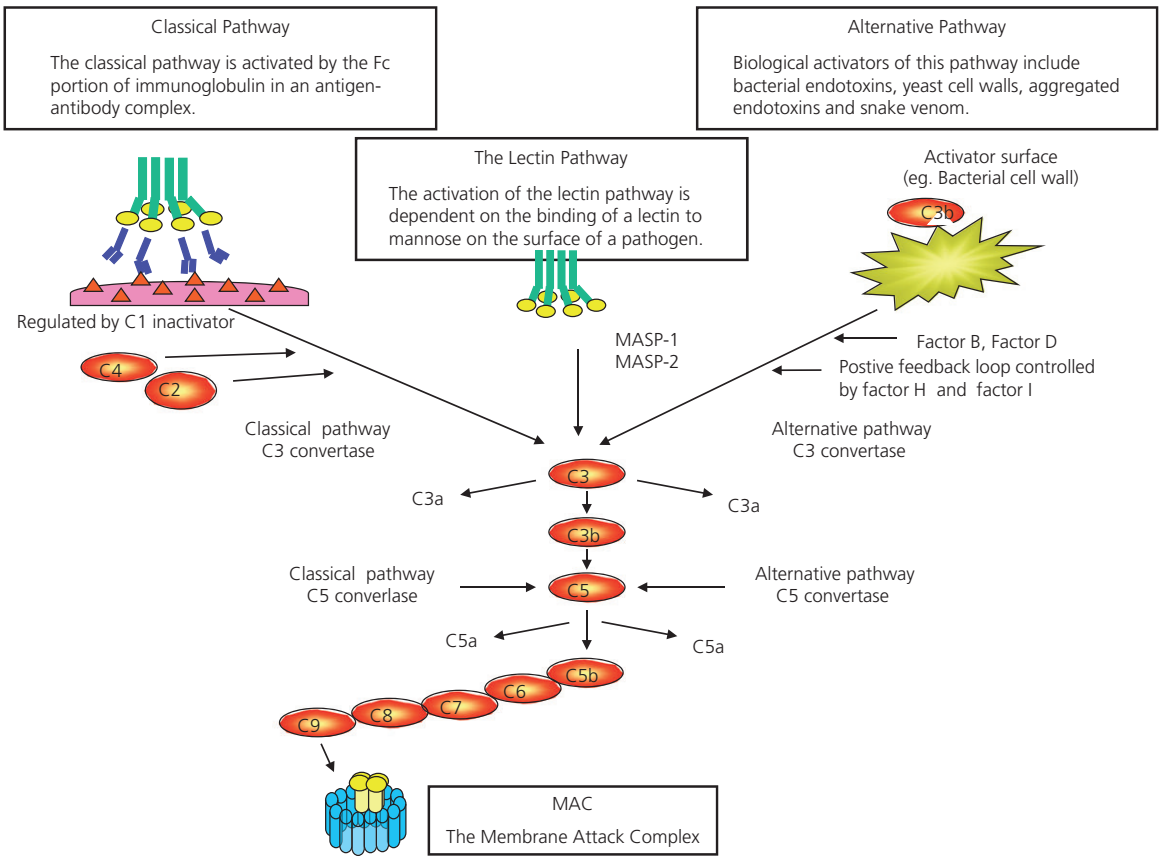

Figure 7.3. Pathways of complement activation. Complement activation is mediated by three pathways: the classical pathway, which is initiated by antigen–antibody complexes, the alternative pathway, and the lectin pathway. All pathways converge on the activation of C3 convertase, and end with the formation of membrane attack complex (MAC), which mediates cell lysis by breaking down cell membranes.

Innate responses to transplants

The immune system responds to allotransplants in strikingly different ways, ranging from ischemia reperfusion injury to rejection, and eventually to tolerance induction, and the innate arm is intimately involved in all processes, thus critically affecting the fate of the transplant.

Ischemia reperfusion injury (IRI)

In solid organ transplantation, surgical trauma, graft ischemia, preservation, and reperfusion are integral parts of the procedure, which inevitably results in tissue injury. The imbalance of metabolic supply and demand during ischemia also creates an intense intragraft hypoxia and vascular dysfunction, leading to further graft damage. Damaged or stressed graft constituents first mobilize the innate immune cells via a variety of pathways that lead to tissue inflammation, production of reactive oxygen species (ROS), and proinflammatory cytokines, APC activation, and ultimately, greater risk of graft loss. This process involves virtually all innate immune cells, molecular sensors, and pathways.

Graft ischemia reperfusion injury (IRI) triggers rapid influx of monocytes, macrophages, neutrophils, and NK cells, as well as certain memory T cells, into the grafts; IRI also mobilizes graft interstitial DCs and migration of host DCs. This massive cellular infiltration, probably in response to potent inflammatory cytokines, chemokines, and complement products, creates an ideal ground for collaboration, activation, and amplification of cellular responses. In this context, key processes include (1) stimulation of TLRs by DAMPs from damaged cells; (2) production of additional inflammatory, cytotoxic cytokines and ROS by activated innate cells; (3) maturation of APCs capable of inducing adaptive responses; and (4) induction of stress markers on otherwise healthy cells, which are recognized by NK cells and trigger NK-mediated killing. All of these responses have been extensively studied in preclinical models, and certain pathways are becoming attractive therapeutic targets for further clinical development. For example, damaged cells release a large amount of the nuclear protein HMGB1, which engages TLR2, TLR4, or RAGE to activate innate immune cells. Thus, mice lacking TLR4, MyD88, and/or TRIF were protected from IRI [41]. Similarly, in liver IRI and myocardial infarction models, the degree of tissue damage was markedly reduced in TLR4-deficient mice [42,43]. At a cellular level, depletion of macrophages with liposomal clodronate or inhibiting their trafficking to the graft ameliorates tissue damage. Furthermore, in models of renal IRI, depletion of kidney DCs in CD11c-DTR reporter mice with diphtheria toxin protected against tubular cell necrosis, leading to less renal dysfunction [44]. Additionally, NK cells readily kill renal tubular epithelial cells through recognition of an induced molecule Rae-1 after ischemia reperfusion, and NK deficiency or antibody-mediated NK depletion protected against kidney IR injury [45]. These findings provide solid evidence to support the importance of the innate immune system in graft IRI.

Complement also contributes to ischemia reperfusion injury, and seminal studies using cobra venom factor to inhibit complement demonstrated mitigation of IRI [46]. Other studies have since shown the importance of various complement components in IRI [47]. For instance, complement receptor 1, complement receptor-related gene Y, C1 esterase, C3 [48], C5 [49], factor B, and decay-accelerating factor (DAF) [35,50] have all been implicated in IRI in various animal models. Indeed, treatment of mice with DAF, which inhibits C3 convertase, prior to reperfusion protects them from IR injury [51]. This protective effect was due to the decrease in complement activation and proinflammatory cytokine release. While serum complement derived from the liver is responsible for antibody-initiated mediated injury, it appears that the graft-derived complement also contributes to IRI [40,52]. It has been shown in murine models that recipients with normal serum complement levels show prolonged graft survival when transplanted with C3-deficient kidneys. Additionally, transplant of these grafts into syngeneic recipients prevented IR injury [53]. Further proof of the importance of local complement to tissue injury came from studies where over-expression of a complement regulatory protein DAF on graft endothelium reduced the extent of IRI following transplantation [54].

Acute rejection

In most cases, innate immune cells, though possessing various effector mechanisms, do not directly mediate acute and complete allograft rejection themselves. However, innate immune cells are fundamentally involved in the control of adaptive T-cell programs, thus indirectly affecting the nature of the rejection response. This aspect of the innate immune system plays important roles in transplant outcomes and is being increasingly appreciated.

The transplanted grafts provide an ideal environment for the innate immune system to act. The "danger signals" generated by IRI stimulate the production of potent proinflammatory cytokines, such as IL-1, IL-6, TNF, and chemokines (e.g. MIP-1α, MIP-1β, MCP-1, IP-10, RANTES, etc.), which mobilize graft interstitial DCs and also mediate rapid influx of host monocytes, macrophages, neutrophils, and host DCs, as well as adaptive T cells and B cells (see Chapter 2). The innate cells infiltrating the grafts produce additional inflammatory cytokines and chemokines upon activation, further amplifying the inflammatory milieu in the graft. This rich inflammatory milieu drives proliferation and maturation of APCs, which includes up-regulation of MHC class I and class II molecules on the cell surface and induction of costimulatory molecules (CD80, CD86, CD40, OX40L, etc.). Matured APCs effectively engage T cells and B cells to initiate the rejection response [55]. Thus, innate immune cells partner with adaptive T cells and B cells to mediate acute rejection. In other words, adaptive immune cells require activated and mature APCs to present alloantigens to trigger their activation. This interdependence in graft rejection was shown in some preclinical animal models. In a minor alloantigen mismatched mouse model (male to female skin transplantation), genetic deficiency for the adaptor molecule MyD88, which prevents TLR signaling and therefore maturation of APCs, led to indefinite skin graft survival [56]. In a more stringent setting, deficiency of both MyD88 and TRIF prolonged the survival of MHC mismatched allografts [57]. These findings also highlight the importance of innate sensors in APC maturation and allograft rejection.

During acute rejection, there is reciprocal migration of APCs, especially DCs. Host DCs can infiltrate the transplanted graft and graft DCs can home to the host's draining lymph nodes. The relative importance of these pathways in transplantation is a matter of continuing debate, but either pathway can trigger acute rejection. Although donor DCs and host DCs present alloantigens differently to T cells, the same mechanisms (i.e. innate sensors, proinflammatory cytokines) are involved in driving their activation and maturation, which allow them to optimally engage T cells. Some innate immune cells, such as macrophages and NK cells, once acquiring effector functions, contribute significantly to the destruction phase of an acute rejection response. Macrophages have long been identified in allograft biopsies of human kidney transplants and in animal

models; they may account for 40–60% of infiltrating leukocytes [58] as detected by immunohistochemical staining for CD68, an intracellular lysozyme-associated glycoprotein used most commonly to detect human macrophages. There are also observations that macrophage infiltration is also seen with acute vascular rejection with endothelilitis or intimal arteritis [59]. As viewed from animal models of acute rejection where macrophage depletion or antagonism [60–62] was explored, it is clear that these cells are critical components of the acute response against the allograft. This conclusion is also supported by clinical studies. In renal allograft recipients in whom T cells have been depleted with alemtuzumab, acute graft rejection occurs despite exceptionally low peripheral T-cell counts, and is often associated with massive infiltration of monocytes [63]. Similarly, eosinophil-driven acute rejection has been observed in intestinal transplantation using alemtuzumab or thymoglobulin [64]. Along the same line, NK cells also contribute to acute rejection. For example, in CD28KO mice, antibody-mediated blockade of NKG2D, an NK-cell-activating receptor, significantly prolongs cardiac graft survival [65], suggesting that NK cells facilitate rejection [66]. Additionally, if NK cells are activated by IL-15, they themselves are capable of mediating acute rejection in the absence of T or B lymphocytes [67].

Among the innate molecules, complement has a unique role in allograft rejection. In transplant models where complement activation is induced, graft destruction is incredibly fast (it can be within hours) and is always irreversible. Graft rejection in this setting often involves vascular endothelial damage, blood coagulation, and activation innate cells without the participation of adaptive T cells. The best example is humoral rejection or acute antibody-mediated rejection (AMR) triggered by antidonor alloantibodies (see Chapter 6) [68]. The antidonor antibodies (mostly against donor HLA molecules, ABO antigens, and endothelial antigens) form immune complexes with donor antigens in the grafts, activate complement cascade via the classical pathway. This process is robust, initiated by the activation of C1 and resulted in formation of MAC (C5b-C9 complex), generation of chemoattractants C3a and C5a, and consequently massive cell death, intragraft inflammation, and extensive blood coagulation in the grafts. In addition, recent studies indicate that T cells and APCs themselves produce complement components, and also express receptors for selected complement elements (e.g. receptors for C3a and C5a). Thus, they can employ the locally produced complement to optimally function, through effects on maturation of APCs and costimulation of T-cell activation upon alloantigen stimulation [52,69]. Furthermore, the engagement of C3aR and C5aR on APCs induces the release of innate cytokines (IL-12, IL-23) and up-regulates costimulatory molecules, again amplifying the T effector response [37,39]. This provides another example of how innate molecules enhance adaptive immune response in the transplant settings. The role of complement in alloreactive T-cell immunity and IR injury explains, at least in part, the fact that murine kidney allografts deficient in C3 exhibit long-term survival [40], whereas those deficient in DAF have worse outcomes [70].

Chronic rejection

Chronic allograft rejection involves mainly the graft vasculature [71,72]. Morphologically, chronic rejection is characterized by concentric neointimal proliferation and eventual occlusion of blood vessels, and this lesion affects vessels of all sizes in the graft. Also, extensive graft interstitial fibrosis is frequently accompanied by vasculature changes. These features are unique to transplants, and therefore also called transplant vasculopathy. In contrast to acute rejection, chronic rejection takes much longer to develop and often requires years following transplantation in patients. This has become a major cause of graft loss impeding long-term transplant success in the clinic. The exact mechanisms of chronic rejection remain elusive, but the current belief is that chronic rejection is perhaps a manifestation of graft injury and remodeling over a long period of time in which both immune and non-immune pathways are critically involved. Importantly, current studies suggest the importance of innate immune mechanisms in the pathogenesis of chronic transplant rejection.

There are distinct features in chronic rejection. In contrast to acute rejection, innate immune cells, mostly monocytes and macrophages, dominate the cellular infiltrates in the lesions, and alloantibodies and complement depositions are also frequently detected. Furthermore, molecular profiling studies often demonstrate ongoing tissue inflammation in chronic lesions, as shown by the heightened expression of multiple inflammatory cytokines. Thus, it is conceivable that tissue damages driven by innate cells, pathways, and innate molecular sensors may contribute significantly to the development of transplant vasculopathy.

It has been shown that TLR signals are strongly associated with the development of atherosclerosis, which is a hallmark of chronic allograft vasculopathy [73,74]. Recently, TLR2, TLR4 and the adaptor proteins MyD88 and TRIF have all been found to be key mediators of chronic rejection in a fully mismatched mouse kidney transplant model [75]. Additionally, in heart transplant patients with evidence of allograft endothelial dysfunction, TLR4 expression and secretion of IL-12 and TNF, which are downstream targets of TLR signaling, were found to be at higher levels than in heart recipients without endothelial dysfunction [76], suggesting the involvement of innate sensors in cellular activation. At a cellular level, macrophages infiltrate heart allografts and contribute to transplant vasculopathy in an animal model of chronic rejection [77]. In this model, partial depletion of macrophages using carrageenan reduced the severity of chronic vasculopathy. This was independent of phagocytosis, as treatment with gadolinium, which inhibits phagocytosis, had no effect on the severity of the disease. In another study, targeting macrophage function using an adenoviral strategy ameliorated the histological features of allograft dysfunction in a rat model of interstitial fibrosis and tubular atrophy (IF/TA) [78]. Mechanistically, monocytes/macrophages, by infiltrating the damaged allograft parenchyma under the influence of chemoattractants, secret growth factors and profibrotic cytokines such TGF-β and IL-13 [12]. They can also directly differentiate into fibrocytes [79], thus promoting synthesis of extracellular matrix proteins and stimulating transition of tubular epithelial cells into fibroblasts [12]. In human transplant recipients, presence of macrophages in early biopsy specimens is predictive of IF/TA development [80,81].

The presence of circulating alloantibodies against HLA, MHC class I polypeptide-related sequence A (MICA), autoantigens, and endothelial antigens increases the risk of long-term graft loss [82,83]. The effector response initiated by alloantibodies in graft damage involves primarily innate pathways. Of central importance is the activation of complement. Indeed, in kidney transplantation in humans, the glomerulopathy and arteriopathy seen in chronic rejection are closely associated with C4d deposition in the graft, supporting a role of complement activation in chronic graft injury [68]. In animal models, C6 deficiency, which affects the formation of the terminal membrane attack complex of the complement

cascade, reduces the severity and onset of graft arteriosclerosis [84]. Studies also demonstrate the importance of locally derived complement (rather than systemic complement) in chronic kidney graft injury. For example, C3-deficient kidney transplants are resistant to adriamycin-induced tubular damage when transplanted into wild-type recipients [85]. In C3a-receptor-deficient mice, adriamycin induced less kidney injury with lower expression of interstitial type 1 collagen and α-smooth muscle actin. Injury by graft-derived complement is also thought to impact long-term graft outcome in humans.

There are circumstances where alloantibody-induced chronic allograft rejection can still arise independent of complement, and a study identified NK cells as key effector cells in driving chronic graft damage [86]. In a mouse model of heart transplantation in which graft recipients are deficient for T cells and B cells (i.e. recombinase-activating gene (RAG)-deficient mice), infusion of donor alloantibodies into transplant recipients induced prominent allograft vasculopathy. Similar findings were observed using either non-complement fixing alloantibodies or recipient mice deficient in complement activation. This suggests that antibody-mediated transplant vasculopathy can still occur via a **complement-independent pathway**. In fact, there are observations in the clinic that some kidney transplant patients develop arteriopathy or glomerulopathy in the absence of C4d deposition is the grafts [87], again supporting a complement independent mechanism of chronic rejection. Interestingly, NK cells are identified as key mediators in transplant vasculopathy independent of complement, as depletion of NK cells or deficiency of NK cells in transplant recipients completely prevented the incidence of chronic rejection induced by donor alloantibodies [86]. Another observation is that in a cohort of MHC compatible kidney transplant patients, KIR mismatches between donors and recipients, which generates alloreactive NK cells, are associated with the worst graft outcomes, thus indirectly suggesting a role for NK cells in clinical chronic graft loss [88]. Together, these findings call attention to innate immune cells and mechanisms in chronic allograft rejection.

Graft acceptance or tolerance

Transplant tolerance is an actively acquired state in which cytopathic responses to allotransplants are absent while those to pathogens are well preserved. Importantly this tolerant state can be stably maintained without prolonged immunosuppression. This is covered in depth in Chapter 11. Traditionally, cells of the innate immune system are thought as initiators of immune responses and, therefore, associated with rejection. However, emerging data have revealed that such cells can also be required for induction and maintenance of transplant tolerance.

A key principle in transplant tolerance is the promotion of death of effector T cells and stimulation of regulatory cells (see Chapter 8) [89]. Innate immune cells and pathways can have significant impacts on both arms of the tolerant induction process (Figure 7.4). For example, DCs are required not only for robust immunity but also for tolerance, as in vivo deletion of $CD11c^+$ DCs in naïve mice breaks down tolerance and induces widespread autoimmunity [7]. This revelation led to the characterization and application of "tolerogenic DCs" in tolerance induction. In fact, DCs contribute to

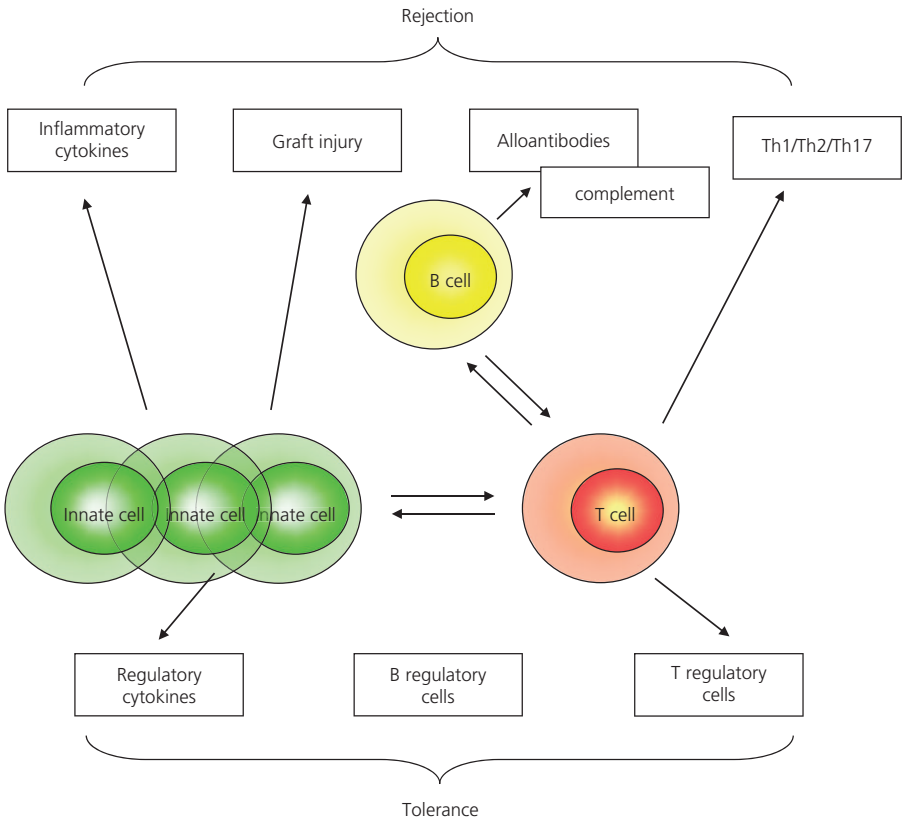

Figure 7.4. Interaction of innate and adaptive immune cells in transplantation. The innate immune cells, similar to their adaptive counterparts, regulate both graft rejection and tolerance induction via a variety of mechanisms, depending on context, models, and tolerizing therapies.

tolerance in several different ways. Mature DCs can drive apoptotic deletion of cytopathic effector T cells following a proliferation burst; subsets of DCs also mediate the induction of Foxp3$^+$ Tregs or homeostasis of natural Tregs. Additionally, DCs, particularly CD8$^+$ DCs, are very efficient at phagocytosis of apoptotic cells, owing to their expression of DEC205, Clec9A, CD36, Tim-1, and Tim-4 [90–92], and clearance of apoptotic cells is a critical process for maintaining tolerance [93]. Also, phagocytosis of allogeneic apoptotic cells inhibits DC maturation, which down-modulates their allostimulatory function, whilst promoting Treg cells [94]. Thus, "tolerogenic DCs" as a cell therapy approach continues to hold promise in transplant tolerance.

Other innate cell types exhibit similar features in tolerance induction. In certain settings, monocyte/macrophages can exert potent anti-inflammatory and immunosuppressive effects that help maintain peripheral tolerance. For example, alternatively activated M2 macrophages or regulatory macrophages (Mregs, see Chapter 2) are capable of secreting anti-inflammatory cytokines, such as IL-10 and TGF-β that are involved in tapering immune responses and resolution of graft inflammation [12]. In fact, some studies demonstrate that adoptive transfer of Mregs can ameliorate the induction of experimental autoimmune encephalitis (a mouse model of multiple sclerosis), and prevent autoimmune colitis by inducing and expanding Foxp3$^+$ Tregs [16]. Additionally, adoptive transfer of donor-derived Mregs in a cohort of human kidney transplant recipients allowed for significant reduction in the use of immunosuppressive drugs [95]. Similarly, NK cells also employ different mechanisms to promote transplant tolerance. NK cells, guided by "missing self-recognition," can eliminate graft-derived allogeneic DCs, thus reducing T-cell priming by the direct pathway of antigen presentation [25]. Killing of donor cells by NK cells favors the indirect antigen presentation, which is implicated in tolerance induction. Also, some NK cells exhibit regulatory function through IL-10-dependent mechanisms and contribute to tolerance by tipping the balance towards regulation [96].

The striking dichotomy of innate immune cells in transplant settings (rejection versus tolerance) is most likely context dependent, in that different cell subsets can exert divergent effects depending on their maturation status and interactions with other cell types. For example, in many instances NK cells can be tolerogenic; however, further NK maturation by IL-15 signaling promotes rejection; M1 macrophages are proinflammatory and M2 macrophages are immunosuppressive. Additionally, TLR signaling mediates maturation of DCs and monocytes in rejection. However, Tregs also express certain TLRs, and TLR stimulation can increase the suppressive properties of Tregs and thus facilitate tolerance. This context-dependent function of innate pathways and context-dependent regulation of innate immune cells constitute a major challenge in manipulating the immune responses to allotransplants.

Summary

In summary, cells of the innate immune arm become activated early following ischemia reperfusion injury and are implicated in the pathogenesis of acute and chronic rejection in both animal models and humans. A significant challenge is that the complex interactions amongst diverse subsets of innate immune cells in vivo in transplant settings are poorly understood. In addition, the dynamic cross-talk between innate and adaptive immunity necessitates a further understanding of both arms for the purpose of tolerance induction.

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CHAPTER 8

Mechanisms of Immune Regulation

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Introduction

The majority of the leukocyte subsets that can contribute to the destruction of a transplant through rejection can also play a role in regulating or controlling immune responsiveness [1]. In some cases, the regulatory cells comprise specialized populations that are selected or differentiate to have functional regulatory or suppressive properties. In other situations, the microenvironment present within the allograft or the lymphoid tissue will enable the immune response at that site to be controlled. In transplantation, T cells have often taken centre stage, both in immune responses that lead to rejection and the destruction of transplanted cells, organs, or tissues, as well as in controlling these unwanted aggressive immune responses. However, other populations of leukocytes can also have this dual functionality, including, for example, B cells where antibody-secreting as well as regulatory B cells have been identified and dendritic cells that can both stimulate immune responses as well as induce immunological tolerance [2]. In this chapter, we will review both the mechanisms of regulation that can be used by the immune system to prevent unwanted immune responses, as well as some of the different cell populations that have been shown to play a role in the regulation of alloimmune responses in vivo. While specific manipulation of these mechanisms remains a future potential, it is likely that regulation plays a substantial role in determining the ultimate effect of conventional immunosuppressants and influences the balance of graft acceptance and rejection both in experimental and clinical practice. Discussions in this chapter complement those on the mechanisms of tolerance found in Chapter 11.

Cell death

The death or deletion of lymphocytes that can recognize and respond to self-antigens or, after transplantation, donor alloantigens is a very effective mechanism for eliminating lymphocytes from the immune repertoire that have the potential to damage the graft, thereby creating unresponsiveness or tolerance to donor alloantigens. Both T cells and B cells can be deleted from the repertoire in this manner.

Central deletion of alloantigen reactive T cells

The ability of the immune system to shape the T-cell repertoire by eliminating or deleting T cells that express a T-cell receptor that will either be of no use or could be dangerous to the host in the future is a critical part of the process of T-cell development in the thymus. T-cell precursors differentiate in the bone marrow from haematopoietic stem cells and then migrate to the thymus. Upon entry into the thymus, these progenitor cells begin to rearrange their T-cell receptor (TCR) genes and undergo a programme of thymic selection. This involves a complex sequence of events whereby $CD4^+CD8^+$ thymocytes expressing a complete $\alpha\beta$TCR undergo positive and negative selection before thymocytes leave the thymus, emigrating to the periphery as mature single-positive (i.e. expressing either CD4 or CD8) T cells. The process of positive selection identifies or selects T cells that express a TCR of sufficiently high affinity to self peptide major histocompatibility complex (pMHC) complexes such that the cell receives survival signals. T cells that express a TCR with no affinity for self pMHC complexes are not selected and die by apoptosis. Cells with TCRs that have too high an affinity for self pMHC complexes, and therefore could react with self-antigens expressed by tissues in the periphery, are deleted in a process known as negative selection. Through these mechanisms, T cells capable of recognizing self MHC molecules bound to foreign peptides that are potentially useful to the host immune system are maintained and populate the periphery, while ensuring that the majority of dangerous self or autoreactive T cells are eliminated.

To ensure that negative selection of T cells is as complete as possible and thereby reduce the potential of autoimmune disease, a wide a range of self pMHC complexes must be present or expressed in the thymus. As some proteins are expressed exclusively in the periphery, this process requires a special mechanism and is controlled by the autoimmune regulator, AIRE. AIRE is a transcriptional regulator that promotes expression of a large repertoire of mRNA transcripts encoding different proteins in the thymus that would normally only be expressed in tissues in the periphery. In this way, T cells undergoing selection in the thymus will encounter a wide range of self pMHC complexes. Importantly, mutations in AIRE can impair its function and as a consequence the deletion of self-reactive T cells in the thymus is affected. Defects in AIRE function result in the emergence of self-reactive T cells into the periphery where they can attack specific organs, leading to autoimmune disease [3]. This system is very sensitive and the absence of just one AIRE-induced peptide in the thymus can lead to autoimmunity [4].

While AIRE is very effective at ensuring a wide range of pMHC complexes are expressed in the thymus, the process of negative selection remains incomplete and therefore some T cells that are potentially harmful to the host may escape negative selection and

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be present in the peripheral immune repertoire. To guard against harm coming to the host, the immune system has developed a fail-safe mechanism to control the activity of these unwanted T cells by also selecting in the thymus a population of so-called thymus-derived or naturally occurring regulatory T cells (nTreg) [5]. This process of selection allows T cells with an intermediate, rather than a high, affinity for self-pMHC complexes to express the transcription factor Foxp3 [6]. Following selection, Treg migrate to the periphery alongside other T cells, where they can control any potentially harmful self-reactive T cells.

The phenomenon of central tolerance (i.e. deletion of unwanted T cells) has been exploited to facilitate the induction of transplantation tolerance. Central deletion of alloreactive T cells has been achieved via direct delivery of alloantigen into the thymus via intrathymic injection [7,8] and by the establishment of allogeneic mixed bone marrow chimeras in experimental models [9]. These studies provide evidence that mechanisms of deletion of antigen reactive cells in the thymus may be harnessed to facilitate operational transplantation tolerance.

Peripheral deletion of alloantigen reactive T cells—activation-induced cell death or immune exhaustion

Apoptosis of antigen-reactive T cells via activation-induced cell death (AICD) is another mechanism for controlling immune reactivity. AICD is a process through which T cells undergo cell death in the periphery [10]. Following restimulation through TCR, a number of different molecules, including CD95 (FAS), tumour necrosis factor receptor 1 (TNFR1), and tumour necrosis factor related apoptosis inducing ligand receptor (TRAILR), can play a role in AICD depending on the circumstances, triggering a complex series of signalling events, which ultimately lead to caspase activation, DNA fragmentation, cytoskeletal degradation, and cell death.

Deletion of alloantigen reactive B cells

The elimination of B cells from the repertoire is also a mechanism that has evolved to ensure that B cells that are polyreactive and capable of binding self-antigens are eliminated in the bone marrow before they enter the periphery. As rearrangement of immunoglobulin genes occurs at random to enable as many foreign protein and carbohydrate antigens to be recognized as possible, it inevitably leads to the generation of B cells that express B cell-receptors (BCR) that can recognize self-antigens. Estimates vary, but suggest that up to 70% of the immature B cells produced are self-reactive. Of the order of one-third of these immature B cells are eliminated by receptor editing, whereby new gene rearrangements result in the production of an alternate light chain that can pair with the existing heavy chain, altering the antigen recognition properties of the expressed BCR. When an immature B cell recognizes self-antigen with high avidity it rapidly internalizes the antigen and undergoes a period of developmental arrest. Lymph node homing receptors, such as CD62 ligand (CD62L) are not expressed, and the receptors for B-cell activating factor (BAFF), a cytokine required for B-cell survival, are not induced. In addition, recombinase-activating genes remain switched on, which allow the B-cell receptor (BCR) to be replaced by editing the light chain. Any B cell undergoing this process will die after 1–2 days if it fails to express a non-autoreactive receptor. Death through this pathway does not require FAS and is, in part, due to antigen-induced expression of the Bcl-2 interacting mediator of cell death (Bim), which inhibits B-cell survival proteins from the Bcl-2 family.

Receptor editing is a mechanism that could be used to delete immature B cells capable of recognizing donor alloantigens from the repertoire, particularly in situations where the repertoire is reshaped following leukocyte depletion or the induction of mixed chimerism, as well as when organs are transplanted into young infants.

If receptor editing fails to eliminate all of the self-reactive B cells generated, residual immature B cells expressing highly self-reactive receptors are triggered to die by interaction with self antigen. This mechanism of control or regulation has been studied using immunoglobulin transgenic mice and the data obtained suggest that B cells are deleted efficiently when the antigen they recognize is membrane bound. The efficiency of this process was found to be dependent on the probability that the immature B cells encountered the relevant self-antigen and therefore its efficiency is clearly related to antigen density or frequency.

Deletion of B cells capable of recognizing donor alloantigens from the repertoire of a transplant recipient is a mechanism that can be harnessed in transplantation. This mechanism of regulation is most efficient when the antigens recognized are present at high doses. Infant recipients of ABO-incompatible heart allografts have been shown to delete B cells capable of making antibodies to blood group antigens presented on the heart transplant [11]. Mixed chimerism strategies that result in the co-existence of donor and recipient bone marrow should also enable donor reactive B cells to be deleted if the level of chimerism achieved is sufficient to ensure that B cells encounter donor cells [12].

Anergy

Anergy is a term used to describe the functional inactivation of lymphocytes resulting in the cells becoming refractory or unresponsiveness to further stimulation. Anergy is characterized by biochemical changes that lead to some degree of developmental arrest in the responding cells and an increased threshold for activation when they re-encounter their cognate antigen. This mechanism of controlling immune responsiveness is applicable to T cells and B cells and may contribute in some settings to the mechanisms that can be brought into play to control immune reactivity to alloantigens after transplantation. However, it is important to note that anergy is a reversible state of unresponsiveness.

T-cell anergy

T cells in the periphery are activated as a consequence of signals delivered from the TCR following recognition and binding of a relevant pMHC complex (signal 1) and from ligand–receptor interactions between molecules on the T cell and antigen-presenting cell (APC), which are collectively known as costimulatory pathways (signal 2). In vitro, antigen recognition (i.e. TCR ligation by the relevant pMHC complex) in the absence of costimulation has been shown to induce T-cell anergy [13]. The absence of costimulation, through CD28, at the time of antigen recognition was demonstrated to be critical for the induction of T-cell anergy in these in vitro systems. In vivo, induction of T-cell anergy has also been shown to be dependent on ligation of CTLA-4 (CD152).

As knowledge of costimulatory pathways has evolved, it has become clear that other ligand–receptor interactions may also contribute to the development and/or maintenance of T-cell anergy. The costimulatory molecule programmed death 1 (PD-1) is highly expressed on anergic T cells and in some tissues and its interaction with its ligand, PDL-1, has also been shown to be involved in the

induction and possibly maintenance of T-cell anergy. Hence, in vitro and in vivo, T-cell anergy can result from ligation of TCRs in the absence of the correct additional costimulatory signals, resulting in the attenuation of the T-cell activation process thereby limiting the ability of T cells to respond.

B-cell anergy

As highlighted above for T cells, the mechanisms of central B-cell tolerance are also incomplete. Thus, self-reactive B cells can escape central tolerance in the bone marrow and emerge into the periphery. Peripheral self-reactive B cells that bind their cognate antigen through the BCR with lower affinity become anergic. This is due to altered intracellular signalling when the B cells are chronically exposed to low levels of antigen [14]. In experimental models, B-cell anergy has been demonstrated in B cells that bind soluble antigens with low avidity.

In a normal heterogeneous B-cell repertoire, both anergic B cells that bind self-antigen as well as recirculating naïve follicular B cells that bind foreign antigen localize to the T-cell zones of the secondary lymphoid organs. In the absence of T-cell help and the activation of additional signalling pathways in the anergic B cells, these cells die within 1–5 days. In contrast, when the naïve B cells engage antigen in the presence of T cell help they collaborate with CD4 cells and differentiate into antibody-secreting plasma cells.

Costimulation by toll-like receptor (TLR) ligands has been found to be blocked in anergic B cells. In the early phase after transplantation this may be helpful, as it is clear that TLRs are activated by damaged cells present in the transplanted organ or tissues and contribute to the rejection process. After transplantation, it would clearly also be advantageous to maintain low avidity, alloreactive B cells in an anergic state to prevent them from secreting donor-specific antibodies (DSA) later in the post-transplant course. Strategies for achieving this are still being sought. It is interesting to note that immunosuppressive agents, such as cyclosporine A (CsA), have not been particularly effective at preventing antibody-mediated chronic allograft rejection [15] where clusters of B cells have been identified in allografts undergoing chronic rejection [16]. This lack of effectiveness may be due to the inability of CsA to mimic the signalling events that occur in anergic B cells.

Immunoregulation

As strategies of inducing and maintaining immunological unresponsiveness or tolerance to self-antigens, deletion and anergy are either incomplete or unstable. The immune system has therefore developed a number of fail-safe mechanisms to ensure that tolerance is maintained despite the presence of self-reactive T cells and B cells in the periphery. These same fail-safe mechanisms, which include populations of regulatory or suppressor cells capable of controlling any potentially autoreactive lymphocytes, can be used to control immune responsiveness to alloantigens after transplantation.

Regulatory T cells

Many different types of T cells with regulatory activity have been described including: $CD4^+$ T cells [17,18]; $CD8^+$ T cells [19–21]; $CD4^-CD8^-$ double-negative T cells [22]; NKT cells [23]; and γδT cells [24]. As $CD4^+$ T cells with regulatory functions have been studied in greater depth to date, this section of the chapter will focus on this population of regulatory cells.

$CD25^+FOXP3^+$ T regulatory cells (Treg) can arise via two distinct developmental pathways. First, as mentioned above, thymus-derived or naturally occurring (nTreg) differentiate in the thymus and are thought to function primarily to suppress responses to self-antigen and hence prevent autoimmune disease. Evidence for this comes from studies in patients with rare genetic defects and in mice with either naturally occurring or genetically engineered defects. For example, profound immune dysregulation leading to autoimmunity is observed in patients with the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. IPEX patients have been found to have a point mutation in the gene encoding factor forkhead box P3 (*FOXP3*) [25,26], the master gene for T-cell regulation, resulting in functional impairment of Treg activity in vitro [27]. Scurfy mice also have mutations in *foxp3* and a related immune profile [28]. Second, when $CD4^+$ T cells encounter antigen in a tolerogenic microenvironment in the periphery, such as when antigen is presented by immature dendritic cells (DCs) or in the presence of immunosuppressive cytokines, the $CD4^+$ T cells differentiate into 'adaptive' antigen induced Treg (iTreg) [6]. In the context of transplantation, it can be argued that this pathway may be the more important route to generating donor alloantigen reactive Treg after transplantation [29]. Interestingly, despite their distinct developmental origins, both nTreg and iTreg rely on sustained expression of high levels of the transcription of *FOXP3* for their suppressive function (Figure 8.1).

Importantly, transplantation provided some of the earliest evidence for suppression or immune regulation by T cells in vivo. Data from neonatal tolerance studies suggested that additional mechanisms beyond deletion of donor-reactive cells were involved [30,31]. Later, a clear indication that long-term allograft survival in the absence of long-term immunosuppression, a status referred to as operational transplantation tolerance, involved the presence of T cells with the ability to regulate the function of naïve alloreactive T cells, thereby preventing the rejection of a fresh graft, was reported [32,33]. Subsequently, this form of cellular regulation was found to be associated with $CD4^+$ T cells and Hall and colleagues were the first to suggest that CD25 might be a useful marker for identifying

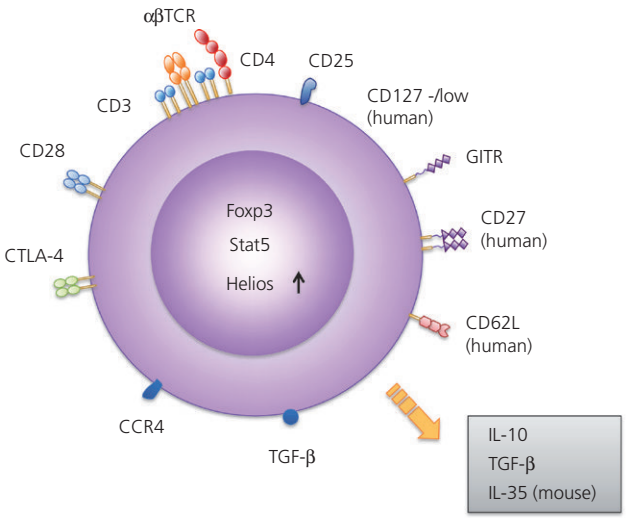

Figure 8.1. Regulatory T-cell characteristics. Shown are the intracellular transcriptional characteristics, surface molecules and defining cytokines of T cells with specialized regulatory potential.

$CD4^+$ T cells with regulatory activity [34]. Similar data were obtained in a rat renal allograft model where operational tolerance was induced by donor-specific blood transfusion [35,36]. As a direct demonstration that $CD4^+$ T cells expressing high levels of CD25 could regulate rejection, Hara and colleagues showed that co-transfer of $CD4^+CD25^+$ T cells from tolerant animals led to indefinite skin graft survival in 80% of immunodeficient mice reconstituted with naïve CBA effector T cells [37].

In the absence of any previous exposure to alloantigen, there are usually insufficient numbers of nTreg to prevent rejection of a fully allogeneic (MHC + minor histocompatibility antigen mismatched) graft as the frequency of T cells present in the repertoire capable of making a destructive response to the graft far out numbers the relatively small number of nTreg present and rejection occurs [37]. The fact that nTreg cannot prevent destruction of an allograft in the absence of immunosuppression does not mean that the cells do not function. However, under these circumstances, the balance between rejection and regulation is against regulation, as the suppressive activity of any Treg present is overwhelmed by the destructive response mediated by effector T cells. The presence of pre-existing donor alloantigen reactive memory T cells in the recipient can also overwhelm immune regulation as the kinetics of activation of the memory cells is very rapid and unless very high numbers of Treg are present at the outset of the response, the balance between rejection and regulation is pushed markedly in favour of rejection [38]. Importantly, this critical balance between graft destruction and acceptance can be shifted in a number of ways, notably by employing strategies that increase the relative frequency and/or the activation status and consequently the functional activity of aTreg that can then respond to donor alloantigens before or in the early period after transplantation [39,40], or by inhibiting or reducing the activity of the effector cells.

Whilst the observation that mice with long-term surviving allografts contain populations of alloantigen-reactive $CD25^+$ Treg was important, these experiments were unable to distinguish between Treg that were generated by the induction strategy itself and those that arose simply by the presence of the accepted allograft. In terms of developing potential clinical approaches, it is important to clarify whether induction strategies that ultimately lead to long-term operational tolerance can drive Treg development independently of the graft itself. Data demonstrating that exposure to alloantigen in the absence of a transplant can lead to the induction of $CD4^+CD25^+$ Treg were obtained in a number of studies. For example, when $CD4^+CD25^+$ were isolated from mice 28 days after pretreatment with donor alloantigen in combination with non-depleting anti-CD4 therapy, these cells prevented rejection of a test skin graft in a sensitive adoptive transfer model [41]. Critically, protection of the test graft was not observed with similar populations isolated from naïve mice, or mice treated with anti-CD4-only or DST-only demonstrating that tolerance mediated by $CD25^+$ Treg can indeed be induced in vivo before transplantation if recipients are exposed to donor alloantigen under permissive conditions. Moreover, data demonstrating that the presence of the allograft alone can lead to the development of T cells with regulatory properties that can protect a challenged graft from rejection [42], even when the allograft itself has been rejected [43], have been reported. Both of these examples demonstrate that T cells with regulatory activity can be induced in the presence of alloantigen in the form of the allograft or an infusion of alloantigen or indeed both, and that these cells can contribute to controlling subsequent responses to alloantigen in vivo.

Another important issue that has been under investigation is where Treg that can control allograft rejection function most effectively and where they can be found or detected in vivo. There is evidence that the location in which Treg function may change with time after transplantation. Early in the response after transplantation, Treg have been shown to be present and functionally active in the draining lymph nodes, while later in the post-transplant course Treg have been shown to function within the allograft itself [44]. Indeed there is increasing evidence that an important site of immune regulation is within the allograft itself where Treg function to create an environment that is permissive of control [45,46]. Moreover, re-exposing Treg to antigen in a tissue may enable them to become more potent suppressors and, therefore, more effective at controlling rejection [47].

Although a significant body of work has demonstrated that Treg can control responses to alloantigen, most studies have used either in vitro assays or experimental models whereby cells are adoptively transferred into immunodeficient recipients where allograft rejection is driven by relatively small numbers of effector T cells compared to the number that would be found in a full immune repertoire in vivo. Arguably, a much more relevant question for clinical application of this approach is what role do Treg play in an intact immune system? In transplantation, Treg-specific inactivation was used to show that in the anti-CD4/DST tolerance induction model described above, the survival of primary heart allografts in normal, lymphoreplete recipients is also unequivocally dependent on iTreg driven by the tolerance induction protocol [48]. These data suggest that it should indeed be possible to boost the function of Tregs in non-lymphopenic transplant patients.

Regulatory B cells

B cells with the capacity to suppress the development of autoimmune diseases in mice were first described in the 1980s. Regulatory B cells (Breg) express high levels of CD1d, CD21, CD24 and IgM and moderate levels of CD19, although some heterogeneity has been described, suggesting that different subsets of Breg may exist [49] (Figure 8.2). One of the characteristics of B cells with regulatory activity is their ability to secrete IL-10. CD40 stimulation appears to be required to stimulate IL-10 production and has been reported to be necessary for activation of Breg, enabling them to manifest their functional activity and suppress Th1 differentiation.

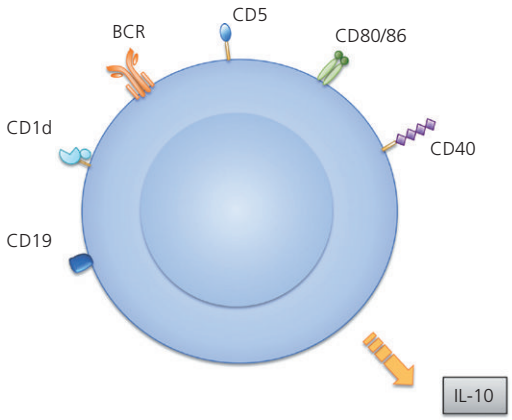

Figure 8.2. Regulatory B-cell characteristics. Shown are the surface molecules and defining cytokines expressed by B cells with specialized regulatory potential.

It has been suggested that there is a link between regulatory B cells and T cells, with Breg acting as potent generators of Treg. Breg have been described in both mouse and human. Mouse Breg express T cell Ig domain and mucin domain protein 1 (Tim 1) [50]. While human regulatory B cells share some properties with their mouse counterparts, including an immature phenotype, and comprise a small subset of the total B cell pool.

In transplantation, there is only indirect evidence that Breg may play a role in controlling immune responses to alloantigens. Interestingly, in renal transplant recipients who have a functioning graft in the absence of immunosuppression, a B-cell signature was found to be associated with this state of operational tolerance to donor alloantigens [51,52]. A distinct, but also B-cell dominated signature of tolerance was identified in a separate cohort of long-term immunosuppression free renal transplant recipients [53].

Experimental studies on the role of Breg in transplantation are limited at present. In a rat model of long-term kidney transplantation tolerance, a shift in both peripheral and intragraft gene expression from IgG to IgM was observed, as well as IgM^+, but not IgG^+ B-cell clusters within the graft [54]. In mice, Tim-1 ligation on B cells induced Tim-1^+ B cells with regulatory activity [50], suggesting a potential therapeutic strategy for increasing the number of Breg *in vivo*. More work is required to determine and characterize the contribution of Breg in the regulation of alloimmune responses and the relationship with the production of DSA [55].

Dendritic cells

DC are central to the activation/priming of an immune response, but paradoxically they can also promote the development of tolerance [1,56,57]. Initially, immature myeloid DC that express low levels of MHC class II and costimulatory molecules at the cell surface were identified as the dominant form of DC that had the capacity to induce T-cell tolerance. In contrast, mature myeloid DC that express much higher levels of both MHC and costimulatory molecules were found to prime T-cell responses most efficiently. However, subsequently, mature DC have also been shown to have the capacity to induce tolerance and, therefore, the relationship between the state of maturity of a DC and its tolerogenic potential is now not thought to be a significant factor in determining whether a DC stimulates or inhibits the development of an alloimmune responses.

There are many studies demonstrating that DC can promote tolerance to solid organ allografts and bone marrow grafts [56]. For example, a single injection of donor-derived DC 7 days before transplantation of a MHC-mismatched heart allograft extends [58] or prolongs survival indefinitely [59] in a donor-specific manner. The potential tolerogenic effects of DC can be potentiated by the co-administration of immune-modulating agents such as costimulation blockade [60]. 'Alternatively activated' or 'regulatory' DC, which have low costimulatory ability, can also, when administered 7 days before transplantation, protect MHC-mismatched skin grafts from rejection [61] and mice from lethal acute graft-versus-host disease when administered 7 days before transplantation [62].

Plasmacytoid DC (pDC) are arguably the best characterized of the DC subsets that can influence the course of an immune response towards regulation. pDC were originally defined by their capacity to secrete large amounts of type I interferons in response to viruses and to play an essential role in protecting individuals against inflammatory responses to harmless antigens, but they have now also been shown to be able to induce human regulatory T cells in vitro. These regulatory cells produce significant amounts of interleukin IL10, low IFN-γ, but no IL4, IL5, or transforming growth factor (TGF)-β [63]. Non-lymphoid tissue pDC, such as those residing in the airways, gut, and liver, play a significant role in regulating mucosal immunity and are critical for the development of tolerance to inhaled or ingested antigens.

The molecular mechanisms used by pDC to promote tolerance are complex [64], including evidence that they can promote the differentiation of $Foxp3^+$ Treg. In transplantation models, tolerizing pDCs can acquire alloantigen in the allograft and then migrate through the blood to home to peripheral lymph nodes, where they induce the generation of $CCR4^+CD4^+CD25^+Foxp3^+$ Treg [65]. Significantly, infusion of donor-derived pDC 7 days before transplant were found to be capable of prolonging subsequent heart allograft survival (from 9 to 22 days) in the absence of immunosuppressive therapy [66], but this effect was markedly enhanced by anti-CD154 mAb administration [67]. In mice, pre-pDC appear to be the principal cell type that facilitates haematopoietic stem cell engraftment and induction of donor-specific skin graft tolerance in allogeneic recipients [63]. The combination of DC, administered with costimulatory blockade, may be the most promising approach identified thus far.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSC) have been associated with many suppressive functions, both antigen specific and non-specific. These include regulation of innate immunity, T-cell activation and tumour immunity. MDSC are a heterogeneous population of progenitor cells that can accumulate in tissues during an inflammatory immune response. The expansion and activation of MDSC is regulated by factors produced by other cells that are present in the same microenvironment; the other cell types involved include stromal cells, activated T cells and in tumours, the tumour cells themselves.

A number of MDSC subsets have been described in both mice and humans [68] (Figure 8.3; adapted from data summarized in [69]). Despite the heterogeneity, common phenotypic markers are expressed including Gr1 and CD11b in mice and CD33, CD11b, CD34 and low levels of MHC class II in humans. Activated MDSC suppress proliferation and cytokine production by T, B and NK cells in vitro through mechanisms that include the enzyme inducible nitric oxide synthase 1, which induces nitric oxide production, and arginase 1, which depletes arginine. Both of these pathways can induce several mechanisms that inhibit cell function. MDSC also appear to be able to be modify T-cell differentiation pathways and it has been reported that they promote Treg differentiation.

In experimental models, MDSC can play a role in the induction of tolerance to alloantigens. For example, transplantation tolerance could not be induced in mice that did not express MHC class II on circulating leukocytes; a finding consistent with a requirement for recipient MHC class II^+ cells for the induction of tolerance to alloantigens [70]. Moreover, data suggesting direct evidence of a tolerogenic role for $CD11b^+CD115^+Gr1^+$ monocytes for heart allografts in mice [71] and, in the rat, evidence for iNOS-expressing MDSCs in kidney have been reported [72].

Regulatory macrophages

Macrophages are inherently plastic in terms of the functional outcome of activation. Classically activated macrophages are highly proinflammatory and are generated in response to ligation of TLRs, resulting in the activation of inflammatory signalling cascades and the production of inflammatory cytokines including tumour

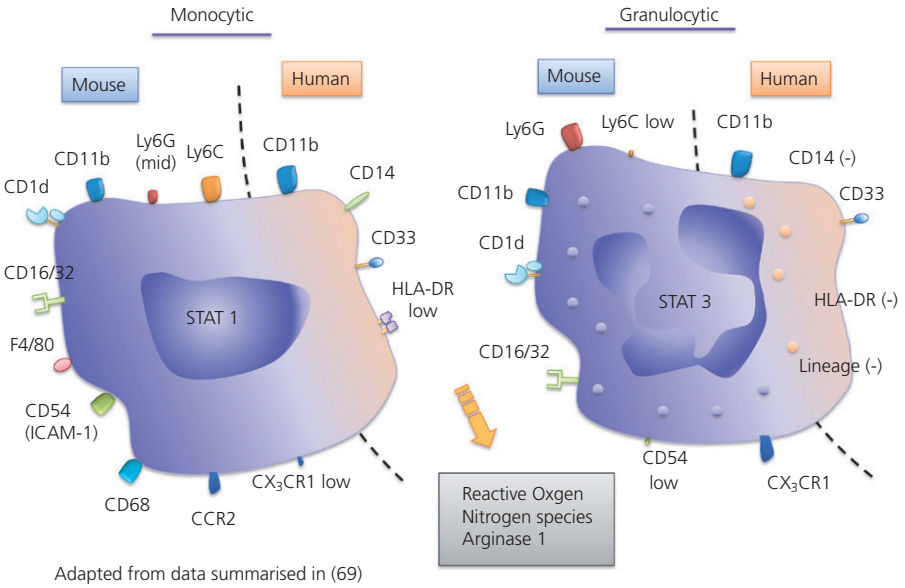

Figure 8.3. Myeloid-derived suppressor cell characteristics. Shown are the intracellular transcriptional characteristics, surface molecules and defining cytokines of myeloid-derived cells (left, monocytic; right, granulocytic) with specialized regulatory potential.

necrosis factor-α and IL-1β. Alternatively activated macrophages, induced by exposure to the cytokine IL-4, are less proinflammatory and in some settings can create a microenvironment that modulates immune responses that would otherwise be deleterious to the tissues in which they reside [73]. Regulatory macrophages may represent an additional population, which is distinct from the other two forms whose main physiological role is to dampen inflammatory immune responses and prevent the immunopathology associated with prolonged classical macrophage activation [74].

Regulatory macrophages produce high amounts of IL-10 usually following costimulation with two ligands, for example TLR and immune complexes. They do not express arginase and are not dependent on STAT6 signalling, as are alternatively activated macrophages. At present, there are no well-established stable or convenient surface markers specific for regulatory macrophages (Figure 8.4 [75];). In tissues, regulatory macrophages are anatomically positioned to interact with lymphocytes, and therefore there is evidence that these cells can influence the activity of each other. In vitro, regulatory macrophages are efficient APCs that induce highly polarized antigen-specific T-cell responses dominated by the production of Th2 cytokines.

Human regulatory macrophages isolated from the peripheral blood have the capacity to regulate immune responses to alloantigens, and in a pilot study have been administered intravenously to living donor kidney transplant recipients [76].

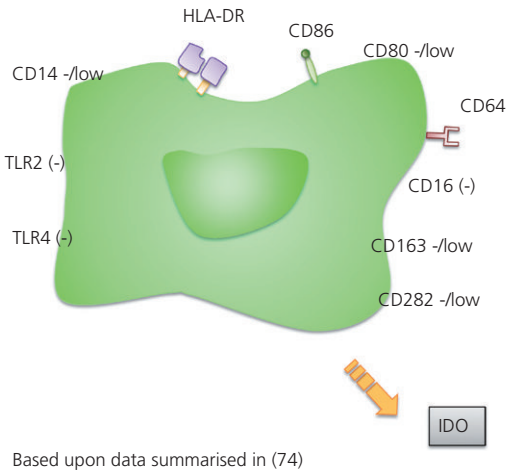

Figure 8.4. Regulatory macrophage (human) characteristics. Shown are the surface molecules and defining products of human macrophages with specialized regulatory potential. Based upon data summarized in [74].

Ignorance

Arguably, the simplest mechanism that facilitates the maintenance of peripheral tolerance is T-cell ignorance of self-antigen. In this setting, autoreactive T cells escape negative selection within the thymus and enter the periphery, but such T cells are either prevented from accessing sites that express their cognate peptide or they never see enough peptide to enable them to overcome activation thresholds [77]. In transplantation it is hard to envisage how this mechanism could operate to regulate the alloimmune response effectively in a sustained manner.

Summary

Immunoregulation is a complex process involving multiple mechanisms and often more than one cell type that work together to contain and control immune responses. These processes are necessary to avoid unwanted immune responses and limit peripheral effects of wanted immune responses. While there are not methods to manipulate these mechanisms precisely, they no doubt play a role in determining the ultimate fate of an allograft and will be increasingly important in finely managing alloimmune responses in the future.

Acknowledgments

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CHAPTER 9

The Spectrum of Alloimmunity, Heterologous Immunity, and Relevant Autoimmunity

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Introduction

Immune alloreactivity is the sum of the actions of multiple effector cell types and their secreted products responding to foreign human antigens within the context of the trauma, cellular damage, and ischemia reperfusion injury that accompanies surgical transplantation. Although the fundamental underpinnings of alloreactivity are similar between individuals, the magnitude and character of an alloimmune response varies considerably from one person to another, with changes in the alloantigen disparity involved, and over time as the responder changes their immune history. Indeed, these changes are increasingly recognized as being critical to our understanding of the variability seen in outbred animals and in clinical transplantation. In this chapter we will review the factors underlying the variability inherent in alloreactivity, and highlight mechanisms by which the immune history of a given recipient can fundamentally shape their alloreactive potential.

The spectrum of alloreactivity

At the core of ones alloreactive potential rest three primary cell types that recognize alloantigen and initiate the alloimmune response; they are natural killer (NK) cells and T and B lymphocytes. These cells are activated following recognition of donor-derived foreign antigen, in the case of B and T cells, or the absence of self-MHC, in the case of NK cells. While the details regarding the mechanisms of activation and mediation of graft rejection will be covered specifically in Chapters 5 and 6, it is important to note that the alloreactive potential, or strength and intensity of alloreactive immune response, varies widely between individuals and between different donor and recipient pairs. Indeed, it varies over time within a single individual against a defined donor. Many factors underlie this differential degree of alloreactivity, the most conspicuous of which is the degree of MHC matching between the donor and recipient. However, the character and functionality of the recipient's immune system likely plays an important role in determining the magnitude and character of the alloreactive response to a given donor tissue. Given the central involvement of the above-mentioned cells, a review of their contributions is appropriate.

Lymphocytes as effectors of alloimmunity following transplantation

As discussed above, three main subclasses of lymphocytes can participate in the alloimmune response following transplantation. While the role of NK-cell-mediated rejection in solid organ transplantation remains controversial [1–5], and will be covered in depth in Chapter 7, recent evidence suggests that the infectious history of a given individual can impact the nature of the NK-cell compartment [6,7]. Specifically, it is now known that although NK cells do not undergo rearrangement in the genes encoding their activating receptors, they do undergo a clonal expansion-like process during viral infection which results in the generation of long-lived progeny that meet the criteria of immunologic memory [6,7]. Importantly, these memory NK cells have been shown to mediate more efficacious secondary responses against previously encountered pathogens [6,7], raising the possibility that prior immune events resulting in NK-cell activation might increase the NK-mediated barrier to transplantation. Further work to assess the impact of virally induced NK-cell memory on alloimmunity is required.

The spectrum of pre-existing humoral alloimmunity in patients awaiting transplantation is better elucidated, and covered in Chapter 6. The degree of humoral sensitization is defined using panel reactive antibody (PRA, described in depth in Chapter 36). PRA assess the frequency of HLA molecules in a defined panel to which serum antibodies from a given patient will react, and is generally regarded as a given patient's overall risk of experiencing humoral rejection. For example, a PRA of 20% would suggest that patient would be incompatible with 1 out of every 5 donors. In practice, PRA values span a wide range across individuals, but it is estimated that approximately 30% of dialysis patients exhibit a PRA of greater than 20%, which is defined as highly sensitized [8], and half of those possess PRAs of >80% [9]. Furthermore, it is general assumed that high pretransplant PRA portends post-transplant risk for antibody-mediated graft injury; although this is most relevant for PRA that includes donor-specific alloantibody [9]. Interestingly, a study comparing PRA and panel-reactive T-cell responses in potential renal transplant recipients revealed that the level of pretransplant alloantibodies did not always correlate with the frequency of pretransplant donor-reactive T cells, suggesting that the presence of T-cell

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alloreactivity did not routinely imply B-cell sensitization and vice versa [8]. In general alloantibody is considered to be T-dependent, but T cells can respond to processed antigen, whereas alloantibody is by its nature directed toward the quaternary structure of an antigen. Thus, it is understandable that the spectrum of these two processes is non-identical. The origin of alloantibodies in patients that have never before been transplanted is most commonly explained by occult exposure to alloantigen through transfusion or pregnancy and it is well established that prior pregnancy may influence the generation of alloantibody [8]. In addition, however, it also is known that systemic viral infection can result in polyclonal activation and subsequent secretion of antibody with broad specificity unrelated to the inciting organism [10]. While this polyclonal antibody is T-independent and not the result of a germinal center reaction (also see Chapter 2), it has the potential to contain antibodies of alloreactive specificity [10]. Again, the contribution of this mechanism to the observed spectrum of humoral alloimmunity across individuals warrants further investigation. In contrast, given the predominant role of cell-mediated immunity in transplant rejection and more in-depth research regarding the nature of alloreactive T cells, much more is known about the spectrum of alloimmunity among T cells, and the mechanisms by which external influences such as exposure to environmental antigens, pathogens, and aging can impact the alloimmune response. For this reason, the remainder of the chapter will focus primarily on T-cell alloimmunity.

The T-cell repertoire and its recognition of pathogens and alloantigens

To defend against an ever-changing panoply of pathogens, the mammalian immune system has evolved the capacity to generate an extraordinarily diverse repertoire of T cells bearing receptors, which are capable of discriminating foreign peptides presented in the context of self-MHC. While the theoretical upper limit of diversity may exceed 1×10^{13}, it is estimated that the actual number of different clonotypes expressed is closer to 2×10^6 in mice and 2.5×10^7 in humans (reviewed in [11]). Theoretical and experimental evidence suggest that the size of the clonal population of T cells bearing identical receptors is approximately 100–200 in mice and between 1000 and 4000 in humans. Studies of the response to lymphocytic choriomeningitis virus (LCMV), a small RNA virus, indicate that the T-cell precursor frequency (defined as the number of naïve T cells specific for a given peptide–MHC combination in mice before antigenic challenge) responding to immunodominant class I and class II restricted epitopes have similar precursor frequencies in the range of 1–2 per 10^5 $CD8^+$ or $CD4^+$ T cells [12,13].

While the immune system did not evolve to reject transplanted tissues, it is nonetheless apparent that immune responses against tissues from non-genetically identical individuals within a species are particularly potent. This is due, at least in part, to the high frequency of T cells that respond to this allogeneic stimulus [14–16]. In fact, in experimental systems, it has been found that 0.1–10% of a naïve individual's T-cell repertoire is capable of reacting with alloantigens expressed by a given fully MHC-disparate donor [14], a figure that is 2–3 logs greater than the estimated precursor frequency of virus-specific responses [12,13]. By combining the estimates of the number of distinct clones in the repertoire noted above with an intermediate estimate of frequency of alloreactivity (~1%), mice and humans would bear approximately 20 000 and 250 000 distinct alloreactive clones, respectively. Thus, the response to pathogens and transplants are initiated from fundamentally different ranges of initial precursor frequencies, a fact that is likely to have major implications on the outcome of the T-cell response.

Impact of T-cell precursor frequency on T-cell behavior

High T-cell precursor frequency is one of the hallmarks of alloreactivity. It is known that this high T-cell precursor frequency fundamentally alters the quality, in addition to the quantity, of responding T-cell populations. Specifically, a study by Marzo et al. revealed that naïve T-cell precursor frequency strongly influenced the outcome of $CD8^+$ T-cell differentiation, with higher precursor frequencies resulting in a net increase in central memory $CD8^+$ T cells [17]. High T-cell precursor frequency also accelerated the kinetics of T-cell expansion [18], but paradoxically shortened the life-span of both naïve and memory T-cell populations [19]. Furthermore, other investigators have made the intriguing observation that a high frequency of $CD8^+$ precursors can convert a helper-dependent response into a helper-independent and CD154-independent response [20], and studies have demonstrated an important role for naïve $CD4^+$ and $CD8^+$ T-cell precursor frequency in determining the degree donor-reactive T-cell proliferation and differentiation following transplantation, and in mediating costimulation blockade-resistant rejection [21,22]. Specifically, naïve T cells present at very high frequencies pretransplant appear to obviate the requirement for CD28 and CD154-mediated costimulation. This critical role for high precursor frequency in determining the potency of alloreactive T-cell responses has been demonstrated for both naïve and memory T cells [23–26]. Alloreactive T cells responding from an initial high precursor frequency exhibit increased expression of CD25, the high-affinity IL-2 receptor [27], decreased expression of the inhibitory receptor programmed death-1 (PD-1) [25], a reduced requirement for $CD4^+$ T-cell help [22], and must undergo fewer rounds of cell division in order to reach a critical threshold number of effectors in order to mediate graft rejection (Figure 9.1) [24]. The major role of high-frequency T-cell responses in mediating allograft rejection is further exemplified by observations that increased MHC matching, which reduces the number of allogeneic epitopes present and thus the number of alloreactive T-cell clones recruited into the response, results in increased 5-year graft survival (Figure 9.2). Taken together, these studies demonstrate that high-frequency T-cell responses, present either as naïve or memory alloreactive T-cell populations, play an important role in mediating allograft rejection [28].

The molecular basis of alloreactivity

During the study of allorecognition over the last half-century, this high precursor frequency has led to speculation that allorecognition is fundamentally different from TCR recognition of a nominal antigen. What is the molecular basis of this high precursor frequency? Early studies raised the possibility that due to the evolutionarily selected propensity of the germline-encoded TCR gene segments to bind to MHC molecules, perhaps in the absence of negative selection TCRs were ligating MHC in a peptide-independent manner. If all alloreactive TCRs could bind allogeneic MHC regardless of the particular peptide presented, this might lead to a very high precursor frequency. However, the peptide independence of allorecognition failed to be supported by crystal structures of allospecific TCR contacting its ligand; these studies revealed the same orthogonal orientation in which the TCR CDR3 regions contact the MHC peptide-binding groove [29]. Subsequent studies

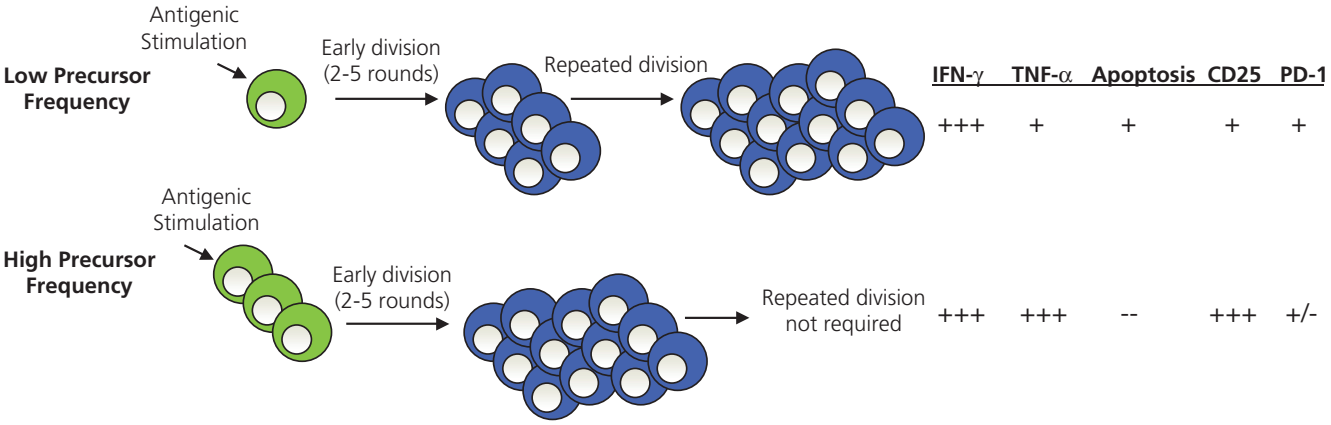


Figure 9.1. Impact of high T-cell precursor frequency on the quality of the alloreactive T-cell response. T cells responding from an initial low precursor frequency must undergo many more rounds of division in order to reach a critical threshold number of alloreactive effectors and mediate rejection of the graft, while T cells responding from an initial high precursor frequency require many fewer rounds of division in order to achieve this threshold. T cells stimulated at higher precursor frequencies also have a greater proportion of high-quality dual cytokine producing cells, decreased frequencies of apoptotic cells, increased expression of CD25, and decreased expression of IL-2.

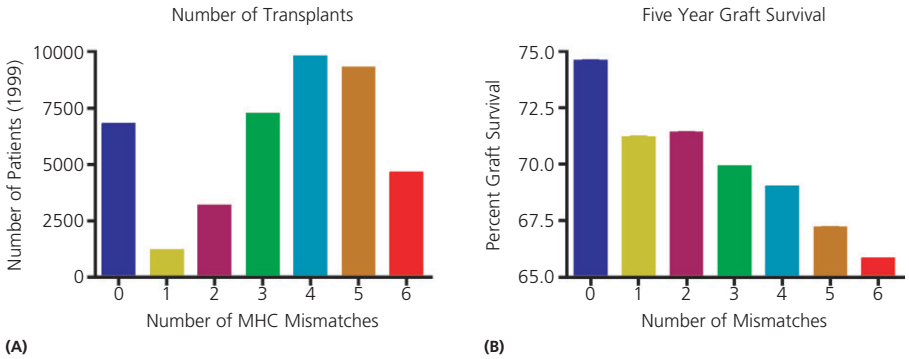
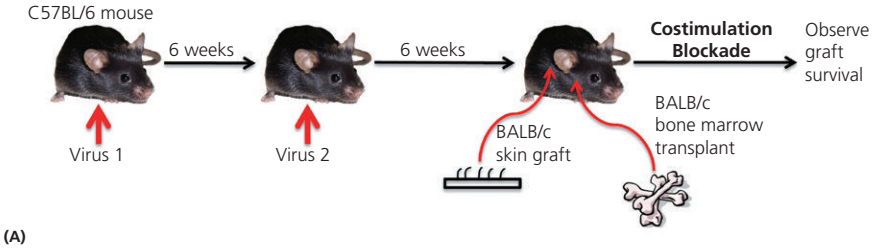


Figure 9.2. Impact of MHC matching on 5-year renal allograft survival. (A) The percentage of surviving grafts at 5 years post transplant is shown for recipients that received a deceased donor, non-extended criteria donor (ECD) renal allograft that were mismatched at 0, 1, 2, 3, 4, 5, or 6 MHC loci. (B) The number of transplant recipients from the data set in (A) that received transplants mismatched at 0–6 MHC loci. Data are derived from the 2006 OPTN/SRTR annual report [118].

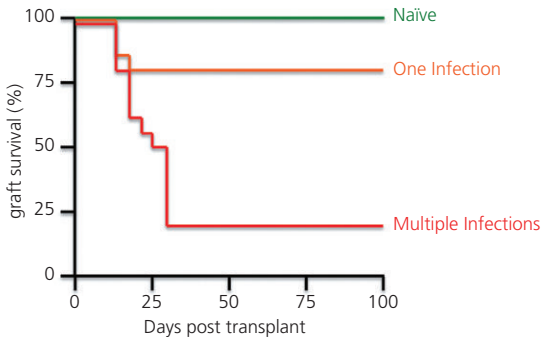
have gone on to definitively demonstrate the peptide dependence of alloreactivity [30,31]. While it is likely that the high alloreactive precursor frequency stems in part from the large number of alloepitopes that can be presented by a given donor-derived APC, there is also another factor that likely contributes to high alloreactive precursor frequency; namely, the polyspecificity of alloreactive T-cell clones. This was demonstrated in a seminal study interrogating the specificity of alloreactive $CD4^+$ T cells for peptide:MHC complexes, where the authors found that alloreactive T cells have the inherent propensity to respond to multiple, distinct peptide epitopes that do not share sequence homology [32]. A similar observation was made in $CD8^+$ human T-cell clones, in that when human T cells were primed with a single tumor antigen presented on self-MHC, the responding T-cell clones were highly specific for that particular antigen, as would be expected [33]. Strikingly, however, when T-cell populations were primed with the tumor antigen presented on allogeneic MHC, the responding T-cell clones recognized not only the immunizing antigen, but also a wide range of distinct peptide epitopes [33]. Taken together, these data demonstrate the polyspecificity of alloreactive T cells relative to conventional T cells, a property that allows them to respond to many unrelated peptide sequences, thereby increasing the frequency of T-cell clones recruited into a given antidonor response [30].

Heterologous immunity: Interactions of pathogen exposure and alloreactivity

Many studies conducted over several decades have documented the ability of pre-existing immunity to pathogens to impact alloreactivity, a phenomenon termed heterologous immunity. In the vast majority of cases, pathogen exposure functions to enhance the cellular alloimmune response and in some cases can precipitate graft rejection (Figure 9.3). In broad strokes, there are two potential mechanisms by which pathogen exposure can augment alloimmunity: (1) antigen-dependent activation of alloreactive T cells, and (2) antigen-independent augmentation of alloimmunity in the setting of a concurrent transplant. Thus, while early studies documented the phenomenon of pathogen-mediated augmentation of alloimmunity [34], they failed to differentiate between these two hypotheses as an explanation for the observed results. More



(A)



(B)

Figure 9.3. Experimental evidence for pathogen-induced heterologous immunity [23]. (A) The experimental design used to demonstrate the influence of viral pathogen exposure on allograft survival. Mice are serially exposed to various viral pathogens and allowed to fend off the infectious. Transplants (e.g. skin and or bone marrow) are then performed using a costimulation blockade-based immunosuppression regimen and graft survival is observed. (B) Animals receiving transplants after multiple infections have worse allograft survival than those that are viral naïve or relatively less virally experienced.

recently, however, new experimental evidence has emerged identifying true molecular mimicry as a potential explanation for the observed alloreactivity within heterologous immune responses [35,36]. We will consider each of these mechanisms of heterologous immunity separately below.

Evidence for alloreactivity among memory

The immune repertoire of a given individual is fundamentally altered over the lifetime of that individual as a result of the particular set of immune challenges that person is faced with. Responses to environmental antigens, viruses, and bacteria fundamentally alter both the composition and character of the T- and B-cell repertoires. It is known that while laboratory mice contain only 2–10% antigen-experienced memory T cells [37], approximately 50% of the peripheral T-cell compartments of adult humans are $CD45RO^+$ $CD45RA^-$ memory T cells [38], as a result of infection with, and response to, multiple agents over the life of the host. These memory T cells possess a great deal of heterogeneity within their phenotypes, functionalities, trafficking patterns, and requirements for optimal recall responses [39]. But does alloreactivity exist within these memory T compartments in normal healthy unsensitized individuals? For many years, there was considerable debate concerning the relative representation of alloreactive clones among memory T-cell populations [38,40]. This important question was first asked using an approach that involved cord blood-derived T-cell preparations, which could be shown to contain very few memory T cells [38], and later using ELISPOT-based experiments to identify those cells that were capable of rapidly producing interferon (IFN)-γ in response to brief stimulation with alloantigen, which is a hallmark of T-cell memory [41]. More recently, polychromatic flow cytometric techniques have been used to both identify and sort subsets of naïve ($CD45RO^-CD62L^+$), central memory ($CD45RO^+$ $CD62L^+$), effector memory ($CD45RO^+$ $CD62L^-$), and terminal effector memory ($CD45RO^-$ $CD62L^-$) T cells within both the $CD4^+$ and $CD8^+$ T-cell compartments, and to assess the precursor frequencies of alloreactive T cells within these subsets [42]. These data have revealed that alloreactivity may manifest differently in different T-cell subsets. For example, a higher precursor frequency of alloreactive T cells is detected among naïve as compared to memory $CD8^+$ T cells when proliferation is used as a measure of alloreactivity, but $CD8^+$ Tem appear to contain a higher precursor frequency of alloreactive T cells as compared to naïve $CD8^+$ T cells when perforin or granzyme expression is used as a read out [42]. It is therefore important to consider that notion that despite the fact that frequencies of alloreactive T-cell clones might be comparable between naïve and memory T-cell compartments, the immunological manifestation of recognition of alloantigen might be very different, with potential profound impacts on graft acceptance versus rejection. In this regard, determining the precise effector functions of alloreactive T cells that are most critical for graft destruction remains an important goal.

Heterologous immunity and the generation of alloreactive T-cell memory

Donor-reactive memory T cells can arise through multiple independent mechanisms (Figure 9.4), many if not all of which are likely at play in an individual transplant recipient. The origin of allospecific memory T cells can generally be categorized into three main subdivisions: (1) "traditional" donor-reactive memory T cells generated following sensitization with alloantigen (i.e. a prior transplant, transfusion, or pregnancy, (2) memory T cells that arose through antigen-independent mechanisms, and (3) allocross-reactive memory T cells that were elicited following exposure to non-alloantigens. First, it is clear that donor-reactive memory T cells are generated in the setting of a failed prior allograft (Figure 9.4). Evidence from both experimental models [43,44] and clinical patients [41] consistently demonstrates poorer outcomes in recipients of a failed prior graft, consistent with "second set" rejection. Alloreactive memory T cells can also arise during pregnancy, during which time (despite the presence of multiple immunologic mechanisms functioning at the fetal/maternal interface to promote tolerance of the fetus) a female can become primed against paternal

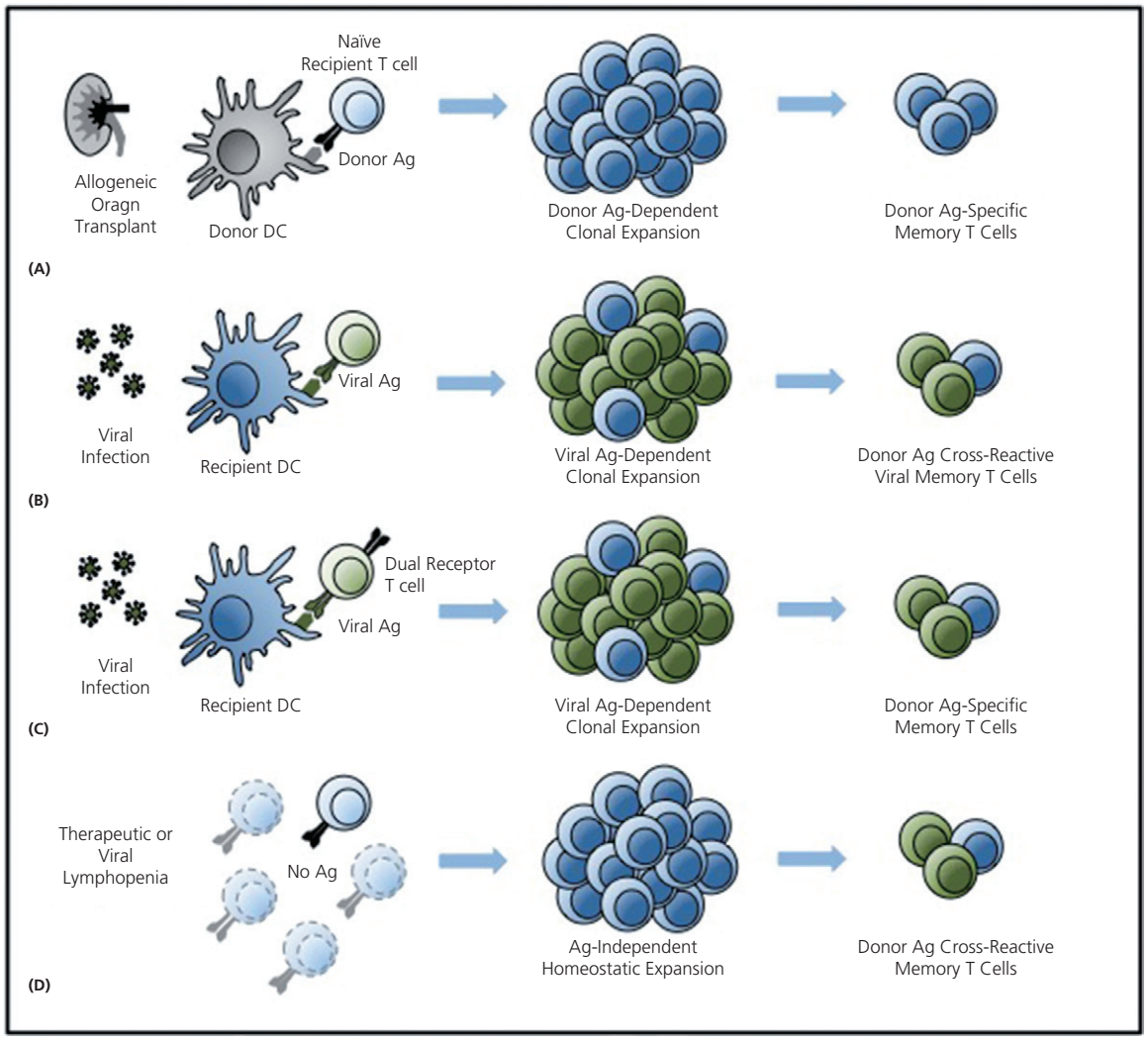


Figure 9.4. Mechanisms by which donor-reactive memory T cells may arise. Alloreactive memory T cells can be generated through at least four independent mechanisms. (A) "Traditional" donor-reactive memory may arise via sensitization with alloantigen in the form of prior transplant, blood transfusion, or pregnancy. (B) Alloreactive memory T cells may arise following sensitization with pathogen-derived antigens that elicit memory T cells, which, by virtue of the degenerate nature of their TCR, cross-react with alloantigen. (C) Alloreactive memory T cells may also arise following activation of dual-receptor T cells. These cells possess one receptor specific for a non-alloantigen, and, when activated by non-alloantigen, result in memory T cells. If these cells bear a second receptor that is alloreactive, they may respond to alloantigen as a memory T cell. (D) Alloreactive memory T cells may also be generated via homeostatic reconstitution following virally or pharmacologically induced lymphopenia. Light blue and green T cells represent naïve T cells. Clonally expanding and memory T-cell populations contain combinations of pathogen-reactive (green) and alloreactive (blue) cells. Ag, antigen; DC, dendritic cell. Reproduced from Krummey SM and Ford ML. Heterogeneity within T Cell Memory: Implications for Transplant Tolerance, 2012. Frontiers in Immunology. 3:36. Epub 2012 Mar 1. PMCID: PMC3342058 [119]. With permission from Frontiers subject to a Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/).

antigens carried by the fetus [45]. With regard to allosensitization following blood transfusion, it has been shown that immunity generated by platelet transfusion is sufficient to induce rejection following subsequent bone marrow transplantation in murine recipients [46]. Because of the high rate of prior platelet transfusion in liver transplant recipients in particular, these data indicate that highly transfused transplant recipients may be at an increased risk for memory T-cell-mediated graft rejection.

A second mechanism by which alloreactive memory T cells might be elicited is the scenario in which a lymphopenic environment exists in the host (Figure 9.4). Under these conditions, naïve $CD4^+$ and $CD8^+$ alloreactive precursors can be induced to undergo antigen-independent, but IL-7-dependent, homeostatic proliferation and subsequent differentiation into memory T cells [47,48]. This phenomenon was demonstrated by seminal studies from Turka and colleagues, which showed that transfer of naïve lymphocyte populations into T- and B-cell deficient severe combined immunodeficiency (SCID) recipients, as well as antibody-induced depletion of lymphocytes in murine transplant recipients, resulted in rapid reconstitution of the peripheral T-cell compartment with memory T cells [49]. The clinical relevance of these findings lies in the fact that lymphopenia occurs following infection with viral pathogens such as HIV, following interferon therapy for the treatment of HCV, and following therapeutic depletion of T cells for the

treatment of autoimmunity or transplantation [50]. Studies from the basic immunology literature have shown that "pseudomemory" T cells arising from lymphopenia followed by homeostatic reconstitution have both phenotypic and functional characteristics of memory T cells, and behave as memory T cells during secondary rechallenge [47,48]. Thus, lymphopenia-induced immunologic memory is essentially indistinguishable from true memory that arose via antigen-dependent mechanisms. Memory cells generated following homeostatic expansion have also been shown to constitute a barrier to transplantation tolerance induction, discussed further below [49].

Next, alloreactive memory T cells can theoretically arise following prior activation of dual-receptor T cells (Figure 9.4), a mechanism that is often overlooked. Alloreactive dual receptor memory T cells can arise through a situation in which two distinct TCRs, one specific for an alloantigen and one specific for a pathogen or other non-alloantigen, co-exist on the same T cell. If that T cell encounters the non-alloantigen for which it is specific, it would become activated and differentiate into a memory T cell. If at some future time that same T cell were to encounter its cognate alloantigen via the second receptor, it would respond as with the kinetics and activation threshold of a memory T cell despite never having previously encountered alloantigen. What is the evidence that dual receptor T cells exist in mice or man? While the vast majority of peripheral T cells express a single alpha and single beta chain owing to the principle of TCR gene alleleic exclusion, it has been shown that up to 30% of mature peripheral human T cells express a second alpha chain at the mRNA level, and up to 8% express one at the protein level [51]. Given the existence of these dual receptor T cells, what is the evidence that these cells possess alloreactivity and can therefore contribute to allograft rejection? Studies in a murine model of graft-versus-host disease (GVHD) revealed that a higher frequency of dual-receptor $CD4^+$ T cells were observed in the alloreactive T-cell compartment as compared to the non-alloreactive T-cell compartment [52]. However, the precise role these cells may play in either GVHD or rejection of solid organ transplants, both in mice and in humans, remains to be fully elucidated.

Existence of alloreactive memory as a result of TCR cross-reactivity

In addition to the mechanisms discussed above, evidence exists in both mouse and human systems indicating that in a process termed heterologous immunity, alloreactive memory T cells are generated following previous exposure to non-alloantigens [23,53,54]. For example, in certain instances, it is known that microbial pathogens activate antigen-specific T cells that then cross-react with allogeneic tissue and result in graft rejection [36,53,55–57]. This phenomenon occurs when TCRs present on virus-specific memory T cells cross-react with alloantigen, due to the inherent ability of TCR to recognize a bind to a range of related peptide:MHC ligands [58–60]. Indeed, it has been estimated that a single TCR may be capable of recognizing up to 10^6 distinct ligands, and computational analysis of the T-cell repertoire suggested that this high degree of TCR cross-reactivity is in fact required for complete coverage of the multitude of potential peptide epitopes that could be generated by pathogens or other foreign antigens [11]. Experimental evidence to suggest that such cross-reactivity between pathogen-derived and graft-derived epitopes exists is derived from data showing that $CD8^+$ T cells specific for EBV-EBNA3A restricted by HLA-B8 are cross-reactive against HLA-B44 complexed to an unrelated self-peptide [55–57,61]. Cross-reactivity between viral and alloepitopes has been demonstrated in several other examples as well, including the fact that $CD4^+$ T cells specific for a tetanus toxoid epitope presented by HLA-DR3 are cross-reactive with the allogeneic MHC molecule HLA-DR4 [62], and a T cell specific for herpes simplex virus (HSV)-VP13/14 presented by HLA- A2 cross-react with the allo-MHC molecule HLA-B44 [63]. As mentioned above, alloreactive T cells have also been shown to be more promiscuous, or have increased "polyspecificity" as compared to cells specific for a nominal antigen [30,31]. This finding of "polyspecificity" of alloreactive T cells was further confirmed by crystallographic studies that interrogated the molecular mechanisms underlying the observed cross-reactivity of EBV-EBNA3A/HLA-B08 restricted TCR with HLA-B44 molecules [35], and found that the binding orientation of a single monoclonal TCR to two discrete, unrelated cognate and allogeneic peptide:MHC complexes were virtually identical. Furthermore, the authors of this study proposed an induced-fit model to explain TCR recognition of alloepitopes, because TCR ligation was required for the viral and allopeptides to acquire the same conformation [35,64]. These insights into the biochemical mechanisms by which pathogen-elicited memory T cells cross-react with alloantigens have therefore definitively demonstrated that heterologous immunity can function at the level of molecular mimicry, facilitated in part by the intrinsic promiscuous nature of alloreactive T-cell clones.

These very specific examples therefore confirm the existence of molecular mimicry as a potential mechanism underlying heterologous immunity, but exactly how common do pathogen-elicited allocross-reactive memory T cells actually occur in humans? A 2010 study revealed that cross-reactive pathogen-specific T-cell clones specific for alloantigens is actually quite common. Using MHC tetramers for FACS-purification of T-cell lines and clones specific for the latent viral pathogens EBV and CMV, the authors tested the reactivity of the resulting cells against a large panel of HLA-typed antigen presenting cells. Interestingly, 80% of $CD8^+$ T-cell lines and 45% of pathogen-derived antigen-specific $CD8^+$ T-cell clones were allocross-reactive against at least one MHC allele [65]. In this study, both the pathogen- and alloreactivity could be conferred by a single TCR, thus demonstrating TCR cross-reactivity at the level of molecular mimicry and excluding a role dual receptor T cells [65]. Furthermore, the potential for pathogen infection or immunization to augment alloimmunity has been demonstrated in case reports, including one in which varicella zoster virus vaccination precipitated the generation of an alloreactive T-cell response [66]. These studies reveal that pathogen-elicited allocross-reactive T-cell memory is not uncommon and therefore may have profound effects on outcomes in clinical transplantation [67]. Specifically, if a large proportion of memory T-cell clones within an individual T-cell repertoire possess intrinsic alloreactivity, and a single individual possesses tens of millions of memory T-cell clones, it is therefore important to ask not simply if donor-reactive memory T cells exist in any given individual, but rather the extent to which they exist, and to determine the extent to which their frequency, phenotype, and functionality poses a threat to long-term graft survival. In this way, the prior immune and infectious histories of individual patients fundamentally shape the spectrum of alloimmunity within the population of transplant recipients.

Specific donor/recipient combination impacts degree of alloreactive T-cell memory

Given the existence of alloreactivity among memory T cells, it had previously been hypothesized that due to the lower activation

threshold of memory T cells, many different alloantigens might be capable of stimulating memory T cells, and that recipients possessing high frequencies of alloreactive memory T cells may exhibit sensitization to a wider range of potential donors. However, while it is true that alloreactive TCRs possess intrinsic cross reactivity, the above-mentioned studies using virus-specific human memory T-cell clones demonstrated that this cross-reactivity was usually confined to a single HLA allele [65]. Thus, while several studies have now shown that that alloreactivity exists among memory, the extent to which donor-reactive memory T cells are present appears to be highly dependent on the donor tissue tested. For example, in a 2007 study, $CD8^+$ memory T cells from 11 different non-human primates were stimulated with a panel of cells expressing 14 different HLA molecules, and results indicated that donor-reactive $CD8^+$ memory T-cell precursor frequencies within an individual memory T-cell compartment spanned an over 40-fold range, depending on the HLA type of the allostimulator [68]. Quite striking was the finding that the precursor frequencies spanned a range far greater than those observed for naïve alloreactive T cells, a finding which may imply that the wide range in donor-reactive memory T-cell precursor frequencies was not due to intrinsic differences in the alloreactive T-cell repertoires of these animals, but instead was a result of differential expansion of alloreactive T-cell clones due to differences in their immunologic histories [68].

Impact of bystander activation on allospecific immunity and graft rejection

The studies discussed in the previous section conclusively demonstrate that the generation of alloreactive memory T cells via TCR cross-reactivity is an important mechanism through which prior pathogen infection can result in increased alloimmunity. However, pathogen infections can also impact alloimmunity via so-called "bystander activation," an antigen-independent process. The effect of bystander activation is variable based on the type and timing of the infection relative to the time of transplantation. Specifically, concurrent infection of a murine transplant recipient with an acute viral (LCMV Armstrong) or bacterial (*Listeria monocytogenes)* infection can result in augmented alloimmunity, resulting in more highly differentiated alloreactive effectors, accelerated allograft rejection, and a loss of tolerance induction [69,70]. However, LCMV Armstrong infection after tolerance is already established has no effect on the maintenance of graft survival [69], while *Listeria* infection induces a loss of tolerance and precipitates graft rejection in 100% of recipients in an IFN-β and IL-6 dependent manner [71]. Interestingly, another bacterial infection, *Staphylococcus aureus*, fails to break established tolerance [71]. Furthermore, prior infection with *Listeria* has no effect on subsequent tolerance induction, whereas prior infection with LCMV Armstrong abrogates tolerance induction in approximately 7% of recipients [72]. In contrast, prior infection of transplant recipients with LCMV clone 13 or murine homolog of human Epstein–Barr virus (EBV), which both persist for the life of the host and presumably induce some low level of inflammation either at baseline or during episodes of viral reactivation, completely inhibited tolerance induction in 100% of the recipients [72,73]. Another example in which pathogen-associated inflammation functions to enhance an alloimmune response is seen in the case of experimental infection with murine polyoma virus, a relative of human BK virus that infects the kidney, resulting in an experimental model of polyoma virus-associated nephropathy (PVAN) [74], which affects some transplant recipients. In mouse models, polyoma virus infection resulted in loss of allogeneic but not syngeneic transplanted kidneys [74], along with a concomitant increase in alloantigen-specific $CD8^+$ T cells [74]. In this system TCR cross-reactivity of viral-specific T cells with alloepitopes has failed to be detected, suggesting instead that the increased inflammatory milieu generated by the polyomavirus infection increased the activation and differentiation of the alloreactive T-cell clones in an antigen-independent manner. This hypothesis was confirmed in a subsequent study in which immunodeficient animals with very high viral loads failed to undergo graft loss, suggesting a non-infectious etiology for graft failure [75]. Indeed, adoptive transfer of alloreactive, but not polyoma virus-specific, T cells resulted in graft loss [75]. In summary, the inflammatory milieu established following a particular viral or bacterial infection can profoundly impact the intensity and outcome of alloimmune responses in an antigen-independent manner [76]. The mechanisms underlying this phenomenon likely involve the ability of a particular infectious agent to activate the innate immune response (see Chapter 7), by inducing the adjuvant effects of cytokines such as IL-6 and type I interferons [71,77], and engaging toll-like receptor (TLR) [78] or other pathogen-associated molecular pattern (PAMP) receptors in order to license alloepitope-presenting dendritic cells [79,80].

Aging and alloreactive immune competence

Alloimmunity can also be impacted by age [81]. The aging immune system is populated by an increasing frequency of memory T cells, in particular Tem cells [82,83], and numerous studies have shown that allograft rejection proceeds with slower kinetics in aged mice [84,85]. This delayed rejection is attributable to altered T-cell responses, in that adoptive transfer of aged alloreactive T cells into young T-cell-deficient hosts still results in delayed rejection kinetics, whereas adoptive transfer of young alloreactive T cells into aged T-cell-deficient hosts does not [85]. However, aging does not seem to impair the ability of donor-derived DCs to initiate alloimmune responses in experimental models [85]. These findings in experimental models are supported by clinical observations that recipient age is associated with decreased incidence of acute rejection [86–90], but that donor age is associated with increased incidence of acute rejection [86]. Furthermore, latent viral infections, particularly CMV, induce inflationary T-cell responses that can achieve frequencies of 50–80% of the peripheral $CD8^+$ T-cell compartment in aged individuals [91–95], a phenomenon that may have the effect of "squeezing out" alloreactive T cells. Thus, in these ways, aging and the associated impact of latent viral infections can have a profound impact on alloreactive immune competence.

Impact of pre-existing autoimmunity on alloreactivity

Alloreactivity may also be impacted by the presence of pre-existing organ-specific or systemic autoimmunity, which very often was the underlying disease that necessitated transplantation in the first place. Recurrent autoimmunity can manifest in the setting of renal, liver, and islet/pancreas transplantation. Patients undergoing renal transplantation may have concurrent autoimmune conditions such as lupus, Goodpasture's disease, IgA nephropathy, etc., which result in pretransplant glomerulonephritis [96,97]. In terms of liver transplantation, there are three main autoimmune conditions for which transplantation is a potential eventual therapeutic option: primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune

hepatitis. Recurrent disease occurs in up to 20–40% of liver transplant recipients undergoing orthotopic liver transplantation for these indications, most commonly during the second or third year post transplantation [98]. Unmasking of underlying autoimmunity is thought to occur following reduction in maintenance immunosuppression, and is characterized by abnormal liver function tests and a lymphocytic infiltrate upon liver biopsy [98]. Finally, patients with type 1 diabetes mellitus may undergo renal, combined renal/pancreas, or islet transplantation for their disease. Overall, recipients transplanted for autoimmune indications are likely at increased risk of acute rejection [99], and control of recurrent autoimmune disease usually requires an increase in immunosuppression in order to attenuate the survival and/or function of autoreactive lymphocytes.

Increased alloreactivity as a result of cross-reactive autoreactive T cells or autoantibody

Underlying autoimmunity may impact alloreactivity through three distinct mechanisms. First, patients with pre-existing autoimmunity often possess effector/memory T cells and/or antibodies specific for autoantigens, which can recognize these antigens in the context of donor tissue and thus mediate destruction of the graft. For example, the presence of circulating autoantibodies specific for glomerular antigens can directly result in kidney transplant damage in patients with IgA nephropathy and Goodpasture's disease [96,97]. Furthermore, it is well known that type I diabetic recipients of islet allografts possess memory, and perhaps even activated effector, T cells directed against β cell antigens that persist after the autoimmune response that resulted in destruction of the β cells has subsided. Given the existence of very well-characterized murine models of type 1 diabetes, extensive work has been done to explore the effect of pre-existent β cell-specific T-cell responses on islet allograft survival in diabetic recipients and responsiveness to various immunosuppressive regimens. For example, in NOD mice that spontaneously develop type 1 diabetes through the peripheral activation of β cell antigen-specific T cells, early treatment with either CTLA-4 Ig [100], anti-CD154 [101], or anti-LFA-1 [102] prevents the onset of insulitis and overt diabetes. However, delaying treatment until after disease has been established (>9–10 weeks of age in most studies), does not inhibit the disease process [100,101]. Therefore, these results suggest that therapies that have efficacy in inhibiting the generation of de novo antigen-specific T-cell responses may prolong islet allo- or xenograft survival in non-autoimmune recipients, but may do little to attenuate pre-existing β-cell-specific T-cell responses in type 1 diabetic recipients.

In this regard, patients with pre-existing autoimmunity may behave as presensitized patients in that these effector/memory T cells and organ-specific antibody that cross-react with alloantigen may make them more refractory to immunosuppression. This point raises interesting questions about the importance of MHC matching in transplant recipients with pre-existing autoimmunity, in that close MHC matching may raise the likelihood of recurrent autoimmunity due to the increased probability of pre-existing autoreactive T-cell populations recognizing autoantigens in the context of well-matched allogeneic MHC. In contrast, MHC discordant grafts, and to a greater extent xenografts, may be far less susceptible to recurrent autoimmunity due to the decreased likelihood of autoreactive TCR recognition of autoantigens in discordant MHC molecules.

Second, the presence of an inflammatory state initiated by the underlying autoimmunity can potentiate the generation of a more aggressive alloimmune response following transplantation. For example, the presence of TLR ligation and DC activation from antinuclear antibodies in patients with lupus nephritis and IgA nephropathy can potentiate the de novo alloimmune response in these recipients [96,97]. Furthermore, perivascular complement deposition resulting from these pre-existing autoimmune conditions can also facilitate the development of antibody-mediated rejection following renal transplantation [96,97].

Third, genetic factors resulting in the propensity to develop autoimmunity may also render a given transplant recipient more likely to develop aggressive alloimmunity. This is best appreciated in studies of mouse models, where strains of mice that are genetically predisposed to develop autoimmunity, including SJL and C57BL/6 (which develop experimental autoimmune encephalomyelitis), and NOD (which develop type I diabetes) also exhibit more aggressive alloimmune responses and are less susceptible to many therapeutic interventions [103]. In sum, the mechanisms underlying the observed increased incidence of allograft rejection in autoimmune recipients are likely multifactorial. Dissecting the contributions of each of the above mechanisms to the observed increase in clinical rejection in this patient population remains an important goal.

Therapeutic interventions targeting alloreactive and autoreactive T-cell memory

No matter which of the above mechanisms graft-specific memory T cells arise through, these cells are likely to negatively impact long-term allograft survival due to their reduced threshold for activation. For example, studies using either standard calcineurin inhibitor-based immunosuppression [41] or an experimental tolerance induction regimen [104] have observed direct correlation between donor-reactive memory T-cell precursor frequency (measured by IFN-γ production following short ex vivo restimulation) and increased risk of acute rejection. However, in vitro analysis of alloreactive memory T-cell responses revealed a strong inhibitory effect of tacrolimus on alloreactive memory T-cell proliferation [105]. Given the known toxicities associated with the use of calcineurin inhibition [106], identification and therapeutic targeting of alternate pathways to inhibit alloreactive memory T-cell recall responses is an important area of investigation. In addition, it is now well-appreciated that memory $CD8^+$ T cells largely resistant to the effects of CD28 costimulation blockade, a fact that may in part underlie the increased incidence and severity of acute rejection episodes observed in patients treated with the CD28 blocker belatacept [107] versus cyclosporine in Phase II and Phase III clinical trials [108–110].

Several additional pathways have been identified in experimental systems as being important for alloreactive memory T-cell responses (Figure 9.5) (see Chapter 18). For example, Vu et al. demonstrated in a murine transplant system that blockade of OX40, but not ICOS, CD70, or 41BB synergized with CD28/CD40 blockade to memory T-cell-mediated graft rejection [111]. However, other studies have found that anti-CD70 could inhibit rejection mediated by memory cells in mice lacking lymph nodes [112]. In addition, blockade of the inducible costimulatory molecule (ICOS, CD278) has been shown to effectively inhibit the effector functions of graft-infiltrating memory T cells [113].

Another potential strategy to limit the pathogenicity of memory T cells might be to block their ability traffic into transplanted tissue. Studies have shown that antagonism of LFA-1 and VLA-4, both of which are integrins that facilitate leukocyte entry into inflamed

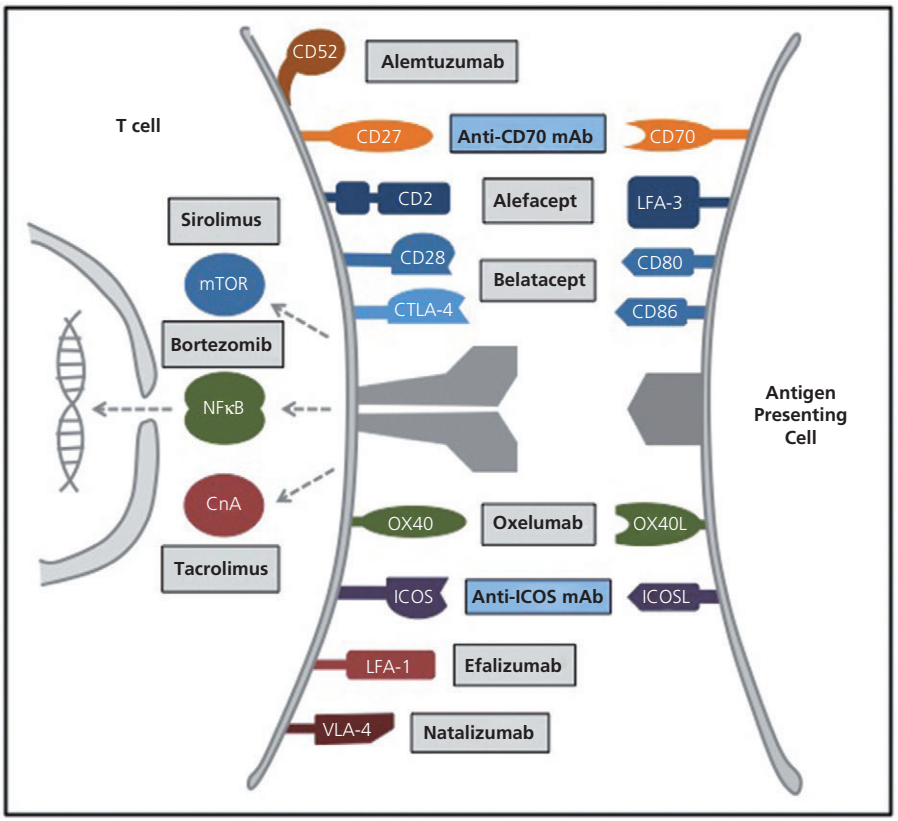


Figure 9.5. Current and experimental therapeutic targets for inhibition of alloreactive memory T-cell responses. Given the fact that donor-reactive memory T cells can arise via prior sensitization, heterologous immunity, and autoimmunity, therapeutics are being developed to specifically target alloreactive memory T cells. These include: alemtuzumab, anti-CD52 monoclonal antibody; alefacept, LFA-3 Ig fusion protein; anti-OX40L mAb, anti-OX40 ligand monoclonal antibody; oxelumab, anti-OX40 monoclonal antibody; efalizumab, anti-LFA-1 monoclonal antibody; natalizumab, anti-VLA-4 monoclonal antibody; bortezomib, protease inhibitor; sirolimus, mammalian target of rapamycin (mTOR) pathway inhibitor; and tacrolimus, calcineurin A (CnA)-NFAT pathway inhibitor. Reproduced from Krummey SM and Ford ML. Heterogeneity within T Cell Memory: Implications for Transplant Tolerance, 2012. Frontiers in Immunology. 3:36. Epub 2012 Mar 1. PMCID: PMC3342058 [119]. With permission from Frontiers subject to a Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/).

tissues, inhibited infiltration of memory T cells into donor tissue and synergized with costimulation blockade in inhibiting memory T-cell-mediated graft rejection in experimental models [114,115]. Furthermore, Kirk and colleagues reported that targeting the CD2/LFA-3 adhesion pathway abrogated the ability of $CD2^{hi}$ Tem cells to mediate CD28 costimulation blockade-resistant rejection. Finally, memory T cells may be at least partially susceptible to the effects of regulatory T cells, especially when their ability to induce neutrophil migration into allografts is simultaneously blocked [116,117].

Summary

In sum, the immune history of a given transplant recipient profoundly influences the quantity and quality of pretransplant alloimmunity and in many cases has a direct impact on graft survival. Identification and quantification of immunologic parameters underlying the relative strength of alloimmunity in a given transplant recipient may allow tailored immunosuppression in order to minimize infectious complications and maximize overall patient health following transplantation.

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CHAPTER 10

Fundamental Concepts Regarding Graft Injury and Regeneration: Tissue Injury, Tissue Quality, and Recipient Factors

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Introduction and definitions

> Who has seen the wind? Neither you nor I but when the trees bow down their heads, the wind is passing by.
>
> *Christina Rossetti, 1830–1894*

Injury is like the wind. We perceive it by its effects: dysfunction, inflammation, histologic changes, and molecular changes of the "injury-repair response". These are manifestations of the response to wounding in the tissue, rather than direct consequences of the injurious agent. The concept is that each tissue has a programmed injury-repair response that is its own variant of wound healing, highly organized and stereotyped. The extent of this response tells us whether injury has occurred recently. Most of the dysfunction is due to the dedifferentiation of the tissue that is necessary for the repair of injury: it is difficult to repair a machine while it is working. The key teaching points in this chapter are outlined in Table 10.1.

We reviewed the problem of delayed graft function (DGF) more than a decade ago [1] and will concentrate on new knowledge since that time. In the interim, donor aging/tissue quality and recipient aging and co-morbidities have emerged as progressively more serious concerns, and use of donation after cardiac death (DCD) donors have introduced a new level of acute injury. However, improved cross-matching and recognition of early antibody-mediated rejection (ABMR) [2] has largely eliminated the influence of undetected sensitization and immunologically mediated DGF, except when the decision is taken to transplant positive cross-match organs (Table 10.2). The consequences and clinical approaches to alloantibody sensitization are dealt with specifically in Chapters 68 and 89. This leaves a triad to consider: acute injury, tissue aging, and recipient factors. The present chapter focuses on this triad, in the context of kidney transplants, but the principles are relevant to all transplants. The kidney has proven to facilitate our understanding of acute injury and recovery given the ready availability of functional markers (e.g. creatinine), its accessibility for biopsy, and the availability of a support system (dialysis) to allow patient survival during periods of severe dysfunction and ultimate recovery.

Acute kidney injury (AKI) per se can induce irreversible changes (particularly when it is severe and leads to infarction) but usually has relatively little impact on long-term graft survival once it recovers. Biopsy studies have shown that late graft loss is primarily due to recipient death and to kidney failure from diseases that develop post-transplant: ABMR, rejection from non-adherence, recurrence of primary diseases, BK, etc [3]. Thus AKI that recovers to normal function is usually not a risk factor for late graft loss, once covariates such as donor aging and recipient co-morbidity/aging are considered [4,5]. For example, among potential standard criteria donors (SCD), elevated donor terminal creatinine is not a risk factor for late graft loss [6]. Similarly, prolonged cold ischemia time (CIT) increases the rate of DGF, but has no effect on long-term outcomes after adjusting for covariates [7]. Even among extended criteria donor (ECD) kidney transplants, there is little or no effect of cold ischemic time on late graft survival [8].

Brain injury and brain death place stress on all tissues, including severe hemodynamic and endocrine-metabolic disturbances in the donation after brain death (DBD) and perhaps hormones and other factors are released into the circulation by severe brain injury [9]. Brain death leads to an initial hyperdynamic stage from the Cushing reflex—a response to hypothalamus ischemia, causing an increase in sympathetic outflow that results in increased systolic blood pressure, widening pulse pressure, and bradycardia. This hyperdynamic response would present in a short period, due to catecholamine effects increasing systemic vascular resistance, and functionally reduces left ventricular output followed by impaired cardiac performance and multiple organ dysfunction [10]. In addition, there would be alteration of the hypothalamic–pituitary–adrenal axis, resulting in the depletion of antidiuretic hormone (ADH), cortisol, and thyroid hormone. Inadequate ADH production can cause central diabetes insipidus, resulting in hypotonic polyuria (urine volume >3 L/day or >5 mL/kg/h) [11] and hypernatremia (serum sodium >145 mEq/L with plasma osmolality >295 mosmol/kg) [11]. Evidence suggesting adrenal insufficiency with low baseline cortisol levels and improper response to ACTH stimulation is common following brain death. In addition, "euthyroid sick syndrome" (low free T3 but normal TSH) can also be found after brain death. In an attempt to counteract all of the effects described above, administration of triple therapy with desmopressin (or vasopressin), methylprednisolone, and a single dose of

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Table 10.1. Key points on acute injury, tissue quality/aging and recipient aging/co-morbidity: the DGF triad and the ECD paradox

1 "DGF ≠ AKI": Early graft dysfunction as observed clinically is a complex differential diagnosis, including infarction, technical problems, and ABMR
2 AKI
- recovery following AKI is usual, with few late consequences; however, occasionally, adequate function is not recovered if AKI is severe
- AKI triggers a complex injury-repair program ("wound healing") with parenchymal, stromal, microcirculation, and inflammatory cell components, involving many thousands of molecular changes
- Inflammation in AKI is inherent in wound healing and probably does not "extend" injury
- AKI probably does not increase immunogenicity and rejection of MHC mismatched tissue

3 Quality/aging
- Older brain dead donor organs are often "older" than their age suggests due to age-related disease, e.g. hypertension
- Older organs are smaller (loss of mass) and the remaining elements are "old": fragile, with impaired repair ability

4 Recipient age is a powerful factor that is underestimated because co-morbidities (e.g. left ventricular dysfunction) are hard to capture
5 The ECD paradox: tissue aging/quality and recipient aging/co-morbidity interact
- The outcomes of ECD kidneys are heavily influenced by the practice of allocating them to high-risk recipient. The understandable practice of allocating only low-risk organs to young persons with no co-morbidities means that high-risk organs are intrinsically linked to high-risk recipients, making risks difficult to dissociate, i.e. some apparent donor risks are actually recipient risks

6 Graft survival is not programmed by early function once organs establish function. Most kidney failures are now due to diseases: antibody-mediated rejection, rejection from non-adherence, recurrent disease, and miscellaneous diseases (BK, PTLD, etc.)
7 Animal models are misleading and must be regarded as an unreliable basis for conclusions
8 Registry analyses are useful but their inherent limitations must be borne in mind

Table 10.2. Delayed graft function: biological variables, outcomes and potential interventions

Biological variables	Early phenotypes	Late function e.g. 1-year GFR	Late deterioration of function and progression to failure (mostly due to recipient death or post-transplant diseases (ABMR, rejection from non-adherence, recurrent disease, BK, PTLD, etc.)	Potential interventions
Kidney quality (age, hypertension, diabetes, etc.)	"DGF" "SGF" Histologic	Reduced late function	Not usually progressive loss of function	Selection; allocation
Recipient age, co-morbidities, LVEF	Imaging changes Molecular AKI signal in biopsies	Patient survival Graft survival Not typically loss of function except during intercurrent recipient illnesses	Not usually progressive loss of function	Selection; allocation
AKI: brain death hypotension organ removal preservation implantation post implantation		AKI per se may cause permanent loss of nephrons that fail to recover	No progressive loss of function	Prevent AKI; manage AKI to permit and optimize recovery; no current treatment

either triiodothyronine or levothyroxine has been advocated to help promote hemodynamic stability in deceased donors. This has been claimed to be useful in cases of cardiovascular instability with adequate volume status and where high doses of vasoactive agents are used [12]. However, convincing evidence of benefit from such therapies is lacking [12–14].

Brain injury can damage the kidney by circulatory disturbances (ischemia) and possibly by humoral factors such as cytokines, but whether such cytokines can cause renal endothelial or parenchymal injury is not proven. Brain injury induces an inflammatory response, perhaps mediated through NF-κB activation, leading to IL-6 production in glial cell with subsequent release of IL-6 into the systemic circulation. Mitogen-activated protein kinases (MAP kinases) are likely involved in the process of the systemic inflammatory signal, leading to the local changes in the kidneys that resemble the acute-phase response [9,15]

Post-operative vasospasm due to arterial manipulation and hypotension due to under-filling, third spacing, and poor left ventricular performance all contribute to kidney ischemic injury, although they are difficult to capture in databases. Thus the donated kidney brings with it injuries and stresses that begin at brain death but continue post-operatively. Moreover, some measures taken to protect other organs—volume depletion to protect the heart and prevent pulmonary edema—may adversely affect the kidney. Ultimately many influences converge to create tissue injury, which is likely more complex than simply oxygen deprivation (hypoxia) and hypoperfusion (ischemia).

One of the challenges of organ transplantation is the necessity of evaluating and transplanting tissue that has been subjected to both chronic injury (e.g. aging and diseases) and acute injury into older recipients with co-morbidities, and supporting it during the injury-repair period to obtain a favorable outcome. This creates the **ECD paradox**: the clinical necessity of avoiding allocation of older organs into young person, plus the rising unmet need for organs in older people on waiting lists, links acute injury, donor and recipient aging, and co-morbidity together. Moreover, these three problems may interact in synergy rather than simple addition: the older recipient with poor left ventricular ejection fraction has difficulty perfusing the transplant kidney with high resistance due to aging and AKI. New understanding and reliable tests for predicting

post-operative organ performance in relationship to biologic donor aging/tissue quality are needed: demographic factors and biopsy histology correlate with post-operative course but actually have poor predictive value [16–20].

Definitions

Acute kidney injury (AKI) is defined here as the phenotypic changes induced by stresses such as ischemia or toxins, including the molecular changes reflecting the response to injury (wound healing). AKI manifests as dysfunction, but there are other causes of dysfunction, including some pure vasomotor states that do not induce parenchymal damage, for example the effect of ACE inhibitors and vasoconstrictor effects of calcineurin inhibitors (CNIs). According to the definition used here, AKI implies reversible injury. More extreme forms of ischemia can induce infarction: patchy cortical necrosis, complete cortical necrosis, or global infarction. As it is used here, AKI specifically excludes such conditions, which are nevertheless in the differential diagnosis, along with disruption of the arterial and venous anastomoses, renal artery stenosis, ureteric obstruction, etc.

The injury-repair response: the transcript changes in the tissue induced by injury are a response of the tissue. The injury-repair response is a state in the tissue best assessed by molecular examination of **biopsies** (molecular assessments) [21,22] but which can be inferred by **biomarkers** in urine or blood, at least some of which are products of the genes induced by injury in the kidney. Many biomarkers for transplants are derived from those used in native kidney AKI, but they need to be validated and calibrated in the transplant setting.

The strategies of the injury-repair response are:

1 stabilize the site to protect the host;
2 restore the tissue to its previous state if possible;
3 achieve the best possible stable state (e.g. atrophy–fibrosis) if it is impossible to restore the previous state;
4 activate inflammation/innate immunity for host defense and repair.

Delayed graft function (DGF) is defined as any observed serious impairment in function of the kidney initially after transplantation from any cause. The causes of DGF include anastomosis problems, cortical necrosis, ureteric obstruction, and antibody-mediated rejection, as well as AKI. Thus, *DGF is not synonymous with AKI*. A typical convention in registry analyses is to define DGF as any dialysis in the first week because these data are easily collected by registries, but the clinical definition is more comprehensive. DGF can be defined clinically by many functional assessments: urine output, GFR assessments, imaging, or dialysis dependency. Remember that some recipients have underlying residual native kidney function and are dialysis independent, and thus may not meet dialysis dependency definitions for DGF, even if the transplant has no function.

Slow graft function (SGF) is defined as a significant degree of early dysfunction from any cause that fails to meet DGF criteria. SGF has most of the same correlates as DGF, and usually reflects AKI and displays a typical injury-repair response, albeit milder than that seen in dialysis-dependent kidneys [1]. Patients have urine output but allograft function is impaired without requirement for dialysis. Serum creatinine may rise then slowly decrease, reflecting the lack of a steady state, in the first week after transplantation [23]. Some useful definitions for analysis include serum creatinine >3 mg/dL at day 5 or 7 after transplantation [23]; or serum creatinine of the recipient decreasing less than 70% on day 7 after transplantation (compared to the predialysis level before transplantation) [24]. However, dialysis independence is part of all definitions of SGF, but the decision to dialyze is to some extent arbitrary.

Actually, function is a continuum and dichotomizing continuous data is not a good idea, because this loses information. DGF is a continuum with SGF, and every kidney transplanted has some degree of injury, as we showed in functioning mouse kidney isografts from live donors [22]. Thus interventions that convert DGF to SGF (e.g. pulsatile perfusion) may not represent any major change in the state of the transplanted tissue [1].

Primary non-function (PNF) is defined as failure to ever achieve enough allograft function to avoid dialysis. It is thus a retrospective diagnosis, and should be broken down to indicate whether the etiology is ABMR, surgical complications, or simply failure to recover from apparent AKI, often after 3 months of observation [25]. When investigators describe DGF, it is important to know whether they are including PNF, and if not how they are excluding it.

Graft ischemic time describes the time between organ cooling or vascular clamping and the time of vascular clamp release during implantation procedures. It can be divided into three periods: **warm ischemia time (WIT); cold ischemia time (CIT); and anastomosis time**. WIT refers to the time from asystole (in cases of donation after cardiac death; see below), or from the time of cross-clamping of the renal artery (in cases of living donation or donation after brain death) until cold organ preservation. CIT refers to the time from when the organ is cooled with preservation solution (after organ procurement) to the release of the vascular clamp of anastomosis [26]. It is also useful to record the time taken for the vascular anastomosis, which when accurately recorded is a powerful predictor of DGF [27].

Extended criteria donor (ECD) and the ECD paradox: ECD, as defined by the Scientific Registry 3 of Transplant Recipients, are deceased donors older than 60 years or donors aged 50 to 59 with at *least two* of the three following criteria: (1) serum creatinine >1.5 mg/dL prior to transplantation, (2) history of hypertension, (3) brain death due to cerebrovascular causes [28]. Kidneys with good characteristics, not fulfilling the definition of ECD, are called **standard criteria donor (SCD)** kidneys.

ECD kidneys were found to have >1.7 times more risk of graft loss than SCD kidneys [28], but this was partly due to the recipient co-morbidities and the ECD paradox (the policy of allocating ECD kidneys into high-risk older recipients). Because recipient co-morbidities are difficult to capture in databases (e.g. left ventricular dysfunction), the ECD paradox overestimates the impact of ECD donor issues and underestimates older-recipient issues.

Marginal donor kidney (MDK): the term MDK not only describes donor tissue quality, as in ECD, but also includes clinical injury variables in its definition. In general, MDK means kidneys with one or more of the following pretransplant factors: donor age >55 years, donor history of hypertension/diabetes longer than 10 years' duration, donation after cardiac death (DCD), or cold ischemia time longer than 36 hours [29]. However, some have considered the risk of transmitting infectious disease and/or malignancy, the histology on biopsy (glomerulosclerosis and interstitial fibrosis), and pretransplant renal function as the criteria for MDK [28]. In this way, MDK can describe both living and deceased donors, whereas ECD only applies to deceased donors [30]. An organ not fulfilling the criteria for MDK is referred to as an **ideal donor kidney (IDK)** or optimal donor kidney.

Thus, the definition of ECD is based on age and age-related co-morbidity, while MDK includes other risks. We will confine our comments here to ECD. Additional clinical considerations of ECD donations can be found in Chapter 53.

Donation after cardiac death (DCD) or **non-heart-beating donor (NHBD):** DCD refers to donation after pronounced irreversible circulatory arrest, which results in prolonged warm ischemia and damage to the organs. In contrast to a deceased donor who has been pronounced brain dead and the heart is still beating (**donation after brain death; DBD**), DCD places more stress on the procured organs.

Five categories of DCD donors were proposed by the International Workshop on DCD Donation in Maastricht [31]:

I dead/cardiac arrest outside the hospital;
II dead (outside the hospital) after resuscitation attempted without success;
III awaiting cardiac arrest (situation after brain death in which active care, particularly inotropic drugs or ventilator, was discontinued);
IV cardiac arrest while brain dead (situation of cardiac arrest during the process of brain death declaration or just before organ retrieval);
V unexpected cardiac arrest in a hospital inpatient.

Another clinical classification based on the timing of circulatory death is simplified for use in

the general practice. They are classified into "uncontrolled DCD donors" and "controlled DCD donors". The **uncontrolled DCD** refers to a donor who died suddenly and unexpectedly before or during transfer to hospital, or unsuccessful cardiovascular resuscitation for a hospital inpatient. This corresponds to donors in categories I, II, or V of the Maastricht classification. Meanwhile, **the controlled DCD** corresponds to donor in category III or IV of the Maastricht classification, which refers to donors who underwent imminent cardiac death due to termination of active care, cardiac arrest during the process of brain death declaration, or immediately prior to organ retrieval in the operating room [32]. The uncontrolled DCD have uncertain time of brain and cardiac death, and they would have longer WIT. On the other hand, the controlled DCD have potentially shorter WIT and experience lower rates of DGF and PNF [32].

Outcomes of transplantation of DCD kidneys have been evaluated for short- and long-term graft function compared to DBD kidneys. The results concluded that the incidence of DGF is significantly higher in DCD (40–80%) [33–36] when compared to DBD kidneys (17–39%) [33–36]; also the incidence of PNF is as high as 15–25% in DCD kidneys [37], while it has been reported that DBD kidneys experience PNF with an incidence of 2–6% [34,38]. However, the long-term graft function (1 and 5 years), slope of GFR decline, and percentage of graft survival are similar in DCD and DBD kidneys [33–36]. This indicates that sublethally injured organs in DCD can recover and achieve function as well as DBD kidneys.

The main concern in DCD kidney results is PNF or severely compromised long-term function due to various degrees of infarction, from focal cortical necrosis to global infarction. The clinical decisions in the use of DCD kidneys, particularly category I and II, would be helped by tests to identify irreversibly injured organs, because changes such as infarction take time to be manifest. Proposed viability tests include enzymatic indicators of proximal tubular cells such as glutathione-S-transferase (GST), which is markedly higher in DCD kidneys that will never function. However, whether such tests are valid for DCD is unclear, because the injury in DCD is so proximal to organ removal that the injury changes do not have time to appear. (See Section Are AKI biomarkers useful in the clinic?). Additional discussions of DCD donations can be found in Chapters 22.

Population lessons—causes and consequences of delayed graft function

DGF represents a complex interaction between acute injury, pre-existing aging, and recipient aging, including the ECD paradox. This complexity cannot be studied in experimental animals, because aging involves different processes in those species compared to human tissue. In general, the understanding of transplant AKI benefits from the available population studies, from native kidney AKI and aging studies, and from experimental models, provided the interpretation is very conservative and the limitations are acknowledged [39,40]. Compared to these early studies, contemporary data places increasing emphasis on donor and recipient aging and on the use of ECD.

The injury-repair response following ischemia in the transplant and native kidney are similar. One example in support of this is the common kidney injury biomarker findings in both disease states [41,42] (see Section Are the AKI biomarkers useful in the clinic?). However, the injury-repair response in kidney transplants may be altered by tissue perseveration/handling (e.g. hypothermic storage, preservation solution, and pulsatile machine perfusion) as well as by composite risk of donor and recipient factors.

Many features of the associations of DGF in renal transplantation were established before 1990. Hypothermic storage at 0 to 4 °C is still used to decrease tissue metabolic rate during the ischemic process. However, hypothermic techniques require use of preservation solutions to protect organs against ischemic injury. Preservation solutions have been developed based on biologic changes of ischemia and aim to:

1 prevent cellular edema by minimizing the osmotic gradients between extracellular and intracellular compartments [43];
2 delayed cell destruction by providing oxygen free radical scavengers and buffers to maintain physiologic pH [43];
3 maximize organ function by using the correct potassium (K^+) concentration to prevent cellular depolarization and diminished cellular ATP due to high concentration of extracellular K^+ [44,45]. This led to the development of solutions such as UW (University of Wisconsin) solution and HTK (histidine, tryptophan, and ketoglutarate). However, studies comparing the use of UW and HTK have failed to show a difference in graft or patient survival between the solutions [46].

Pulsatile machine perfusion reduces observed DGF compared with cold storage but has little effect on survival [47]. Long-term differences in survival reported in some studies may be due to accidental imbalance in covariates [48].

The relationships among injury, inflammation, DGF, and outcomes are complex. Injury induces inflammatory changes, which may simulate T-cell-mediated rejection (TCMR) and can be misinterpreted [49]. Thus AKI can produce tubulitis, interstitial inflammation, and probably endothelial lesions ("v-lesions") [49]. While immunologic factors played a role in the past due to poor cross-matching [50], such factors are only important now when a choice is made to deliberately transplant across a positive cross-match. The recognition of early ABMR in sensitized renal transplant recipients

as a syndrome distinct from TCMR and from AKI [51], and advances in cross-matching have largely eliminated DGF due to undiagnosed ABMR. DGF due to immune factors or tissue quality (older donors) has associations with reduced long-term outcomes, whereas pure AKI does not, for example CIT [1]. For example, studies of paired kidneys from one donor reveal that both kidneys generally perform badly when either has DGF, emphasizing the importance of donor-related factors, rather than the DGF phenotype per se [52].

The DGF phenotype has long-term associations with reduced late graft and patient survival but associations must not be interpreted as "consequences" [53]. Observed early dysfunction (DGF or SGF) [54] includes many adverse covariates that give strong correlations with late graft and patient survival but are not consequences of the DGF or SGF, for example the ECD paradox. These associations complicate the assumption that interventions that improve early function will affect late outcomes [53]: an increase in GFR from 5 to 10 may mean that the definition of DGF is not met but this is actually not a major change in the injury state of the parenchyma, and does nothing to alter the recipient's risks due to the ECD paradox.

Independent of covariates impacting tissue quality and recipient health, DGF has no long-term impact once the kidney recovers [4,5]. CIT is a case in point. Multivariate analysis of factors affecting functional graft survival suggested that even though CIT is a powerful risk factor for DGF, it was not an independent risk factor for graft survival [7]. Analysis of a paired DBD kidney cohort, derived from the national Scientific Registry of Transplant Recipients between 2000 and 2009, suggested that paired donor transplant graft survival, with and without DGF, was similar. These results suggest that DGF, specifically induced by prolonged CIT, has limited effect on long-term outcomes [7]. Lesson from other paired kidney analyses revealed many recipient characteristics were associated with DGF: recipient sex (male), African-American race, obesity, diabetes, HLA mismatch, and duration on waiting lists, as well as panel reactive antibody (more than 10%).

While clinicians hope that better management of modifiable recipient and transplant risk factors, such as obesity, waiting time, and CIT, will reduce the occurrence of DGF, the effects of interventions are often disappointing. For example, waiting time may reflect co-morbidity (e.g. ischemic heart disease) that is not reduced by simply reducing the time [55]. Perfusion should provide benefits over cold storage, but its impact on observed early, reversible graft dysfunction is rather limited and not accompanied by improvement in late survival or function [56,57].

AKI probably does not program late graft deterioration

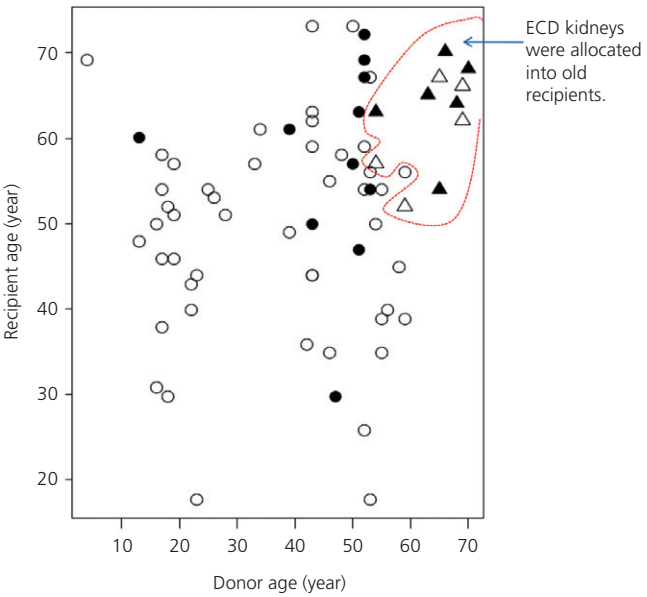
The causes of late graft loss are now better understood due to the use of prospective biopsy studies since 2005. The main causes of late graft loss in kidneys that are biopsied for clinical indications are ABMR (sometimes complicated by non-adherence) and recurrent disease, with smaller numbers being due to polyoma virus nephropathy and other causes [3,58,59]. In other words, late deterioration is due to diseases, not mysterious fibrosis [60]. The finding of atrophy–fibrosis without a disease is common because disease diagnoses are easily missed in kidneys with extensive atrophy–fibrosis, but this does not imply a primary fibrosis process.

Role of the interaction of donor age with recipient age and the ECD paradox

Donor and recipient age may act together to affect renal allograft survival: risk of late allograft failure increases via an interaction between donor and recipient age. Donor aging is undoubtedly important: kidneys lose cells/tissue elements (e.g. loss of renal mass and function, i.e. nephrons and GFR) [61]. We found that AKI molecules measured in implantation biopsies predict only early function in kidneys (manifested by DGF), but tissue quality/aging affects early and late function [4], influenced by donor age and age-related diseases.

The role of donor tissue aging/quality and of recipient aging/co-morbidities in graft survival is complex [4]. The ECD paradox means that ideal kidneys from young donors are generally allocated to young recipients to maximize utility [62]. Thus older donor age carries a major burden of recipient co-morbidity that cannot be fully captured in registries. The older kidneys, which are most likely to have DGF, are selectively given to the highest-risk recipients (Figure 10.1). The relationships among AKI, donor age, recipient age, and graft survival in population studies [63,64] must be interpreted with caution because of the hard linkage of high-risk kidneys from older donors used as the only solution for many high-risk older recipients with co-morbidities.

Transplant survival was lowest in elderly recipients [65], mainly because of death with a functioning graft, as expected. However, recipients' intercurrent illnesses and ICU admissions are also a risk to the survival of the graft, and may compromise immunosuppressive management. Thus recipient co-morbidities increase not only the risk of patient death but of graft failure during medical illnesses.



Legend; ○ =SCD with early graft function, ● = SCD with graft dysfunction, Δ = ECD with early graft function, ▲ = ECD with graft dysfunction.
SCD - standard criteria donors, ECD - extended criteria donors

Figure 10.1. The ECD paradox. Ideal kidneys from young donors are generally allocated to young recipients. Meanwhile, transplants from older donors allocated into older recipients are at a high risk for early dysfunction. Thus, interpretation of early graft dysfunction (DGF or SGF) is confounded by a relationship between the donor and recipient age.

Graft failure in young recipients who received older-donor kidneys is increased compared to young recipients who received young donor kidneys (relative risk = 1.97; $P = 0.001$) [66], although the reason for this is not clear. Recipients of older-donor kidneys have inferior graft function, which may have a greater negative impact on young recipients (i.e. increased risk of cardiovascular-related morbidity and mortality, because of their longer life expectancies compared to older recipients). Some evidence suggests renal dysfunction is an independent risk for coronary artery disease, congestive heart failure, and hypertension [67–70], although it is not clear whether renal function is an independent risk factor after Framingham risks [71] have been adjusted. However, the decision to give an older kidney to a young recipient may be driven by medical risks in the recipient.

Because transplant recipients have survival and quality-of-life advantages over patients remaining on waiting lists, the organ shortage forces clinicians to transplant from ECDs. Recognizing the detrimental effects of older-donor kidneys on graft survival, preferential allocation of young organs to young recipients is understandable, thereby improving graft and patient survival. The justice, utility, and fairness of such practices are beyond the scope of this chapter. Problems of older-donor tissue and older recipient age are strongly linked and probably interact, for example the high-resistance kidney from an ECD may be poorly perfused in the elderly recipient with reduced left ventricular ejection fraction.

In summary, donor age (particularly that of deceased donors), must be taken into consideration:

1 Young adult kidneys are best for every recipient, young or old. Transplanting "old for old" [72] makes sense to utilize older kidneys but it is not ideal for the older recipient, although it is usually better than dialysis.
2 Older deceased kidneys should be allocated (not discarded), but are not a good solution for young recipients, giving rise to the ECD paradox.
3 The phenotype of aging includes loss of functioning mass and aging changes in the remaining tissue, which could be manifest as fragility ("injurability") and poor repair on injury.

Biology of aging is not linear

The effects of time on human tissue include growth and development and aging. The ideal donor is not an infant but a young adult—hence the U-shaped distribution of risk. Empirically derived equations (e.g. Schold [73] and Kidney Donor Risk Index [KDRI] [74]) that express risk of failure based on kidney characteristics all include donor age but expressed as a complex equation to describe the U-shaped distribution. The complex effects of aging on the graft reflect factors such as graft mass [75], somatic cell senescence [76], vascular resistance, function, vulnerability to injury, and potential for repair. For this discussion, aging is considered to begin when young adults have completed their growth and development.

Experimental models

Lessons and limitations of experimental models

Animal models, both small and large, can be misleading because they do not simulate the risks observed in humans, for example tissue quality/aging and recipient factors. Even the biology of aging and somatic cell senescence is different in small animals compared to humans. Thus interventions that have produced beneficial effects in rat and mouse models of AKI seldom translate into successful human trials.

One strong assumption based on small animal models is that "inflammation" is injurious. In fact inflammation is essential to wound repair. Manipulation of experimental models can be misleading in this regard: many concepts arising from manipulation of experimental animals do not translate into human applications. Although in rats and mice injury sometimes triggers long-term kidney deterioration, this involves processes such as focal glomerulosclerosis that are unique to these models and not relevant to humans. Moreover, contrary to the widespread assumption, it is not proven that injury predisposes to rejection: in most circumstances rejection and injury are additive (see Section Clinical treatment of AKI).

The biology of injury repair at tissue and organ level

The nephron is a worm-like unit, subject to highly sophisticated developmental and homeostatic programs, responses, and regulation, including renal hemodynamics. Autoregulation is the mechanism that maintains the renal plasma flow (RPF) and GFR constant over a wide range of arterial blood pressure, mediated by the interaction of myogenic stretch receptors response and tubuloglomerular feedback [77].

Cessation of movement of columns of fluid caused by interruption of glomerular filtration alters the function of renal primary cilia (another sensor of movement in renal tubules) [78]. Fluid movement through these tubes provides signals to renal epithelial cells to maintain their normal differentiation state. The ability of renal primary cilia to sense fluid flow also has an important role for epithelial differentiation during renal injury and repair. They contain the movement-sensing mechanoreceptor-like proteins (e.g. polycistins), which play an important role in communicating changes in extracellular milieu from cilia to the intracellular environment, leading to the regulation of epithelial cell differentiation, proliferation, migration, and adhesion [78].

Cessation of arterial blood flow causes immediate oxygen deprivation in cells (i.e. hypoxia with accumulation of metabolic products), but also many other effects such as interruption of the movement of columns on fluid inside capillaries and tubules. In the kidney, reduction in arterial blood delivery causes relatively greater ischemia in the outer cortex compared to the inner cortex and medulla. (Thus after relatively severe ischemia a rim of cortical necrosis can result in the outer cortex.) Severe reduction in blood flow causes high-energy phosphate depletion and subsequent failure to maintain physiological ion gradients across the cell membrane. ATP depletion stops ATP-dependent transport pumps, resulting in mitochondrial swelling and outer membrane rupture, with release of cytochrome c and apoptosis-inducing factor (AIF), which is also involved in initiation of apoptosis [79].

After ischemic renal injury, the modes of cell death—necrosis and apoptosis—depend primarily on the severity of the insult and the resistance of the cell type. Necrosis, histologically characterized by loss of membrane integrity, cytoplasmic swelling, and cellular fragmentation, usually occurs after more-severe injury [80]. Apoptosis is characterized by cytoplasmic and nuclear shrinkage [80]. Apoptosis predominates after less-severe injury and leads to the formation of apoptotic bodies that are rapidly cleared by phagocytosis. This process is initiated by signaling from proapoptotic proteins (Bax and Bid) in response to cell stress and/or proapoptotic cytokines [81].

Of the regulatory factors, the proapoptotic transcription factor p53 has been shown to be induced at the mRNA and protein levels. Inhibition of p53 by pifithrin-α suppresses ischemia-induced

apoptosis by inhibiting transcriptional activation of Bax and mitochondrial translocation of p53. However, pifithrin-α is an unlikely candidate for therapeutic consideration in humans because systemic inhibition of p53-dependent apoptosis is likely to promote survival of damaged or mutation-bearing cells in other organ systems [81,82].

Injured renal tubular epithelial cells respond by secreting chemokines (e.g. CXCL1), cytokines (e.g. IL6, IL18), and other inflammatory mediators [83], and express ITGB6, which activates latent TGF-β [84–86] bound to the extracellular matrix. The inflammation in AKI is probably beneficial as a component of wound repair, but is often assumed to be an extension of the injury—often termed "maladaptive" but more appropriately termed "injurious". It actually makes little sense that a tissue that is wounded would automatically increase its wound. It is more appropriate to conceptualize the early inflammatory changes as the activation of innate immune mechanisms in response to wounding—stabilizing the site, clearing debris, and looking for infection. In mouse kidney transplant isografts, macrophages accumulate over the first week [87] and persist in decreasing numbers for many weeks. They have many potential roles, for example removing debris. Effector memory T cells also accumulate in injured tissue, but their role is not clear.

The histological changes associated with AKI are most pronounced in the corticomedullary junction of the kidney. Some morphologic features of ischemic AKI in humans include: (1) proximal tubular regeneration, with loss of brush borders; (2) patchy distal tubular necrosis; (3) regeneration; (4) pigmented (heme) granular casts; (5) Inflammation; and (6) nucleated cells in the vasa recta [88]. Compared to the histology of native kidneys with AKI, transplanted kidneys with AKI are said to have fewer tubular casts, occasional necrosis of complete tubular cross-sections, and less tubular dedifferentiation and regeneration. In addition, there might be more calcium oxalate crystals, and more microcalcification and isometric vacuolization (possibly reflecting calcineurin inhibitor toxicity in some cases). Arteriolar hyalinization and arterial fibrous intimal thickening are increased in older donors and thus are associated with DGF [89], although the chronologic age is probably a stronger association. In general, histologic changes of aging in renal biopsies at implantation correlate with, but do not reliably predict, transplant outcomes. Imaging by ultrasound detects increased echogenicity of cortex and loss of corticomedullary differentiation in kidneys with AKI, probably reflecting edema, but this feature is non-specific [90,91].

In indication biopsies of transplanted kidneys with AKI, there are characteristic AKI transcript changes that correlate with depression of GFR, whereas the tubular changes called "acute tubular injury" by histology do not correlate [21] (see below).

Thus the injury-repair response probably does not cause additional stress on the tissue any more than the inflammation in a wound produces stress on the wounded tissue—it is a component of the program. Renal tubular cells continue to undergo repair, migration, and proliferation, and attempts at cellular and tubule integrity are re-established. The GFR begins to improve as cellular differentiation continues and normal cellular and organ function returns [92]. Some morphologic changes persist into the recovery phase and are not correlated with function, whereas brush border changes and detachment of individual tubular epithelial cells subside as function returns [93,94].

The response to damage in renal cells is integrated by the response of the nephron. The nephron is best conceptualized as having an intelligence system, receiving information and responding with a series of potential responses including:

1. temporary shutdown without a wound repair response, e.g. pure vasomotor dysfunction;
2. temporary shutdown and a wound repair response that brings the unit back to quality standards;
3. permanent shutdown and atrophy–fibrosis and global sclerosis of the glomerulus, possibly with ultimate disappearance.

Figure 10.2 depicts the processes discussed above over time.

Molecular changes in AKI

The injury-repair response of kidney is analogous to wound repair, with epithelial and matrix remodeling being essential to restoring homeostasis. Indeed, the renal regeneration/repair process shares some features with renal carcinoma [81,95–99], including synthesis of extracellular matrix components cell growth and proliferation. (Cancer has been called "the wound that does not heal".) The inflammatory cascades are initiated by mediators such as proinflammatory cytokines, for example, TNF-α, IL-6, and TGF-β [83,100]. The principal elements of this injury-repair response are the dedifferentiation of the parenchyma, appearance of repair and embryonic programs in the parenchyma, remodeling of the matrix and microcirculation, a wave of cell cycling, and inflammation.

Although most dysfunction is due to massive de-differentiation, not cell death, various forms of cell death accompanies this process. Cell death is of several types: apoptotic, autophagic, and necrotic, and it is likely that injury can result in any of these. However, cell death may be more merely a direct than a consequence of injury: it may be part of the injury-repair process and critical for remodeling of damaged tissue elements. Apoptosis is critical in embryonic development programs and may sometimes be a part of the recapitulation of these developmental programs to remodel the tissue after injury [101].

Experimental transplant models: Gene expression profiles associated with the injury of kidney isografts

AKI induced by transplantation can be modeled in rodent kidney isografts, as reviewed [22]. The injury-repair induced transcripts (IRITs) in mouse kidney transplants reflect different types of injury suffered by the isografts (i.e. early, intermediate, and late) [22]. The early IRITs reflected the systemic influences (wounding, anesthetic), and the intermediate and late IRITs represented the local stress changes in the epithelium, reminiscent of ATN injury of a native kidney (Figure 10.3). In allografts, the IRIT expression profile (the early, intermediate, and late IRIT) was initially similar to isografts but diverged later due to TCMR [101].

Transcripts associated with the stress response, kidney development, cell cycle, and ECM processes were enriched in intermediate and late IRIT expression profiles. Transcripts associated with embryonic kidney and ureteric bud development were over-represented in intermediate IRITs, whereas the mesenchyme-associated transcripts were prominent in late IRITs, alongside ECM components and TGFβ1/fibrogenesis-related transcripts.

A detailed inspection of the post-transplant time course of gene expression showed that the isografts displayed a mild but complex active injury-repair response and loss of epithelial features (KT1, KT2), accompanied by inflammatory features (QCATs, GRITs, QCMATs, and AMATs). Similar changes were observed in day 7 ATN (Figure 10.4) [102,103]. Note that the active injury-repair response is accompanied by secondary inflammation, particularly

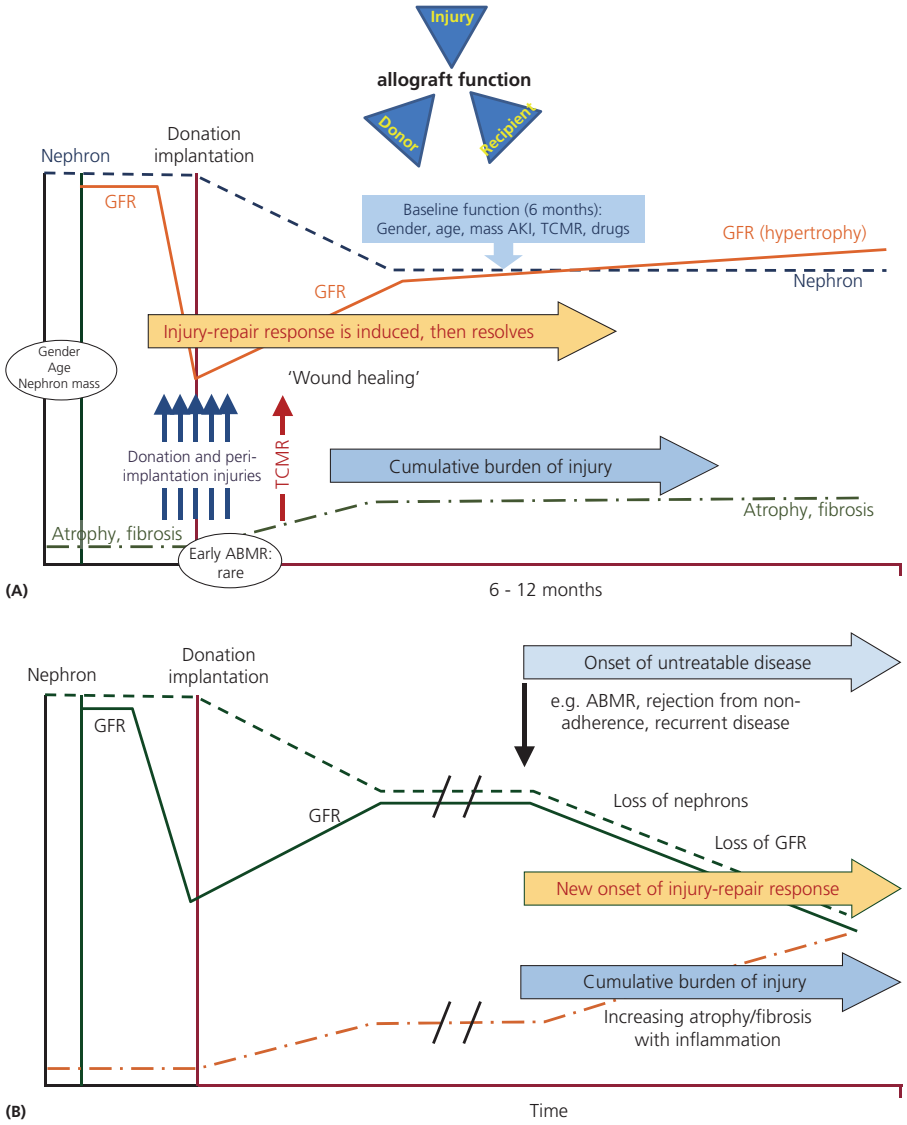


Figure 10.2. Conceptualizing the time course of a kidney transplant. (A) Stresses of the early transplant period induce the injury-repair response. The majority of changes in the kidney transplant reflect the resolution of the injuries associated with donation and implantation, plus damage during AKI, TCMR, and drug toxicity, followed by stabilization. Injury is often followed temporally by the injury-repair response and later nephron loss; the nephron loss is caused by the injury, not by the injury-repair response. The injury-repair response is adaptive and self-limited, like wound healing, and the early injury does not trigger relentless deterioration, but the active injury-repair response will usually leave at least some permanent burden of injury. (B) Despite early injuries, kidney transplants are stable until a disease appears. Kidneys that present with late (>1 year) onset of dysfunction can usually be shown to have a new disease operating. In addition, the issue of non-adherence should always be considered as a condition that must be corrected to prevent graft loss. Late deterioration should never be explained as the result of early remote injury. Atrophy scarring reflects the failure of the efforts of the injury-repair response to restore the damaged tissue to its previous state.

the IFNG effects and macrophage features even in the absence of rejection. However, as the healing process continues, their expression returns toward normal.

Allografts (Figure 10.4) display all the features of isografts, but after day 3 accumulate progressively more T-cell and macrophage transcripts accompanied by an augmented active injury-repair response (intermediate and late), ENDATs, and decreased KTs, coinciding with the emergence of the interstitial infiltrate and then rejection. Importantly, this study demonstrated that the increase in expression of the intermediate and late IRITs in the rejecting allografts with TCMR was independent of T-cell cytotoxic molecules, B cells, or immunoglobulins. Thus the mechanism of TCMR seems to involve triggering of the inherent injury-repair response to the nephron.

Accumulation and activation of monocyte–macrophage–dendritic cell populations (MMDC) is complex and closely related to the extent and type of injury [104]. The MMDC population has a number of potential roles in inflammation, phagocytosis, response to injury, and tissue remodeling [105]. There are two phenotypes of macrophage activation in the injury-repair response. Classical macrophage activation (CMA) is essentially driven by IFNG, whereas the alternative macrophage activation (AMA) is more complex. AMA is usually modeled in vitro by IL4 and IL13 [106] but can also be driven by activin A [105,107]. Each has a role in

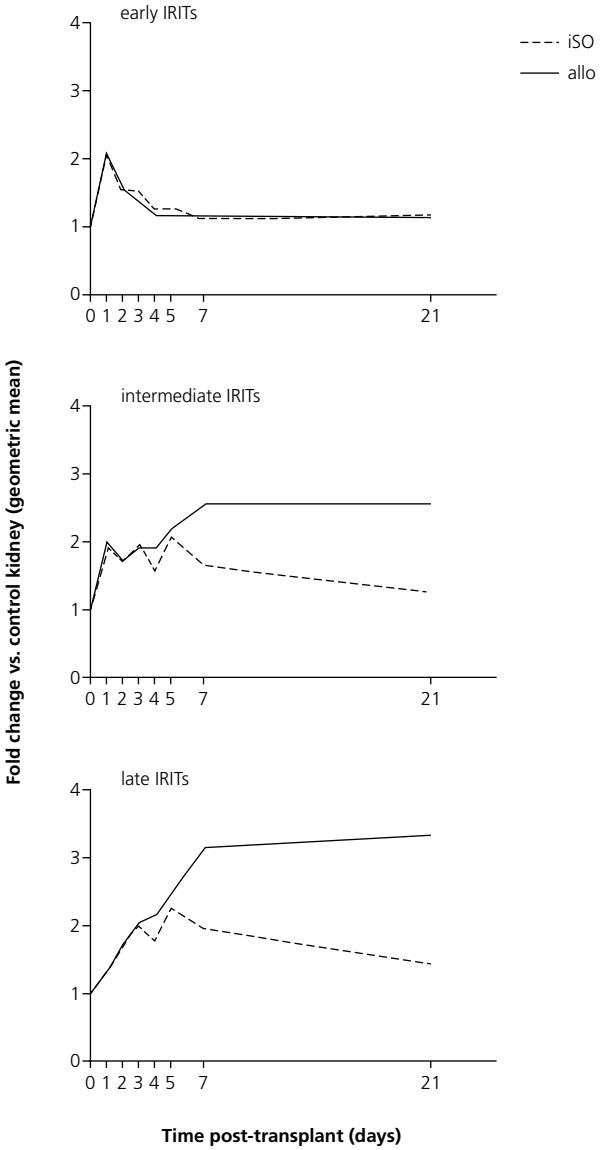


Figure 10.3. The heterogeneity of the injury-repair response in mouse isografts and allografts. Expression time course of injury and repair-induced transcripts (IRITs): early IRITs, intermediate IRITs, and late IRITs. Dashed lines indicate isografts, solid lines indicate allografts. Early IRITs showed transient peak at day 1 and are induced equally in isografts and allografts. They represent the systemic effects of anesthesia and wounding. Intermediate and late IRITs showed a broad peak of expression on days 1–5 in isografts and then decreased. In allografts, expression of intermediate and particularly late IRITs was markedly elevated after day 5 post-transplant. (Reproduced from Famulski et al. 2007 [22], with permission from John Wiley and Sons Ltd.)

the innate response against certain pathogens. There is a strong interest in AMA features because of their relationship to tissue injury [108] and tissue remodeling after injury [109]. In humans, the summarized expression of IFNG effects and alternative macrophage activation is seen in severe AKI to some extent, but it quantitatively characterizes the TCMR, perhaps due to the tissue injury [21].

Molecular changes associated with AKI in human kidney transplants

Post-transplant biopsies

We investigated the relationship of gene expression to kidney function in post-transplant indication biopsies [21], focusing on kidneys with AKI that had biopsies taken early post-transplant (within the first 6 weeks), and excluding those with histological evidence of rejection or specific diseases. These kidneys had poor function (eGFR) at the time of biopsy, but recovered their function after 6 month.

We identified the top 30 candidate genes of the AKI signal (Table 10.3), which represent stereotyped mechanisms in the injury-repair response (response to wounding) such as tissue development and cancer, particularly invasive and metastatic cancers, as well as fibrosis. The AKI signal correlated well with GFR and the AKI phenotype, while the histological changes of AKI had no associations with either kidney function or the phenotype. Thus kidney transplants with an early AKI represent a successful repair of the non-immune injury and their transcripts reflect a snapshot of the active injury-repair response that occurs in recovering kidneys [110].

Surprisingly, the AKI signal is a general feature of injured and diseased renal parenchyma, and thus is detected in transplants biopsied late post-transplant. The injury signal in late transplants was a better predictor of future graft loss than fibrosis, inflammation, or expression of collagen genes. At least 19 individual genes of the AKI signal were predictive of the future graft loss [111]. Thus the acute injury signal, defined in reversible injury, is present in many progressive diseases as a reflection of parenchymal distress. The association of the AKI signal with the risk of failure is dictated by the inducing insult, that is treatable/self-limited versus untreatable and sustained. Thus progression in troubled transplants is primarily a function of ongoing parenchymal injury by disease, not fibrogenesis.

Implantation biopsies

Both the local and systemic responses in the implanted kidneys had an early DGF phenotype. Furthermore, the high incidence of DGF was associated with the expression of genes associated with injury-repair response (IRITs) and inflammation [112].

The AKI signal has a predictive value for DGF in implantation biopsies, as shown in DBD kidneys [113]. The AKI signal score was significantly higher in kidneys with a future poor function compared to kidneys with a good function. Living donor kidneys and control kidneys had the lowest AKI signal scores.

In some reports, detection of inflammation at the time of implantation has been associated with post-transplant clinical outcomes, but inflammation is a feature of older kidneys with patchy scarring. Thus these reports need confirmation to adjust for such factors as ECD donors, high-risk recipients, and the ECD paradox. African-American recipient race, donation after death, increased donor age, acute rejection during the first three transplant months, the degree of HLA mismatching, and increased *CD25* and decreased *BCL2L1* gene expression individually predicted risk for poor graft function (serum creatinine $\geq$2.0 mg/dL) 6 months after transplantation [114]. In one study, high CD3 and low platelet endothelial cell adhesion molecule (PECAM) were associated with worse 2-year allograft function, and high TNF-α predicted worse death-censored and overall 2-year graft survival [4], but, after adjustment for clinical variables, none of the gene expression profiles was associated with distinctive clinical outcomes at 3 or 4 years post-transplantation.

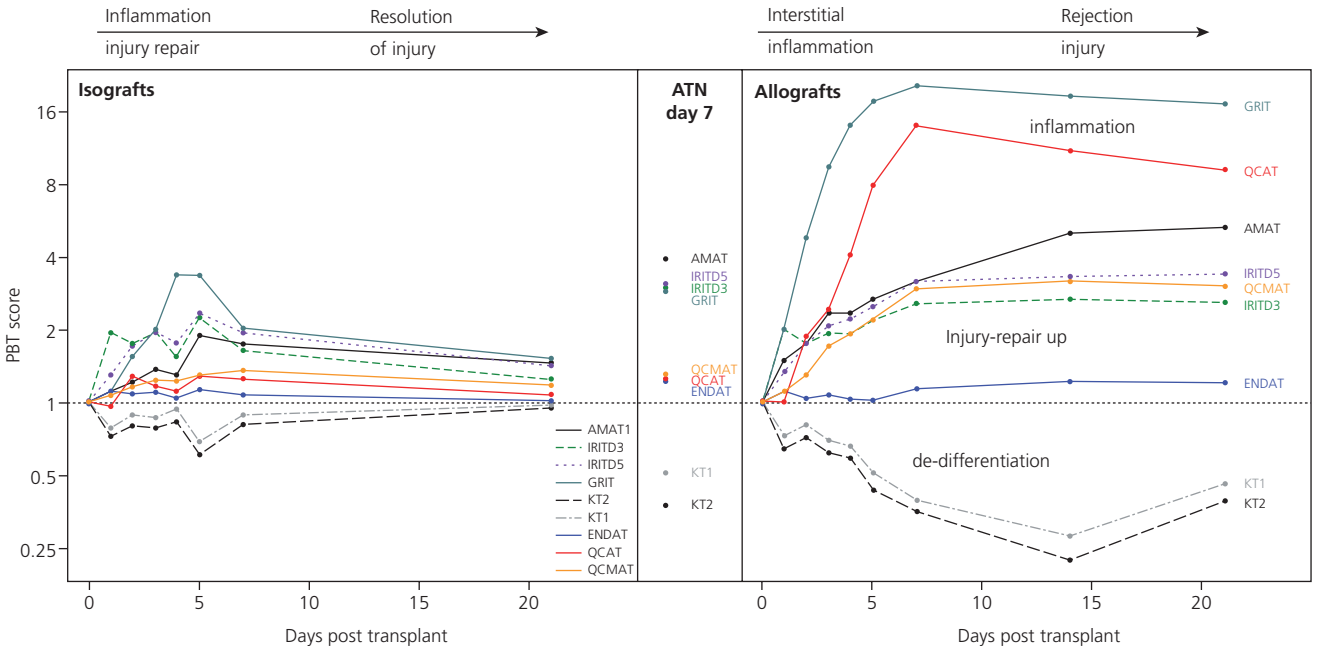


Figure 10.4. Time course of expression of pathogenesis-based transcript sets demonstrates close relationship between inflammation, injury-repair response, and tissue dedifferentiation. T-cell mediated rejection (right) triggers the same injury-repair (AKI) response as seen in isografts (left). When the injury resolves in the isografts, inflammation, injury response, and tissue dedifferentiation abate. However, in the highly inflammatory state (allografts) injury response and tissue dedifferentiation is prominent and continuous. Note that ischemic injury (ATN) demonstrates the injury response similar to that in allografts. AKI–QCAT, burden of effector/effector-memory T cells; AMAT1, alternatively activated macrophages; ENDAT, microcirculation (endothelial cell) response to injury; GRIT, interferon-gamma effects on the tissue and inflammatory cells; IRIT3, injury and repair-induced transcripts during the injury-repair response, peaking at day 3 in mouse isografts (intermediate); IRIT5, injury and repair-induced transcripts during the injury-repair response, peaking at day 5 in mouse isografts (late); KT1, parenchymal transcripts that decrease during the injury-repair response; KT2, solute carrier transcripts that decrease during the injury-repair response. QCMAT, burden of macrophages; (Reproduced from Halloran et al. 2010 [101], with permission from John Wiley and Sons Ltd.)

The belief that pure AKI in transplants leads to poor long-term graft outcomes is not supported by the molecular data. The impaired long-term survival of kidneys with DGF in registry data is not an effect of pure AKI, which is undesirable but inherently reversible.

Some key issues in AKI

Does inflammation increase/extend injury?

There has been a suspicion that inflammatory cells, as part of the injury response in the kidney, contribute to the tissue injury. As a result, many investigators have attempted to manipulate the injury response to mitigate the injury in experimental transplant models. At present, there is no evidence from humans that inflammatory cells create injuries in the minutes, hours, or days after organs have been injured. Just as in any wound-healing site, these cells are present and presumably have been recruited because they have benefits in the healing response. Wound healing is such a major program in multicellular organisms that it is difficult to improve, and apparent phenotype "benefits" from an intervention either may not be reproducible in other models or in the clinic, or are associated with adverse events at another time. However, given the strong interest in inflammatory cells, it seems likely that efforts to manipulate wound healing in the context of an injured organ will continue.

Does injury increase immunogenicity and either TCMR or ABMR?

In models of ischemia–reperfusion injury, AKI induces the proinflammatory compartment [115], which increases MHC expression and thus increases the "antigenicity" of the kidney, but whether this increases allorecognition of MHC "immunogenicity" is doubtful. ABMR can also masquerade as DGF and early AKI. However, AKI and DGF have not been shown to induce de novo HLA antibody formation and ABMR.

The DGF phenotype has many covariates (donor age, recipient age, brain death, sensitization, etc.) that render its interpretation difficult. The DGF phenotype is associated with more histologic diagnosis of TCMR [116], but these may be false positives due to injury-induced tubulitis and interstitial infiltrate, detected because kidneys with DGF are biopsied much more frequently than those with good function. Protocol biopsies of kidneys with the AKI phenotype show an increased frequency of inflammation resembling rejection [117], because injury induces interstitial inflammation and tubulitis and probably even endothelialitis (v-lesions) [49], all of which will give false-positive diagnoses of TCMR. The inflammation present in protocol biopsies, which some call "subclinical rejection", is in fact primarily a reflection of AKI [118]. In addition, rejection is not primarily determined by the characteristics of the donor tissue. The concept of rejection associated with DGF might be an observational artifact due to a high number of biopsies.

Table 10.3. Top 30 genes associated with AKI ordered by fold change in AKI biopsies versus pristine protocol biopsies

Gene* symbol	Gene name	Fold change: transplants with AKI vs. normal kidneys	Correlation with eGFR at biopsy in transplants with AKI†	Predictor of the AKI phenotype (AUC ≥0.7)	Predictor of future graft loss
ITGB6	integrin, beta 6	7.9	−0.69	yes	yes
SERPINA3	serine (or cysteine) proteinase inhibitor, clade A	6.1	−0.71	yes	
MTND6	NADH dehydrogenase, subunit 6 (complex I)	5.6	−0.51	yes	
OLFM4	olfactomedin 4	5.5	−0.74	yes	
PTX3	pentaxin-related gene, rapidly induced by IL-1 beta	5.3	−0.58	yes	
LCN2	lipocalin 2 (oncogene 24p3)	4.3	−0.57	yes	yes
VCAN	chondroitin sulfate proteoglycan 2 (versican)	4.1	−0.35‡	yes	yes
LTF	lactotransferrin	4.1	−0.63	yes	yes
SLPI	secretory leukocyte protease inhibitor (antileukoproteinase)	4.1	−0.70	yes	yes
ADAMTS1	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	4.0	−0.64	yes	
MEGF11	multiple EGF-like-domains	3.8	−0.68	yes	yes
CDH6	cadherin 6, type 2, K-cadherin (fetal kidney)	3.7	−0.72	yes	yes
PI15	peptidase inhibitor 15	3.6	−0.40	yes	
FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor for (CD16)	3.4	−0.30‡	yes	yes
NNMT	nicotinamide N-methyltransferase	3.3	−0.56	yes	yes
S100A8	S100 calcium binding protein A8 (calgranulin A)	3.2	−0.11‡	yes	
SOD2	superoxide dismutase 2, mitochondrial	3.2	−0.61	yes	yes
ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	3.0	−0.74	yes	yes
NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	3.0	−0.67	yes	yes
AKAP12	A kinase (PRKA) anchor protein (gravin) 12	3.0	−0.60	yes	yes
VMP1	likely ortholog of rat vacuole membrane protein 1	2.9	−0.71	yes	yes
ADAM9	a disintegrin and metalloproteinase domain 9 (meltrin gamma)	2.9	−0.57	yes	
PTPRC	protein tyrosine phosphatase, receptor type, C	2.9	−0.53	yes	yes
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	2.9	−0.48	yes	
OSMR	oncostatin M receptor	2.8	−0.80	yes	yes
C9orf71	chromosome 9 open reading frame 71	2.8	−0.41	yes	
EGR1	early growth response 1	2.8	−0.48	yes	
CTSS	cathepsin S	2.7	−0.50	yes	yes
RARRES1	retinoic acid receptor responder (tazarotene induced) 1	2.7	−0.55	yes	yes
EVI2A	ecotropic viral integration site 2A	2.7	−0.32‡	yes	yes

* Non-redundant and annotated genes are shown. In case of multiple transcripts per gene, the one with highest fold change was selected.
† Spearman correlations of gene expression with eGFR at the time of biopsy were considered significant at $P < 0.05$.
‡ P value not significant.

Aging and somatic cell senescence in relation to organ injury

Older donor age is an issue for DGF and for long-term impairment of GFR, and is considered here in relation to DGF. Older organ age is a major determinant of early organ dysfunction, and a major concern leading to the discard of organs. Moreover, the ECD paradox mandates that organs from older donors are put into higher-risk recipients, making recipient death and co-morbidities complex associations of donor age. With living donors, kidneys from older donors do well, except for their decreased GFR, as expected. However, live donors with hypertension or renal disease are commonly excluded. (Some centers accept kidney donors with well-controlled hypertension and without any evidence of end-organ damage.)

In deceased donors, the duration of hypertension is an independent risk factor for graft survival. The 3-year graft survival rate was inferior when compared to donor-age-match controls (65% vs. 75%, relative risk = 1.36 for hypertension >10 years, $P = 0.001$) [20] (but again the ECD paradox must be considered). The negative impact of hypertension and diabetes on transplant outcome is probably a matter of severity and duration, for example donor hypertension duration >10 years.

Histology lesions from preimplantation biopsies, including glomerulosclerosis, interstitial, and fibrosis, and fibrous intimal thickening of small arteries have questionable predictive value for transplanted organ function beyond that of donor aging and demographic equations, leading some to argue that biopsies should not be performed to assess aging and/or quality [119]. In addition,

most published studies that evaluate tissue quality assessment methodology lack appropriate validation [119].

The problem of what to do with ECD kidneys has no easy answers: transplant two into one recipient, two into two recipients, or discard both [120]. The practice of transplanting two kidneys from an older donor into one recipient is not well supported by evidence: there are no trials of randomly transplanting two kidneys into one recipient versus transplanting them into single recipients. Studies of the outcome of double kidney transplants from ECDs into one recipient compared to transplantation of a single ECD kidney usually find patient- and graft-survival rates of double kidney transplant are similar to single kidney transplant (95% and 90%, respectively) [121,122]. In view of the donor shortage, ECD kidneys should probably be transplanted as singles (into two recipients) rather than doubles into one recipient, if at all possible, although two into one is preferable to discarding both kidneys.

This puts the biology of aging on the agenda for understanding organ transplant performance and organ injury. In essence, the problem is that older organs are "smaller"—they have lost functioning mass—and their remaining cells are "old"—their cells are more fragile and vulnerable to injury [123]. Older kidneys that are functioning adequately in the donor after brain death manifest more DGF than younger organs. The older deceased donor population is qualitatively different, with more hypertensive vascular disease, and thus has a second issue in organ quality, that is more advanced changes preceding death. In contrast, the living donor of the same age can be selected to avoid hypertensive disease and avoid renal insufficiency. These choices cannot be made in the deceased donor, where evaluation is very limited.

The molecular changes of aging include the progressive DNA damage in telomeres, TP53, and mitochondria [124]. As cells undergo senescence, their mitochondrial function is compromised, which leads to the protein, lipid, and DNA modifications [125], including glycation [126].

There are two main theories of somatic cell senescence [127]: the telomere shortening theory [128] and the P16INK4A (cyclin-dependent kinase inhibitor 2A, CDKN2A) theory [129]. Telomeres are the ends of chromosomes and they are shortened as the cells replicate because the ends cannot be completely regenerated in cell replication. The problem is the absence of telomerase in somatic cells.

In experimental animals, such as rats and mice, the telomeres are very long and do not shorten significantly with age. In the humans and in phylogenetically higher animals, telomeres are short and shorten with age. At a certain point, they become limiting and evoke the DNA damage response [130,131]. This is beneficial because it keeps somatic cells from excessive replication and thus acts as a defense against cancer. Indeed, any cancer must find a way of bypassing somatic cell senescence mechanisms, by strategies such as re-expression of telomerase or deactivation of TP53.

The main cell cycle regulator used by somatic cells as they enter senescence is CDKN2A. CDKN2A is expressed in somatic cells in culture, such as fibroblasts, as they experience replication-induced arrest. Somatic cells have a limited number of cycles, which is known as "the Hayflick limit". The Hayflick limit is caused by telomere shortening. Other limitations on cells in culture include oxidative stress, which damages many aspects of the cell. As cells enter replicative arrest, they express CDKN2A, which acts as an irreversible inhibitor of the cell cycle. Epigenetic factors also contribute to the aging process and CDKN2A expression [132–134]. A number of studies have shown that replicative senescence occurs in human, mouse, and rat kidneys. These issues have been reviewed [76,135]. Cellular senescence in pretransplant kidney biopsies is predictive of postoperative organ function [136].

Markers of somatic cell senescence as a measurement of biological age of the organ at the time of transplantation have yet to be proven to give more information than chronological age itself. Donor age strongly predicts injury and function during the first weeks. In order to use telomere length or CDKN2A measurements at the time of transplantation, to assess the true age of the organ, one would have to show that these measurements they are superior to demographic factors alone. The commonly used risk indices (Schold, KDRI), expressed as complex weighted equations, are strongly influenced by the donor age.

Is somatic cell senescence a factor in organ survival?

The stresses of organ transplantation induce replication and oxidative stress adds further somatic cell senescence to that caused by pre-existing aging. Thus organs with extensive atrophy and fibrosis manifest high levels of expression of CDKN2A, as shown by staining. Moreover, the expression pattern is abnormal, and is very extensive in the atrophic tubule cytoplasm, as well as nucleus. Expression of CDKN2A in renal transplants and native kidneys is associated with impaired organ function and supports the concept that some cell senescence changes that accompany aging are also induced by stresses due to injury and disease [4]. In addition, a study of implantation [4] indicated that only donor age and donor-age-related histology (particularly interstitial fibrosis) is associated with long-term graft function. This demonstrated that somatic cell senescence is a factor in organ survival. However, this study also found that injury/ischemic stress at implantation failed to correlate with long-term function [137].

Can the somatic cell senescence mechanisms be bypassed or treated by drugs?

The possibility of a temporary suspension of some of our anticancer controls to secure additional replicative capacity in acute injury should be considered [138] but the potential risks must be weighed. There is a link between somatic cell senescence mechanisms and cancer defense: the tradeoff for not getting cancer is that somatic cells are not immortal. Attempts to induce telomerase to prevent telomere shortening and to bypass CDKN2A are theoretically attractive to giving cells extra replicative ability. There are some encouraging results in CDKN2A knockout mice, but one must be concerned about bypassing the limits imposed on somatic cells as cancer defenses. Both at the whole animal level and in the organ, there could be consequences and risks for bypassing either the telomerase mechanism or the CDKN2A mechanism.

Clinical treatment of AKI

Are the AKI biomarkers useful in the clinic?

Studies have examined the aim of using of the molecular tools to correlate with and diagnose AKI, ideally before the onset of the functional disturbance. As mentioned before, the functional classification of molecular changes in AKI revealed changes in immunity and defense response, ligand-mediated signaling, cell proliferation, and differentiation, as well as apoptosis. Some genes are up-regulated but some are down-regulated after AKI [139,140]. For example, expression of IL-6 and IL-18 is elevated (the inflammatory biomarkers), as well as the expression of HAVCR1 (KIM-1),

Table 10.4. Potential biomarkers reported in the context of AKI

Biomarker name	Gene name	Testing Specimen	Molecular function	Biological process	Study type
Neutrophil gelatinase-associated lipocalin (NGAL)	*LCN2* Synonym: *NGAL*	Serum, urine	Iron ion binding Transporter activity	Regulation of apoptosis Innate immune response Siderophore transport	Native AKI Post-KT AKI
γ-glutamyl transpeptidase (γGT) (gamma-GGT 1)	*GGT* Synonym: *GGT1*	Urine	Acyltransferase activity Gamma-glutamyltransferase activity Protein binding	Glutathione biosynthetic process	Native AKI
Sodium/hydrogen exchanger 3 (Na(+)/H(+) exchanger 3)	*SLC9A3* Synonym: *NHE3*	Urine	Sodium :Hydrogen antiporter activity	Ion transport	Native AKI
Interleukin-6 (IL-6) (B-cell stimulatory factor 2 (BSF-2)) (Hybridoma growth factor interferon beta-2 (IFN-beta-2))	*IL6* Synonym: *IFNB2*	Serum	Cytokine activity Growth factor activity Il-6 receptor binding	Acute-phase inflammatory response	Native AKI Post-KT AKI
Kidney injury molecule 1 (KIM-1) (Hepatitis A virus cellular receptor 1 homolog (HAVcr-1))	*HAVCR1* Synonym: *KIM-1*	Urine	Receptor activity	Host–virus interaction	Native AKI Post-KT AKI
Interleukin-18 (IL-18) (Interferon gamma-inducing factor)	*IL18* Synonym: *IGIF*	Serum, urine	Cytokine activity Signal transducer activity	T-helper 1 type immune response Angiogenesis Cell–cell signaling Granulocyte macrophage colony-stimulating factor biosynthetic process Interferon-gamma biosynthetic process IL-2, IL-13, IL-17 biosynthetic process Positive regulation of NK T cell, activated T-cell proliferation Positive regulation of tissue remodeling Regulation of cell adhesion	Native AKI Post-KT AKI

the receptor for a signal generated by apoptotic cells. Meanwhile, GGT and SLC9A3 are down-regulated probably due to dedifferentiation of the epithelium. Biomarkers of AKI were detected either as the product in urine or blood [4] or as mRNA expression in human kidney [110]. Most of the candidate markers had been initially discovered in native kidney diseases and were subsequently found in the human post-transplant AKI (Table 10.4).

In transplanted kidneys, the top 30 up-regulated genes in post-transplant AKI are potential markers for predicting AKI [4]. In addition, HAVCR1 and IL-18 were elevated in kidney tissue in response to injury, suggesting a potential role as a biomarker of renal injury. Molecular markers of AKI have also been studied in preimplantation biopsies. The results indicated that individual gene members of the AKI signal (*OSMR*, *SOD2*, *NNMT*, *ITGB6*, *CTSS*, *ITGB3 CDH6*, *LCN2*, *SLPI*, and *VCAN*) also showed significant predictive ability of DGF at implantation (area under the curve, AUC >0.7) [141].

In DCD kidneys, lactate dehydrogenase (LDH) levels have been assessed as indicators of the extent of injury [142]. However, LDH is not commonly used because it is not specific to renal tissue (it can also be released from damaged erythrocytes). In current practice, viability markers based on glutathione-S-transferase (GST) and α-GST iso-enzyme, a lysosomal protein from proximal renal tubular cells, are more extensively used. These correlate with warm ischemic time and are markedly increased in DCD kidneys that will never function [110]. Nevertheless, no single biomarker is an ideal marker for AKI, which should have the ability to predict injury phenotype, be unique to the kidney, easy to detect at an early phase of injury, and be highly accurate, sensitive and specific, and inexpensive [4].

Many of the top 30 AKI transcripts are used by independently derived, molecular risk score classifications of graft failure, such as NMMT, ITGB6, and VCAN [143]. This indicates that the parenchyma and stroma reaction to acute stress is also evoked in the context of ongoing progressive diseases. The association between the molecular changes of AKI and progressive disease underscores the need to identify aggressive processes that are disrupting the parenchyma [144,145] and becomes an important tool for clinical progress [146]. The prominence of the AKI signal in kidney transplants with progressive disease, such as ABMR, has encouraging implications for potential treatments. If progression is due to injury from the ongoing effects of the primary disease, progression will stop if the disease-causing injury is arrested. If progression is an autonomous secondary process, such as dysfunctional fibrosis (or FSGS, as in rodents), it would be necessary to treat both the primary disease and the secondary autonomous process.

In particular, the clinicians charged with making decisions about the use of ECD kidneys would be helped by new tests of organ aging/quality, whether that is based on biopsies or body fluids. We have analyzed whether the AKI signal in implantation biopsies could be clinically useful as a predictor of future poor kidney function, in addition to the clinical risk (e.g. donor age). The addition of the AKI signal in implantation biopsies to demographic risk scores, such as the Kidney Donor Risk Index, was better than either using histology or clinical risk alone [147,148]. It remains to be seen whether biomarkers in body fluids will reflect the molecular changes in the tissue and permit quantitative estimates of AKI.

Therapeutics of AKI: Lessons from failure

Many agents and experimental compounds have been investigated for their potential prevention or treatment of AKI, but virtually all have failed in subsequent clinical trials (as summarized in Table 10.5). The strategies for managing injury in experimental models are based on a rationale consisting of three main points:

Table 10.5. Clinical trial results for prevention and treatment of ischemic AKI

Intervention	Pharmaceutical/ intervention categorization	Rationale	Experimental trial (animal study) results	Clinical trial (human study) results
Objective : To increase oxygen delivery to the injured renal tissue				
Dopamine	Vasodilator	Vasodilator	Prevention and treatment [158–160]	No benefit from treatment [161–163]
Fenoldopam	Vasodilator	Vasodilator	Prevention and treatment [163]	No benefit from treatment [164,165]
Furosemide	Diuretics	Decreased oxygenation requirement of renal tubules	Treatment [166–168]	No benefit from treatment [169–171]
Hemoxygenese, carbon monoxide release compounds, and bilirubin	Vasodilator/antioxidant	1. Vasodilator 2. Scavenging peroxy radicals and inhibiting lipid peroxidation	Prevention and treatment [172,173]	Lack of clinical trial
Endothelin antagonist	Vasodilator	Vasodilator	Prevention and treatment [174]	Lack of clinical trial
Atrial natriuretic peptide	Vasodilator	Dilating afferent arterioles while constricting efferent arterioles	Prevention and treatment [175]	No benefit from treatment [176]
Remote ischemic preconditioning (RIPC)	Procedure	Flow reperfusion mechanism of transient non-lethal ischemic organ protects another organ or tissue from a subsequent episode of lethal ischemia and reperfusion (AKI)		No benefit to protect against AKI [177] (The actual benefit by NCT00975702 trial will be finished at the end of 2012)
Objective : To decrease inflammation via the inhibition or elimination of proinflammatory cytokines, free radical oxygen species, apoptosis, expression of adhesion molecules, or leukocyte function				
Mannitol	Diuretics	A free radical scavenger	Prevention [178]	No benefit to protect against AKI [179–181]
Statin	Anti-inflammatory	Statins can induce the cellular accumulation of endothelial nitric oxide synthase; inhibit the expression of adhesion molecules and chemokines that recruit inflammatory cells; inhibit expression of procoagulant factors and ameliorate platelet hyper-reactivity Pathways/factors implicated in the cellular effects of statins include the cholesterol biosynthesis pathway, Ras/Rho, nuclear factor-kappaB, and activator protein-1-mediated proinflammatory pathways, and nuclear factors such as peroxisome proliferator-activated receptor and Kruppel-like factor-2	Prevention [182]	Insufficient clinical data (Lack of RCT [183])
Alkaline phosphatases	Anti-inflammatory/antisepsis	Alkaline phosphatase (AP) attenuates inflammatory responses by lipopolysaccharide detoxification and may prevent organ damage during sepsis Inhibits the up-regulation of renal inducible NO synthase, leading to subsequent reduced NO metabolite production	Treatment [184]	Insufficient clinical data (Lack RCT [185,186])
N-Acetylcysteine (NAC)	Antioxidant	Reduces oxygen free radical	Prevention and treatment [187–189]	No benefit to protect against AKI [190,191]
Caspase inhibitors	Antiapoptosis/necrosis	Inhibition of caspase	Prevention and treatment [192,193]	Lack of clinical trial
Minocycline	Antiapoptosis/necrosis	Inhibition of p53 and BAX	Prevention [82,194]	Lack of clinical trial
p53 Inhibitor	Antiapoptosis/necrosis	Inhibition of p53	Prevention and treatment [195]	Lack of clinical trial
PARP inhibitor	Antiapoptosis/necrosis	Inhibition of poly ADP-ribose polymerase (PARP) activation	Treatment [196]	Lack of clinical trial
Soluble thrombomodulin	Antisepsis	Down regulates VCAM-1, ICAM-1, E-selectin	Prevention [197]	No benefit from treatment [198,199]
Ethyl pyruvate	Antisepsis	Inhibits tumor necrosis factor-α (TNF-α), down-regulates iNOS	Treatment [200]	No benefit to protect against AKI [201]
Activated protein C	Antisepsis	Down-regulates VCAM-1, ICAM-1, E-selectin	Treatment [201]	No benefit from treatment [202]
Sphingosine 1 phosphate analog (FTY720)	Anti-inflammatory/ immunosuppression	1. Direct activation of S1P1Rs results in a reversible redistribution of lymphocytes (B and T cells) from the circulation to secondary lymph tissue and thus away from sites of inflammation 2. Protected from apoptotic cell death through activation of mitogen-activated proteinkinase/extracellular regulated kinase (MEK/ERK) and phosphatidylinositol 3-kinase (PI3K)/Akt signaling	Prevention [203,204]	No benefit to protect against AKI [205]

Table 10.5. (*Continued*)

Intervention	Pharmaceutical/ intervention categorization	Rationale	Experimental trial (animal study) results	Clinical trial (human study) results
α -Melanocyte-stimulating Hormone	Anti-inflammatory	Inhibits proinflammatory cytokine from leukocyte, inhibition of iNOS	Treatment [206,207]	Lack of clinical trial
Interleukin 10	Anti-inflammatory	Inhibits proinflammatory cytokine from leukocyte, inhibition of iNOS	Treatment [208]	Lack of clinical trial
Peroxisome proliferator (PPAR) -activated receptors	Anti-inflammatory	Peroxisome proliferator (PPAR) -activated receptors inhibit NF-κB, and down-regulate VCAM-1 expression	Prevention [209,210]	Lack of clinical trial
Inducible nitric oxide synthase inhibitors	Anti-inflammatory	Inhibits iNOS	Treatment [211]	No benefit from treatment [212]
Objective : To enhance renal tubular regeneration via growth factors or mesenchymal stem cell therapy				
Erythropoietin	Growth factors	Down-regulation of NF-κB and activator protein 1 (AP-1)	Prevention and treatment [148,213,214]	Lack of clinical trial
Hepatocyte growth factor	Growth factors	Hepatocyte growth factor (HGF) is a potent regenerative factor for many cell types including renal tubule, with mitogenic, motogenic, and morphogenic activities. Renal expression of HGF and its receptor, c-met, increase after ischemic ARF HGF modulates neutrophil-endothelial interaction by suppressed TNF-mediated endothelial ICAM-1 induction. HGF is also capable of suppressing de novo induction of E-selectin, another key molecule for leukocyte-to-endothelial adhesion	Prevention and treatment [215–217]	Lack of clinical trial
Insulin like growth factor (IGF-1)	Growth factors	IGF-IR signaling also activates phosphatidylinositol 3-kinase (P13-K), which in turn activates protein kinase B (PKB/Akt) that also prevents apoptosis. These pathways converge on the inhibition of caspases, especially caspase-3, which is then blocked from performing an apoptosis-initiating cleavage of poly(adenosine diphosphate ribose) polymerase (PARP) and blocked from degrading β-catenin, part of the cadherin cell-adhesion system	Prevention and treatment [218,219]	No benefit from treatment [220]
Stem cell	Procedure	Amplify adult mesenchymal stem cells in the laboratory or identify factor (or factors) and then reintroduce them into recipients at the time of injury to promote proliferation of renal tubular cells	Prevention and treatment [221–223]	Lack of clinical trial

1 to increase oxygen delivery to the injured renal tissue by improved renal blood flow or decreased oxygen demand in the repairing renal tubular tissue;
2 to decrease inflammation via the inhibition or elimination of proinflammatory cytokines, free radical oxygen species, apoptosis, expression of adhesion molecules, or leukocyte function;
3 to enhance renal tubular regeneration via growth factors or mesenchymal stem cell therapy.

The patterns from these models have been misleading. In fact, interventions in AKI models have produced essentially no successes in predicting human interventions. The recent enthusiasm for erythropoietin treatment of kidneys with AKI is a case in point, where experimental models indicated efficacy [149] and clinical trials failed [150].

Perhaps it is time to change this paradigm: the interventions that "improve" function in young rodent tissue now seem very unlikely to improve outcomes with human tissues, although we do not know why. Perhaps much of the dysfunction in these rodent models is actually due to a different event (from the human point of view, an irrelevant event) from what we see in adult humans, where the problems of injury, donor aging, and recipient morbidity are so much more complex.

Having said that, treating and even preventing AKI in the clinic is difficult because much of the serious injury occurs in the donor before organ removal and cannot be prevented. Moreover, the major limitation of successful therapeutics in clinical trials of AKI is the complexity of AKI biology and the parenchymal remodeling. Interventions that directly address the cell cycle, and thus anticancer defenses, raise concerns about safety and adverse effects. The injury-repair process is closely related to cancer pathways, and bypassing somatic cell senescence also bypasses mechanisms such as telomere shortening and CDKN2A, which are part of our anticancer defenses. This must be kept in mind during efforts to modify or "improve" the repair processes by suspending these mechanisms. Future strategies may require drugs targeting more specific disease pathways rather than broad treatments, but it will be important to anticipate the consequences of manipulating the repair mechanism.

Injury repair wound healing is a remarkable program in every tissue, and we have achieved very little in the clinic to actually improve on the natural program, even in terms of accelerating the healing of the sterile surgical wound. This should not be a source of discouragement, only of appreciation that the study of this area at the molecular level continues to be challenging.

Management of high-risk early allograft dysfunction

Detailed strategies to reduce and manage AKI are beyond the scope of this chapter, but obviously include the general principles of ICU care of the donor and recipient, for example fluid replacement with crystalloid or colloid for a central venous pressure 8–10 cmH_2O for maintaining organ perfusion while avoiding aggressive hydration [5,151]. Other pretransplant, recipient-directed therapies, such as ischemic preconditioning with carbon monoxide [152], vasodilatory agents (e.g. endothelin receptor antagonist, a selective adenosine A1 receptor antagonist, and calcium channel blockers) [153–155], have yet to be proven in clinical studies for treatment of allograft AKI [152–155].

Calcineurin inhibitors (CNIs; cyclosporine and tacrolimus) are nephrotoxic at least in part by dose-dependent reversible preglomerular vasoconstriction, reducing renal blood flow and GFR. They probably seldom induce DGF but can prolonged recovery time [156] and complicate management and interpretation of functional disturbances. Many clinicians delay introduction of CNIs, often with antibody induction, which is more difficult to diagnose in patients with early allograft dysfunction [156]. Studies that compared early and delayed CNI initiation after transplantation generally have found that the incidence of DGF, acute rejection, graft failure, and kidney function was similar in either early or delayed CNI initiation protocols [157]. Mammalian target of rapamycin inhibitor (mTORi) based regimens (sirolimus and everolimus) have been proposed as a replacement for CNIs, but they delay injury repair and prolong recovery from DGF. Based on the current available information, the combination regimen of antibody induction, minimization dosing of CNI, and use of mycophenolate mofetil and steroids is commonly used for patients who receive kidneys with high risk of, or manifesting, early dysfunction.

Summary

Early graft dysfunction (DGF or SGF) as observed clinically is a complex differential diagnosis, including infarction, technical problems, and ABMR. The causes of DGF include anastomosis problems, cortical necrosis, ureteric obstruction, and antibody-mediated rejection, as well as AKI. AKI is defined here as the reversible phenotypic changes induced by stresses such as ischemia or toxins, including the molecular changes reflecting the response to injury (wound healing). Thus DGF is not synonymous with AKI**.** There is usually recovery from AKI with few late consequences; however, occasionally, adequate function is not achieved following severe AKI. Inflammation in AKI is inherent in wound healing and probably does not "extend" injury. The allograft function is affected by the following triad: donor factors (tissue aging/quality), the injury insult at implantation, and the recipient aging/co-morbidity interaction. The outcomes of ECD kidneys are heavily influenced by the practice of allocating them to high-risk recipients (the ECD paradox). The interventions in AKI models have produced essentially no successes in clinic. Perhaps this is due to the situation that we see in adult humans, where the problems of injury, donor aging, and recipient morbidity are so much more complex. This must be kept in mind during efforts to modify or "improve" the repair processes by suspending these mechanisms. Future strategies may require drugs targeting more specific disease pathways rather than broad treatments, but it will be important to anticipate the consequences of manipulating the repair mechanism.

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CHAPTER 11

Mechanisms of Allograft Tolerance

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Introduction

The last 60 years have seen clinical transplantation evolve from an experimental therapy with poor outcomes to the standard of care for most end-stage organ diseases. However, despite outstanding 1-year graft survival rates exceeding 90% for most organs and associated excellent control of acute rejection, the field continues to be plagued by late graft loss and immunosuppressive toxicity-related morbidity and mortality [1]. The need for chronic immunosuppression combined with its adverse effects is evidence we have likely entered a period of diminishing returns, such that increased immunosuppression will minimally increase graft survival and markedly augment morbidity. As such, gains in graft survival will best be achieved by pursuing strategies promoting immune tolerance and favoring therapies that enhance physiologic immune adaptation to transplanted tissues rather than those that suppress immune effector function.

Tolerance, or the maintenance of allograft function without an evident immune suppressed state, has been a long sought goal of allotransplantation. It is known to be clinically possible in selected circumstances, as it has been observed that rare allograft recipients can be withdrawn from immunosuppression without apparent clinical consequence [2,3]. Indeed, several series of tolerant kidney transplant recipients have been reported in the literature [4–7], and up to 20% of highly selected liver transplant recipients achieve successful immunosuppression withdrawal [8–13]. These exceptional cases have demonstrated that operational tolerance is possible but stochastic; the ability to prospectively and reliably induce durable, allospecific tolerance remains elusive.

Although clinically rare, tolerance is readily achievable in controlled experimental animal models. The underlying immune mechanisms of allograft rejection and acceptance have become increasingly defined and exploited, such that experimental allograft tolerance is now commonly achieved in rodents and has been demonstrated in higher animals including pigs [14,15], dogs [16–18], and non-human primates (NHPs) [19,20] with increasing regularity. However, success in animal models has not reliably translated to humans. Indeed, human tolerance has been observed primarily in the setting of patient non-compliance or drug withdrawal due to complications [21]. Some generalizations have, however, emerged. We know tolerance tends to evolve with time, occurring with increasing frequency late after transplant. It likely requires some resilience of the targeted organ, as younger donor organs and organs with regenerative capacity, such as the liver, tend to be favored; this implies that there is an early engagement with the immune system that has the potential to damage the graft. Tolerance is facilitated by lesser degrees of MHC mismatch, suggesting that reduced donor-specific precursor frequency is salutary. Nevertheless, reliable methods of predicting tolerance do not exist [22], making the risk of rejection with weaning or discontinuation of immunosuppressive therapy prohibitive in stable transplant recipients on minimal immunosuppression. Thus, some patients likely are tolerant, but remain on immunosuppression for lack of a suitable means of recognizing their condition. The clinical application of tolerance is therefore in need of assays to recognize tolerance when it occurs.

This chapter will provide an overview of the predominant mechanisms by which tolerance is induced and maintained: chimerism, depletion, costimulation blockade, and regulation; and will introduce B-cell mechanisms of tolerance. It will highlight those therapeutic maneuvers that are translatable, and provide a framework with which to interpret clinical tolerance trials. Additional insights into tolerance with specific commentary on clinical tolerance trials can be found in Chapter 76.

Conceptual considerations

In 1953, Billingham, Brent, and Medawar published their landmark work showing that tolerance could be purposefully induced under experimental conditions [23], arguably giving birth to the discipline of transplant immunology and laying the foundation for essentially all subsequent research in the field. They showed that two of five adult mice injected as fetuses in utero with alloantigen from a donor strain failed to reject donor-specific skin grafts. Additionally, the passive transfer of sensitized lymph node cells into the tolerant mice led to rapid rejection of the tolerated skin grafts. The three non-tolerant mice rejected their skin grafts, two shortly after transplant and one following a long period of acceptance. These findings led Medawar to several prophetic conclusions that remain salient to current concepts of tolerance in transplantation.

Medawar summarized that "The conferment of tolerance is not of an all-or-nothing character; every degree is represented, down to that which gives the test-grafts only a few days of grace . . . ." Indeed, immunity is not an all-or-nothing phenomenon, but rather a spectrum of immunologic responses which fall between rejection and tolerance. Physiologic immunity has subsequently been found to require contextual signals that augment or suppress effector

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responses to increase the appropriateness of an immune response. As in Medawar's experiments, tolerance in humans has been observed, but the natural history of most allografts is rejection—indicating that the conditions at the time of transplantation favor an aggressive immune posture. This is not to say that those conditions cannot be manipulated to evoke a "perfect storm" of tolerance, but rather that rejection is more likely given the combination of high allospecific precursor frequency, reperfusion injury, and established allospecific memory (all discussed below). Rejection likely looms even in operationally tolerant patients, because, by definition, a competent immune system must retain the ability to adapt to changing contexts, maintaining its dynamic nature as determined by innumerable and at times immeasurable variables [21]. In essence, tolerance is observational, unpredictable, and likely not indefinite in a high percentage of operationally tolerant individuals unless substantial alterations to the recipient immune response are made.

Medawar also declared, "The conferment of tolerance is immunologically specific." That is, mice made tolerant to allografts from one donor strain retained the ability to reject grafts from another strain. This specificity is crucial to any sustainable form of tolerance, as indiscriminate hyporeactivity would, in essence, manifest as a state of gross immunosuppression, and indiscriminate immunity would be best characterized clinically as septic shock. Specific immune engagement of antigen by antigen receptors must occur in the appropriate context to maintain the balance between tolerance and immunity [24]. The development of antigen-specific tolerance facilitates the maintenance of protective mechanisms and the capacity to respond to other antigens or potential pathogens.

Lastly, Medawar concluded, "Acquired tolerance is due to a specific failure of the host's immunological response. . . .The host itself retains the power to give effect to a passively acquired immunity directed against a homograft, which has until then been tolerated by it." In other words, allograft rejection or acceptance is determined by the immune system's interpretation of donor antigen, not ignorance of the donor antigen or an alteration of its properties by "residence in a tolerant host." It has been a common misconception that tolerance is a state of immunologic unresponsiveness, when in reality it is best defined as the absence of a detrimental immune response, but a response nonetheless, recognizing that immune activity can either augment or hinder graft acceptance. Antigen recognition must occur but with a non-aggressive outcome. It is not that antigen recognition fails, but rather the outcome resulting from recognition changes—a change in interpretation, not occurrence.

These concepts outlined by Medawar more than 60 years ago underscore our understanding of mechanisms of tolerance today. Tolerance requires specificity to alloantigen and results from a combination of effector and regulatory immune responses that can manifest as rejection at any time following transplantation. While protective immunity is theoretically maintained by selective suppression of effector reactivity to donor alloantigen alone, some component of overlap between the allo- and protective immune repertoires is inevitable. In such instances, the coexistence of tolerance and immunity will rely on a multitude of cross-reactive combinations, where allo-specific cells dispersed in a sufficiently stochastic manner allow for the elimination of the allospecific repertoire without commensurate elimination of pathogen-specific immunity (Figure 11.1). Because this is a random process, some cross-reactive cells will be very related and the potential for loss of a pathogen-specific response present, but more often than not immunity will be maintained.

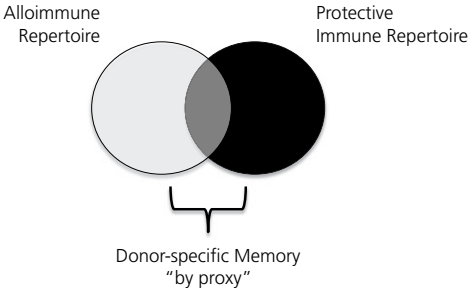


Figure 11.1. The relationship between alloimmunity and protective immunity. Overlap between the allo- and protective immune repertoires is inevitable, thus creating donor-specific memory "by proxy." In these instances, selective suppression of donor-specific effector reactivity has the potential to eliminate pathogen-specific immunity, and maintenance of protective immunity will depend on the random distribution of cross-reactive allospecific cells to facilitate coexistence of tolerance and immunity.

Successful induction of true tolerance will require the introduction of alloantigen to illicit specificity under circumstances that favorably tip the balance from rejection to tolerance. Initial alloantigen exposure with control of the destructive donor-specific response needs to be offset by a sufficient level of effector cell activation that results in compensatory responses favoring allograft acceptance. Current prevailing strategies of tolerance induction include chimerism, lymphocyte depletion, and costimulation blockade, with emerging interest in immune regulation techniques and B-cell-targeted therapies. These methods aim to manipulate the immune response through central and/or peripheral mechanisms of tolerance, essentially altering the context in the thymus, secondary lymphoid tissues, and periphery to favor acceptance (Figure 11.2). Given the complexity of the human immune response, and the required need to control the entirety of the immune response, it is likely that successful induction of clinical tolerance will involve a combination rather than a single one of these immune modulatory strategies. Indeed, as the mechanisms below are discussed, one will see that the practical implementation of these approaches frequently involves combinations of the various mechanisms (e.g. T-cell depletion and costimulation blockade to achieve chimerism, etc.).

Chimerism

Depending on context, hematopoietic stem cells (HSCs) can function in two ways: as expendable alloantigen to drive antigen-induced cell death (AICD), or for engraftment to induce chimerism. The former purpose is a peripheral mechanism that selectively eliminates alloreactive T cells via mechanisms such as costimulation blockade leading to AICD (discussed in the respective section), and the latter a central mechanism (requiring thymic or other primary lymphoid organ function) that relies upon donor HSC engraftment to achieve recipient marrow replacement. Both are intended to induce transplant tolerance.

Complete chimerism classically occurs in bone marrow transplantation (BMT), where all bone marrow-derived cells in a recipient are eliminated and replaced by donor cells. This form of hematopoietic cell engraftment provides the ideal condition for allograft acceptance, as evidenced by the successful persistence of

renal allografts with no requirement for immunosuppression in patients who developed renal failure after undergoing allogeneic BMT for treatment of hematologic malignancies [25]. However, the significant infectious and graft-versus-host disease-related morbidities of the myeloablative conditioning required for marrow transplantation precludes the application of this approach to induce tolerance in solid organ transplantation, with morbidity clearly exceeding that of standard immunosuppressive regimens. To achieve the same outcome with less morbidity, investigators have developed mixed chimerism [26,27], in which milder forms of non-myeloablative preconditioning do not completely ablate the recipient hematopoietic system. In instances of mixed chimerism, the recipient marrow is largely preserved but modified such that both donor and recipient hematopoietic components coexist [28].

Initial proof-of-concept studies in mice using lethal total body irradiation [26,29], and then more translatable non-myeloablative [30] techniques consisting of T-cell depletion and local thymic irradiation, successfully produced mixed chimerism and donor-specific tolerance. These strategies inspired subsequent successes in swine [31,32] and NHP models [33,34] and facilitated extension of a non-myeloablative preparative regimen to induce mixed lymphohematopoietic chimerism and solid organ allograft tolerance in humans [35]. In these instances, it is thought, by avoiding donor marrow rejection for some period of time, transferred hematopoietic cells populate the recipient thymus and marrow and facilitate central deletion of donor alloreactive T and B cells [29,36]. This non-myeloablative approach has the advantages of being less toxic, preserving immune competence, and lessening the risk for graft-versus-host disease. It does, however, depend on a rigorous early regimen that variably includes T-cell depletion, transient maintenance immunosuppression, and thymic or total lymphoid irradiation (TLI) to prevent marrow rejection [35,37].

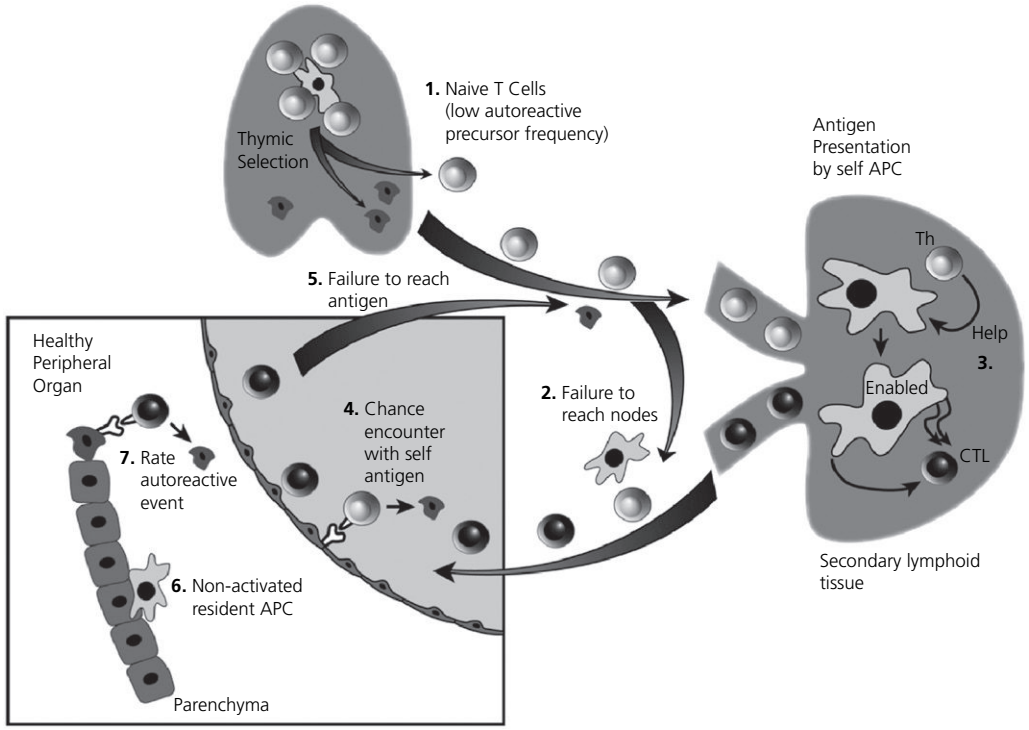
Interestingly, although persistent mixed chimerism has generally been shown to be required in the murine studies that gave rise to this field, primate and human experience has established a tolerant phenotype despite the loss of chimerism. Thus, it remains unclear what mechanisms are dominant in humans. In mice, donor-specific tolerance requires permanent mixed chimerism and is maintained by central, intrathymic clonal deletion and not peripheral suppression or anergy, as the loss of donor-specific tolerance in the absence of donor antigen was thymus-dependent [38]. A costimulation blockade-based regimen has been used to achieve mixed chimerism and tolerance without T-cell depletion or myeloablation [39,40]. Following tolerization of the peripheral T-cell repertoire to achieve mixed chimerism, long-term tolerance is also maintained by intrathymic deletion, and regulatory cells do not play a role in maintaining tolerance in this model [41,42]. However, other mouse models using costimulatory blockade as conditioning for allogeneic BMT have been associated with less complete deletion of pre-existing donor-reactive T cells and appear to involve long-term peripheral regulatory mechanisms [36,43].

In contrast to the permanent mixed chimerism required in mice, the multilineage chimerism achieved in NHPs using an analogous regimen plus splenectomy has been shown to be transient [33]. Nevertheless, in the seminal studies of this approach, recipients have not rejected their allografts and developed donor-specific tolerance. Without splenectomy, all recipients developed alloantibody-mediated rejection [44], introducing the specter of humoral immunity as a barrier to tolerance. Long-term survival and more durable chimerism were salvaged by replacement of splenectomy by costimulation blockade (anti-CD154) [45]. The persistence of tolerance after loss of chimerism suggested that peripheral mechanisms might be responsible for the maintenance of tolerance in primates. Furthermore, sustained operational renal allograft tolerance achieved in multiple myeloma patients with ESRD after HLA-identical bone marrow and kidney transplantation [46], despite the loss of detectable hematopoietic chimerism, also suggests the presence of peripheral mechanisms in addition to (or instead of) the deletional tolerance demonstrable in mice. The detection of antidonor alloreactivity in vitro after loss of chimerism and the enrichment of $CD25^{+}CD4^{+}$ T cells both have implicated immune regulation as a mediator of tolerance in humans [47].

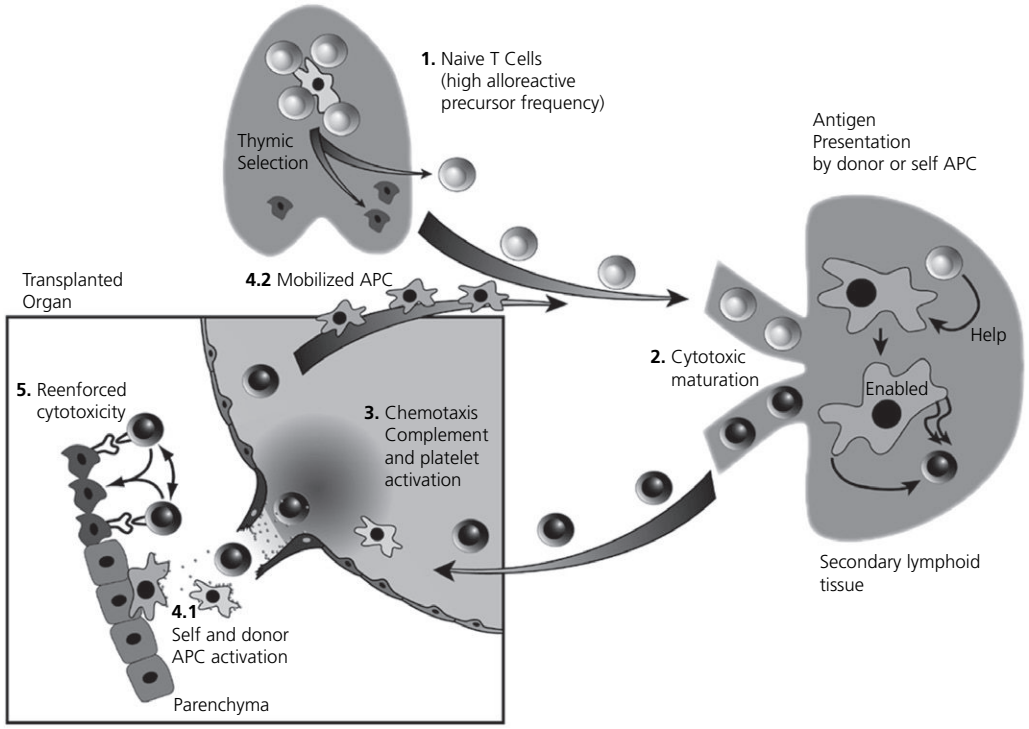
In 2008, Kawai et al. published the first consecutive series of HLA-disparate allografts intentionally transplanted without maintenance immunosuppression [48]. Following variable conditioning regimens and donor bone marrow infusion, four of five recipients of haploidentical live donor kidney transplants were weaned off immunosuppressive medication for greater than 2 years without rejection. In contrast to preparatory mouse studies where chimerism was a prerequisite for success, and similar to preceding NHP studies, durable mixed chimerism was not achieved. Four patients experienced humoral complications, highlighting the critical but poorly understood role of B cells. Interestingly, high levels of Foxp3 in the renal allografts of these patients suggest a role for regulatory T cells in the maintenance of unresponsiveness.

Several additional reports have linked chimerism and tolerance in humans. Scandling et al. described one of three patients rendered tolerant after HLA-identical marrow and kidney transplantation with persistent mixed chimerism [49], and Alexander et al. reported on an HLA-mismatched liver allograft recipient who spontaneously developed multilineage chimerism and tolerance in the setting of lymphopenia and infectious complications without HSC transplantation [50]. Despite these remarkable results, no true single tolerance regimen exists [51]. The only commonality amongst the various reports of chimerism and tolerance in humans has been profound and prolonged lymphopenia in all patients during initial alloantigen exposure. As such, a better understanding of lymphocyte behavior during lymphopenia and repopulation remains an important research focus (discussed below in the Depletion section).

The relationship between the actual induction and maintenance of chimerism and solid organ allograft tolerance remains a conundrum in the field. It is generally accepted that microchimerism (<1% donor chimerism) is distinct from macrochimerism (>1% of circulating cells), and is a property of transplant recipients and not a mechanistic harbinger of tolerance [52]. It is also important to recognize that all transplant recipients are chimeric, in that the organ itself is a massive amount of donor antigen residing within the recipient. What makes hematopoietic cells particularly important to the process when organs have many antigenic properties of these cells remains unresolved. Regardless, the persistence of macrochimerism, or lack thereof, has been an important variable in both preclinical animal models and clinical protocols using combined HSC and renal transplantation. While NHPs, unlike mice, do not seem to require long-lived macrochimerism for tolerance to solid organ allografts, both circumstances have been reported in humans [48–50]. A study from Northwestern University and the University of Louisville reported use of a non-myeloablative conditioning HSC transplant approach to establish complete chimerism and tolerance in renal transplant recipients [53]. The authors achieved durable chimerism in HLA-disparate HSC and renal


Thymic Selection
1. Naive T Cells (low autoreactive precursor frequency)
Antigen Presentation by self APC
Th
Help
3.
Enabled
CTL
5. Failure to reach antigen
2. Failure to reach nodes
Healthy Peripheral Organ
4. Chance encounter with self antigen
7. Rate autoreactive event
6. Non-activated resident APC
Parenchyma
Secondary lymphoid tissue

(A)


Thymic Selection
1. Naive T Cells (high alloreactive precursor frequency)
Antigen Presentation by donor or self APC
Transplanted Organ
4.2 Mobilized APC
Help
2. Cytotoxic maturation
Enabled
5. Reenforced cytotoxicity
3. Chemotaxis Complement and platelet activation
4.1 Self and donor APC activation
Parenchyma
Secondary lymphoid tissue

(B)

Figure 11.2. Mechanisms of (A) self tolerance and (B) alloimmunity to overcome for tolerance induction. (A) (1) A naïve T-cell pool is tailored for appropriate interactions with self-MHC through thymic selection. The result is a T-cell precursor frequency of autoreactive cells that is exceptionally low relative to the allospecific precursor frequency. (2 and 3) As productive antigen presentation is best achieved in the secondary lymphoid tissues, the stochastic meeting of a rare autoreactive cell that has escaped thymic selection with an activated APC derived from the periphery and presenting autoantigen in the secondary lymphoid organs is infrequent. (4) In the event that autoreactive T cells are successfully activated through autoantigen presentation, their migration back to their target antigen is also rare and stochastic in the absence of endothelial activation or chemotactic signals homing the activated cell to its peripheral antigen target. Chance encounters of non-activated autoreactive T cells with autoantigen in the periphery without the appropriate APC delivered presentation and costimulation results in anergy or apoptosis. (5) Activated T cells that fail to reach their target antigen eventually undergo apoptosis. (B) (1) A naïve T-cell pool with high allospecific precursor frequency derived from thymic selection in the absence of alloantigen brings any T-cell-activating event close to threshold for generating a significant immune response. Introduction of donor antigen in the recipient thymus through chimerism induction and/or depletion of donor-specific lymphocytes will counteract this mechanism of immunity. (2) Alloantigen presentation by self or donor type APCs in secondary lymphoid organs is easily enabled by a high frequency of allospecific T-cell help and can also be controlled by chimerism and/or lymphocyte depletion. Costimulation blockade has potential to interfere with T cell:APC interactions and prevent T-cell activation by inducing anergy or apoptosis. (3) Injured endothelium serves as a site of platelet binding and complement activation, thus activating endothelial cells and initiating chemotactic signals to attract lymphocytes for alloantigen encounter. Efforts to limit ischemia and reperfusion injury, and cellular adhesion attenuate this step of the alloimmune response. (4) Activated platelets encounter APCs (4.1), thus initiating mobilization (4.2) to regional secondary lymphoid tissues where costimulation blockade serves to disrupt activation. (5) CTLs encounter their target organ in sufficient numbers to cause rejection in a milieu rich in inflammatory cytokines (e.g. IL-2), both as a result of precursor frequency and chemotaxis. Depletion and costimulation blockade limit the productiveness of this response and prevent the attainment of a milieu that is supportive of cytotoxic lymphocyte activity. The role of regulatory and B-cell mechanisms are not depicted in this figure.

transplant recipients, and found that T-cell chimerism was associated with persistent donor chimerism and the ability to successfully wean subjects from immunosuppression while maintaining stable renal function. In addition, Ramakrishnan et al. have recently reported that durable whole blood (non-T cell, non-B cell) chimerism is insufficient at prolonging allograft acceptance or facilitating immune tolerance to renal transplants in an MHC-matched NHP model [54]. This study underscores the need for a multilineage requirement in that durable chimerism that lacks T-cell chimerism failed to protect subsequently transplanted renal allografts in primates. Thus, it may be prudent to closely adhere to the lessons learned from murine models and pursue strategies that reproducibly lead to chimerism that is both multilineage and stable.

Although the above referenced existing clinical protocols have been inspired by mixed chimerism, tolerance has been achieved without it, and rejection observed with it. There are differences in both the methodology and the mechanisms by which tolerance has been induced in the different animal models. In mice, where complete T-cell depletion is possible, mixed chimerism is long lasting and tolerance is based on central, clonal deletion. The maintenance of such tolerance does not depend on the presence of an organ allograft and it persists even after such a graft is removed. In monkeys and in humans, T-cell depletion is less complete and while central deletion may be involved early after HSC transplantation, maintenance of tolerance long-term, after peripheral chimerism has disappeared, may depend on the presence of the donor organ allograft and appears to involve control of peripheral donor-specific responses by regulatory T cells.

The last few decades of experience with BMT for the induction of chimerism and allograft tolerance has certainly begun to inform our understanding of the mechanisms necessary to achieve tolerance. However, chimerism has yet to reliably predict success in humans or primates, and the antibody response distinct from the T-cell response remains incompletely addressed (see B cell section). The relationships described above between chimerism and tolerance highlight the interdependence of the following individual methods of tolerance induction, and how a combination of these is most likely to lead to true, intentional tolerance.

Depletion

Targeted irradiation to lymphoid tissue crucial for the development of alloimmunity has been used to control the immune response after transplantation in animals [16,55–57] and in patients [58–60]. Lymphoid irradiation alone has had the effect of vigorous T-cell depletion, but more recently has been used as a means of immunosuppression to facilitate marrow engraftment and subsequent mixed chimerism [60] as discussed above. As a pretransplant conditioning regimen, TLI has induced tolerance in chimeric and non-chimeric humans, whereas post-transplantation irradiation has failed to produce stable chimeras or tolerance [61].

Depletional tolerance strategies all have a common aim of controlling allospecific T-cell precursor frequency. Between 1 and 10% of peripheral T cells are able to recognize alloantigen [62,63], and this far exceeds the frequency of T cells for any given nominal antigen. Lymphocyte depletion with polyclonal or monoclonal antibodies has been envisioned as a strategy to non-specifically reduce the precursor frequency, allowing peripheral mechanisms to proceed with less risk for allograft injury. $CD4^+$ and $CD8^+$ antigen-specific precursor frequency has been shown to impact T-cell proliferation, differentiation, and overall quality of the effector immune response [64,65]. However, the compensatory response to depletion, homeostatic repopulation, also has been shown to influence the character of an immune response, typically engendering a more activated phenotype [66]. These concepts are expanded upon in Chapter 9. To date, depleting antibodies have been clinically used both as induction and as rescue therapy of acute rejection [67]. The availability of depletional antibodies makes depletional strategies logistically attainable, but as we have learned more about the myriad types of, and responses to, depletion, its proper place in tolerance regimens has remained incompletely defined. In surveying the literature, it is important to

recognize that every approach to depletion has unique properties to be considered.

In 1997, Knechtle et al. reported that T-cell depletion by a CD3-specific immunotoxin in NHPs led to prolonged renal allograft survival and tolerance to donor skin grafts in five of six recipients, despite rejection of third-party skin grafts [68]. Prior to this study depletion with rabbit antithymocyte globulin (ATG) had been used to promote tolerance, but only in conjunction with donor bone marrow infusion [69,70]. The ability of immunotoxin to promote tolerance alone in contrast to ATG and TLI was attributed to improved selectivity and more efficient T-cell lysis in both the peripheral circulation and lymph nodes [33,71]. Interestingly, despite rodent studies to suggest that the presence of donor MHC class I or class II antigens in the recipient thymus given in combination with anti-T-cell therapy might promote tolerance [72–74], no additional tolerogenic effect was achieved by concomitant thymic donor lymphocyte injections.

Although immunotoxin showed initial promise as a singular tolerogenic agent, subsequent studies rendered it best as an adjunct to tolerance induction methods. As a single agent administered pretransplant it promoted allograft survival, inducing tolerance in at least one-third of recipients, but administered post-transplant (more clinically relevant) in combination with steroids and deoxyspergualin or mycophenolate mofetil (MMF), long-term unresponsiveness was produced more reliably than with immunotoxin alone [75]. Mechanistic examination of NHPs treated with immunotoxin revealed that donor-specific cytotoxic T-lymphocyte precursor frequency and responsiveness were suppressed, but not mixed lymphocyte reaction responsiveness or alloantibody production [76,77]. Histologic and serologic examination of NHPs receiving post-transplant immunotoxin showed alloantibody-mediated glomerular and arterial damage associated with expression of the co-inflammatory cytokines INF-γ and IL-2 [78], introducing the possibility of early T-cell allosensitization and promotion of antibody-mediated graft injury. Post-transplant immunotoxin-treated animals also were able to mount donor-specific antibody responses, further implicating humoral mechanisms as responsible for the failure of tolerance induction and providing rationale for the successful use of the B-cell-suppressive agent deoxyspergualin in combination with steroids and immunotoxin that reliably produced tolerance in NHPs [79].

These preclinical studies using the CD3-specific immunotoxin established the principle of profound T-cell depletion at the time of transplantation as a platform for promoting long-term unresponsiveness to allografts. Calne et al. used campath-1H (alemtuzumab), a CD52-specific antibody that depletes both T and B cells in human cadaveric kidney recipients in combination with cyclosporine to achieve allograft acceptance on low-dose maintenance immunosuppression [80,81]. Several subsequent randomized control trials validated the use of alemtuzumab as a depletional induction agent to achieve *prope*, or almost tolerance, followed by very-low-dose maintenance immunosuppression to guard against rejection [82,83]. The authors point out that operational tolerance has been difficult to achieve with any given protocol due to the enormous variation between donors and recipients of organ grafts of tissue matching, innate immune reactivity, and susceptibility to disturbance of a tolerant state by infections or allergic reactions; thus, they make the case for *prope* tolerance in which graft acceptance is maintained by a low, non-toxic dosage of maintenance immunosuppression [84]. Although this is a very acceptable clinical outcome, it is not clear if the mechanisms involved are related to tolerance, or just efficient immunosuppression. Regardless, it is clear that lymphocyte depletion alone of the level achievable with antibody preparations such as alemtuzumab, will not induce tolerance. This has been tested prospectively in a series of living-donor renal transplant recipients treated aggressively pretransplant with alemtuzumab to achieve complete elimination of T cells in the peripheral blood and marked depletion in the secondary lymphoid tissues [85]. Despite profound lymphopenia after alemtuzumab induction, all patients in this trial developed rejection episodes characterized by predominantly monocytic (not lymphocytic) graft infiltrates. These data underscore a prominent role for non-lymphocyte cell populations in allograft rejection regardless of lymphocyte depletion, or perhaps a need for very few T cells to incite a rejection response. Another seminal point drawn from these studies is that non-specific, polyclonal depletion is not homogeneous in that effector memory T cells have been found to be relatively resistant to depletion [86]. These cells are, however, sensitive to calcineurin inhibitors (CNIs), in part explaining the results of CNI-based *prope* tolerance trials. In addition, remaining T cells after depletion undergo homeostatic repopulation, and this has been recognized as a barrier to the development of tolerance [66,87]. B cells too have been implicated in depletion-resistant rejection due to elevated levels of BAFF (a factor integral to B-cell homeostasis) in renal transplant patients treated with alemtuzumab [88]. Thus, vigorous depletion alone is not a reliable means toward tolerance.

Depletional studies have informed us of important mechanisms salient to the development of tolerogenic protocols. We have learned that allograft dysfunction is not solely determined by T-cell cytotoxicity, and that depletional agents have inhomogeneous effects on macrophages, T cells and B cells. Differences in the immune repertoire present prior to depletion (memory vs. naïve) and differential homeostatic expansion kinetics likely influence the ultimate effect of depletion. Overall, lymphocyte depletion is best viewed as a component of other approaches to tolerance, yet it does facilitate reduced maintenance immunosuppressive requirements in many patients. The lessons learned from depletional strategies to tolerance are likely to lead to improved strategies toward clinical tolerance in organ transplantation.

Costimulation blockade

In 1975, Lafferty and Cunningham's two-signal hypothesis of T-cell activation offered at least the possibility that an immune response under the proper circumstances could promote tolerance rather than being obligatorily hurled toward antigen destruction [89]. This conceptual breakthrough inspired decades of subsequent work amassing considerable experimental evidence that costimulation blockade facilitates tolerance induction [90–93]. Costimulation blockade is based on the paradigm that specific immune responses require two signals for optimal activation [94]. In the absence of a facilitating costimulatory signal, antigen stimulation induces anergy or apoptosis. Thus the provision of signal 1 via antigen exposure, combined with signal 2 or costimulation molecule inhibition, has the effect of eliminating cells in an antigen-specific manner. Costimulation blockade has been used in robust preclinical models to facilitate the protolerant mechanisms at play during peripheral antigen encounter, either with the graft itself [95–98] or with infused hematopoietic cells [99], and has also been used as a means to facilitate chimerism [45,100]. This model has subsequently been modified to include a "signal 3" [101], that being cytokine-induced signals controlling postactivation expansion.

As it has been increasingly recognized that costimulatory signals are critical for optimal T-cell activation, proliferation, and differentiation, there has been an explosion in the study of costimulatory molecules and their roles in enhancing antidonor T-cell responses and facilitating tolerance following transplantation. Amongst the multitude of costimulatory pathways described [102], the best-characterized and perhaps most important interactions occur between CD40 and CD154 (CD40L) in the TNF:TNFR family, and CD80 (B7-1)/CD86 (B7-2) and CD28 of the immunoglobulin superfamily. The exact mechanism by which these molecules exert their effects remains incompletely defined; however, inhibition of these pathways using transgenic knockout mice, monoclonal antibodies, or fusion proteins has demonstrated profound effects on the immune response [103].

Lenschow et al. first reported that CTLA4Ig, a soluble fusion protein of CTLA4 that binds the B7 molecules, blocked pancreatic xenoislet rejection and induced donor-specific tolerance in mice [104]. Similar survival of mouse pancreatic islet allografts was subsequently obtained targeting the CD40:CD154 pathway with an anti-CD154 antibody [105]. Larsen et al. then showed that long-term acceptance of skin and cardiac allografts in a more stringent mouse model required simultaneous but not independent blockade of the CD28 and CD40 pathways [95]. These instances of tolerance induction in select rodent transplant models following blockade of the CD28 and CD40 costimulatory pathways have been replicated in NHP transplant models [96,97,106], but identification of clinically applicable strategies that consistently induce tolerance in large animal preclinical models has so far been elusive [19,20,107]. Furthermore, a pilot study using a humanized CD154-specific antibody in human renal transplantation raised concerns about the efficacy of this strategy, as data showed that five of seven patients experienced rejection episodes [108].

The reasons for the apparent discrepancies between the results in rodents, primates, and humans remain unclear, but may, in part, be due to the increased complexity of the human immune system and the more diverse and longer-term environmental exposure that humans experience compared with laboratory animals [19,20,107]. Though not completely understood, further studies have identified mechanistic barriers to the development of tolerance by way of costimulation blockade [102,109]. They include duplicitous positive and regulatory effects of some costimulatory pathways and heterologous immunity mediated by alloreactive memory T cells [110], the nature of which is covered in detail in Chapter 9.

The discovery that CTLA4, which binds CD80 and CD86 more avidly than CD28 and competitively inhibits rather than enhances T-cell activation, presented one of the first mechanistic challenges for tolerance induction by B7 costimulatory blockade [111,112]. Because blockade of B7 might inhibit differential signaling pathways, modulation of T-cell costimulation in attempts to promote allograft tolerance proved to be more complex than simply blocking primary positive costimulatory interactions. As a result, it is not surprising that blocking CD28:B7 alone has neither been universally effective in generating tolerance in rodent transplant models nor has it been able to induce tolerance in NHPs [20,113]. Blockade of the CD40:CD154 pathway has also had its share of challenges. Despite initial enthusiasm for CD154-specific antibodies, thromboembolic complications in NHPs and humans almost led to abandonment of strategies aimed at targeting the CD154 side of this pathway [108,114]. Fortunately, alternative targeting of CD40 has been efficacious in preclinical studies [115–119] and CD40-specific agents are currently in clinical development [120]. However, the CD40:CD154 story highlights that although costimulatory molecules are generally viewed through their effects on T cells, receptor-ligand interactions are bidirectional with potential for significant immune and non-immune consequences. Both inadvertent inhibition of negative regulatory mechanisms or non-T-cell and non-immune bystander effects of costimulation blockade could negatively impact tolerogenesis.

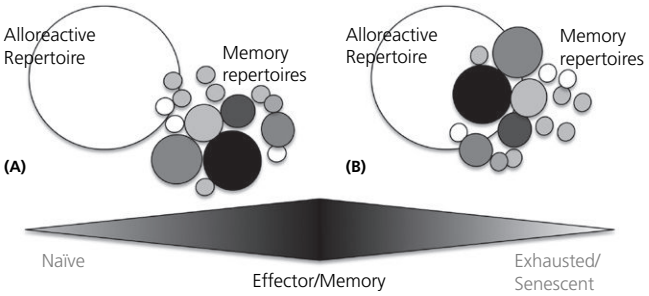


Figure 11.3. High frequencies of donor-specific memory present a barrier to tolerance induction. In scenario A, a low frequency of alloreactive memory repertoires favors tolerance induction, as costimulation blockade potently blunts naïve donor-specific T-cell responses. Conversely, the high frequency of donor-specific memory in scenario B presents a barrier to costimulation blockade-based tolerance induction strategies.

Blockade of T-cell costimulatory pathways can potently blunt naïve donor-specific T-cell responses following transplantation, but the presence of donor-reactive memory T cells poses a sometimes insurmountable barrier to long-term graft survival and tolerance induction [109] (Figure 11.3). Memory T cells can reject allografts independent of secondary lymphoid organs [121] and are known to be relatively resistant to a variety of therapeutic interventions [86,122], including costimulatory blockade [110,123–125]. In addition, $CD4^+$ and $CD8^+$ precursor frequency plays a critical role in determining the ability of T cells to mediate rejection in the setting of costimulation blockade following transplantation [64,65]. More recently, the presence of high frequencies of donor-reactive T-cell memory have been reported to impair tolerance induction to kidney allografts in NHPs [126]. Thus, a high frequency of alloreactive memory T-cell populations in transplant recipients may obviate the need for costimulation and play a large role in mediating costimulation blockade-resistant allograft rejection and tolerance prevention [109,127].

The complexity of costimulatory pathways presents both challenges and opportunities in employing costimulation blockade to promote transplant tolerance. Costimulatory molecules regulate multiple aspects of the alloimmune response, from priming of naïve cells to the development of memory and regulatory T cells, and have both negative and positive effects that may function to either augment or attenuate antigen-specific T-cell responses. It has become clear, as with other immunosuppressive methods, that a costimulation blockade alone approach to the generation of tolerance is not feasible. However, with the recent introduction of belatacept [128], a high affinity CTLA4Ig for clinical use, costimulation blockade can be folded into clinical regimens to be tested in humans, and like depletional antibodies, the clinical availability may drive investigation over more theoretically attractive approaches.

Several possible alternative approaches may harness the therapeutic potential of targeting T-cell costimulation to achieve tolerance. Negative costimulatory molecules present a physiologic

means of dampening or terminating immune responses and maintaining peripheral tolerance. The best-studied is CTLA4, as it plays a key role in immune regulation [129] and its signaling has been required for tolerance induction in several studies [130–132]. Strategies aimed at enhancing CTLA4 signaling may prove useful given the potency of this endogenous tolerogenic pathway. Alternatively, selective targeting of memory T cells in conjunction with costimulation blockade has successfully neutralized this costimulation-resistant population to prevent allograft rejection in NHP renal and islet transplant models [133,134]. The targeting of numerous other costimulatory molecules has shown in rodents to evoke at times dramatic effects. However, the cataloging of each costimulation blockade approach is beyond the scope of this chapter, and these additional pathways have been extensively reviewed elsewhere [93,102,109,135,136].

Recent history has seen a shift in the use of costimulation blockers primarily as a tool for tolerance induction toward exploitation of their potential as primary maintenance drugs in CNI-free regimens [92]. Encouraging clinical results from studies in renal transplantation targeting the CD28 pathway using belatacept [128,137,138] suggest that costimulation blockade may someday be a component of the future therapeutic regimen that provides transplantation tolerance.

Regulation

As Peter Medawar conjectured from his early description of "actively acquired tolerance," the mechanisms of tolerance are probably reversible, continually being threatened by emerging effector cells, inflammatory cytokines, and revolving contexts with general inflammatory conditions and pathogen exposures. Thus it is logical that active suppressive mechanisms are involved in the maintenance of tolerance. For allograft survival following transplantation, let alone tolerance induction, it is critical that both the innate and adaptive immune responses be controlled. Whether immune cells promote rejection or facilitate tolerance depends on both the origin of the cells and temporal conditions in transplant recipients. Although leukocyte populations such as macrophages, dendritic cells, B cells, and T cells can mediate destruction of transplanted tissues, they can also promote graft survival and tolerance [139]. The topic of regulation is considered in depth in Chapter 8.

Early experiments over 20 years ago showed that, under certain circumstances, $CD4^+$ T cells could prevent damaging immune reactions [140–143]. Studies showed that rats with long-term allograft survival off drug-based immunosuppression had T cells that could actively prevent graft rejection [144,145], and that the adoptive transfer of such a population of cells in a rodent model conferred donor-specific tolerance [146]. This form of cellular regulation in transplantation was later found to be associated with $CD4^+$ T cells that express CD25, the α-chain of the IL-2 receptor [147–149], and that $CD4^+CD25^+$ T cells could suppress T-cell proliferation [150]. Ultimately, murine studies implicated Foxp3 as the transcription factor needed to direct $CD4^+$ T cells to become regulatory cells [151–153]. These regulatory cells have been found to be increased in the circulation and within the grafts of operationally tolerant liver and kidney transplant recipients [154–159].

$CD8^+$ T cells [160–162], $CD4^-CD8^-$ T cells [163], natural killer T cells [164], and $\gamma\delta$ T cells [165] comprise more newly identified T-cell populations with regulatory activity, but $CD4^+CD25^{hi}Foxp3^+$ regulatory T cells (Tregs) remain the best characterized with the most promising experimental results. They function to maintain immune tolerance to self-antigens and provide negative feedback mechanisms to keep immune reactions in check [166]. Tregs are classified as either naturally occurring cells centrally selected in the thymus [167], or induced cells that encounter antigen in the periphery in a tolerogenic environment and differentiate into induced Tregs [168]. Various cellular mechanisms by Treg cells to inhibit the effector immune response have been described [169]. They include direct cellular contact inhibition of effector T-cell proliferation [170] and the induction of cytolysis or cell cycle arrest and apoptosis of target cells such as antigen presenting cells (APCs) [171–173]. Tregs also indirectly induce suppression via paracrine regulation by secreting the suppressor cytokines IL-10, TGF-β, and IL-35 [148,174], or consumption of the proliferative cytokine IL-2 [150,175]. Furthermore, constitutively expressed CTLA4 on Tregs can block T-cell access to necessary costimulatory signals for activation due to a higher affinity for CD80/CD86 than CD28, and can lead to attenuated T-cell proliferation via CD80/CD86-mediated activation of indoleamine 2,3-dioxygenase in APCs [176].

Upon initial alloantigen encounter after transplantation there are usually insufficient Treg cells to prevent the rejection of an allograft, particularly in the presence of a high frequency of donor-specific memory T cells [122]. This imbalance favoring alloantigen-reactive T cells will shift the immune response toward allograft destruction. However, with initial control of the effector alloimmune response, Treg cells can be peripherally induced or thymus-derived cells expanded in the prolonged presence of alloantigen—thus theoretically tipping the balance toward regulation [177–179]. This balance between destruction and regulation provides an opportunity to manipulate the alloimmune response in favor of tolerance either before or after transplantation by inhibiting the activity of effector T cells and/or increasing the relative frequency or functional activity of donor-specific Tregs [180–183].

Non-T-cell regulatory immune cell populations have arisen as potentially important mediators of allograft tolerance. Interest in B-cell-mediated tolerance arose from clinical findings of higher B-cell-associated gene expression in tolerant human kidney transplant recipients [184–186]. Human regulatory B cells identified as $CD19^+CD24^{hi}CD38^{hi}$ have the ability to secrete the proregulatory cytokine IL-10 [187–189], but their exact role in alloimmunity remains to be defined. Regulatory macrophages characterized by their morphology, cell-surface phenotype, and their ability to suppress T-cell proliferation in vitro also produce IL-10 and are thought to dampen proinflammatory immune responses [190,191]. Though dendritic cells play a critical role in the generation of antigen-specific effector T cells, they too have been described to promote tolerogenic responses [192,193].

It is probable that the overall interactions of the regulatory-cell-mediated mechanisms described above, rather than their individual effects, determine their promotion of allograft protection and tolerance. As reviewed by Wood et al. [194], hypothetically, early in the immune response to an allograft, hematopoietic progenitor cells attracted to the graft by innate inflammatory mechanisms may initiate immunomodulatory activity and create a microenvironment favoring the generation of regulatory macrophages and dendritic cells. Such a tolerogenic microenvironment could then serve to peripherally induce Tregs upon the arrival of returning activated T cells from secondary lymphoid organs, aided by modulation from thymus-derived Tregs and regulatory B cells. These processes are all dependent on aid from immunosuppressive therapy to help tip the balance away from destruction of the allograft to tolerance

while alloantigen-specific regulatory mechanisms are allowed to mature.

Key to the discussion of regulatory mechanisms of tolerance is the net in vivo effect of current immunosuppressive strategies on effector cell populations and the generation of immunomodulatory cell populations, as it is variable and dependent on the combination and timing of agents used. For example, lymphocyte depletion has the potential to reduce the number of effector cells and allow for repopulation with regulatory cell subsets to prevent rejection, as has been observed with alemtuzumab induction [195–197]. On the other hand, maintenance therapy with CNIs and antiproliferative agents (e.g. MMF) can inhibit both effector and regulatory cells. The effect of anti-CD25 antibodies on Tregs is debated, and sirolimus has been shown to support Treg generation ex vivo and promote function in vivo [198,199]. Data suggests that CTLA4Ig worsens outcomes in Treg-dependent models of transplantation by blocking CTLA4-mediated inhibition of T-cell activation [200–202]. Thus, the potentially competing components of different immunosuppressive protocols need to be thoroughly considered in efforts targeted at promoting regulatory mechanisms of tolerance.

Attempts at clinical translation of regulatory cell therapies in transplantation based on proof-of-concept preclinical animal studies [203,204] are underway and revolve around the transfer of Treg cells into transplant recipients with or without the aid of various immunosuppressive agents. The infusion of Tregs into transplant recipients, however promising experimentally, is clinically limited by their low frequency in vivo, prompting the need for isolation and expansion in vitro [205]. In vitro strategies include expansion of natural Tregs, induction of adaptive Tregs from precursor cells, and Treg generation by ectopic gene expression [206]. Current clinical experience has been limited to the BMT population [207–209], but the upcoming European Union "ONE Study" proposes the use of immunomodulatory cells, including expanded recipient natural Tregs in living-donor kidney transplantation. Nonetheless, the first trials of Treg cellular therapy in clinical HSC transplantation have been promising and provide a basis for future trials in solid organ transplantation.

B cells

Traditionally, T cells have been primarily blamed for allograft rejection, but humoral mechanisms mediated by alloantibodies have been recognized to play a significant role in antibody-mediated rejection (AMR) [210,211], the mechanisms of which are covered in depth in Chapter 6. AMR is thought to account for 2–10% of rejections with a greater incidence in sensitized patients [212], is characterized by donor-specific antibody formation [213] and histopathologic complement deposition [214], and correlates with poor long-term allograft survival. Studies within the last few years have provided increasing evidence that antibody-dependent and antibody–independent B-cell-mediated immune responses are a formidable barrier to the development of allograft tolerance.

Much of our knowledge of mechanisms of B-cell tolerance derives from studies of B cells in autoimmunity [215,216]. The random assembly of B-cell receptors through V(D)J genetic recombination leads to large receptor diversity, with as much as 20–50% autoreactivity [217–219]. Physiologic self-tolerance is achieved via both central and peripheral mechanisms. Central tolerance tends toward the elimination of high-affinity autoreactive B cells by clonal deletion or receptor editing [220–222]. Self-antigen stimulation of autoreactive B-cell receptors leads to receptor internalization and arrest of B-cell maturation. Alternatively, cell death of autoreactive cells can occur through increases in proapoptotic factors such as Bcl2-like protein 11 and inhibition of B-cell survival proteins [223,224]. For autoreactive B cells that escape clonal deletion, continued expression of *RAG* genes responsible for V(D)J recombination lead to receptor editing and new receptor combinations that overcome autoreactivity [225–227]. Peripheral tolerance mechanisms include anergy induction in low-affinity transitional B cells that escape central mechanisms via well-described molecular changes that raise activation thresholds and turn on intrinsic regulatory pathways of autoreactive B cells [228,229]. Low-affinity autoreactive B cells can also be rendered clonally ignorant by emerging extrinsic B-cell mechanisms responsive to homeostasis of the peripheral B-cell population [230], such as competition for a follicular growth factor with limited availability known as TNFSF13B [231]. Further insight into these central and peripheral mechanisms of physiologic B-cell regulation will guide the development of methods to achieve B-cell tolerance.

Current B-cell-targeted therapies for AMR fail to effectively control B-cell-dependent effector immune responses. Non-selective alloantibody removal by plasma exchange is limited by a compensatory homeostatic response of antibody production induced by hypogammaglobulinemia, and generalized humoral suppression with agents such as rituximab (anti-CD20), bortezomib (proteasome and plasma cell inhibitor), and eculizumab (anti-C5) does not achieve stable immune suppression or tolerance amid safety concerns [232–234]. Thus, the need for emerging therapies for B-cell tolerance is clear, but as with T cells, B-cell tolerance has been more difficult to achieve in humans than in animal models. Nonetheless, observations derived from animal models of allograft transplantation have served as the foundation for modern clinical strategies that aim to achieve allograft tolerance by targeting B cells [19,20,235].

Neonatal tolerance has been observed between cattle with shared placental circulations [236] and in mice after fetal infusions of alloantigen, possibly by clonal deletion of alloreactive B cells [23]. Human monochorionic dizygotic twins with different blood groups also develop neonatal tolerance to each other's blood group in utero [237]. Similar B-cell tolerogenic mechanisms presumably facilitate successful ABO-incompatible transplantation in infants, as they do not produce ABO-specific antibodies until 5–6 months of age and therefore are at low risk of hyperacute allograft rejection [238]. Human ABO-incompatible heart transplantation has been performed in infants without hyperacute rejection or ABO-incompatibility related problems [239]. The mechanisms responsible for this observation seem to involve transient deletion of ABO-specific B cells dependent on the presence of alloantigen, as B cells specific for the previously mismatched ABO epitope reappeared in recipients subsequently transplanted with an ABO-compatible heart graft [240]. Thus, in contrast to neonatal tolerance, which seems to involve centrally based mechanisms, allograft tolerance seems to require peripheral antigen. Additional treatment of this topic can be found in Chapter 112.

A 2004 study of ABO-incompatible heart transplant recipients suggested that B-cell tolerance to donor-specific blood group antigens can develop spontaneously [241]. Although these patients did not develop tolerance to donor HLAs, the development of B-cell tolerance to ABO blood group antigens provided the first demonstration that neonatal tolerance could be acquired in humans. ABO-incompatible transplantation has also been attempted in renal and liver transplantation [242,243], with most success in

pediatric recipients less than 1 year of age [244,245]. Further understanding of tolerogenic mechanisms in infants will greatly enable the development of strategies for achieving B-cell tolerance to allografts in adult patients.

The role of B cells in mixed chimerism protocols is unclear. Mixed chimerism of multilineage hematopoietic cells is associated with centrally mediated tolerance of T cells and B cells to donor allografts [246]. In one of the clinical mixed chimerism studies discussed above [48], renal allograft tolerance was achieved in four of five patients but one recipient experienced allograft loss due to acute AMR and two others developed alloantibodies despite transient mixed chimerism, indicating that tolerance can coexist with some level of B-cell reactivity to donor alloantigens. Though the relationship between transient mixed chimerism and B-cell-mediated tolerance has not been established, given the transient nature of the chimerism achieved, long-term tolerance is unlikely to result from clonal deletion of B cells alone. In humans, therefore, it is probable that other mechanisms of peripheral B-cell tolerance are involved.

Lymphocyte depletion non-specifically reduces the number of T-cell and B-cell precursors specific for alloantigen. However, plasma cells and some B-cell subsets resistant to lymphocyte-depletion therapy might pose an important barrier to the induction of allograft tolerance [85,247]. While depletional therapies have generally reduced rejection rates, their effects on B-cell tolerance have not been evaluated. In fact, some depletional agents might be associated with increased rates of AMR [248,249], as homeostatic proliferative activation of T cells might promote T-cell-mediated B-cell antibody production. Thus, the linear notion that partial B-cell depletion can reduce B-cell function and minimize AMR might be fundamentally flawed and requires further study.

Moreover, substantial depletion of T cells and B cells has been associated with increased production of TNFSF13B [88], a cytokine critical for B-cell survival, maturation and differentiation [250–252]. Increased TNFSF13B production is perhaps a response to homeostatic pressure to replace depleted lymphocytes and might lead to paradoxical B-cell activation in the setting of B-cell lymphopenia. Modulating B-cell homeostasis and survival via targeting of TNFSF13B has potentially important implications for transplantation tolerance. For example, ablation of B-cell clones in combination with controlled repopulation using TNFSF13B inhibitors might facilitate the induction of transplantation tolerance, because the newly emerged B cells could undergo induction of tolerogenic mechanisms in response to exposure to donor antigens.

An exploratory mechanistic study analyzed the gene-expression profiles in subsets of peripheral blood lymphocytes in 25 spontaneously tolerant kidney transplant recipients from the Immune Tolerance Network registry [184]. In comparison to patients with stable graft function on immunosuppression and healthy controls, gene expression of tolerant patients suggested that allograft tolerance was strongly associated with an increased expression of multiple genes involved in B-cell differentiation. This subset of B-cell-related genes or "B-cell signature" was consistent with the up-regulation of CD20 messenger RNA in urine sediment cells and the elevated numbers of naïve and transitional B cells in spontaneously tolerant kidney transplant recipients. Similarly, a study from the Indices of Tolerance European Union consortium [185], which analogously screened 11 operationally tolerant kidney transplant recipients, cohorts of immunosuppressed recipients exhibiting allograft injury and healthy controls, found that peripheral blood from tolerant patients contained higher levels of B-cell-related genes than peripheral blood from the other patients. These results suggest a critical role for B cells in the regulation of the alloimmune response in transplant-tolerant kidney graft recipients.

Summary

The field of transplantation remains burdened by a requirement for chronic immunosuppression, making immunologic tolerance a most appealing solution to combat poor long-term allograft outcomes. Rejection is an aggregate phenotype influenced by multiple interrelated pathways, all of which must be mollified to achieve donor-specific tolerance. Thus, a combination of the current strategies reviewed in this chapter is most likely to comprise the first consistent tolerance-inducing protocol. While purposeful mixed chimerism approaches have had early successes that thus far appear unrelated to durable mixed chimerism, a single proven regimen has not been developed. Lymphocyte depletion alone does not lead to tolerance and, in fact, alters homeostasis in ways that may promote immunity. Costimulation pathways have clear and potent influence over immune responsiveness, but are most relevant for naïve responses. Although the CD28:B7 pathway has advanced to the clinic in the form of belatacept, it will surely require adjuvant therapies if it is to be used as a tolerogen. Increasingly, identified regulatory and B-cell mechanisms are bound to factor significantly in future tolerance studies, and ongoing coordinated clinical efforts to identify larger numbers of operationally tolerant transplant recipients and study them should shed light on the mechanisms responsible for transplantation tolerance.

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CHAPTER 12

Xenoimmunity

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Introduction

Xenotransplantation involves the transplantation of organs across species lines. While technically inclusive of all combinations of donor and recipient species combinations, the field of xenotransplantation has driven almost exclusively toward the pig to human combination as the most clinically relevant combination. Clinical xenotransplantation using pigs as donors has great potential to solve the shortage of human organs, tissues and cells that currently limits allotransplantation. The major barrier to clinical xenotransplantation is immunological rejection of the graft. Unless this key obstacle can be overcome, other important issues such as physiological compatibility [1] and infectious risk [2] become irrelevant.

Most of this textbook is about allotransplantation, and when we think of immunity in transplantation our minds naturally focus on those mechanisms that are critical in allotransplantation, specifically cellular and humoral adaptive (or acquired) immunity (discussed in depth in Chapters 5 and 6, respectively). In such a transplant into a non-sensitized recipient, the first obvious sign of the immune response is the onset of what was initially termed first-set rejection, typically manifesting after about a week, and the main mechanism is a T-cell-mediated delayed type hypersensitivity response. That xenotransplantation should be approached with a different mindset should be obvious when we note that the timing of the first manifestations of rejection is completely different, occurring within minutes. This is due primarily to the binding of preformed antibodies and a heightened innate immune response. Elements of the innate immune system are known participants in both allo- and xenotransplantation (see Chapter 7), but their involvement in xenotransplantation is far more primary. While an allograft excites some activation of the innate immune system due to the tissue damage associated with the transplant procedure, the xenograft is itself a source of molecular differences that are powerful and rapid activators of innate immunity. However, that is just the beginning of a complex story in which there are not only critical differences but also important similarities between xenoimmunity and alloimmunity. As such, the student of xenotransplantation will find this chapter to be complemented by the chapter on physiologic immunity (Chapter 2), and the chapters on fundamental alloimmunity (Chapters 5–7), particularly when concerning the later forms of xenograft rejection.

Dissecting the mechanisms of xenoimmunity has relied on a number of small and large animal models, each with their own blend of contributing factors and with widely varying degrees of relevance to clinical pig-to-human xenotransplantation. As an exhaustive discussion of these models is beyond the scope of this chapter, most of the focus here will be on the pig-to-non-human primate (NHP) preclinical model. These discussions complement those in Chapter 15, which outlines pig and primate large animal models in general.

Rejection of porcine vascularized xenografts

Trying to prevent the rejection of solid organ xenografts is like peeling an onion—it is only as each layer is removed that the next one becomes fully apparent. The study of porcine heart and kidney xenografts has been pivotal to understanding the rejection process because they are the only organs that can be coaxed to survive for longer than a week in NHP recipients. This section will draw mainly from the cardiac and renal pig-to-NHP literature to describe the phases of rejection, the mechanisms responsible and the approaches being taken to promote long-term xenograft survival.

Hyperacute xenograft rejection

Anti-αGal antibodies and complement: the key mediators of hyperacute rejection

Hyperacute rejection (HAR) is an extremely potent process in which porcine xenografts sustain terminal injury within minutes to hours of transplantation into untreated recipients (Figure 12.1). Like HAR of blood group ABO-incompatible allografts, HAR of xenografts is due to differences in glycosylation between the donor and the recipient. Most species, including pigs, express a glycosyltransferase called α1,3-galactosyltransferase (also known as GalT) that synthesizes the terminal carbohydrate structure galactose-α1,3-galactose-β1,4-*N*-acetyl-glucosamine (Galα1,3-Galβ1,4-GlcNAc) on glycoproteins and glycolipids. This moiety, called the αGal epitope, differs from the blood group B antigen only by the absence of a fucose residue attached to the penultimate galactose. αGal is present in abundance on the surface of most pig cells, notably on vascular endothelial cells where the estimated density is more than 10 million epitopes per cell [3]. In contrast, humans and their closest relatives, the apes and Old World monkeys, lack αGal expression due to frameshift and nonsense mutations in the coding region of the GalT gene [4]. Continual exposure to αGal on gut bacteria induces the development of anti-αGal IgM and IgG antibodies in these species [5]. In humans, anti-αGal is made by up to 1% of B cells [6], comprises about 80% of preformed ('natural')

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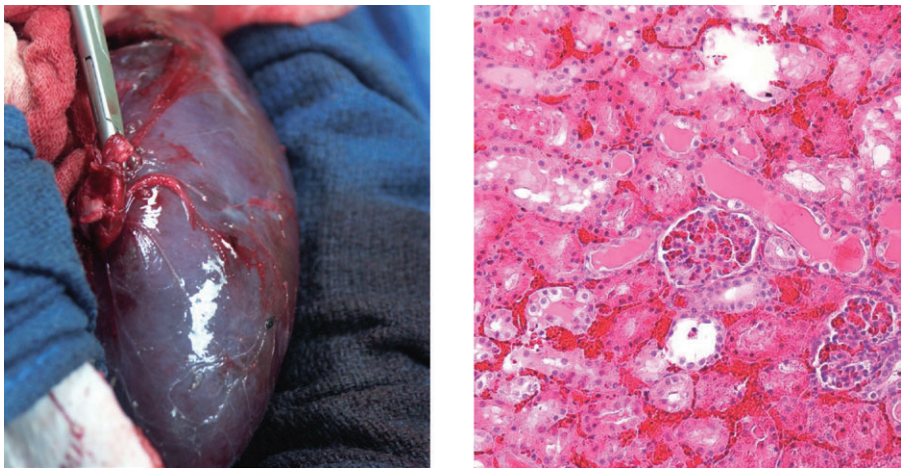

(A) (B)

Figure 12.1. Hyperacute xenograft rejection. The gross (A) and histological (B) appearance of hyperacute rejection of a pig-to-baboon kidney xenograft is shown, minutes after implantation. Note the vascular congestion seen grossly and indicative of pangraft thrombosis, and the substantial haemorrhage evident histologically indicative of a primary disruption of vascular integrity.

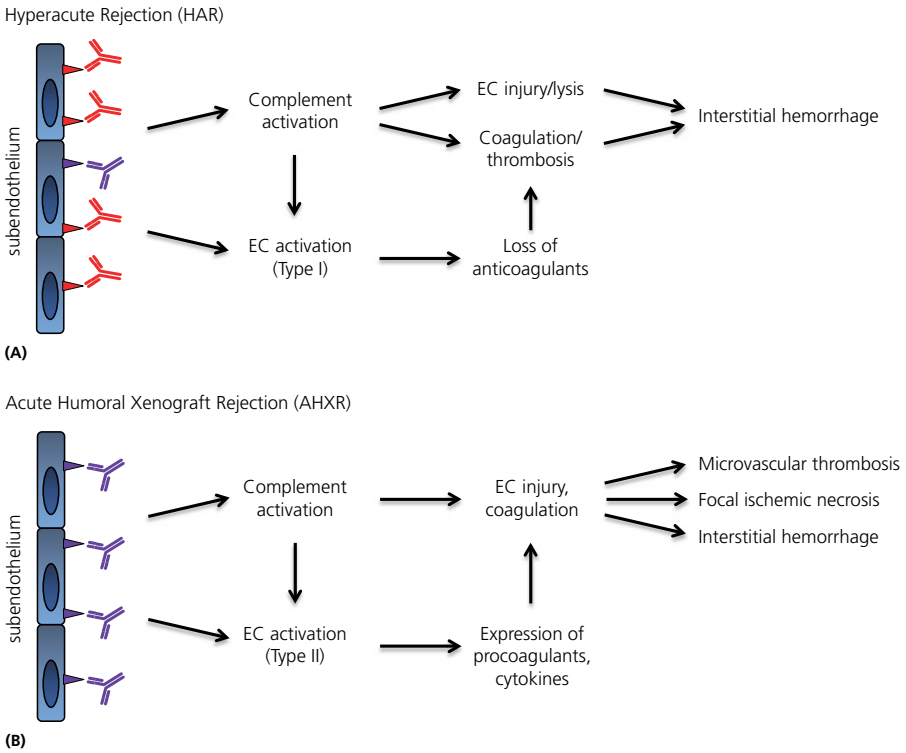

Figure 12.2. The phases of organ xenograft rejection. (A) Hyperacute rejection is initiated by the binding of preformed anti-αGal antibodies (red) to xenograft endothelial cells (EC), triggering a chain of events that culminate in vascular injury and massive interstitial haemorrhage. (B) Acute humoral xenograft rejection, exemplified here in a GTKO xenograft, is an extended process in which binding of elicited non-Gal antibodies (purple) drives a gradual conversion of the endothelial environment from anticoagulant to procoagulant.

antipig IgM [7], and is the most abundant naturally occurring IgG [8]. The corollary is that all humans possess high levels of preformed cytotoxic antibodies to an antigen that is densely expressed on the surface of pig blood vessels—a recipe for disaster for porcine organ xenografts.

Binding of anti-αGal IgM antibodies to the vascular endothelium of the xenograft triggers the classical pathway of complement activation, which in turn activates the coagulation cascade (Figure 12.2A). The resulting pathogenesis is striking at both the macroscopic and microscopic levels. Grafts change colour soon after

reperfusion, becoming mottled and dark, and initial signs of function (if any) rapidly disappear. A detailed histopathological analysis of hyperacutely rejected cardiac xenografts identified thrombosis of veins and venules as the initiating event, followed by the development of congestion, oedema and massive interstitial haemorrhage [9]. These features are the hallmarks of HAR. Immunohistochemical analysis of the early stages of HAR showed significant deposition of IgM, activated complement components and platelet aggregates, with expression of P-selectin but little evidence of endothelial cell lysis or apoptosis [10]. The overall picture is consistent with type I activation of endothelial cells, a protein synthesis-independent process involving the opening of cellular junctions, exocytosis of the platelet ligand P-selectin from Weibel–Palade bodies and loss of cell-surface anticoagulants [11]. The mediators of type I activation of xenograft endothelial cells are antibody binding and sublytic activation of complement [12,13]. At the later stages of HAR, numerous apoptotic endothelial cells are observed [10], potentially promoting further thrombosis by exposure of phosphatidylserine [14] and release of tissue factor-bearing microparticles [15].

Homologous restriction and membrane complement regulators: quality versus quantity?

One could speculate that cross-species incompatibilities contribute to the failure to efficiently regulate complement activation in HAR. In general, complement efficiently lyses 'non-self' cells from different species but not 'self' cells [16]. An early hypothesis to explain this phenomenon, known as homologous restriction, was that membrane-bound complement regulatory proteins (CRPs) from one species are unable to inhibit complement of another species [17,18]. Like humans, pigs express CD46 (membrane cofactor protein), CD55 (decay accelerating factor or DAF) and CD59; perhaps the devastating rapidity of HAR could be partly explained by an inability of these porcine CRPs to inhibit activation of primate complement. However, more recent studies have shown that homologous restriction is a patchy rather than a universal phenomenon, and that the three pig CRPs regulate human complement with efficiency similar to their human homologues [19,20]. It is also salient that ABO-incompatible human allografts can be hyperacutely rejected [21] in the presence of an autologous complement regulatory system. The ability of human (h)CRPs to prevent HAR of transgenic porcine xenografts (see below) is probably due to an elevated overall capacity to regulate complement, rather than the correction of an intrinsic molecular incompatibility.

Prevention of HAR

Conventional immunosuppression is not designed to deal with high levels of preformed antibodies and is thus ineffective against HAR [22]. A number of studies have shown that treatment of the recipient to prevent HAR requires targeting one or both of its essential components, complement and anti-αGal antibodies. For example, systemic treatment with cobra venom factor to deplete complement delayed the rejection of a cardiac xenograft from less than 1 hour to 6 days [23]. Similarly, depletion of anti-αGal antibodies by immunoadsorption delayed the rejection of renal xenografts to a mean of 7 days, although all grafts ultimately rejected when circulating anti-αGal returned [24]. While these approaches have provided valuable insights into the mechanisms of HAR, they are of limited practical benefit in the longer term because they usually afford only transient protection and may compromise the recipient's resistance to infection.

A technique that is theoretically superior to systemic therapy—and that is unique to xenotransplantation—is genetic modification of the donor. Two groups pioneered this approach in 1994 with the generation of transgenic pigs expressing hDAF [25] or hCD59 [26], followed later by pigs expressing hCD46 [27,28] or combinations of the hCRPs [29–32]. With rare exceptions [33], organs from hCRP-transgenic pigs are protected from HAR, whether or not other treatments are employed [27,28,30,31]. There are theoretical arguments for the use of particular hCRPs: DAF and CD46 because they regulate complement upstream of CD59; CD46 because it regulates the alternative pathway more effectively than DAF [28]; CD59 because of the importance of the membrane attack complex, and because thrombin can bypass regulation by DAF and CD46 by directly activating C5 [34]. However, it is not yet clear which hCRP or combination thereof will provide optimal complement regulation beyond HAR.

The second major breakthrough in genetic engineering of the donor pig was the deletion of the GalT gene [35–40]. The resulting GalT knockout (GTKO) pigs lack αGal expression but otherwise appear to be completely normal, and as expected develop anti-αGal antibodies [41]. GTKO renal and cardiac xenografts are protected from HAR in the pig-to-NHP model [42–47], confirming the central role of αGal in the rejection process.

Preformed antibodies to non-αGal determinants

While anti-αGal is the predominant natural antipig antibody in humans and higher primates, preformed antibodies with other specificities have been detected by incubating sera from non-sensitized individuals with endothelial cells or peripheral blood mononuclear cells from wild-type and GTKO pigs [48–51]. It has been suggested that many of these natural 'non-Gal' antibodies recognize carbohydrate targets expressed in pigs but not in humans [52], such as the Hanganutziu–Deicher antigen [53]. Preformed non-Gal IgG and IgM antibodies vary considerably in titre and are capable of inducing complement-dependent lysis and antibody-dependent cellular cytotoxicity (ADCC) to porcine cells [50]. However, the consistent survival of GTKO xenografts beyond 24 hours in the pig-to-NHP model suggests that they rarely play a role in HAR.

Acute humoral xenograft rejection

When HAR is averted, conventional immunosuppression usually prolongs the survival of pig-to-NHP cardiac and renal xenografts for days to weeks. Most xenografts are rejected during this period by a second antibody-dependent process known as acute humoral xenograft rejection (AHXR) (Figure 12.2B), also referred to as acute vascular rejection (AVR) or delayed xenograft rejection (DXR) [54–57]. The interchangeable terms for this phase of rejection reflect a degree of uncertainty about the underlying mechanisms, but a role for even low levels of antigraft antibodies seems quite well established. The immunopathological and histopathological signs of AHXR include antibody and complement deposition, microvascular thrombosis, focal ischaemic necrosis and interstitial haemorrhage, and endothelial cell changes ranging from swelling to severe damage and apoptosis [47,56,58]. Perivascular infiltration by lymphocytes and macrophages may also be observed, but usually only when immunosuppression has been relatively ineffective. The occurrence and severity of the features of AHXR are somewhat variable depending on the genetic background of the donor (e.g. hCRP-transgenic and/or GTKO), the immunosuppressive regimen and even the type of organ.

Just as type I activation of xenograft endothelial cells is integral to HAR, type II activation is a key element in the pathophysiology of AHXR [59]. Type II activation is a protein synthesis-dependent process triggered by a range of factors, typically proinflammatory cytokines such as TNF-α and IL-1 [11]. The mechanism involves activation of the transcription factors NF-κB and AP-1 and subsequent expression of proinflammatory proteins including chemokines and adhesion molecules; the resulting cytokine- and leukocyte-mediated injury to endothelial cells favours thrombosis [11]. Type II activation of porcine endothelial cells can be initiated by the binding of xenoantibodies even in the absence of complement activation [60].

The T-cell-dependent xenoresponse

As discussed in Chapter 5, donor antigens can be presented to recipient T cells by two pathways: direct, in which the T-cell receptor (TCR) engages mismatched MHC–peptide complexes on donor antigen-presenting cells (APCs); and indirect, in which recipient APCs take up and process donor molecules and present antigenic fragments within self-MHC to the TCR. The T-cell response in allotransplantation stems from a mixture of direct and indirect presentation, with the direct pathway predominating in acute allograft rejection [61]. (The significance of a third pathway [semidirect presentation] to allograft rejection remains unclear [62].) In contrast, the balance in acute xenograft rejection appears to be shifted in favour of indirect presentation [63,64]. Numerous porcine proteins and carbohydrates released by injury to xenografts provide the source material for the processing of xenoepitopes that can be presented by the indirect pathway. That is not to say that direct presentation does not play a role—the human TCR is capable of recognizing swine leukocyte antigen (SLA, the porcine equivalent of MHC) [65], and in vitro data suggest that porcine lymphocytes can directly activate human T cells [66]. The overall stimulus for T-cell activation is powerful, and among the consequences, T-cell help to B cells will be of major significance to the development of AHXR.

Identity of the antibodies and antigens responsible for AHXR

Elicited anti-αGal antibodies are the predominant cause of AHXR of αGal-positive xenografts [67]. When these antibodies are neutralized, AHXR can be induced by non-Gal antibodies, either preformed high-titre IgG or elicited IgG and IgM [68,69]. Non-Gal antibodies are also responsible for AHXR of GTKO organs [70]. The relevant non-Gal xenoantigens have not been unequivocally defined. Candidate carbohydrate xenoantigens include *N*-glycolyl-neuraminic acid (NeuGc), *N*-acetyl-neuraminic acid (NeuAc), and the T and Tn antigens [71]. The number of protein xenoantigens, on the other hand, could number in the hundreds or even thousands. Almost all pig proteins exhibit amino acid sequence differences to their human homologues and are thus potentially immunogenic. The induced non-Gal antibody response has been examined in NHP and human recipients of porcine xenografts. One NHP study showed that induced non-Gal antibodies to GTKO cells are encoded by a restricted number of germline progenitors and may bind to a carbohydrate epitope structurally related to αGal [48]. In human recipients, non-Gal antibodies to a large number of unidentified pig proteins were continuously produced, apparently from multiple B-cell clones, for as long as the xenograft remained in the recipient [72].

Possible effects of non-Gal antibodies on endothelial cell function

In addition to activating endothelial cells and mediating ADCC, elicited non-Gal antibodies may contribute to AHXR by interfering with critical vasculoprotective mechanisms. One group searched for xenoantigens by using sera from sensitized baboons, enriched in non-Gal IgG, to screen porcine endothelial cell expression libraries [73]. Four targets of non-Gal that were consistently identified were endothelial membrane proteins involved in the regulation of complement (CD46 and CD59) and thrombosis/ inflammation (endothelial protein C receptor and CD9). Although the ability of the sera to inhibit the function of these proteins was not assessed, it seems conceivable and perhaps even likely that the non-Gal antibody response can promote AHXR by blocking the natural defence systems of xenograft endothelial cells. This strengthens the argument for expressing the corresponding human proteins in pigs, as has been done for CD46 and CD59.

Prevention of AHXR

The deletion of αGal significantly prolongs the survival of cardiac and renal xenografts [74], but AHXR of GTKO organs has proven extremely difficult to prevent. Conventional immunosuppression capable of protecting renal allografts did not protect GTKO xenografts, which succumbed to the induced non-Gal antibody response [44]. This outcome highlights that drugs designed to suppress the acute T-cell alloresponse, which involves predominantly direct presentation, are less effective against the mostly indirect xenoresponse. More success has been achieved with costimulation blockade-based regimens, in particular those employing anti-CD154, with survival of GTKO ± hCD46 hearts for up to 8 months [43,75] and GTKO composite kidney–thymus grafts for nearly 3 months [42]. However, immunohistochemical analysis of the xenografts at rejection or recipient death indicated that non-Gal antibodies were not completely negated by these protocols. It is conceivable that molecular incompatibilities unique to the xenograft context, which will be discussed in the following two sections, promote a disproportionate response to even relatively low levels of xenoreactive antibodies.

Role of innate immune cells

As discussed in Chapter 7, an important component of innate immunity is the ability of innate immune cells to recognize and respond to pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs, respectively), primarily through Toll-like receptors [76,77]. The process of transplantation generates DAMPs irrespective of whether the donor is of the same or a different species. Where xenotransplantation differs from allotransplantation is that human innate immune cells appear to directly recognize porcine cells as foreign, largely because of differences in cell surface glycosylation [78,79]. There is also evidence that molecular incompatibilities affect particular cellular interactions, which will be discussed further below.

NK cells

Natural killer (NK) cells injure and lyse target cells by a variety of mechanisms. Although the role of these cells in solid organ allotransplantation has not been fully defined, data suggest that they are activated by the transplant process and contribute to acute rejection by producing inflammatory cytokines such as interferon gamma (IFN-γ) [80–82]. NK cells express a set of activating and inhibitory receptors, and their cytolytic activity is determined by

the balance of interactions between these receptors and ligands on the target cell. In the human allograft setting, the key NK receptors include the inhibitory killer-cell immunoglobulin-like receptor (KIR) family and NKG2A, which bind self but not allogeneic MHC class I, and the activating NKG2D receptor, which recognizes several 'stress' ligands that are up-regulated on allografts [83–85].

Several in vitro studies suggest that the human NK cell response to xenografts is more pronounced than that to allografts. FcγRIII-mediated ADCC, while considerably reduced by the elimination of αGal [86], is likely to remain a problem due to elicited non-Gal IgG antibodies [87]. Human NK cells are also capable of direct perforin/ granzyme B-mediated cytotoxicity to porcine endothelial cells [88]. Furthermore, there is evidence that pig cells provoke an exaggerated human NK cell cytotoxic response [89]. Gene sequence analysis indicates that SLA class I will not transmit an inhibitory signal to human KIRs and NKG2A [90]. In contrast, several activation pathways are fully operational (Figure 12.3). Human NKG2D efficiently binds ULBP1 expressed on pig endothelial cells, triggering NK cytotoxicity [91], and may bind an additional, as yet unidentified, porcine ligand(s) [92]. NKp44 also transmits an activating signal from an unknown porcine ligand [93]. The involvement of the human C-type lectin NKRP1A, which binds *N*-acetyllactosamine (NAcLac) and (with apparently lower affinity) αGal [94], is less clear. As the substrate of α1,3-galactosyltransferase, NAcLac might be expected to be exposed at increased levels in GTKO animals, but this has been demonstrated only in mice [94,95] and not in pigs [96]. However, irrespective of the role of NKRP1A, the balance between inhibitory and activating NK signals in xenotransplantation appears to be tilted towards activation.

A logical genetic strategy to attenuate human NK cell xenoreactivity is to change this balance by expressing inhibitory ligands on porcine tissue and/or deleting activating ligands. The latter is a more challenging proposition because of the number and diversity of activating ligands, some of which remain unknown, so most work in this area has focused on the expression of inhibitory signals. The most advanced example of this approach is the generation of transgenic pigs expressing the non-classical human MHC class I molecule HLA-E [97], following promising in vitro proof-of-concept studies [98,99]. HLA-E was used because it binds the inhibitory NK receptor NKG2A and is a poor inducer of allogeneic T-cell responses. Endothelial cells from the transgenic pigs were partially protected from human NK cell-mediated cytotoxicity in vitro in an NKG2A-dependent manner [97]. Determining in vivo efficacy will probably require crossing the HLA-E transgene onto an appropriate genetic background in which other more profound xenoimmune mechanisms have been addressed (e.g. GTKO ± hCRP-transgenic). The use of GTKO donors may itself reduce the NK cell xenoresponse, because several studies have demonstrated that human NK cells directly recognize αGal on pig endothelial cells, although this remains controversial [87].

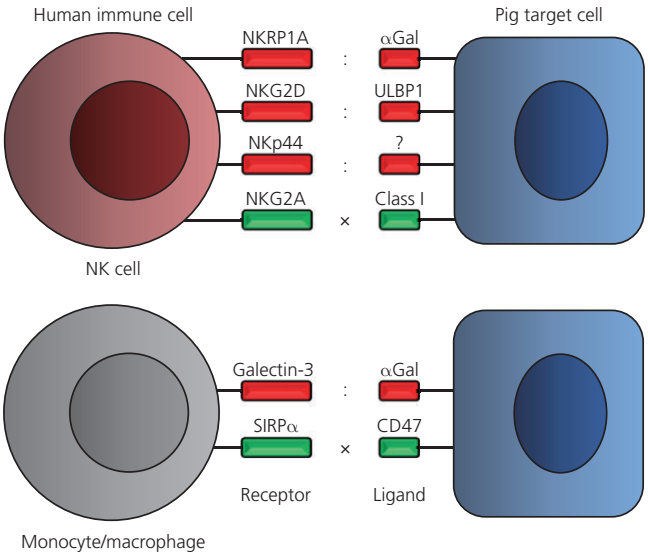

Figure 12.3. The heightened reactivity of human innate immune cells to porcine targets. Interactions between activating receptor/ligand pairs (red) are preserved, while key inhibitory interactions (green) appear to be non-functional due to molecular incompatibilities.

Macrophages

Infiltration by macrophages is a common feature of acute antibody-mediated renal allograft rejection [100], although evidence for a direct role in the rejection process is lacking [82]. Macrophages express receptors for Fc and complement and are therefore likely to be involved in antibody- and complement-dependent mechanisms of xenograft rejection. Human monocytes have been shown to directly recognize αGal on pig endothelial cells via the galectin-3 receptor [79]. Furthermore, as is the case for NK cells, cross-species molecular incompatibility may reduce the threshold for activation of human macrophages by porcine cells (Figure 12.3). SIRPα is a highly polymorphic macrophage inhibitory receptor that recognizes CD47 as a marker of 'self' on other cells [101]. In the absence of a functional SIRPα/CD47 interaction, macrophages are capable of phagocytosing haematopoietic cells [102]. Although human SIRPα was shown to bind porcine CD47 in vitro [103], subsequent studies indicated that this interaction does not send a negative signal and thus does not prevent human macrophages from engulfing porcine cells [104]. Consistent with this, expression of human CD47 on porcine cells inhibited their phagocytosis by human macrophages in vitro [104] and protected them from rejection in a mouse tumour model [105]. These results suggest that transgenic expression of human CD47 in pigs may be a useful strategy to prevent macrophage-mediated destruction of xenografts. The benefits of this approach are likely to be greatest for cellular xenografts such as haematopoietic stem cells (for tolerance induction) and islets.

Neutrophils

The role of neutrophils in allograft rejection remains largely unknown [82], but the fact that they are recruited and activated by antibody- and complement-mediated mechanisms argues for at least a secondary involvement in the rejection of xenografts. In addition, in vitro studies have shown that human neutrophils directly recognize, adhere to and activate pig endothelial cells in the absence of antibody and complement [106,107]. Should this interaction prove to be significant in vivo, it will be difficult to prevent. There are few treatment options to control neutrophil number and/or activity that do not leave the recipient susceptible to opportunistic infection. Genetic modification of the donor to express human CD47 may be beneficial because neutrophils, like macrophages, are known to express SIRPα, although the incompatible human

SIRPα/pig CD47 interaction seems to be of greater significance for macrophage activation. Identification and deletion of the porcine molecule(s) directly recognized by human neutrophils, which does not appear to be αGal [107], is another avenue.

Dysregulated coagulation

Graft endothelial cells are activated by a range of mechanisms including ischaemia–reperfusion injury (IRI), antibody binding and complement activation [108]. In extreme circumstances, the procoagulant effects of endothelial cell activation can result in allograft loss. Thrombotic microangiopathy (TMA), a complication of renal allotransplantation, is an example of this. TMA in renal allografts is triggered de novo by immunosuppressive drugs or acute antibody-mediated rejection, and is characterized by vessel wall thickening and microvascular thrombosis [109]. It is frequently associated with a reduced capacity to regulate the alternative pathway of complement activation. Renal allograft recipients with mutations affecting the circulating alternative pathway regulators factor H and factor I appear to be at increased risk of de novo TMA [110]. This not only reflects the interplay between the complement and coagulation pathways, but also points to some of the unique problems that may be faced by organ xenografts. The routes to activation of complement in xenotransplantation are more numerous and varied than in allotransplantation, and induced antibodies have been shown to target regulators of complement expressed on porcine endothelial cells [73].

Indeed, while severe coagulation is rare in allotransplantation, it is relatively common and difficult to deal with in xenotransplantation. Dysregulated coagulation is one of the major current challenges in organ xenotransplantation [74,111–113]. TMA is frequently observed in rejected cardiac and renal pig-to-NHP xenografts [114,115]. Even when the humoral response appears to have been effectively suppressed, TMA still develops and is a major cause of xenograft failure [43,45,116]. TMA in xenografts is often accompanied by the development of consumptive coagulopathy, characterized by thrombocytopenia (often profound), a falling fibrinogen concentration, increased levels of D-dimer and thrombin-antithrombin complex, and prolonged clotting time. Coagulopathy is common in renal xenograft recipients [30,117–120], and can only be treated by removal of the graft [118]. Recipients of cardiac xenografts seem to be less prone to developing the condition [121,122], although not without exception [116]. It has been suggested that these different outcomes may be due to differential gene responses in the endothelium of renal and cardiac xenografts, with up-regulation of von Willebrand factor (vWF) and P-selectin (favouring platelet deposition) in the former and of plasminogen activator inhibitor-1 (favouring fibrin deposition) in the latter [123].

Dysregulated coagulation also plays a major role in lung and liver xenotransplantation. Long-term function of lung xenografts has not yet been achieved in the pig-to-NHP model. Xenoreactive antibodies, complement activation, pulmonary macrophages, shedding of vWF by pulmonary endothelium and poor control of coagulation have all been suggested to play a role in the rejection process [124,125]. The maximum reported graft survival, achieved using vWF-deficient donor lungs depleted of macrophages, was 4.5 days [126], but in most cases survival has been measured in hours [127–129]. Even the combination of GTKO/hDAF-transgenic donors and depletion of pulmonary macrophages failed to extend graft survival beyond 2 days [130]. The main histological features of pulmonary xenograft rejection are micro- and macrovascular thrombosis, neutrophil infiltration and oedema [126,129]. The development of consumptive coagulopathy is even more rapid and consistent than in kidney xenotransplantation, although why pig lung xenografts should be particularly susceptible to coagulation remains unclear.

Coagulopathy in the form of severe thrombocytopenia is a major problem in pig-to-NHP liver xenotransplantation. Baboons transplanted with GTKO/hCD46 livers died or had to be euthanased within 7 days, not due to rejection but rather to bleeding problems resulting from a sharp and prolonged drop in platelet count within an hour of transplantation [131]. At necropsy, the grafts showed only patchy injury at the macroscopic and microscopic levels [131,132] and liver function was relatively normal [133]. The degree of platelet deposition in the livers did not appear to be extensive enough to account for the severity of thrombocytopenia, which was instead hypothesized to be due to rapid platelet activation and subsequent formation of platelet–leukocyte aggregates in the circulation [134]. An alternative mechanism, proposed on the basis of ex vivo perfusion studies, is that pig liver sinusoidal endothelial cells and Kupffer cells rapidly and efficiently phagocytose human platelets in an antibody- and complement-independent manner [135–137].

Expression of tissue factor by recipient platelets and monocytes

In vitro and in vivo studies indicate that tissue factor is up-regulated on pig endothelial cells activated by elicited anti-αGal and/or non-Gal antibodies (Figure 12.4), even in the absence of complement, contributing to intravascular coagulation and the development of consumptive coagulopathy [138]. More recently, it has been proposed that the induction of tissue factor expression on recipient platelets and monocytes may also play a significant role. Human platelets and monocytes up-regulate tissue factor expression and activity simply by contact with pig endothelial cells, in the absence of antibody and complement [139]. Rejected GTKO pig-to-baboon cardiac xenografts showed tissue factor expression on recipient macrophages in thrombosed vessels and in the interstitium [116]. Time-course analysis of circulating cells in baboon recipients of GTKO/hCD46 porcine renal xenografts revealed several quite

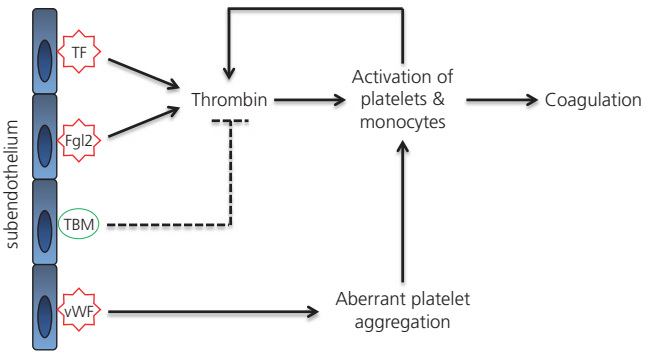

Figure 12.4. The potential mechanisms of dysregulated coagulation in solid organ xenotransplantation. Expression of tissue factor (TF) and the direct prothrombinase fgl2 by activated xenograft endothelial cells drives thrombin generation, which is poorly controlled by pig thrombomodulin (TBM). In addition, human platelets aggregate in response to pig von Willebrand factor (vWF) in the absence of shear stress, and are activated by contact with pig endothelium.

distinct stages: up-regulation of tissue factor on platelets within a day of transplantation, followed by the appearance of increasing numbers of platelet–leukocyte aggregates, and finally expression of tissue factor by monocytes, accompanied by the onset of thrombocytopenia and other features of coagulopathy [120]. Recipient tissue factor was also detected in the native heart and liver at necropsy. These events occurred in the apparent absence of a significant humoral response, leading the investigators to hypothesize that: (1) platelets are activated to express tissue factor soon after transplantation in response to inflammation and contact with xenograft endothelium; (2) monocytes subsequently express tissue factor after being activated by thrombin and/or by adhesion to activated platelets; and (3) the resulting systemic exposure of recipient tissue factor triggers consumptive coagulopathy [120]. However, the failure of other groups to observe this very early and profound coagulopathy in GTKO renal xenograft recipients [140] suggests that it may be model dependent rather than universal.

Tissue factor-independent coagulation: direct prothrombinase activity

Pig endothelial cells express an enzyme called fgl2, which directly converts human prothrombin to thrombin [141] (Figure 12.4). Fgl2 expression and activity is induced in porcine endothelial cells by proinflammatory cytokines including human TNF-α [141,142]. Rejected pig-to-baboon renal xenografts showed up-regulated fgl2 expression in association with fibrin deposition in small vessels and glomerular capillaries [141]. Interestingly, co-localization of fgl2 and fibrin was also observed in rejecting human renal and mouse cardiac allografts [143,144]. This suggests that fgl2 is a common inflammation-mediated mechanism for tissue factor-independent coagulation in acute rejection of solid organ transplants, although it should be noted that this view is not universally shared [144].

Molecular incompatibilities in the control of coagulation and inflammation

Coagulation and inflammation are powerful interlinked processes that require multiple levels of regulation [145,146]. The vascular endothelium maintains an anticoagulant and anti-inflammatory surface by expressing a range of regulatory proteins. Tissue factor pathway inhibitor-1 (TFPI-1) sequesters factor Xa and factor VIIa/tissue factor and is the primary physiological regulator of the initiation phase of coagulation [147]. Antithrombin inhibits multiple coagulation serine proteases including factor Xa and thrombin [148]. Thrombomodulin (TBM) binds thrombin and alters its specificity from procoagulant and proinflammatory substrates, such as fibrinogen and protease activated receptors, to protein C; activated protein C (APC) inhibits the propagation phase of coagulation by inactivating factors Va and VIIIa [149]. The endothelial protein C receptor (EPCR) enhances APC generation by the TBM/thrombin complex and mediates many of APC's anti-inflammatory and cytoprotective effects [150]. TBM also has direct, APC-independent anti-inflammatory effects via its lectin-like domain [151,152].

Some of these regulators (TFPIα and antithrombin) are circulating proteins that associate with the endothelial cell surface via heparan sulphate proteoglycans, while others are integral endothelial membrane proteins. In transplanted organs, the latter group (TFPIβ, TBM and EPCR) will be of donor origin—a distinction that is irrelevant in allotransplantation, but potentially critical in xenotransplantation if the regulators do not function efficiently across the species barrier. This was first highlighted by in vitro studies in the 1990s suggesting that pig TFPI does not bind human factor Xa and that this failure may contribute to uncontrolled coagulation in xenografts [153,154]. More recently, however, a recombinant form of pig TFPI, expressed in transfected cells, was shown to bind and inactivate human factor Xa and factor VIIa/tissue factor as efficiently as human TFPI [155]. Furthermore, even if there is a degree of incompatibility, it should be corrected to some extent by association of recipient-derived TFPIα with xenograft endothelium.

There is more compelling evidence for a molecular incompatibility affecting the protein C pathway. Pig TBM binds human thrombin but is a poor cofactor for activation of human protein C, with only 1–10% of the activity of human TBM [156–158]. It is quite conceivable that this level of impairment will have a detrimental impact in vivo (Figure 12.4), considering that the protein C pathway is a key regulator of coagulation and inflammation within the microvasculature [149] and that microvascular thrombosis is a feature of xenograft rejection [9,58]. Expression of human TBM in pig endothelial cells in vitro suppressed classical and direct (fgl2) prothrombinase activity and delayed cell-mediated clotting of human plasma [142,159]. Pig EPCR has been less well studied, although it has been reported to bind human protein C in vitro [160]; targeting of this regulator by induced antibodies [73] may prove to be a more significant problem than molecular incompatibility.

Another incompatibility defined in vitro may promote platelet adhesion and aggregation in xenografts. Human platelets spontaneously aggregate upon contact with porcine vWF (Figure 12.4), in the absence of shear stress, due to an aberrant interaction between human platelet glycoprotein Ib (GPIb) and the *O*-glycosylated A1 domain of vWF [161–163]. The in vivo significance of this heightened platelet response is not known. The use of donors severely deficient in vWF expression had no obvious impact on coagulation or graft survival in a pig-to-baboon renal xenograft model [164], suggesting that porcine vWF is not a major contributor to dysregulated coagulation. However, the only treatment of the recipients in this study was depletion of natural antipig antibodies by immunoadsorption prior to transplantation. Any effect of the vWF deficiency may have been difficult to distinguish in the face of the ensuing humoral response, which was severe and caused rejection of all grafts within 5 days [164].

Approaches to control dysregulated coagulation

Intravascular thrombosis in short-term ex vivo pig organ xenoperfusion models can be inhibited by pharmacological antithrombotic drugs including APC [165], desmopressin, which reduces vWF content [166], and tirofiban, a platelet GPIIb/IIIa receptor inhibitor [167]. In vitro experiments suggest that statins may also be useful by blocking the up-regulation of tissue factor in activated pig endothelial cells [168]. Anticoagulation in vivo is more challenging because the dosage must be balanced against an increased risk of bleeding. The pig-to-NHP model has been used to test many anticoagulant and antiplatelet agents, including warfarin, antithrombin, APC, heparin, aspirin, and clopidogrel [45,169–173]. While there was evidence for limited benefits in several of these studies, it would be fair to say that pharmacotherapy will not be the complete answer to controlling thrombosis and prolonging xenograft survival [174]. This may be partly a reflection of progressive damage to xenograft endothelium by mechanisms such as ADCC that are not directly influenced by anticoagulation.

Perhaps the best prospect for solving the coagulation problem is genetic modification of the donor pig. This has already been partly addressed by deleting αGal and expressing hCRPs, which reduce

the stimuli for endothelial cell activation and coagulation. However, these modifications alone appear to be insufficient because dysregulated coagulation is still evident in baboon recipients of GTKO ± hCRP-transgenic xenografts [116,120]. The next logical step is to express human anticoagulant and antiplatelet molecules to correct molecular incompatibilities and/or to provide a supraphysiological level of control of thrombosis within the xenograft. The beneficial effects of expressing human TFPI and TBM have been demonstrated in murine transplant models [175,176]. Both proteins have also been expressed in transgenic pigs [168,177–179], but in vivo efficacy data are not yet available.

Another molecule of interest is the ectonucleotidase CD39, which plays a critical thromboregulatory and anti-inflammatory role on vascular endothelium [180]. CD39 sequentially degrades extracellular ATP to ADP and AMP, which is then hydrolysed to adenosine by CD73. It therefore removes proinflammatory (ATP) and prothrombotic (ADP) signals, while concurrently promoting the generation of adenosine, which has a generally dampening effect on the activity of both innate and adaptive immune cells [181,182]. Like several other anticoagulant/ anti-inflammatory molecules, including heparan sulphate [183], TBM [184] and EPCR [185], CD39 is lost from the endothelium during inflammation, promoting vascular injury [186]. Over-expression of CD39 is therefore an attractive prospect in xenotransplantation. Proof-of-concept studies using transgenic mice have shown that expression of human CD39 protects hearts and kidneys from transplant-related vascular injury [187,188]. A preliminary report suggests that the addition of human CD39 to a GTKO/hCRP-transgenic background was not sufficient to prevent AHXR of pig-to-baboon renal xenografts, although the level of CD39 expression was reportedly low [189].

Chronic xenograft rejection

Reports of chronic xenograft injury are relatively rare because few organ xenografts have survived long enough for this type of injury to develop. Chronic vasculopathy, considered to be a characteristic feature of chronic allograft rejection, was observed in GTKO pig-to-baboon cardiac xenografts that survived for between 78 and 179 days [47]. In another study, a GTKO pig-to-baboon renal xenograft that survived for 81 days under an immunotolerance protocol showed evidence of chronic glomerulopathy, similar to that observed in clinical allotransplantation [190]. Possible reasons proposed for the development of glomerulopathy were unstable induction of tolerance, injury by preformed non-Gal antibodies and dysregulated coagulation. However, the mechanism of chronic xenograft rejection is likely to remain obscure until more consistent long-term survival is achieved.

Rejection of porcine islet xenografts

Pig islet xenotransplantation covers a broad range of procedures in which there may be different sources of tissue (embryonic, fetal, neonatal, young, adult), different routes of delivery (intraportal, renal subcapsular, intraperitoneal, subcutaneous, omental), and different methods of immunoprotection (immunosuppression, encapsulation). Many of these factors have been discussed in recent reviews [191,192]. This section will focus mainly on intraportal transplantation of neonatal or adult pig islets into NHP, because it is the model that is most widely used and most closely reflects current clinical islet allotransplantation practice. Additional discussions of this topic can be found in Chapter 15.

The instant blood-mediated inflammatory reaction

Intraportal transplantation brings islets directly into contact with the circulation, provoking an immediate thrombotic/ inflammatory response termed the instant blood-mediated inflammatory reaction (IBMIR). This phenomenon, first recognized in allotransplantation [193], is characterized by rapid activation of complement and coagulation, adherence of activated platelets to the islet surface, entrapment of islets in clots, and infiltration by activated neutrophils and monocytes [194]. The proposed mechanisms include binding of natural antibodies, islet expression of tissue factor and chemoattractants such as MCP-1, exposure of thrombogenic molecules such as collagen, and the absence of membrane regulators of complement and coagulation [195–197]. IBMIR is thought to be the major factor responsible for the early destruction of approximately half of the islet allograft mass [198,199].

IBMIR is also well described in pig-to-NHP islet xenotransplantation [200–202] and in pig-to-human in vitro models [200,203,204]. In fact, IBMIR is likely to be more severe in xenotransplantation because of higher levels of natural antibodies and the hyperactive innate immune response to xenogeneic cells [205]. This is supported by the observation that the number of pig islets required to consistently reverse diabetes in NHP (25 000–100 000 islet equivalents per kg body weight) [206–208] is much higher than the number of human islets used to similar effect in clinical allotransplantation.

Acute cellular rejection of pig islet xenografts

Intraportal islet xenografts surviving the initial innate onslaught of IBMIR are subjected to an adaptive immune response that is predominantly dependent on, and mediated by, T cells [191]. In the absence of immunosuppression, adult pig islets are destroyed within 3 days by acute cellular rejection, with $CD4^+$ and $CD8^+$ T cells and macrophages most prominent in the infiltrate [201]. With T-cell-targeted immunosuppression including costimulation blockade, intraportal islet xenografts from wild-type pigs are capable of restoring insulin independence in diabetic monkeys for several months [206,209,210].

The role of αGal in pig islet xenograft rejection

Expression of αGal on pig islet β cells appears to correlate with their maturity, with neonatal β cells expressing significant levels and adult β cells virtually none [211]. Adult pig islet preparations therefore contain little αGal, apart from that expressed on residual contaminating non-endocrine cells, and do not seem to be susceptible to anti-αGal antibodies [191,206]. This interpretation is supported by a pig-to-monkey study that showed no apparent survival benefit for GTKO adult islet xenografts, although the numbers were too small to draw definitive conclusions [207]. In contrast, αGal clearly plays an important role in the rejection of neonatal porcine islets. Xenografts from neonatal GTKO pigs showed significantly improved engraftment and induced less intrahepatic inflammation in diabetic monkeys than wild-type grafts [212].

Strategies to protect pig islet xenografts

Islets are somewhat different to organs in that there are a far smaller proportion of patients for whom the benefits of transplantation outweigh the side-effects of immunosuppressive drugs. It is therefore critical to develop strategies to reduce the level of immunosuppression required in islet xenotransplantation, or even to eliminate it altogether. For intraportal porcine islet xenografts, a major goal is to protect the islets from early inflammatory and thrombotic

mechanisms, as this would enable transplantation of fewer islets and reduce stimulation of adaptive xenoimmunity. A combination of pharmacological and genetic approaches will probably be necessary. One treatment option to regulate complement and coagulation is low molecular weight dextran sulphate [203,207]. On the genetic side, deletion of αGal has clear benefits for neonatal islet xenografts [212], and expression of hCD46, while not preventing early attrition, significantly prolonged adult islet xenograft survival [207]. Certain genetic modifications designed to protect organ xenografts, such as the expression of various human anticoagulant molecules described earlier, should also benefit islet xenografts.

The induction of tolerance is another avenue that has produced promising results recently. Diabetic monkeys transplanted with adult pig islets under the cover of anti-LFA-1, anti-CD154 and low-dose rapamycin showed xenoantigen-specific T-cell tolerance, and maintained insulin-independent normoglycaemia for more than 140 days [208].

Another strategy to protect pig islets from IBMIR and the xenoimmune response is to encapsulate them in alginate-based compounds, allowing passage of nutrients and insulin but preventing entry of antibodies and immune cells. The potential of encapsulated pig islets to correct diabetes for long periods, without immunosuppression, has been demonstrated in monkeys transplanted either intraperitoneally with microcapsules [213] or subcutaneously with a macroencapsulation device [214]. A Phase IIb clinical trial of microencapsulated pig islets is currently in progress in New Zealand, but full results are not yet available.

Rejection of other types of porcine xenograft

Neuronal cell xenografts

Transplantation of pig dopaminergic tissue into the brain has long been proposed as a therapy to relieve the symptoms of Parkinson's disease. In a clinical trial in the 1990s, 12 patients were transplanted with fetal pig neuronal cells, half under the cover of cyclosporine and half receiving grafts that had been treated with the Fab fragment of a monoclonal antibody directed against MHC class I [215]. Although there was evidence of improved function in some recipients, neither immunosuppressive strategy was able to prevent rejection, despite the brain being considered a relatively immune-privileged site. In contrast, a study in mice showed that embryonic pig neuronal cell xenografts were protected from rejection by short-term costimulation blockade with CTLA4Ig, anti-CD154 and anti-LFA-1 [216]. More recently, Parkinsonian monkeys were transplanted with fetal neural tissue from transgenic pigs expressing human CTLA4Ig from a neuron-specific promoter, under immunosuppression with cyclosporine, mycophenolate and steroids. Preliminary data indicated prolonged engraftment (up to 17 months) and significant improvement in locomotor function in monkeys receiving CTLA4Ig-transgenic cells, but not in those receiving control non-transgenic grafts [217]. A relatively high incidence of post-transplant lymphoproliferative disease in the recipients suggests that adjustment of the immunosuppressive regimen and/or further genetic modification of the donor is still required.

Corneal xenografts

The prospects for xenotransplantation of pig corneas have been reviewed recently [218,219]. The relatively few pig-to-NHP corneal xenotransplantation studies performed to date have shown that corneal xenografts are not subject to HAR, as might be expected for an avascular tissue transplanted into an immune-privileged site, but usually succumb to a largely T-cell-mediated rejection process over weeks to months [220–223]. The corneal endothelium appears to be the primary target of the immune response [221], and decellularization of corneal xenografts reduces the frequency of rejection [223]. The role of αGal and non-Gal antigens in corneal xenograft rejection has not been determined, but the expression of αGal on corneal keratocytes and endothelial cells [218] and the sensitivity of the latter to lysis by complement-fixing antibodies [224] suggest that GTKO ± hCRP-transgenic xenografts may have a survival advantage.

Reducing the immunosuppressive burden

Systemic immunosuppression, with its attendant complications and side-effects, is a greater challenge in xenotransplantation than in allotransplantation because of the severity of the xenoimmune response. Xenotransplantation of porcine solid organ xenografts will not enter clinical practice unless the degree of immunosuppression required can be reduced to a manageable level, similar to or less than that used in allotransplantation. Three potential solutions to this problem are outlined below.

Induction of accommodation

Pig-to-NHP xenografts have occasionally been observed to survive and function normally despite the presence of circulating xenoantibodies and immunohistochemical evidence of antibody and complement deposition on graft endothelium [67,225,226]. This is an example of accommodation, an incompletely understood phenomenon in which vascularized organ allografts or xenografts acquire resistance to acute humoral injury and rejection [227,228]. Little is known about the mechanism or durability of accommodation in the pig-to-NHP model. Evidence from other models suggests that sublethal injury to grafts, particularly as a consequence of antibody binding, can trigger the activation of protective pathways that lead to resistance to humoral immunity. These pathways include up-regulation of the complement regulators DAF and CD59 [229,230] and cytoprotective molecules such as A20 and heme oxygenase-1 [231]. Certain genetic strategies designed to inhibit humoral rejection of porcine xenografts, for example transgenic expression of hCRPs, might therefore be expected to promote the development of accommodation. However, there is no evidence so far that this is the case, and accommodation of pig-to-NHP xenografts remains elusive.

Induction of tolerance

The T-cell-mediated xenoresponse has proven to be a formidable barrier in pig-to-NHP solid organ xenotransplantation. Even the combination of genetically modified donors and intensive T-cell-directed immunosuppression has not resulted in a clinically applicable outcome. This has led to the suggestion that the induction of T-cell tolerance (the mechanisms of which are discussed in additional detail in Chapter 11) will be essential to the success of any treatment regimen for clinical xenotransplantation [232]. The two approaches to tolerance induction are mixed chimerism and co-transplantation of thymic tissue. Both methods have been used to establish that human T cells can be tolerized to porcine xenografts, albeit in highly manipulated humanized mouse models [233,234]. In the more challenging pig-to-NHP model, stable mixed chimerism has been difficult to achieve, even with intensive preconditioning of recipients and massive doses of wild-type pig haematopoietic stem cells (HSCs) [235]. There is some evidence that natural

xenoantibodies may present an obstacle to HSC engraftment. The use of GTKO donors reduced the number of cells required to achieve the same level of transient chimerism, and prolonged the period of cellular hyporesponsiveness as measured by mixed lymphocyte reaction [236]. In a more recent study also using GTKO HSCs, the degree of transient bone marrow chimerism and nonresponsiveness to pig cells inversely correlated with the level of preformed non-Gal antibodies in the recipients [237]. Only the two baboons with relatively high pretransplant titres of non-Gal IgG failed to demonstrate chimerism or pig-specific hyporeactivity, and both rapidly rejected pig kidneys transplanted soon after bone marrow infusion [237]. Another possible reason for the failure of porcine HSCs to stably engraft is destruction by macrophages and NK cells due to the previously discussed SIRPα/CD47 and KIR/SLA cross-species incompatibilities, respectively. It remains to be seen whether transgenic expression of human CD47 and HLA-E on donor cells will enhance the establishment of mixed chimerism.

Transplantation of porcine vascularized thymic tissue, either as thymic lobes or 'thymokidneys' (kidneys in which autologous thymic tissue is engrafted before renal xenotransplantation), has produced more promising results. Initial experiments using αGal-positive thymokidneys showed some evidence of thymopoiesis and T-cell hyporesponsiveness in vitro, but xenografts were rejected within 30 days upon the return of anti-αGal antibodies [238]. The use of GTKO donors markedly prolonged thymokidney survival in baboons to up to 83 days, among the longest recorded for a life-supporting xenograft in the pig-to-NHP model [42]. The immunosuppressive regimen included thymectomy, splenectomy, whole body irradiation (WBI), antibody-mediated T-cell depletion, mycophenolate mofetil, anti-CD154 and low-dose steroids. Unfortunately, this regimen caused a high incidence of early post-operative complications, mainly infectious. Several animals died with functional grafts that appeared relatively normal by histology, with minimal signs of rejection [42]. Modifying the protocol to eliminate WBI and steroids reduced the incidence of early complications and increased mean graft survival time from 34 to 51 days, although maximum graft survival was not prolonged [140]. Adjustments to the T-cell depletion strategy did not lead to further improvement, even with recipients selected on the basis of low levels of preformed non-Gal antibodies [239]. Nevertheless, these studies provide a tantalizing hint that the thymic transplantation approach, perhaps in combination with mixed chimerism, will eventually lead to long-term tolerance to porcine xenografts. It will first be necessary to overcome the many challenges of the model and to find a substitute for anti-CD154, which appears to be essential for success but is not currently available in a clinically applicable form.

Graft-mediated (local) immunosuppression

A novel strategy with obvious appeal for xenotransplantation is to engineer transplanted tissue to secrete immunosuppressive proteins in the local environment of the graft. Most of the data supporting the efficacy of local immunosuppression have come from small animal islet allograft models involving adenoviral or transgenic expression of CTLA4Ig [240–243]. Similar data from the pig-to-NHP model are limited to the observation that transgenic expression of CTLA4Ig appears to be required for the long-term engraftment of porcine neurons in Parkinsonian monkeys [217]. On a cautionary note, strong and ubiquitous transgenic expression of porcine CTLA4Ig resulted in a high mortality rate in pigs due to compromised humoral immunity [244]. Tissue-restricted and/or regulated expression of the transgene may be the solution to this problem.

Limitations of the pig-to-NHP model

While demonstration of efficacy in the pig-to-NHP model is generally regarded as an essential prerequisite for moving to the clinic [245], it should be borne in mind that the model is not without limitations. First, NHP may share some antigens with pigs that humans do not possess. For example, NHP and pigs express NeuGc, also known as Hanganutziu–Deicher antigen [96,246], whereas humans do not and develop anti-NeuGc antibodies [247]. The true relevance of these antibodies may not be known until xenotransplantation enters clinical practice [248]. Second, NHP appear to have a more 'hypercoagulable' phenotype than humans [249], suggesting that we may be setting the bar too high when it comes to solving the problem of thrombosis in solid organ xenotransplantation. Finally, NHP have a higher insulin demand than humans, implying that higher doses of pig islets will be required to achieve insulin independence in the pig-to-NHP model than may be needed in human recipients [250]. These and other considerations have led some in the field to call for the commencement of clinical trials sooner rather than later [71].

Summary

When considered as a whole, the immunological challenges that must be overcome before xenotransplantation can enter the clinic seem almost insurmountable. The xenograft is glycosylated in a way that makes it immediately recognizable as foreign, attracting the attention not only of a high level of preformed antibodies but also the cells of the innate immune system. The multitude of protein and carbohydrate antigenic differences provokes a powerful T-cell xenoresponse that seems to be quite different to the alloresponse. Furthermore, xenoimmunity operates on a background in which innate immune cells are more prone to activation, and several key mechanisms that regulate coagulation and inflammation are compromised by molecular incompatibilities. It is therefore hardly surprising that drugs designed to prevent allorejection are considerably less effective against xenorejection.

Balancing these 'cons' is one unique and powerful 'pro'—the ability to genetically modify the donor to remove stimulatory signals, correct molecular incompatibilities and bolster graft-protective defences. Even the relatively few modifications tested in the pig-to-NHP model to date have significantly prolonged maximum survival of heterotopic and orthotopic cardiac xenografts to 8 months [75] and 2 months [251], respectively, renal xenografts to 3 months [42], islet xenografts to 13 months [207], and neuronal cell xenografts to 17 months [217]. Several new transgenic pigs await in vivo testing [97,252–254], and increasingly sophisticated methods are being developed to precisely and efficiently manipulate the porcine genome [255–257]. These advances, together with improved immunosuppressive regimens aimed at inducing tolerance, give cause for cautious optimism that clinical xenotransplantation will indeed be 'the next medical revolution' [74].

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CHAPTER 13

In Vitro Models of Alloreactivity

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Introduction

Transplantation is inherently an in vivo procedure. Allogeneic transplantation performed as a curative procedure for end-stage heart, lung, liver, or kidney disease, or for diabetes is quickly followed by a sequence of complex inflammatory processes and immune interactions invoked to reject the graft. First, the time of ischemic storage imposed on the donor tissue dictates the intensity of early inflammation primarily through production of reactive oxygen radicals that stimulate the production of acute-phase cytokines by graft endothelial cells and other cells in the graft during reperfusion [1,2]. Second, these oxygen radicals and cytokines not only mediate direct graft tissue injury but also potently amplify the inflammatory response by stimulating the production of leukocyte chemoattractants and up-regulating the expression of adhesion molecules on the graft endothelium that synergize to direct the trafficking of cellular components of innate immunity (i.e. neutrophils, monocytes, macrophages, and dendritic cells) into the graft and their activation to express functions mediating graft tissue injury (covered in Chapter 7). Third, this innate response to the initial inflammation in the allograft is quickly followed by the infiltration of endogenous memory T cells expressing receptors specific for donor alloantigens, and the activation of these memory T cells within the graft further exacerbates the intensity of the inflammation and the tissue injury [3,4]. This initial inflammatory response also activates graft passenger leukocytes, primarily interstitial dendritic cells, to emigrate from the graft and traffic to secondary lymphoid organs of the recipient where alloantigen-reactive T and B lymphocytes are activated to clonally expand and differentiate into effector cells [2,5]. These primary effector T cells traffic to the graft where they are re-activated to express programmed functions that mediate acute and/or chronic graft tissue injury. These processes are covered in depth in Chapter 5 [6]. Activation of B cells expressing surface immunoglobulin receptors for donor antigens results in their differentiation into antibody-producing cells, plasmablasts, or plasma cells, and binding of the antibody to the target donor antigens on the graft endothelium and other cells results in tissue injury through several different mechanisms (see Chapter 6 for additional detail) [7,8].

Many of these processes, such as ischemia–reperfusion injury as well as the continuum of the response from innate to donor antigen-specific mediated inflammation and the resulting graft tissue injury, are best studied using in vivo models as they encompass multifactorial components and interactions that cannot be reproduced using in vitro approaches. There are, however, many instances in which in vitro approaches have provided a great many insights into the in vivo responses mounted against transplanted cells and organs. This overview will detail what we have learned from using in vitro approaches to investigate the presence and characteristics of alloimmune responses.

Stimulation of alloreactive T-cell proliferation

Initial studies investigating the nature of T-cell alloreactivity established the ability of cells expressing allogeneic MHC molecules to activate and drive the proliferation of T cells in vitro [9–11]. This resulted in the development of mixed lymphocyte reactions (MLR) to assay the presence and intensity of T-cell reactivity to allogeneic stimulator cells. In this assay, naïve T cells are cultured with stimulator cells expressing allogeneic class I and/or class II MHC molecules. The allogeneic stimulator cells are normally inactivated through irradiation or treatment with a mitotic inhibitor to allow detection of T-cell activation and proliferation more accurately. T-cell receptor engagement of allogeneic class I or class II MHC molecules by $CD4^+$ or $CD8^+$ responder cells results in activation of the T cells to produce growth factors, primarily IL-2, and to express high-affinity receptors for the growth factors. This results in cell cycle progression and proliferation of the alloreactive T cells. T-cell proliferation was initially detected in these assays by adding ^{3}H-thymidine during the last 24 hours of the culture and then assessing the amount of ^{3}H-thymidine incorporation into the DNA of the cells as an indication of the proliferation of the cells. The proliferation of naïve or antigen-unprimed T cells is optimally observed 4–5 days after initiation of the culture whereas proliferation of memory T cells is observed within 1–2 days of culture initiation.

More recently, the use of ^{3}H-thymidine to assess T-cell proliferation has been replaced by labeling the responder T cells with a fluorescent dye that is retained within the responder cells; the most commonly used dye for this purpose is carboxyfluorescein succinimidyl ester (CFSE) [12]. CFSE is a derivative of carboxyfluorescein

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diacetate succinimidyl ester (CFDA-SE). CFDA-SE, which is non-fluorescent, is highly cell permeable and enters the cytoplasm of cells. Intracellular esterases remove the acetate groups and convert the molecule to the fluorescent derivative, CFSE, which is retained within cells by covalently coupling to intracellular lysine residues and other amine sources. As the responder T cells divide, the amount of the dye retained in each daughter cell is decreased by half for each cycle of proliferation (Figure 13.1). Thus, non-dividing and dividing cells are easily detected as indicated by the level of CFSE dilution measured on a flow cytometer. When performed with appropriate controls, where the responder T cells are cultured with autologous/self cells as stimulators so that the level of spontaneous proliferation is assessed, this can be a powerful approach not only for detecting alloantigen-reactive responder T-cell proliferation but also for determining the number of distinct cycles of proliferation and dissecting the characteristics of the non-dividing and dividing T cells. Elegant studies by Wells and colleagues [13], using a polyclonal T-cell receptor (TCR) agonist, plate-bound anti-CD3 mAb, to activate CFSE-labeled T cells, indicated that the frequency of T cells entering the proliferating cell pool is regulated by the TCR stimulus, but that the addition of CD28-mediated costimulation regulates both the frequency of responding cells and the number of mitotic cycles. In addition, this approach can be combined with antibody staining to detect the expression of specific cell surface molecules, for example cellular activation markers such as CD11a, CD44, and/or CD69, or intracellular cytokine production, so that cells in each cycle of the proliferative response can be interrogated for specific markers of activation and differentiation [14]. The CSFE labeling approach has been widely used to investigate the alloantigen-induced proliferation of T cells isolated from various animals as well as human transplant patients [15–17].

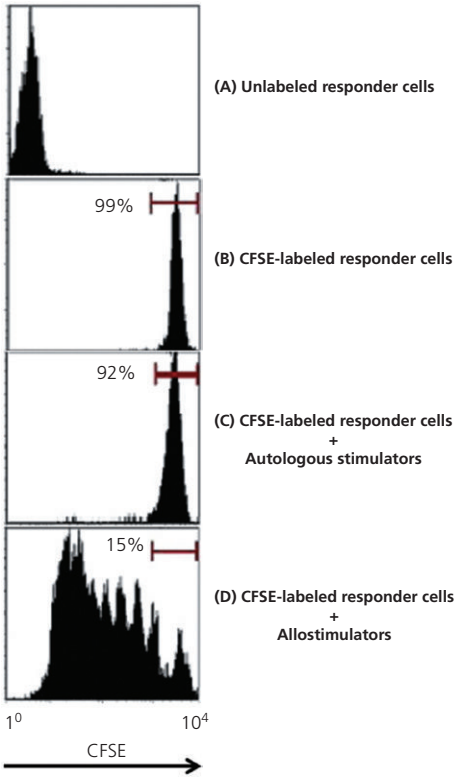

Figure 13.1. Detection of alloreactive T-cell proliferation by in vitro CFSE dilution. Equal numbers of unlabeled (A) or CFSE labeled (B–D) responder T cells are aliquoted into culture plates. Responder T cells are then stimulated with media alone (B), or autologous (C), or allogeneic stimulator cells (D). Three days after culture, responder cells are harvested and their proliferation assessed by dilution of CFSE using flow cytometry.

In addition to proliferation, functional characteristics of T cells following activation by allogeneic stimulator cells in culture can be measured through a number of different approaches. These approaches include assessing the production of cytokines by enzyme-linked immunosorbent assay (ELISA) in the supernatants of MLR cultures or cytolytic assays using radioactive- or CFSE-labeled allogeneic target cells. In addition, enzyme-linked immunosorbent spot (ELISPOT) assays can be performed to enumerate alloreactive T cells producing specific cytokines in response to allogeneic stimulator cells (Figure 13.2). In this assay, the responder T cells and allogeneic stimulator cells are cultured on filters that are coated with an antibody specific for the test cytokine. After 24–72 hours of culture, the cells are washed from the filters and a second antibody to the test cytokine is added, except in this case the antibody is coupled to a chromagen that converts a substrate to a color that is retained by the filter as a spot. A reader is then used to count the spots, which represent the number of alloreactive T cells producing the specific cytokine. These approaches have indicated that in unmanipulated cultures, alloantigen activation of $CD4^+$ and $CD8^+$ T cells promotes differentiation to IFN-γ producing cells and that $CD8^+$ T cells also differentiate to express FasL and undergo perforin/ granzyme B-mediated lysis of allogeneic target cells. It is important to note, however, that the composition of the MLR cultures can be manipulated to drive alloantigen-specific T cells to perform other functions, as is discussed below.

Seminal studies by Lafferty and Cunningham [18] led to the hypothesis that alloreactive T-cell activation requires the delivery of two signals. In vitro and in vivo studies provided support for this

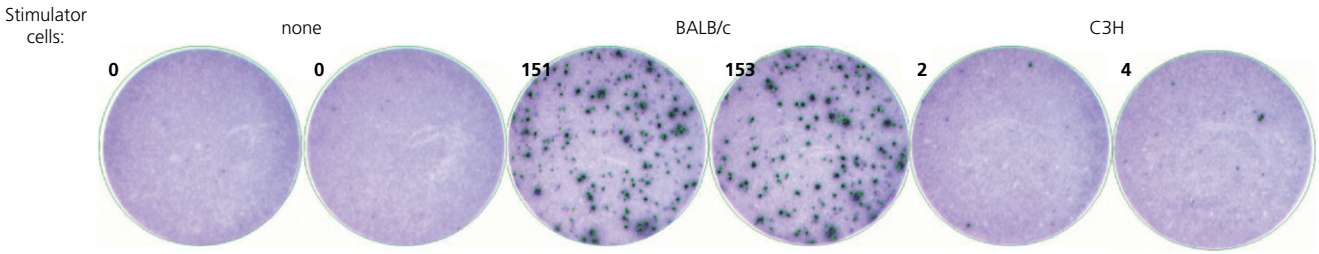

Figure 13.2. ELISPOT assay. Spleen cells isolated from B6 (H-2^b) recipients of BALB/c (H-2^d) heart allografts were tested in a recall response against BALB/c and third-party SJL (H-2^s) stimulator cells by IFN-γ ELISPOT assay. Duplicate wells are shown with 100 000 cells plated in each well. Numbers represent spots per well.

hypothesis in that the activation of naïve T cells to proliferate and differentiate into cells expressing distinct functions required both signaling through the TCR complex and through what are now termed costimulatory molecules [19]. In vitro approaches have been used to identify the key costimulatory molecule interactions that are required for the activation (or inhibition) of alloantigen-specific naïve and memory T cells during stimulation with alloantigen-presenting cells.

In vitro approaches were initially used to demonstrate the synergistic activity of an agonist monoclonal antihuman CD28 antibody with anti-CD3 antibodies in activating human T cells to produce IL-2 and proliferate, thus identifying the requirement for CD28 stimulation for optimal activation of human T cells [20–22]. Subsequent studies demonstrated that alloreactive human T-cell activation was dependent on CD28-B7 (i.e. B7-1/B7-2 or CD80/CD86) interactions by transfecting B7-deficient allogeneic stimulator cells to express the ligand for CD28 [23]. A second ligand for B7 molecules, CTLA-4, expressed by T cells was identified by Peter Linsley and colleagues [24] and a generated fusion protein, CTLA-4Ig, inhibited human alloreactive T-cell proliferation in MLR and the delivery of CD4 T helper cell signals for in vitro antibody production by B cells. These studies provided seminal clues leading to the initial observation of Turka and colleagues that administration of CTLA-4Ig could prolong the survival of MHC-mismatched Norway cardiac allografts in Lewis rat recipients [25]. Over the course of 20 years, these initial in vitro studies, defining the molecular structures and properties of CD28 and CTLA-4 expressed by T cells and their interactions with CD80/86 molecules on antigen-presenting cells, have led to the development of a new modified form of CTLA-4Ig, belatacept, that has shown efficacy in improving graft outcomes in renal transplant patients [26,27].

Since the identification of CD28-CD80/86 mediated costimulation, it has been further appreciated that there are many more costimulatory ligand–receptor pairs that can positively or negatively influence the activation of alloreactive T cells [19]. The identification and function of these additional positive and negative costimulatory molecules on T cells and their ligands on antigen-presenting cells has required extensive in vitro investigation using approaches similar to those used to delineate the structures and functions of the CD28/CTLA-4 molecules and their cognate ligands, CD80/CD86.

Presentation of alloantigens to T cells

Alloantigen is recognized by T cells through three different presentation pathways to induce proliferation, expression of activation markers, and expression of specific functions that contribute to graft injury [28–32]. Depending on the specificity of the TCR, alloreactive T cells can recognize peptide/ allogeneic class I or class II MHC complexes on donor antigen-presenting cells through the direct pathway or can recognize donor allogeneic peptides that are presented by MHC complexes on self-antigen-presenting cells through the indirect pathway. In the latter case, peptides from graft allogeneic MHC molecules or allelic variants of other proteins are acquired, processed, and presented by host- or self-antigen-presenting cells. In addition, host-antigen-presenting cells may acquire intact peptide/ allogeneic MHC complexes, most likely through exosomes released by graft cells, which are incorporated into the membrane and presented through the semidirect pathway to T cells expressing receptors specific for the allogeneic peptide/ MHC complex.

The presence of T cells specific for alloantigen presented through the direct versus indirect presentation pathways can be detected and investigated through many different in vitro approaches. Essentially, these approaches must involve the use of purified T cells that will respond during culture with the appropriate antigen-presenting cells and source of antigen. For alloreactive T cells responding through the direct pathway, this simply involves culture of the purified T cells with antigen-presenting cells expressing allogeneic class I and/or class II MHC molecules, for example those isolated from an allogeneic donor or allogeneic tumor cells. For detection and/or measurement of T cells responding through the indirect pathway, purified syngeneic antigen-presenting cells are added with a source of allogeneic proteins or peptides, cell-free tissue homogenates from allografts, or synthetic peptides from allogeneic MHC molecules or other target proteins. These cultures can be used to measure proliferation of the responder T cells as discussed above or their activation to produce cytokines that can be detected by ELISA or ELISPOT assay. Using these in vitro approaches to investigate T cells isolated from MHC mismatched allograft recipients, Benichou and colleagues [31] demonstrated that the majority (>90%) of alloreactive T cells isolated from the time of transplantation to the time of acute graft rejection responded through the direct alloantigen presentation pathway.

For reasons discussed below, the frequency of T cells in a repertoire that will respond to peptide/ allogeneic MHC complexes is in the order of 1 in $10–10^4$ cells, whereas the frequency of T cells that will respond to allogeneic peptides presented by self-MHC molecules is the same as that for T cells responding to other foreign peptides presented by self-MHC molecules, that is $<1:10^5$ cells. Thus, the alloreactive T-cell response through the direct presentation pathway is much stronger than the response through the indirect presentation pathway and is normally the major component of the strong, initial alloimmune response to allografts which constitutes the need for potent immunosuppression in transplantation.

Analysis of alloreactive precursor frequencies

The number of available α and β chain gene segments for somatic recombination and the additional diversity generated by junctional insertion of nucleotides can result in the generation of more than 1×10^{13} different α/β TCRs, or T-cell clones, in the thymus [33]. This high number of T-cell clones ensures the optimal chance of immune protection against most pathogens that an individual will encounter. In addition to generating such a vast diversity in potential protective reactivity to pathogens, however, the repertoire must be culled to remove those clones that have high reactivity to self-peptide/MHC complexes so that the risk of autoimmune disease is attenuated. During development of the T-cell repertoire in the thymus, TCRs that engage self-peptide/MHC complexes with high affinity induce signaling that results in thymocyte death, preventing the T-cell clone from escaping the thymus and seeding the peripheral T-cell repertoire. These events are detailed in Chapter 2. Alternatively, thymocytes expressing TCR that cannot engage self-peptide/MHC complexes are not given the signals needed for cellular survival and such "useless" clones are also removed from the repertoire. It is only thymocytes expressing TCR that engage self-peptide/MHC complexes with "intermediate" affinity that are given the appropriate intracellular signaling which promotes cell survival and seeding of the clone into the peripheral T-cell repertoire.

With respect to alloimmune responses, it is important to realize that during development thymocytes are only deleted for strong reactivity to self-peptide/MHC complexes, not for strong reactivity to peptide/allogeneic MHC complexes. Because the evolution of the T-cell receptor has endowed it with inherent affinity for peptide/MHC complexes, the reactivity of TCR selected for engagement of self-peptide/MHC complexes is strong for peptide/allogeneic MHC complexes and accounts for the strong response to antigen-presenting cells expressing peptide/allogeneic MHC complexes in cultures and in allografts. Determining the actual frequency of the peripheral T-cell repertoire in mice and in humans that is reactive to defined allogeneic MHC molecules has been an actively investigated area of T cell and transplant immunobiology. Initial studies were performed using limiting dilution analyses of murine and human $CD4^+$ or $CD8^+$ T cells in proliferation or cytotoxicity assays with defined allogeneic stimulator cells [34–38]. These approaches determined the frequency of T cells responsive to any different MHC haplotype difference to be on the order of 0.2–2%. Using a different approach, T-cell clones generated from T cells responding to immunization with several model protein antigens were tested for activation by proliferation to a panel of allogeneic antigen-presenting cells with different MHC haplotypes and all clones were shown to have reactivity to one or more of the allogeneic stimulator cells [39]. More recently, the CFSE proliferation approach has been used to determine the frequency of T cells for allogeneic MHC molecules [40,41]. Overall, the precursor frequency of T cells for allogeneic peptide/MHC complexes is three to four orders of magnitude greater than the precursor frequency of T cells specific for a foreign peptide/self-MHC complex which is typically $1:10^5$–10^6, and this accounts for the strength of the alloreactive T-cell response. This is covered in detail in Chapter 9.

These studies have prompted investigation of the structural nature of the interaction of the T-cell receptor for a peptide/allogeneic MHC molecule as would occur during direct alloantigen presentation [42]. Until these studies, it was unclear if the TCR heterodimeric protein on $CD8^+$ T cells engaged the allogeneic MHC molecule with some degree of flexibility regardless of the peptide presented in the groove of the α helices of the class I MHC molecule or if the interaction of the T-cell receptor with the allogeneic class I MHC molecule was also dependent on specific peptides presented in the groove. Initial crystallographic studies indicated that the T-cell receptor protein interacts in a similar manner with peptide/self class I MHC complexes as it does with a peptide/allogeneic class I MHC molecule, with the T-cell receptor heterodimer sitting at a diagonal angle on the plane of the peptide/MHC complex and the CDR3 regions of the T-cell receptor engaging the peptide [43]. However, T-cell receptor interaction with the peptide/allogeneic class I MHC complex may occur in some instances with more flexibility in the docking angle [44]. Furthermore, at least one T-cell receptor reactive to a peptide/self class II MHC complex is also reactive to peptides presented by both allogeneic class I and class II MHC complexes [45] (see Chapter 9). Crystallography analyses, performed in conjunction with in vitro T-cell activation assays discussed above, have been used to demonstrate a T-cell receptor from human T cells reactive to viral peptide/HLA-B molecules that also reacts to allogeneic peptide/allogeneic HLA-B complexes with the same engagement structure, indicating that in some cases the structural basis of TCR alloreactivity is through molecular mimicry rather than an alteration in the physical interaction of TCR with peptide/MHC [46].

Naïve versus memory T-cell proliferation to alloantigens

The arsenal of T lymphocytes within an individual comprises both naïve and memory T cells, with the proportion of memory T cells increasing with age, reflecting an accumulation of antigenic experiences. Naïve and memory T lymphocytes are functionally, spatially, and temporally distinct entities in transplantation. Unlike their naïve counterparts, memory T cells are able to mount a faster and stronger immune response upon re-exposure to the same antigen due to their higher frequency, elevated functional avidity for the antigen, and their low requirement for costimulation [4,47]. Whereas naïve T lymphocytes are restricted to secondary lymphoid organs, memory T cells are themselves heterogeneous and two memory subsets have been identified in humans and rodents. These subsets are designated (1) central memory T cells that localize primarily to lymphoid tissue, and (2) effector memory T cells that localize to both lymphoid and non-lymphoid tissues [48]. Importantly, clinical studies have indicated that memory T cells are more resistant to T-cell depleting induction immunosuppression therapies that effectively deplete naïve T cells [49,50].

The presence of pre-existing memory T cells with reactivity to allogeneic MHC molecules in the transplant recipient is now widely recognized as a major barrier to tolerance induction and maintenance [47]. The generation of allospecific memory T cells results from previous exposure to alloantigens via prior transplantation, blood transfusions, or pregnancy. However, individuals without prior alloantigen sensitization also contain alloreactive memory T cells. Generation of allospecific memory independent of direct alloantigen exposure in such individuals is thought to occur via heterologous immunity (covered in depth in Chapter 9), in which memory T cells induced through prior environmental exposures, such as viruses, bacteria, and vaccines, cross-react to and become activated by an unrelated antigen, such as an alloantigen [51,52]. Proof of this principle has been demonstrated in mice after exposure to such pathogens as lymphocytic choriomeningitis virus (LCMV) and *Leishmania* parasites [53,54]. Studies using in vitro approaches in rodents, non-human primates, and humans have provided important insights into how memory T cells differ from naïve T cells in their response to alloantigens.

In vitro studies using mouse models have demonstrated that, on a per cell basis, antigen-specific memory T cells have less stringent activation requirements and have enhanced activation compared with antigen-specific naïve T cells. The development of TCR transgenic mice in which $CD4^+$ or $CD8^+$ T cells express a defined TCR specific for a given peptide antigen/MHC complex has enabled the side-by-side comparison of memory and naïve T cells specific for a defined alloantigen. Using such TCR-transgenic systems, it has been shown that $CD8^+$ memory T cells specific for alloantigen undergo more rapid activation than naïve $CD8^+$ T cells upon in vitro stimulation with cognate antigen, despite equivalent levels of TCR and CD8 [55]. Analyses of culture supernatants by ELISA reveal that memory $CD8^+$ T cells secrete IFN-γ and IL-2 much more rapidly and in greater quantities than their naïve counterparts. Furthermore, in vitro chromium release assays used to measure cytolytic activity have shown that memory, but not naïve, $CD8^+$ T cells are potently cytolytic [55,56]. While naïve T cells require CD28/B7-mediated costimulation provided by professional antigen-presenting cells (APCs), such as dendritic cells, memory cells may be fully activated in the absence of costimulation via the CD28/B7 or CD40/154 pathways and are more permissive to activation by different non-professional APCs, such as resting B

cells and macrophages [57]. These findings have been supported by in vitro work in which memory, but not naïve, allogeneic $CD8^+$ T cells were able to become activated, expand, and differentiate into cytotoxic T cells by co-culture with endothelial cells [58].

In contrast to laboratory mice, significant numbers of alloreactive memory T cells are present in monkeys and humans prior to transplantation. As an example, in a study of 48 cynomolgus monkeys caught in the wild, memory T cells (distinguished by surface CD95 expression) were found to comprise approximately 48% of all T cells and were equally divided between central and effector memory T cells [59]. Naïve and memory T cells were isolated from the peripheral blood of these monkeys and tested for their ability to mount an alloresponse against allogeneic irradiated donor peripheral blood mononuclear cells via in vitro ELISPOT assays to enumerate cytokine-producing cells. While only a few naïve T cells were able to respond, the pre-existing endogenous memory T cells mounted a potent direct alloresponse characterized by high frequencies of T cells secreting IFN-γ and IL-2. Furthermore, similar to the results seen in mouse studies, the kinetics and overall magnitude of inflammatory cytokine production by memory T cells in these in vitro assays was overwhelmingly greater than that seen for purified naïve T cells. These data in non-human primates provide support for the kinetically and functionally enhanced behavior of memory T cells relative to their naïve counterparts in a more physiologic setting by studying the naturally generated endogenous alloreactive memory T-cell repertoire.

Clinically, the presence of donor-reactive memory T cells in the peripheral blood of patients prior to transplant is associated with delayed graft function and poor long-term outcome of the allograft. The detrimental effect associated with the presence of these cells is further complicated by the observation in patients that effector memory T cells are relatively insensitive to induction immunosuppression with T-cell depleting agents such as alemtuzumab or rabbit antithymocyte globulin, which effectively deplete naïve T cells [49]. In 1999, Heeger and colleagues pioneered the use of in vitro ELISPOT to detect donor-specific IFN-γ-producing lymphocytes in the peripheral blood of patients prior to kidney transplantation [60]. ELISPOT assays detect cytokine production at single-cell resolution and only by cells with a history of immunologic priming, thus representing a measure of effector/memory donor-specific immunity. IFN-γ production detected in this assay was specifically a manifestation of endogenous memory T cells and the pretransplant frequency of these donor-specific memory cells has been found to strongly correlate with the post-transplant risk of developing acute rejection episodes [60,61]. The utility of this assay as a measure of the strength of pretransplant alloreactivity and predictor of graft outcome highlights the importance of pre-existing donor-reactive memory T cells independent of the number of antigen mismatches at MHC loci. Nonetheless, while the information collected from ELISPOT assays is clinically valuable, the assay cannot be performed within the time frame required for use in the context of deceased donor transplants.

An alternative in vitro method, the panel of reactive T cells, or PRT, assay has been developed as a variation of the traditional ELISPOT assay. In the PRT assay the peripheral blood T cells of transplant candidates are tested for reactivity to a panel of allogenic stimulator cells rather than to donor-derived stimulator cells. This allogeneic stimulator panel consists of B-cell lines generated from the spleen cells of several different donors, which together encompass greater than 90% of the human HLA antigens. The alloreactive response of patient endogenous memory T cells during culture with each of the allogeneic B-cell lines is measured by enumerating the T cells producing IFN-γ using ELISPOT assays and a positive pretransplant PRT has been found to correlate with acute renal graft rejection even in the absence of humoral allosensitization [62,63].

Skewed functional development of alloreactive T cells in vitro

In addition to interrogating the frequency of T cells for MHC allogeneic stimulator cells, in vitro approaches have been useful for driving alloantigen-reactive T cells to different functional phenotypes in order to test the impact of the expressed functions during alloreactive responses.

Seminal studies by Mossman and colleagues [64,65] had indicated that during in vitro activation of antigen-specific $CD4^+$ T cells, the T cells differentiate into either cells producing IFN-γ and lymphotoxin-α (LT-α) (i.e. type 1 cytokines) that are associated with delayed-type hypersensitivity responses or into cells producing IL-4, IL-5, and IL-10 (i.e. type 2 cytokines), which are associated with allergic responses, and that this differentiation depended on the co-factors included in the T-cell activation cultures. In vitro alloantigen activation of murine $CD8^+$ T-cell clones stimulated IFN-γ and LT-α production, suggesting that alloimmune $CD8^+$ T-cell-mediated responses may share functions associated with delayed-type hypersensitivity [66]. Subsequent studies from other laboratories indicated that factors produced and/or expressed on the membranes of antigen-presenting cells directed this skewing of antigen-specific $CD4^+$ T-cell differentiation into Th1 or Th2 cells [67,68]. The antigen-presenting cell function-dependent T-cell differentiation to Th1 versus Th2 cells was consistent with the model that antigen-presenting cells activating $CD4^+$ T cells for cell-mediated/delayed-type hypersensitivity responses would receive signals at the immunization site to express functions, including production of IL-12, that skew the population towards Th1 differentiation. Similarly, antigen-presenting cells activating $CD4^+$ T cells for allergic responses would receive signals during allergen immunization that induced production of factors, including IL-4, that skew differentiation to the Th2 functional phenotype [67,68]. In each case, the dendritic cell-derived signals activate transcriptional factors that skew the functional differentiation of responding T cells, with T-bet skewing to the IFN-γ-producing Th1 phenotype and GATA-3 skewing to the IL-4-producing Th2 phenotype [69]. More recently, immunologists have defined several other functional subsets of $CD4^+$ T cells, including those that express the transcriptional factor FoxP3 and mediate regulation of immunity (i.e. Tregs) and those that express the transcriptional factor RORγT and produce IL-17 and IL-23 (i.e. Th17) [69].

The discovery of antigen-specific $CD4^+$ T-cell differentiation profiles prompted studies by the transplant immunology community to investigate the ability of different functional phenotypes of alloantigen-specific T cells to mediate graft injury. Culture of purified human $CD4^+$ T cells with allogeneic stimulator cells in the presence of IL-2 or TGF-β promoted alloreactive T-cell development to cells producing IL-2 and IFN-γ whereas the presence of IL-4, IL-10, or anti-TGF-β antibody promoted $CD4^+$ T-cell development to IL-4 and IL-10 producing cells [70]. The impact of this T-cell functional differentiation on allograft outcome was addressed in studies by Orosz and Bishop laboratories comparing the abilities of in vitro differentiated alloreactive Th1 versus Th2 cells to mediate rejection in a mouse model [71,72]. These $CD4^+$ T cells were differentiated in culture with allogeneic stimulator cells and the

appropriate cytokine co-factors and neutralizing antibodies and were then transferred to cardiac allograft-bearing SCID mouse hosts. In each of these studies, Th2 cells were as effective as Th1 cells in mediating rapid rejection of the heart allografts and the alloreactive Th2 cell-mediated rejection was accompanied by heavy eosinophil infiltration of the allograft. Other laboratories used similar approaches to demonstrate the ability of alloreactive T cells skewed to Th1 versus Th2 effector functions to mediate rejection of allografts by different histopathology [73–75]. Recent studies have focused on the ability of donor-reactive $CD4^+$ and $CD8^+$ T cells producing IL-17 to mediate allograft rejection and have shown the ability of such cells to provoke graft injury in experimental models and in clinical transplants [76–78]. Overall, these studies have indicated the abilities of in vitro differentiated $CD4^+$ T cells to mediate graft injury although the specific histopathology of injury may be different based on the functions expressed by the T cells within the graft. However, during alloantigen-priming in allograft recipients, the donor-reactive $CD4^+$ and $CD8^+$ T-cell response is largely, though not entirely, skewed to an IFN-γ-producing Th1 phenotype, most likely because of the inflammatory environment created during surgical tissue stress and ischemia–reperfusion injury imposed on the graft.

It is important to note the many studies that have used in vitro approaches to derive T cells expressing regulatory functions of alloimmune responses [79–81]. These studies have tested factors promoting this differentiation, the specificities of the regulatory T cells, and the functions expressed by the regulatory T cells to inhibit alloreactive responses. A common in vitro approach to test regulatory T-cell functions is measuring their ability to suppress the proliferation of alloreactive effector T cells in response to allogeneic stimulatory cells or polyclonal mitogens. Often this approach uses CFSE-labeled effector T cells to assess decreases in the proliferation of the responding alloreactive T cells. In addition, several laboratories are now using in vitro approaches to grow large numbers of T regulatory cells for infusion into pancreatic islet and organ graft recipients in experimental models as well as in clinical transplant patients [82–85].

Summary

While transplantation remains an inherently in vivo procedure, in vitro models have been a critical tool in investigating and elucidating the fundamental mechanisms of the immune system's recognition of and response to alloantigens. The development of in vitro approaches such as the MLR have been essential to our understanding of the capacity for self-peptide/MHC selected recipient T cells to recognize allogeneic MHC molecules. Modifications of the MLR and its coupling to ELISA and ELISPOT assays have offered powerful tools, which have captured not only the ability of alloreactive immune cells to proliferate in response to alloantigen, but also express potent effector functions including cytokines, growth factors, and cytolytic molecules, which contribute to graft injury and dysfunction in vivo. The knowledge gleaned through use of such in vitro models to study alloreactive T-cell responses have extended well beyond transplantation to encompass basic immunologic principles, including the costimulatory requirements of immune cells, the fundamental differences between naïve and memory T cells, and the development and differentiation of various T-cell subsets. Importantly, not only have such in vitro studies provided novel insights for targeted clinical therapy in transplantation, but the adaptation of these in vitro tools, as in the PRT assays, are now used regularly prior to human transplantation to effectively assess the likelihood of acute rejection and patient outcomes before the surgery is even begun. Though major strides have been made in the field of transplantation immunology during the past few decades, the immune response to a surgically transplanted allogeneic organ is a complex process and many additional questions remain unanswered. In vitro models will continue to be a necessary and valuable asset to fully understand mechanisms of allograft rejection and the development of targeted therapies to improve transplant patient outcomes.

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CHAPTER 14

Small Animal Models of Transplantation

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Introduction

The understanding of alloimmunity in man has been facilitated by experimentation in animals. These studies have provided not only opportunities to develop surgical techniques but also platforms for identifying the mechanisms of alloimmune injury and the effects of immunosuppressive strategies. Initial studies primarily focused on rodent models of transplant injury, in both rats and mice. Rat models have provided significant insights into immune responses in both vascularized and neovascularized settings. Mice have shown great utility, with the strong knowledge of their immune system, the substantial availability of reagents, and, critically, the ability to genetically manipulate these hosts and donors, providing enormous information about the biology of rejection and innate immune injury. In this chapter, we will review the history of the use of animal models in transplantation, the biology and potential for therapeutic intervention in ischemic injury, define the models of alloimmune-mediated injury and agents used to ameliorate those injuries, and, finally, compare and contrast these small, preclinical models with larger animals models, including the use of non-human primates prior to the delivery of agents into humans. This chapter complements Chapters 13, 15, and 16, which deal with in vitro, large animal, in silico, models of transplantation, respectively.

In establishing a model of alloimmune injury to investigate, it is critical to understand that the immunogenicity among the transplanted organ and tissue types varies widely. While the extent of antigen presentation and specificity of tissue antigens are involved in the potency of rejection responses, the microenvironment of the allograft and access to draining lymph nodes have critical impact on the alloresponse. In rodent models of transplantation, the most rigorous rejection response is seen in skin, a non-vascularized transplant, followed in order of decreasing immunogenicity by heart, kidney, and islet grafts [1,2]. Thus findings in the stringent skin model will likely apply to less stringent models, but prolonged graft survival in a less stringent model may not be observed in the context of a more immunogenic organ.

Another key consideration in establishing a model is the critical contribution of the strain of both host and recipient. For example, kidney transplants in mice may enjoy prolonged graft survival in the absence of any immunosuppression depending on recipient strain [3]; when the donor strain is derived from Balb/c strain (H-2^d) and recipient is C57BL/6 (H-2^b), the complete MHC mismatch does not result in vigorous rejection while the reverse combination does. The use of isolated MHC class I or II mismatch results in prolonged graft rejection response with associated vasculopathy in heart allografts [4]. While the use of small animal models, in the context of inbred strains, have provided unique insights and understanding into rejection and non-immune injury, it is also important to recognized their applicability to the outbred human may have limitations. In the following sections, we will attempt to highlight the key features of each model as well as potential applicability to the human condition.

Evolution of solid organ transplant models in rodent models

The history of animal models in transplantation studies is rich, filled with solid discoveries as well as anecdotes. While a detailed discussion of this history is beyond the scope of this chapter, it is covered in some depth in Chapter 8. The use of experimental rodent models for transplant studies was clearly launched by pivotal surgical and medical advances and propelled by a high level of technical skills given the physical challenges of working with small animal organs. The French surgeon Alexis Carrel established the "vascular anastomosis," which allowed his inaugural kidney and heart transplants in dogs, and he was recognized for this by receiving the Nobel Prize in 1912 [5]. The development of inbred mice by George Snell in the 1930s, from consecutive brother–sister mating, minimized genetic variability between mice and allowed more controlled transplant experiments, including Medawar's mouse skin transplants. Snell termed the underlying genes, which remain central to alloimmunity, "histocompatibility (H)" genes. With Peter Gorer, Snell established that the major locus was identical to a locus encoding "antigen II," thus the term histocompatibility locus 2, or H-2 in mice. Jean Dausset working with antibodies in the sera of patients who had received blood transfusions, delineated the genetically determined homologue of the H-2 system in man, namely the major histocompatibility complex or HLA. Alloimmunity research in mice and humans thus became mutually complementary. This association was highlighted by the award of the Nobel Prize to both

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Snell and Dausset in 1980. The severe limitation of transplant research in having only brief acceptance of organs in unmanipulated adult animals was addressed by the availability of the first antirejection drugs. Antimetabolite 6-mercaptopurine (6-MP) and the imidazole derivative azathioprine were identified by Hitchings and Elion and became available in the 1960s [6], followed by cyclosporine [7,8] (see Chapter 17).

The development of rodent models was clearly influenced by technical (i.e. surgical skills) and diagnostic challenges (i.e. rejection), and, as these were overcome, many groups were able to utilize small animal models for their specific organ system and particular areas of interest. Islet transplantation was pioneered by Lacy in 1967, when he established a collagenase-based isolation procedure to procure islets from rat pancreata [9–11]. Importantly, he showed that implanting islets in the subcapsular space of rat kidneys was a relatively simple model that corrected the metabolic defect of insulin production in autoimmune diabetic or streptozocin treated animals. In contrast to islet transplants in which the largest challenge was the isolation of islets, in solid organ mouse transplant models progress was limited by the technical skill required (i.e. heart <kidney <liver, small bowel).

Rodent heterotopic cardiac transplantation was described in 1964 [12] in a rat model that severely restricted blood flow to the lower extremities and resulted in paraparesis and paraplegia. A revised and quickly adopted heterotopic abdominal approach in mice resulted in 90% graft and recipient survival [13] (Figure 14.1). While mouse abdominal cardiac transplants are "non-physiologic" and "non-loaded" due to the unique directionality of intracardiac blood flow, they have been widely utilized for tolerance studies, as they have low surgical costs, relatively low requirement for personnel, and a wide availability of genetically inbred mouse strains. Importantly, the low spontaneous acceptance rate of hearts compared to other solid organs (i.e. liver, kidney) facilitated analyses of potential tolerance-inducing strategies. Assessment of rejection using palpation, as originally described, continues to be used. However, assessment of contraction force from "0" (cessation of graft contraction) to "4" (vigorous graft contraction) has been criticized for being too subjective, insensitive in detecting ear¬ly changes post-transplant, and unsatisfactory for evaluation of long-term graft survival [13,14]. The gold standard for diagnosing cardiac rejection clinically and experimentally remains histology, but is not feasible for serial studies due to the small size of the rodent heart [15]. The addition of electrocardiography [16], nuclear magnetic resonance [17], ultrasound, coronary perfusion studies, and biochemical and molecular markers of rejection have all refined rejection diagnosis [18], but obviously add complexity and cost.

Mouse kidney transplantation was first described by Skoskiewicz and Russell in 1973 [19], with modifications by Kalina [20] and Zhong [21]. However, this model remains confined to relatively few transplantation centers compared to cardiac transplantation in mice, due to the surgical challenge of anastomoses presented by small-caliber vessels (the mean mouse renal artery diameter is 0.3 mm) and higher technical failure rates. The conventional method includes harvesting of the left kidney as a donor graft only, whereas the right kidney is generally considered unsuitable for transplantation due to the short length of the right renal vein and difficulty of anastomosis to the vena cava of the recipient. Anastomoses are performed end-to-side to the abdominal aorta and vena cava and urinary reconstruction is established by suturing a small bladder patch to the bladder dome (Figure 14.2). As the murine kidney is quite sensitive to ischemia–reperfusion injury (IRI), the time of anastomosis and warm ischemia is generally confined to less than 35–40 minutes. While the use of a transplant into a bilaterally nephrectomized recipient (i.e. survival model) simplifies the

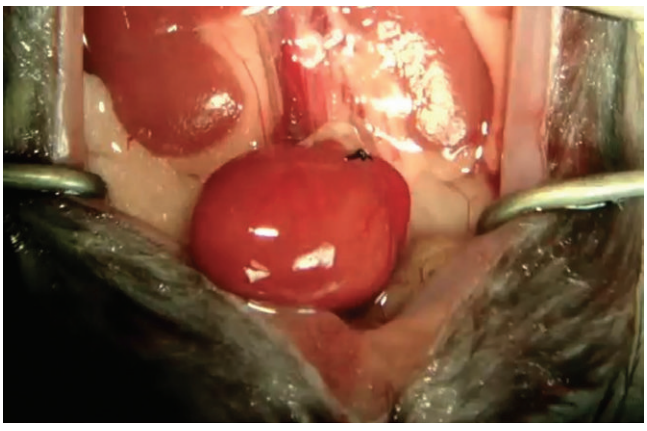

Figure 14.1. Vascularized heterotopic heart transplant in the mouse. This figure demonstrates the infrarenal placement of a cardiac allograft in a mouse. The heart is vascularized through the aortic root, and beats such that survival can be assessed by manual palpation of the animal's abdomen. However, the ventricles do not fill; as such, there is no contribution to cardiac output, and recipient survival is not dependent on graft survival. (Photo courtesy of Jean Kwun.)

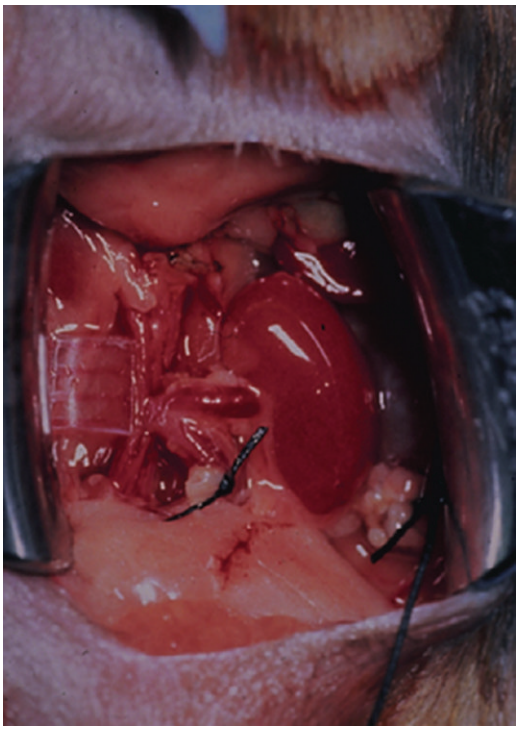

Figure 14.2. Vascularized kidney transplant in the mouse. This figure demonstrates the end-to-side vascular anastomoses and ureter/dome of bladder anastomosis that typifies the technique in mice. This can be a survival model, followed by serum creatinine, if recipient native nephrectomy is performed. If the native kidneys are left in place, the graft can be studied independent of survival.

diagnosis of rejection, this approach is clearly associated with a higher mortality rate if there is prolongation of warm ischemic time and a subsequent delay in graft function. Alternatively, a two-stage procedure can be performed, leaving the right native kidney in place until day 7. This approach, while having a lower mortality rate, may have some subtle effects on the alloimmune response and subsequent graft survival due to intrinsic immunoregulatory factors of the native kidney during alloantigen exposure [22]. While rejection can be determined biochemically through monitoring of serum creatinine, histology also remains the gold standard. This is particularly important with a spontaneous acceptance rate of approximately 30% in mouse kidney transplants using the B6 (H-2^b) to Balb/c B6 (H-2^d) strain combination [2,3].

While rat heart and kidney transplants continue to be the mainstay models in experimental rodent transplantation, the unique physiologies and immunological responses of other organs have resulted in the establishment of nearly all solid organ transplant models in mice. Surprisingly, it was not until 1991 that Starzl's group reported orthotopic liver transplantation in mice [23], based on techniques described in the initial rat liver transplants performed in the 1970s [24–26]. A higher level of spontaneous liver transplant acceptance is also well recognized, particularly in the rat model, in which low responder strains such as PVG or Dark Agouti (DA), accept completely MHC-mismatched livers for greater than 100 days [27].

Other critical models of solid organ transplant models, which have also proven to be particularly formidable challenges for tolerance induction, are those of small bowel and lung. The differential susceptibility of particular organs to tolerance induction appears to occur in organs in which there is a greater microbial exposure and activation of innate immunity [28]. Zhong described development of the heterotopic mouse intestinal transplantation in 1993 [29,30]. However, these grafts were non-functional as they were excluded from normal gastrointestinal passage and a definite endpoint for rejection was more difficult to define as the nutrition of the host was maintained by the native alimentary tract. This was addressed by the development of orthotopic small bowel transplantation in 2005 [31]. Orthotopic lung transplants have also been described in the rat [32,33] and the mouse [34,35].

Availability of strains

The unique ability to manipulate the genome of mice has facilitated an enormous series of studies that have unique opportunities to manipulate the donor and recipient and provide insights into specific arms of the immune response [36]. These genetic manipulations include the specific deletion of various components of the alloimmune response (Table 14.1). A detailed discussion of these strains and available resources for such mice is beyond the scope of this chapter but is reviewed in [37]. These strains can be used as both donors and recipients of transplants, and can test the requirements of host immunity and/or responses to donor antigens or proteins. Additionally, there is an extensive series of knockout strains that have cytokine deletions as well as critical signaling molecules in immunity. These include Janus kinases (JAK), non-receptor tyrosine kinases involved in immune and hematopoietic tissues. Deficiency of JAK3 is the defect in human severe combined immunity [38]. The signal transducers and activators of transcription (STAT) proteins are activated in response to a large number of cytokines, growth factors, and hormones, and bind to the promoters of specific target genes. Through the use of deficient mice, STAT4 and STAT6 have been identified as essential for Thl and Th2-responses and have provided novel agents to dissect alloreactive responses to solid organ transplants [39,40] (see Chapter 5). Lastly, mice deficient in specific cytokines have been similarly utilized to identify the possible roles of Th1 versus Th2 responses [41–46] (see Chapter 2).

Table 14.1. Genetically modified mice with immune defects

Strain name (abbreviation)	Defect	Immune defect	Background strain
Nude	*Foxn1*^{nu} mutation	Absent thymus, T cell depleted	H-2^d
RAG deficient (RAG$^{-/-}$)	VDJ recombinase deficiency or absence	Absent antigen-specific cellular or humoral immunity	H-2^b
Severe combined immunodeficiency (SCID)	Mutation in protein kinase, DNA activated, catalytic polypeptide (*Prkdc*)	Absent B and T cells, complement activation impaired	H-2^b
μT-deficient (μT$^{-/-}$)	Gene for membrane exon for IgM μ chain absent; IgM receptor on pre-B cells absent	B cell depletion Humoral immunity impaired	
β_2 microglobulin deficient (β_2m$^{-/-}$)	β_2 microglobulin gene disrupted	Absence of intact class I expression, CD8 T cell depletion	H-2^b
$A^b\beta$ deficient ($A^b\beta^{-/-}$)	Disruption of $A^b\beta$ gene	Absent class II expression; CD4 T cell depletion	H-2^b
CD4 deficient (CD4$^{-/-}$)	Disruption of CD4 gene	Absent CD4 T cells	H-2^b
CD 8 deficient (CD8$^{-/-}$)	Disruption of the CD8α gene	Absent CD8 T cells	H-2^b
Beige (*bg*^{j}/*bg*^{j})	Spontaneous mutation	NK cell depletion; cytotoxic T cell and macrophage function affected	
op/op	Osteopetrosis	m-CSF and monocytes depleted	
Interleukin 2 receptor, gamma chain deficient (*Il2rg*$^{-/-}$)	Mutation in IL-2 receptor gamma chain	NK cell development defect; abnormal signaling for IL-s, 4, 7, 9, 15, and 21	
Prf1 deficient (Prf1$^{-/-}$)	Perforin 1 mutation	Defect in NK and CD8$^+$ T lymphocyte function	

In summary, compared to rat models, mouse models require a more advanced level of microsurgical skill and are performed by a limited number of laboratories. Allograft rejection is much less intense in mouse models compared to rat models, even when comparing the extent of MHC class mismatches. Moreover, as noted above, there is more variability in graft survival in mice compared to the rat model. However, their relative smaller size makes them a more cost-effective approach and more scientifically elegant due to the large number of genetically modified strains available.

Models of alloimmune injury: acute injury

Acute cellular rejection

In the development of our understanding of the host immune response, acute cellular rejection has been a critical area of investigation. As already alluded to, strain differences can lead to differences in immunoreactivity. Additionally, the type of graft is

important and the microenvironment also impacts on the extent of response.

Skin transplantation remains one of the technically easy models of transplantation. The ease of transplantation allows for rapid screening of immunomodulatory strategies as well as mechanistic insights. Skin grafts have been used as a primary test of donor-specific hyporesponsiveness in preclinical studies of tolerance. Of all tissues and organs transplanted, it is the most immunogenic and provides a high degree of immune stringency. However, these grafts are non-vascularized and may undergo ischemic degeneration and necrosis, making them more susceptible to the immune response [47]. Rejection is also affected by the presence of tissue-specific antigens, which are critical targets of the immune response [48]. In addition, skin contains specific professional antigen-presenting cells (Langerhans cells), which have the capability of migrating from the graft and efficiently activating T cells. Finally, the size of the organ may also impact on graft rejection, such that the quantity of donor tissue as well as donor APCs may affect host responses [49]. More careful and detailed studies using specific mouse strain combinations comparing heart, islet, and skin rejection suggests that neither the number of resident APCs or tissue-specific antigens impact on the quantity of T-cell host response; rather, graft microenvironment and size may be more important in determining susceptibility to rejection [50]. Critical studies by Singer and Rosenberg using skin allografts have demonstrated fundamental insights into the distinct roles of CD4 and CD8 T cells during the rejection of class I disparate skin grafts in mice (reviewed in [51]).

The use of mouse kidney allografts to study rejection is discussed in detail below in the section on chronic graft injury, because, as already noted, these allografts enjoy prolonged survival in the absence of immunosuppression compared to skin and heart allografts amongst the same donor/recipient combinations. These models have provided more of a platform for assessing chronic immune injury. More recently, these models have been utilized to assess antibody-mediated injury and the impact of viral nephritis on graft function and survival. However, with strain manipulation, a more acute pattern of rejection may be seen. Indeed C57BL/6 kidneys transplanted into fully mismatched Balb/c recipients were rapidly rejected over a 2-week period; rejection was mitigated however by treatment with anti-CD45RB, which resulted in improved allograft and recipient survival, histology, and renal function [52]. Only a limited number of these recipients demonstrated true donor-specific hyporesponsiveness to a skin allograft while all rejected third-party skin. This novel blockade strategy is undergoing further studies in non-human primates with the goal of understanding mechanism of action and providing a preclinical platform.

Location of antigen engagement

Another critical concept explained almost exclusively by mouse models is the understanding of where and how antigen is engaged. While for many years the prevailing impression was that antigen engagement occurred within the allograft, it is now understood that secondary lymphoid organs, including spleen, lymph nodes, and mucosal lymphoid tissue, not only provide the proper environment for antigen presentation, but are necessary to initiate rejection. These studies utilized cardiac allografts transplanted into wild type, alymphoplastic (*aly/aly*) mice that lacked lymph nodes and Peyer's patches, and splenetomized *aly/aly* mice, which are totally devoid of secondary lymphoid tissue, as well as *Hox11*$^{-/-}$ mice which are asplenic. In spite of the absence of either lymphoid tissue or spleen, *aly/aly* and *Hox11*$^{-/-}$ mice rejected heart allografts, albeit modestly delayed in the former [53]. However, the splenectomized *aly/aly* mice accepted cardiac allografts indefinitely and failed to develop donor-specific antibodies. Thus, fully vascularized allogeneic organ transplants are unable to induce a productive immune response in the absence of secondary lymphoid tissue. In contrast, skin allografts are not rejected by *aly/aly* recipients, while *Hox11*$^{-/-}$ recipients do reject these allografts, demonstrating the importance of antigen location in defining the immune response. While these studies seem fairly convincing, studies in splenectomized lymphotoxin α-deficient and β-receptor deficient mice (two strains that lack lymph nodes and Peyer's patches) demonstrate efficient rejection of skin and cardiac allografts, albeit with delayed kinetics compared to wild-type recipients, suggesting that secondary lymphoid tissue is not an absolute requirement for allograft rejection [54]. Moreover, in small intestinal allografts, the presence of donor lymphoid tissue is a significant factor in mediating host immune responses [55]. These studies identify a key feature of immunogenicity, that is donor lymphoid tissue in the case of small bowel, but also the minimum requirements to mediate allograft rejection.

Allorecognition

A formidable number of studies have utilized mouse transplant models to identify the process of allorecognition that is necessary and sufficient for transplant rejection. Initially, direct recognition was considered the only mechanism by which an allograft could be rejected. However, it is now clear now that the indirect pathway may mediate rejection, as demonstrated in rat studies utilizing immunization with MHC molecules followed by skin grafting [56,57]. In mice depleted of CD8 T cells, donor grafts lacking class II MHC (I-A^{b} deficient mice) are rapidly rejected with evidence of CD4 T cells sensitized to donor peptides indirectly presented in the context of class II antigens on host recipient cells [58]. Again, using MHC class II deficient mice as well as class II deficient mice engineered to have an intact CD4 compartment due to expression of class II only in the thymus, Steele and colleagues demonstrated that indirect recognition is responsible for B-cell activation and alloantibody production [59]. This pathway has also been associated with chronic rejection responses and is viewed as less amenable to current immunosuppression [60].

Minor histocompatibility antigens

Minor histocompatibility antigens, immunogenic peptides derived from polymorphic self-proteins, are sufficient to trigger rejection responses (reviewed in [61]). In this setting, there is usually considerable identity of donor and recipient MHC antigens such that MHC/peptide complexes are essentially identical whether presented directly by donor, or indirectly by recipient, DCs. Additionally, there is a low frequency of alloreactive T cells that recognize minor antigens, resulting in slower and delayed graft rejection relative to the process of rejection mediated by direct recognition. The classic minor antigen is the male H-Y antigen and has been most frequently studied in mouse skin transplant models. These studies have demonstrated the requirement for CD4C MHC class II-restricted T helper cells to synergize with CD8^{+} MHC class I-restricted cytotoxic T cells [62]. Using IL-17 deficient mice (IL-17$^{-/-}$) as recipients of minor mismatched skin allografts, Vokaer et al. demonstrate that minor skin grafts have prolonged survival, and in this model Tregs can promote a Th17-mediated neutrophil-dependent pathway of graft rejection [63].

Th deviation in transplant rejection

A key consideration studied in mouse models has been the contribution of Th1 versus Th2 T cells in transplantation. Th1-derived cytokines were postulated to promote rejection by mediating delayed-type hypersensitivity (DTH) and cytotoxic responses and via antibody-dependent cellular cytotoxicity (ADCC). Th2 cytokines, on the other hand, were postulated to protect from rejection by suppressing DTH and macrophage activation, with antibody production deviating to IgE and non-cytotoxic IgG subclasses. These hypotheses were initially supported by transplant studies that correlated cytokine expression to transplant outcomes. However, the complexity of immune deviation and redundancy of cytokines in rejection was identified using cytokine knockout mice (reviewed in [64]). For example, IL-2-deficient mice were shown to be perfectly capable of rejecting islet allografts and fully mismatched heart allografts [46], indicating that a signature Th1 cytokine was not necessary for rejection. And interestingly, tolerance induction using CTLA4Ig required expression of IL-2 [65]. Double knockouts of IL-2 and IL-4 were also capable of rejecting islet and heart allografts, suggesting that remaining signaling through the γ-chain of the IL-2 receptor could mediate rejection responses, via IL-15, 7, or 9 [43]. Similarly, long-term survival under the cover of costimulatory blockade (CSB) requires IFN-γ as this therapy was ineffective in IFN-$\gamma^{-/-}$ recipients [66]. Moreover, IFN-$\gamma^{-/-}$ mice rejected completely mismatched or class I mismatched allografts, but were unable to reject class II mismatched grafts [67]. Thus, while IL-2, IL-4, and IFN-γ contribute to acute rejection, they are not required. Similarly, as will be discussed below, the association of Th2 responses with long-term graft survival is another oversimplification, in that the unopposed actions of IL-4 in IL-$2^{-/-}$ or IFN-$\gamma^{-/-}$ mice does not lead to prolonged graft survival [45,46,65,66]. Overall, these studies exemplify how unexpected findings in the mouse models have provided unique insights into the role of Th immune deviation and its contributions to the transplant immune response.

Th17 cells and their function in transplant rejection and tolerance is of recent interest. The prevailing notion is that Th17 cells are a barrier to transplant tolerance and may have an antagonistic response to Treg differentiation. For example, inhibition of IL-17 prolonged rat cardiac allograft survival [68], and there have also been correlations of IL-17 expression with chronic injury in lung transplantation in rats [69]. More direct support comes from the observation that Tbet-deficient mice (Tbet$^{-/-}$) which lack an intact Th1 response, rapidly and vigorously reject cardiac allografts with associated IL-17 producing CD4 T cells within the graft [70]. Similarly, CSB is ineffective in Tbet$^{-/-}$ mice, and is associated with CD8$^+$ IL-17 producing T cells [71]. However, whether IL-17 producing T cells are important mediators of immune injury in clinical transplant remains under debate. It is unclear whether, in the setting of an active Th1 response, Th17 cells play a role in rejection and, moreover, whether Th17 cells are responsive to conventional immunosuppression remain inconsistent (reviewed in [72]). Finally, the ability of Tregs to control Th17 cells in transplantation remains unanswered.

The functional capacity of Tregs has been explored extensively in animal models with the general goal of enhancing the activity of these cells by either (1) ex vivo expansion with adoptive transfer or (2) providing conditions within the host to facilitate this population's role in suppressing allogeneic immune responses. In the former approach, human CD4 T cells were cultured and polyclonally stimulated ex vivo; the resulting Tregs were sufficient to suppress vasculopathy in a chimeric humanized arterial allograft in mice [73]. An alternative approach by the same group involved enrichment of alloreactive induced Tregs by stimulation of recipient CD4$^+$ T cells with donor antigen-presenting cells under defined culture conditions. In this case, the Tregs were effective in prolonging survival of MHC-disparate skin grafts in mice [74]. Similarly, human CD45RO$^-$CD25$^-$CD4$^+$ T cells, cultured under similar conditions, were effective in suppressing vasculopathy in a human arterial allograft model [74]. Treg adoptive transfer following ex vivo expansion and maintenance in culture with indirect allospecificity has also been beneficial in inducing long-term survival of completely MHC-disparate mouse cardiac allografts [75]. Alloantigen-specific expansion of FoxP3$^+$CD25$^+$CD4$^+$ Tregs in the presence of bone marrow-derived dendritic cells led to recipient hyporesponsiveness to cardiac allografts in mice but had no effect on third-party donor grafts [76]. Thus, there are some convincing data in preclinical models to demonstrate feasibility of this approach which may be applicable to man.

The manipulation of the recipient in order to facilitate Treg expansion has been a focus of a number of studies in tolerance induction (see below). These studies demonstrate the importance of recipient immunosuppression in affecting outcome. For example, a number of have implicated the use of mTOR inhibition as beneficial in mediating long-term survival in the setting of CSB [77,78] and in development of Tregs in man (reviewed in [79]), but the benefit is not observed consistently [80]. Alternatively, treatment of recipient mice with antilymphocyte serum and donor pancreatic lymph node cells results in the proliferation and expansion of FoxP3$^+$ Tregs and supports islet allograft survival [81]. Treatment with IL-2/anti-IL-2 complexes was associated with in vivo expansion of FoxP3$^+$ Tregs in mice, and in concert with G-CSF treatment expanded myeloid-derived suppressor cells and led to a significant delay of MHC class II disparate skin grafts [82]. These data support a potential strategy in human subjects, although it may be difficult in practice to support these approaches. A cautionary note is that recent studies indicate inhibition of the B7/CD28 pathway using CTLA4Ig in mice may alter Treg homeostasis and be detrimental in situations where graft prolongation is dependent on Tregs [83].

Innate immune mechanisms of allograft injury

The role of NK cells in acute rejection is an area of intense investigation, and covered specifically in Chapter 7. The mechanisms by which innate immunity triggers and collaborates with adaptive responses is key not only to initial clinical management but long term as well, where innate responses are not the primary focus of immunosuppressive therapy. Studies in rats have shown that depletion of NK cells fails to impact the kinetics of rejection [84,85]. However, NK cell activation has been identified in acutely rejecting rat cardiac allografts [86] and specific subsets may contribute to rejection responses to mouse cardiac allografts [87] through direct recognition of alloantigens. This was functionally demonstrated when CD28-deficient (CD28$^{-/-}$) mice that received anti-CD154 antibody rejected cardiac allografts [88] and depletion of their NK1.1 cells allowed indefinite survival [89]. Blockade of NKG2D, the activating immune receptor expressed on the surface of NK and CD8 T cells, similarly prevented rejection in CD28$^{-/-}$ recipients [90]. NK cells have also been shown to directly mediate graft rejection, as evidenced by the fact that IL-15 (an NK cell activating cytokine) precipitates rejection of skin transplants in RAG knockout mice (which lack B and T cells) but not RAG-common gamma chain (γc, CD132)-deficient mice, which also lack NK cells [91].

Macrophages may also participate in acute allograft rejection (reviewed in [92] and covered in Chapter 7) and a number of studies have utilized mouse models to investigate this pathway. Genetic deletion of CCR1 [93] was associated with prolonged survival of both MHC class I and class II mismatched heart allografts in mice; deletion of CCR5 [94] was associated with improved rejection of class II mismatched heart allografts. In both instances, macrophage infiltration was reduced. Treatment of recipient mice with DTCM-glutarimide, which inhibits macrophage function, prolonged survival of fully mismatched heart allografts [95]. Treatment with liposomal clodronate, an agent that eliminates macrophages, improves renal allograft function and reduces inflammation in a rat model [96]. Conditional depletion of macrophages in the transgenic CD11b-DTR mouse resulted in marked reduction in inflammation of kidney allografts [97]. Collectively, these studies demonstrate the proinflammatory function of macrophages in acute rejection.

Acute antibody-mediated injuries

Antibody mediated-rejection is an increasingly recognized means of graft failure (see Chapter 6) With the recognition that antibody-mediated injury is a critical cause of late kidney allograft loss [98] and also a key feature of ongoing injury in heart transplantation [99], there is now a critical need for the study of mechanisms in animal models of injury. Colvin and colleagues have provided a sequence of events in man based on non-human primate (NHP) models [100]. In the context of small animal models, the focus of this current decade has been on mouse models, to define the pathogenesis of antibody development, injury mechanisms, biomarkers, and potential therapeutic interventions.

Acute antibody-mediated rejection (AMR) is a difficult entity to recapitulate in mice. Passive transfer of antibodies to donor-specific MHC proteins into $RAG^{-/-}$ mice does not result in a dramatic antibody-mediated injury response and this is titer dependent [101]. Use of monoclonal antibody against a specific MHC determinant passively transferred into a $RAG^{-/-}$ recipient with donor-specific cardiac allograft has similarly failed to induce substantial injury, although there is evidence of antibody deposition on capillaries [102].

The passive transfer studies do provide some interesting insights into the development of antibody-mediated injury. There are limitations, however. The use of monoclonal antibodies focuses on either class I or II determinants and in man there is frequently a combination. Moreover, in humans, anti-class II antibodies are the predominant type in late graft failure. In mice, class II molecules are not expressed on resting endothelium and are up-regulated only when there is inflammation; in man, class II protein is constitutively expressed. Finally, $RAG^{-/-}$ lack intact cellular responses, which may facilitate B-cell activation as well as produce key cytokines to alter MHC expression in allograft tissue and activating other inflammatory cells in the rejection response.

An alternative model, reported by Bickerstaff and colleagues, is the use of CCR5 knockout mice ($CCR5^{-/-}$) [101]. In this model, A/J cardiac allografts transplanted into $CCR5^{-/-}$ are rapidly rejected with minimal cellular infiltration but intense deposition of C3d in the large vessels and capillaries of the graft, characteristics of AMR. Donor-specific antibody titers were substantially elevated compared to wild-type recipients and adoptive transfer of this serum led to rapid humoral rejection of donor-specific heart allografts. Similarly, accelerated rejection is also seen in isolated class I differences, and this response is clearly dependent on continual production of antibody and not cytotoxic T cells [103]. These results are not organ specific as A/J kidney allografts transplanted into $CCR5^{-/-}$ mice are rapidly rejected with histologic evidence of antibody deposition [104]. Use of μ-chain-deficient mice $CCR5^{-/-}$ doubly deficient mice as recipients of kidney allografts abrogates this response, demonstrating the dependence of antibody-producing cells to mediate organ rejection.

While these models have focused on capillary deposition of antibody and complement, arterial injury may also be seen. Using segments of human mesenteric artery interposed into the abdominal aorta of beige/SCID mice accompanied by transfer of anti-class I HLA antibody results in complement deposition and neointimal formation [105]. These results are difficult to put entirely into context of a cardiac allograft as there is disruption of the vasa vasorum in the adventitia and this effect is not accounted for in the model of injury development. This will be discussed further below in the context of chronic antibody-mediated graft injury.

Complement activation

The key role of complement activation leading to endothelial injury has been examined as an alternative potential pathway to ameliorate antibody-mediated injury (also see Chapters 2 and 7). Complement inhibition has been the focus of animal studies to mitigate acute injury. First reported in 1995 in a pig-to-primate xenograft cardiac transplant model of hyperacute rejection, anti-C5 antibody prevented the development of hyperacute rejection, allowing normal function and the absence of histologic injury [106]. Subsequent studies in mice demonstrated a similar beneficial effect. In this model, Balb/c mice were first grafted with C3H skin, and 7 days later, received a cardiac allograft from the same donor strain as the skin. This resulted in accelerated graft loss by day 3 post-transplant. A combination of low-dose cyclosporine, cyclophosphamide, and anti-C5 antibody resulted in prolonged graft survival of >100 days, with normal histology despite the presence of systemic and intragraft antidonor antibodies and complement, with immunological alterations in both the graft and the recipient, were required for successful graft accommodation to occur [107].

Blocking complement activation using soluble complement receptor 1 (sCR1) demonstrated similar findings in hyperacute rejection models following presensitization. For example, Pruitt and Bollinger demonstrated an amelioration of vascular injury in Lewis recipients presensitized with ACI skin and subsequently transplanted with ACI kidneys [108]. Similarly, in DA recipients of Lewis kidneys treated with soluble complement receptor 1 (sCR1) there was reduced vascular and cell-mediated injury compared to untreated control allografts [109]. Cobra venom factor, which mitigates C3 activity, suppressed hyperacute rejection in presensitized Lewis recipients of ACI heart allografts [110]. The genetic absence of terminal component of complement C6 also resulted in modification of vascular injury in cardiac transplantation. In this model, PVG rats ($RT1^c$) were genetically modified to lack C6 and were recipients of ACI hearts after sensitization by skin allografts. The lack of this terminal complement component showed reduced antibody and cellular-mediated injuries compared to wild-type recipients [111]. Finally, blockade of C5 convertase, in association with CTLA4Ig significantly prolongs survival of completely mismatched cardiac allografts in mice, by also apparently blocking T-cell-mediated graft rejection [112]. Thus, acute antibody-mediated injury, in the context of pre-existing antibodies in both rats and mice, whether kidney or cardiac allografts, appears to be ameliorated by strategies that limit complete activation of the complement

cascade. Such provocative data have supported recent clinical trials in humans (reviewed in [113]).

It is important to note, however, that early complement components are important anti-inflammatory mediators. Deficiency of C1q is associated with autoimmune phenomena, perhaps due to its role in clearing apoptotic bodies by macrophages [114]. Indeed, deficiency of C1q may alter alloimmune responses as C1q-deficient mice reject cardiac allografts with a tempo faster than wild-type recipients [115]. This study indicates the complex role of early complement proteins.

BK polyomavirus nephropathy

A vexing problem in kidney transplantation is BK polyomavirus infection. The virus has a tropism for urogenital epithelium and may become reactivated or transmitted from the donor allograft in the context of over-immunosuppression. The resulting infection leads to a proinflammatory response within the kidney allograft that histologically mimics acute cellular rejection with associated allograft dysfunction [116]. Treatment is primarily by immunosuppressive withdrawal and, in some circumstances, antiviral therapy, which may include cidofovir, limited in its effectiveness by intrinsic nephrotoxicity [117]. Monitoring has improved the opportunity for earlier detection and intervention. However, critical questions remain in understanding the pathogenesis of this disease, intervening against the strong CD8 cytotoxic T cell and associated profibrogenic responses [118], and identifying new antiviral therapies. These issues could be further addressed using animal models of disease.

Finding an appropriate small animal model has been difficult due to the specificity of BK virus to human tissue. BK infection similar to that in man has been reported in cynomolgus monkeys during studies of novel immunosuppressive strategies [119] and also in squirrel monkeys [120]. A mouse model of polyomavirus nephropathy has been described more recently [121]. In these studies, C57BL/6 mice were subcutaneously inoculated in the hind footpad with mouse polyomavirus (Mpy) wild-type strain A2. Peak infection was detected at day 4 postinoculation and was associated with the expansion of $CD8^+$ cytotoxic T cells, as detected by MHC class I tetramers containing viral peptide epitopes as well as by measuring viral peptide stimulated IFN-γ production. While there was no detectable virus in blood by 6 months postinfection, there was detectable virus in some solid organs including the kidney at that time point.

In the transplant setting, kidneys from either C57BL/6 ($H\text{-}2^b$; isografts) or C3H/HeJ mice ($H\text{-}2^k$; allografts) were transplanted into C57BL/6 recipients with associated bilateral native nephrectomy. Acute infection with MPy at day 1 post-transplant accelerated kidney allograft failure compared to uninfected allograft recipients, and had no effect on isograft function and recipient survival. Examination of the kidney demonstrated increased tubular cell loss, inflammation, and interstitial edema in infected allografts compared to isografts or uninfected allografts, associated with elevated viral loads within the kidney in infected recipients. Moreover, there was an enhanced antidonor cytotoxic T-cell response, which may lead to allograft destruction. This model could provide new insights into the immunologic and viral factors that lead to graft failure in human PVN. Interestingly, IFN-γ inhibits replication of BK in this model by blocking viral replication and diminishing viral protein expression [122]. The use of mouse models of infection may thus provide substantial insights into viral replication, viral persistence, and therapeutic effects, while also defining the pathogenesis of progressive allograft injury in man [123]. While informative, these studies do not precisely mimic human infection, which may be quiescent for years prior to reactivation or may present as a primary infection.

Models of alloimmune injury: "chronic" injury

Long-term graft survival remains a critical issue in all solid organ transplants. Key features of this disease include allograft fibrosis, ongoing inflammation, and vasculopathy. Small animal models have provided a number of unique insights into the human condition, providing further information regarding therapeutics, biomarkers, and pathogenesis (reviewed in [124]). A key issue in this field is finding the proper model to emulate the specific human disorder being studied.

Kidney chronic injury

In the kidney, late allograft failure was referred to for many years as "chronic rejection" and subsequently as "chronic allograft nephropathy" ("CAN") [125]. This term relates to the characteristic histologic features of interstitial fibrosis (IF) and tubular atrophy (TA). Numerous investigators began to consider these findings a disease entity and a cause of late graft failure even in the absence of tissue for histologic diagnosis. Consequently, in current clinical practice, the term "CAN" has been eliminated and specific pathological investigation into the cause of IF/TA and functional failure are emphasized [126]. Thus, finding ideal models may be limited to those with IF/TA but recognizing that for etiologies such as BK or antibody-mediated injury, more specific models as described herein, apply.

The rat models were the first to be developed, likely due to the relative technical ease. As already discussed, strain manipulation is key, providing a more stable model of prolonged allograft failure. First reported in 1969 by White et al., the use of only minor MHC mismatches led to prolonged graft survival [127]. The most common model is the F344 graft into a Lewis recipient coupled with immunosuppression of low-dose cyclosporine A (CsA; 5 mg/kg/d) for 10 days [128]; here, the donor and recipient are matched at class I and class II with minor and non-MHC antigen disparities. The kidney is fully vascularized, heterotopically placed in the abdomen with a full connection of the transplant ureter to the recipient bladder, with a staged or simultaneous bilateral nephrectomy that results in survival on the transplanted graft. Within 12 to 16 weeks post-transplant, segmental or global sclerosis is seen with increasing tubular atrophy, intimal proliferation, and gradual luminal occlusion of cortical vessels. There is renal function deterioration accompanied by the development of nephrotic range proteinuria at 24 weeks. Numerous studies of this model have emphasized a proinflammatory response including T-cell and macrophage activation of the host [128,129]. Prolonged administration of CsA in this model was associated with worse interstitial fibrosis, while mycophenolate treatment was associated with a down-regulation of proinflammatory cytokines [130]. Impressive amelioration of this injury and histologic abnormalities were seen with CSB, specifically a single dose of CTLA4Ig [131,132]. The development of injury in this model may be further mediated by a reduction in nephron supply provided by a single kidney transplant, as retention of one native kidney mitigated the development of proteinuria and the allograft glomerulosclerosis [133], suggesting that in this model inadequate nephron mass is contributory to chronic injury.

However, such studies underestimate the potency of the alloimmune response operating in this model, and, in particular, underestimate the humoral component.

Mouse kidney transplants were limited in development due to the need for microsurgical expertise. The key operative challenges were the relatively small caliber of blood vessels and the ureteral connection, which was successful using a dome to dome anastomosis. While initially developed for the study of acute rejection of the kidney, it became clear that these models rejected with altered kinetics and severity compared to rat models as well as similar strain combination skin allografts [134]. B10.D2 (H-2^{d}) kidneys transplanted into B6AF1 (H-$2^{b/a}$) mice enjoyed prolonged survival over a period of weeks. Some recipients survived >1 year post-transplantation. These animals were shown not be tolerant based on in vitro assays of donor-specific responsiveness and the ability to mediate immunologic injury by treatments that heightened donor reactivity [135].

The factors related to this hyporesponsiveness and the potential use of these models to study more chronic tempo rejection have more recently been studied in depth. Mannon and colleagues demonstrated that prolonged graft survival in mice was associated with poor allograft function and histologic features of chronic immune injury including IF/TA [3]. Studies using class I and class II MHC knockout strains in which intact MHC molecules were absent on donor tissue demonstrated modestly altered kinetics of graft failure, and modest reductions in severity of these responses when either donor class I [136] or class II was absent [137]. The complete absence of donor MHC, however, resulted in significant improvements in function and the reduction in IF/TA changes [137]. These studies further demonstrated the critical components of both the cellular and humoral immune responses to mediate chronic allograft injury. They also utilized renal function as measured by inulin clearance as a more sensitive measure of injury.

A number of studies have examined the role of recipient hyporesponsiveness to kidney allografts, investigating the role of the kidney microenvironment. An interesting phenomenon is that recipient $CD8^{+}$ T cells down-regulate the cell surface expression of the TCR and, as such, may have altered immune activation [138]. Spontaneous acceptance, with graft survival greater than 60 days in the absence of immunosuppression seen in DBA/2 kidneys transplanted into C57BL/6 recipients, is associated with up-regulation of host TGF-β expression but not IL-10 [139]. This expression pattern was not seen in cardiac allografts of the same strains which rapidly rejected [140]. Furthermore, these kidney allografts are infiltrated by $FoxP3^{+}$ regulatory T cells (see Chapter 8), with up-regulated expression of IDO and regulatory dendritic cells [141]. These studies suggest immune regulation in recipient mice with both more immediate and, perhaps, more sustained responses, which allow for prolonged survival but still have ongoing immune responses that result in a chronic injury pattern of IF/TA.

Therapeutic interventions in chronic graft injury in the kidney

Based on data implicating the renin–angiotensin system (RAS) in fibrosis models in other kidney diseases [142], and the stimulation of the profibrogenic cytokine TGF-β by RAS activation [143], numerous studies have evaluated the impact of RAS blockade on mitigating the chronic injury. These studies have typically utilized the rat model of chronic fibrosis and glomerulosclerosis with F344 grafts into Lewis recipients, described above. Treatment with angiotensin receptor blockade, initiated at the time of transplantation, was associated with a reduction in urinary protein, glomerular capillary pressure, and glomerulosclerosis [144,145]. There were also reductions in proinflammatory cytokines and interstitial inflammation [144]. These effects were specific to this class of agent as blood pressure reduction using alternative agents did not show similar benefits [146]. These studies indicate the potential benefit of RAS inhibition in long-term graft failure, a clinical therapy already approved in humans.

It would seem to follow that regardless of injury, the long-term complications of both immune and non-immune mediated injuries are the developments of allograft fibrosis and tubular atrophy. Thus an alternative to treatment would be the use of antifibrotic agents. These agents attack the development of collagen formation by either stimulating matrix degradation or slowing or abrogating deposition. There have been limited preclinical studies in transplantation with these agents and these have been reviewed [147]. However, some are quite encouraging. The use of prolyl-4-hydroxylase inhibition (PHI), to limit formation of active collagen chains, ameliorated chronic injury in a mouse model of kidney allograft fibrosis, with a significant improvement in renal function and reduction in interstitial fibrosis and inflammation [148]. However, these agents have broader impacts within the kidney, including the overexpression of hypoxia-inducible factor and many downstream genes [149], which, in and of itself, may have a modulatory impact on inflammation.

The limitations of this approach may not seem obvious at first, but there are several difficulties in using these agents. Most studies have engaged therapy in the immediate post-transplant period, which prevents fibrotic injury but requires all recipients to be treated de novo. In practice, this would not be acceptable in man. The toxicities of these agents, including failure of wound healing, are obvious. The PHIs have potential effects on other biological pathways, including the induction of erythropoietin [149]. Thus, intervening at the time of developing injury would be more reasonable, but the biomarkers for fibrotic injury in transplant recipients remain an enigma. Finally, the instigating events of fibrosis are not affected by these strategies, leading to an uncompensated activation of profibrotic and repair pathways, a futile situation for the recipient's graft.

Cardiac vasculopathy

Chronic allograft vasculopathy, the leading cause of late heart allograft failure, has been studied extensively using mouse models of cardiac allotransplantation. This lesion is characterized histologically by intimal proliferation with luminal stenosis of epicardial branches and small vessels. In rats, heterotopic cardiac allografts between MHC class I compatible strains (RT1.A and/or RT1.E) develop intimal proliferation if surviving at about 30 days post-transplant [150] to as far as 120 days in the Lewis to Fisher-344 recipient [151]. These combinations likely differ in other MHC loci as evidence of acute rejection and myocardial inflammatory cell infiltration occur. Using ACI donors and (LEW×BN)F_1 recipients, which differ in both MHC and non-MHC antigens, grafts that survived 20 to 50 days developed arterial intimal fibrosis, which was modestly affected by brief CsA treatment [151,152]. A more detailed study of rat strains and the development of this histology was carried out by Cramer et al. [153]. Here, syngeneic control grafts demonstrated minimal abnormalities compared to strain combinations with MHC class I incompatibilities, which displayed variable levels of acute inflammation as well as intimal fibrosis. Non-MHC incompatible combinations had lesions more consistent

with mild-to-moderately severe allograft rejection, with concomitant development of myocardial fibrosis, and concentric myointimal changes in medium-to-large intramyocardial arteries. These differences demonstrate the critical dependence of strain on model outcome, with vascular lesions less prominent in recipients with more active acute rejection and less common in class I disparate recipients or syngeneic controls.

The pathogenesis of these vascular lesions has been studied extensively. Infection with CMV may mediate endothelial cell injury, vascular smooth muscle proliferation, and subsequent intimal thickening [154,155]. As implied above, an earlier inflammatory stage precedes vascular injury, and macrophages play a critical role, responding to up-regulation of MHC class II and ICAM-1 on endothelial cells [156,157] with associated up-regulated intragraft MCP-1 [158] and induction of nitric oxide production [159]. Strategies to limit mononuclear cell infiltration in the rat are limited. Further successful attempts have been utilized in a limited fashion in mouse models discussed below.

While donor-specific antibody has been strongly implicated in the pathogenesis of cardiac vasculopathy in the rat, modulation of T-cell responses have shown significant success. CSB, typically used in the early post-transplant period, has shown dramatic impact in late injury. Using the Lewis to Fisher-344 model, and a single dose of CTLA4Ig 2 days post-transplant, graft survival extended to nearly 70 days with a reduction in proinflammatory and macrophage-related cytokines as well as a reduction in the frequency and severity of arteriosclerosis [160], demonstrating the prominent role of T-cell activation early on in the development of chronic injury. Similarly, blockade of the CD40–CD154 pathway has ameliorated both acute and chronic injury in this rat model with prolonged graft survival [161]. Mitigation of chronic injury in this model has also been successful using a hematopoietic chimerism approach by transplantation of T-cell-depleted allogeneic marrow, demonstration the potential of this approach to affect long-term outcomes [162]. Other successful strategies include the use of genetically engineered dendritic cells that over-express indoleamine 2,3-dioxygenase [163], the oral delivery of donor-specific antigens [164], and treatment with synthetic peptide corresponding to residues 75–84 of the alpha1 domain of the HLA-B7 molecule [165]. These various approaches have all demonstrated significant modulation of the T-cell response and subsequent vasculopathy. However, further development in clinical trials has not occurred.

Murine models of transplant vasculopathy have also been studied and have provided some very specific insights that are available through the use of genetically engineered donor organs and recipients. A number of proposed models currently exist and their utility is apparent. Heterotopic transplantation of B10.A hearts into B10.BR recipients reproducibly develops intimal proliferative lesions, characteristic of transplant coronary vasculopathy in the absence of immunosuppression, over a period of 4–6 weeks [166]. This combination differs in the D and L loci of MHC class I, but are identical for the K locus of class I, class II, and minor MHC proteins (see Chapter 4 for detailed discussions of MHC molecule structure and function). Additional strain combinations that elicit the presence of vasculopathy over 4–7 weeks include transplants with isolated MHC class II differences (bm12 [H-2^{bm12}] into C57/BL6 [H-2^{b}] and bm6 [H-2^{bm6}] into C57/BL6 [H-2^{b}]) as well as multiple non-H-2 differences between donor and recipient (129/J [H-2^{b}] into C57BL/6 [H-2^{b}]) [4,167]. Complete MHC disparity between donor and recipient may also be used but requires adjuvant immunomodulation. For example, transplantation of CBA/CaJ [H-2^{k}] hearts into C57BL/6 [H-2^{b}] will develop vasculopathy under coverage of combined anti-CD4/anti-CD8 therapy [168]. Chronic vasculopathy will also develop in DBA [H-2^{d}] into C57BL/6 [H-2^{b}] recipients following prolonged treatment of anti-CD4 [169] or gallium nitrate [170,171], an immunomodulatory semimetallic element that inhibits T-cell and macrophage activation in part by affecting iron transport. Long-term acceptance of these allografts under gallium treatment is associated with production of TGF-β within the recipient and suppressed donor-specific DTH responses [172].

The critical role of alloantibody has been further evaluated in these models. While a B10.BR heart into a B10.A recipient does not develop arteriopathy, the addition of passive transfer of polyclonal antidonor antibodies leads to coronary lesions in a dose-dependent fashion [173]. B10.BR hearts transplanted to C.B-17 SCID mice results in arteriopathy, again only with the addition of anti-B10.BR serum [173]. Similarly, B10.BR hearts transplanted in $RAG^{-/-}$ mice lacking both B and T cells that further received passive transfer of monoclonal IgG2a anti-H-$2K^{k}$ also develop arteriopathy [174] and this response was dependent on continued administration of donor-specific serum. Moreover, reconstitution with passive transfer of IgG2b antibody into immunoglobulin knockout mice (C57BL/6 Igh-6) may recapitulate antibody-mediated injury of cardiac allografts [175]. While intimal thickening may occur in the context of donor-specific anti-class I antibodies, these responses are not dependent on complement fixation [176]. These data support the notion that chronic injury may occur in humans in the absence of detectable complement activation histologically.

The contributions of both antigen-dependent and independent mechanisms of rejection have been evaluated in these models. The dependence of rejection on CD8 cytotoxic T-cell responses has been demonstrated in both class I and class II mismatched models using CD8-deficient mice [177] or via CD8 depletional antibody therapy [168]. Costimulatory blockade of the CD40–CD154 pathway but not CD28/B7 alone has been effective in ameliorating vasculopathy [178,179], but this is a strain-dependent response [180]. Innate immune responses are also contributory. Carrageenan depletion of macrophages in the recipient ameliorates vasculopathy in C57BL/6 into (C57BL/6xBalb/c) F_1 recipients, suggesting macrophages as end-effector cells in a final common pathway that may be independent of B- or T-cell immunity [181]. Innate immune responses contributed by NK cells was also apparent using CCR4-deficient recipients [182] or in the context of parental to F_1 transplants [183], or in mice genetically deficient in mature NK cells (γ chain knockouts) [184]. Finally, non-antigen-dependent factors include oxidant stress [185], up-regulation of adhesion molecules [186,187], and the presence of viral infection [188].

Chronic vascular and glomerular injury also can be caused by non-MHC specific antibodies, suggesting an autoimmune phenomenon associated with injured tissue. In the rat model, these include cardiac myosin [189], other non-MHC antibodies in cardiac allograft failure in the rat [190], antibodies to perlecan and type IV and VI collagens responsible for glomerulopathy in the F344 into Lewis recipient [191], and antimesangial cell antibodies [192,193]. In the mouse, antibodies to vimentin [194] have also been identified as pathogenic. Further understanding of the contribution of antibody responses to structural proteins in chronic injury is needed.

While these studies highlight the development of vasculopathy, Bishop and colleagues have studied the development of graft

hypertrophy and fibrosis. Their studies, using an MHC complete mismatch cardiac allograft (H-2^b into H-2^d) with anti-CD4 antibody induction, have highlighted a novel pathway of fibrosis, mediated by IL-6 [195] and the downstream effector CTGF [196] and Th17 cells [197]. These studies and the use of this model may help to determine novel therapeutic targets in inflammatory-mediated cardiac hypertrophy. Finally, fibrosis is markedly reduced in heart allografts in IL-$17^{-/-}$ mice treated with [198] or without CSB [199], suggesting a detrimental effect of IL-17 in this development of chronic injury.

Vessel allografts—an alternative model for accelerated arteriosclerosis?

As an alternative to heterotopic cardiac transplants as a model of graft arteriosclerosis, a number of investigators have utilized the implantation of arterial "jump" grafts attached to the recipient aorta. Models exist in both rat and mouse recipients. In the former, combinations have included DA ($RT1^a$) into Wistar Furth ($RT1^u$) without immunosuppression for a 1 to 5-month period [200], Lewis ($RT1^l$) to F344 ($RT1^{lv}$) grafts over a 75-day period without immunosuppression [201], Brown Norway($RT1^n$) into Lewis [202], and PVG ($RT1^c$) into DA ($RT1^a$) [203]. A cellular inflammatory response was commonly seen, with intimal and adventitial infiltrates containing T cells and macrophages. Murine models have provided more intense scrutiny of these immune responses. An initial report using B10.A into C57BL/6 recipients highlighted the vascular injury, and smooth muscle cell proliferation within 30 days in the absence of immunosuppression and further demonstrating the technical feasibility of this mouse model [204]. However, the elegant use of genetically modified mice highlighted the need for both intact cellular and humoral arms of the immune response, particularly B cells, macrophages, and $CD8^+$ T cells [205] to mediate this inflammatory intimal fibrosis.

While these data provide interesting clues to the cellular and antibody-mediated injuries, and this model is easy to reproduce, these grafts have some significant differences from fully vascularized allografts. As already noted, the vasa vasorum, a network of microvasculature in the adventia that supplies nutrients and oxygen to the arterial wall, is disrupted in these grafts. This lack of physiological underpinning has significant implications and may alter the development of this lesion, making the findings less biologically relevant [206]. The lack of interstitial dendritic cells also implies that the direct pathway of allorecognition is relatively absent, necessitating only indirect pathway activation of recipient T cells. This is in contrast to the dual pathways that are activated in vascularized organ rejection. These limitations have made this model less biologically relevant in examining graft arteriosclerosis and the intact organ models are favored.

Bronchiolitis obliterans syndrome and lung allografts

Past mechanistic studies of chronic lung injury, called bronchiolitis obliterans syndrome (BOS), were limited by the availability of small animal models. A commonly used approach is the heterotopic tracheal allograft. Here, a section of trachea is implanted under the skin in a pocket of a recipient mouse [207]. In general, MHC incompatible strains are used and some immunosuppression, typically CsA, is provided over a 4-week period. Tracheal allografts developed epithelial injury and cellular infiltrates with both CD4 and CD8 T cells by 2 weeks post-implantation with epithelial denudation and luminal obliteration by 6 weeks and, finally, dense collagenous scarring by 15 weeks [208,209] with a broad outpouring of T-cell cytokines [210]. Severity of immune response is based on MHC mismatch with a primary response against MHC class I antigens which is both cellular [211] and humoral [212]. Further investigation has identified the epithelium as a key target of immune injury in which epithelial regeneration is an active process, providing a self-renewing source of antigens [213,214]. This targeted injury is further augmented by viral infections, including CMV [215]. TGF-β and IL-13 may also play a role as a profibrogenic cytokine [216,217]. Like other models of chronic allograft injury, costimulatory blockade ameliorates the development of this lesion [218]. Finally, inhibiting fibrocyte migration using anti-CXCL12 attenuates the histologic features of BOS [219].

While this is a facile model to study airway injury, there are limitations to a model which is physiologically passive without ventilation and hence does not recapitulate the intact lung model. Secondly, this is a neovascularized graft, and lacks the development of a microcirculation, which has now been identified as a key contributor to allograft injury [220]. Finally, the morphology of the trachea differs from the bronchioli and cannot be compared to small bronchioli after orthotopic lung transplantation [221]. Hence, a number of laboratories have developed a rat fully vascularized heterotopic lung transplant model and later a mouse model. These models differ in donor and recipient strain combination and include Wistar Kyoto into F-344 [222,223], F-344 into Wistar Kyoto [224], Lewis into F-344 [225], and Brown Norway into Lewis [226,227]. These models typically show a progression of inflammatory T-cell-mediated injury leading to fibrosis and airway occlusion and may require low-dose CsA treatment with injury evolving over months [226,228,229], although this has not been consistently reported [223,230]. Additional insults, including charcoal installation [226] and viral and bacterial infections [225], have stimulated more aggressive injury. The inconsistent results of these models are still unclear but do not appear to relate to surgical technique. The lesions may require two insults and may relate to strain variation from vendors [224].

A more elegant model of orthotopic transplantation in the mouse has been developed. Here, MHC-incompatible C57BL/6 left donor lungs are transplanted into Balb/c recipient [35]. Extensive inflammatory cell infiltration by CD4 and CD8 T cells as well as monocytes are seen in the first 7 days post-transplant, and marked graft edema. Treatment with dual costimulatory blockade using anti-CD154 mAbs and CTLA4Ig results in severe vascular rejection with interstitial fibrosis, while airway epithelium remained intact [231]. Using a staged pneumonectomy and right donor lung, recipient's survival depends on the transplant and both functional and histologic features of rejection may be quantitated [232]. In this model, rejection is independent of secondary lymphoid organs [233]. These studies demonstrate the feasibility of orthotopic lung transplantation in the mouse, with an emphasis on acute rejection. Further changes in the donor and recipient strains has provided more consistent findings of BOS, such as lungs from C57BL/10 (H-2^b) mice transplanted into C57BL/6 recipients, where only minor MHC disparities exist. Here, there was a rapid onset of BOS features within 2 weeks post-transplant which is ameliorated by neutralizing IL-17A [234]. The use of CsA with steroids in a Balb/c allograft into a C57BL/6 recipient resulted in BOS after about 10 weeks with an acute inflammatory injury consistent with rejection, although pulmonary function measurements did not correlate to the extent of severity of late histologic abnormalities [235]. These promising studies in a technically

difficult surgical model may provide new insights into the mechanisms of injury mediating BOS.

The toxicity of immunosuppressive agents

The role of the calcineurin inhibitor CsA as an immunosuppressant and its mechanisms of action are discussed in Chapter 17. In its early clinical use, CsA was found to mediate dose-dependent renal failure and was initially seen in renal transplant recipients (reviewed in [236]). With its accepted use, nephrotoxicity was also demonstrated in the native kidney, in recipients of non-renal organs, as well as patients treated for autoimmune diseases [237]. These clinical observations led to a detailed analysis of a rat model of nephrotoxicity whereby Sprague Dawley rats treated with 12.5 mg/kg CsA daily for 3–10 weeks developed reduced renal function and the histologic features of "striped fibrosis" with tubular degeneration along the thick ascending limb of the loop of Henle, as well as fibroblast proliferation and collagen deposition in the outer medulla [238]. These findings were exacerbated by a low-sodium diet but unaffected by unilateral nephrectomy. Other rat stains have been used including Wistar [239] and PVG [240] with similar responses, indicating that this is not a strain-specific phenomenon. Doses of CsA utilized include 5–25 mg/kg/day, for an average of 4 weeks [239], and nearly every model emphasizes the use of low-sodium diet to enhance the vasoconstrictive process.

Mechanistic studies using this toxicity model have identified several critical classes of mediators, including angiotensin II [241–246]. Indeed, spironolactone [247] or angiotensin receptor blockade [242] treatment ameliorated the renal injury in this model. Similarly, vasoconstrictive eicosanoids like thromboxane A_2 [248–252] and leukotrienes [240,253] have been shown to be up-regulated in this model, and inhibition of these pathways not only improves renal function but histology as well. Other implicated molecules include endothelin [254] and nitric oxide [255–257].

The profibrogenic effects of CsA have been associated with the induction of PDGF and TGF-β within the kidney [239,258–260], as well as CTGF [261]. The functional impact of TGF-β in this model was further demonstrated by mitigation of the CsA-induced fibrosis following treatment with the pirfenidone [262] as well as anti-TGF-β antibodies [259]. Furthermore, as angiotensin II expression has been linked with the induction of TGF-β, several studies have demonstrated a reduction in TGF-β when the angiotensin pathway is antagonized [242,263]. HMG-CoA reductase treatment has also been associated with a reduction in renal fibrosis and interstitial inflammation in nephrotoxicity of this model [264]. While TGF-β is a unifying theme for the development of fibrosis in this model, antagonists of this pathway may have unintended consequences as TGF-β has strong immunomodulatory actions as well.

Due to the tubular injury mediated by CsA, it should come as no surprise that apoptosis occurs during exposure. This has been demonstrated in the nephrotoxic rat model by TUNEL staining [265]. Up-regulation of proapoptotic genes also occurs within the kidney of nephrotoxic rats [266] as well as a mouse model [267]. Reversal of this expression as a potential therapeutic strategy may ameliorate injury [268–270]. Additional modulation of the fibrotic injury may also occur via nestin expression, which may be found in the interstitium of kidneys following CsA nephrotoxic injury [271]. Depletion of macrophages at the time of injury was similarly associated with improved renal function and reduction in interstitial fibrosis following CsA treatment [272].

Endoplasmic reticulum (ER) stress has been identified as a potential mechanism mediating the injury of CsA on human tubular epithelium in vitro [273]. Indeed, prolonged treatment with CsA in the rat is associated with the ER stress that accompanies the apoptosis of the epithelium [274] and autophagy of epithelial cells following CsA treatment [275]. Moreover, protection of ER from CsA-mediated stress improves renal function and the histologic features of CsA toxicity in the rat [276]. These studies suggest a novel mechanism that could be an alternative therapeutic avenue to ameliorate CsA-mediated tubular injury.

While most of the mechanistic data regarding CsA nephrotoxicity has been obtained using rats, there have been a number of reports utilizing a mouse model. Similar to transplant models, a mouse model provides the advantages of smaller animal size, the convenience of genetically manipulated models, and greater understanding of the immune response and reagent availability for the mouse. However, mice are resistant to this treatment [277,278] and as such require additional maneuvers. A reduced sodium diet of 0.01% is combined with 30 mg/kg/day subcutaneous administration of CsA in ICR mice, and, by day 28 of treatment, these animals developed the characteristic lesions associated with CsA nephrotoxicity [279]. In contrast, a normal sodium diet, even with 100 mg/kg/day treatment, had no impact on renal function or structure, even out to 56 days of treatment. Associated with this injury is an increase in apoptotic tubular epithelium and up-regulated gene expression for apoptotic proteins [267], much like that seen in the rat model. These reports provide the possibility of an alternative model to study CsA injury. Whether there are differences in nephrotoxicity mediated by tacrolimus as compared to CsA has yet to be explored.

Small animal models of transplantation tolerance

Animal models, and in particular rodents, have been extensively studied for more than 50 years, in the pursuit of predictable and robust transplant tolerance strategies that might be successfully applied clinically. Discussions on the mechanisms of tolerance and on the clinical application of tolerance protocols can be found in Chapters 11 and 76, respectively. Despite our contemporary focus on rodent models, the field of transplantation tolerance has been critically dependent on both small and large animal models. Indeed our foundation of tolerance understanding has benefited greatly from integration of species observations. J.B. Murphy showed, as early as 1914, that foreign tissues could grow on the chorioallantoic membrane of the chick embryo due to immunoincompetence, which could be reversed by the concomitant administration of adult spleen cells [280]. The evolution of transplant tolerance studies continued with large animals observations in 1945 by R.D. Owen, noting that dizygotic cattle twins display red cell chimerism–mosaicism. This was followed by Medawar's observation that dizygotic cattle twins generally had a natural "tolerance" for each other's skin allografts [281]. The association of transplantation tolerance and utility of small animal (i.e. rodent) models was, however, firmly established in 1953 when Medawar's group reported that tolerance could be established with the adoptive transfer of lymphocytes to fetal mice that could subsequently accept skin transplants from the same donor [282].

Transplant tolerance tends to be operationally defined in small animal models, usually with an allogeneic graft being accepted 100–150 days after cessation of therapy. Tolerance is more firmly

established if the animal is unable to reject a graft from the same donor, yet retains the ability to reject "third-party" tissues or organs. Due to technical limitations in small animals, often a donor-specific skin graft is used a second graft challenge. While the skin graft remains a "gold standard" as it is the most sensitive to rejection, skin tissue is very immunologically different from the first graft. Indeed, it has been suggested that acceptance of a skin graft may be too rigorous a criterion for tolerance, as the number of T cells required to elicit skin graft rejection is 6000 times smaller than that required for heart rejection [50].

While the use of small animals in transplant studies has greatly expanded our understanding of tolerance, it is clear that they have not provided a "Rosetta stone" to unravel the tolerance phenomenon, nor have they become a substitute for large animal (i.e. swine), non-human primate (NHP), or clinical studies in patients. While tolerance in mice was demonstrated by Medawar's experiments, it was evident even in 1953 that transplant tolerance in inbred strains of mice was influenced by complex factors beyond his mouse model, as graft survival could be permanent or transient and it was highly strain specific. Furthermore the induction of tolerance by Medawar in mice depended on the presentation of allogeneic antigens to fetal mice before they were immunologically mature, a situation that may be rarely clinically relevant. However, his pioneering experiments in the 1950s unequivocally demonstrated that alteration of recipient immunity could lead to acceptance of adult tissues, and, in doing so, launched the field of "immunoregulation." Despite their limitations, small animal models continue to provide valuable insights in transplantation tolerance. Advanced technologies continue to provide not only novel therapeutics that require validation in small and large animal models, but also unique genetically modified mouse models that can be used to test specific potential pathways or more accurately reflect human systems.

Limitations of small animal models in tolerance studies

There is little doubt that tolerance insights have come from mice and transplant studies have been revolutionized by the use of genetically engineered transgenic, knockout, and mutagenized mice [283–288], as well as monoclonal antibody probes for lymphocyte membrane markers [289]. However, tolerance appears to be more readily achieved in small, inbred animals compared with large, outbred animals and even robust animal models seem less challenging than patients. The biology of tolerance, even in mice, is complex. Of note in Medawar's classic experiments of central tolerance, only three of five mice became tolerant of skin grafts after perinatal injection of donor tissue, and one of these three animals lost the skin graft between days 75 and 91 after transplantation [290]. It is apparent that while small animals and rodents remain "economical" for identifying potential strategies of tolerance induction, there are complex factors that alter their validity for human transplant tolerance approaches. Immune systems of rodents differ from those of larger animals and certainly primates (Table 14.2), with differences in MHC organization, age-related decline in T-cell regeneration, T-cell repertoire diversity, B-cell and T-cell development in response to different regulatory factors, differences in cytokine and chemokine repertoires, cytotoxic effector cell bias in rejection, and homeostatic T-cell proliferation and memory T-cell expansion in response to depletional strategies [291–293]. It is also well known that many inbred mouse strains commonly used for experimental purposes represent extreme immunological phenotypes, including their tendency to generate Th1 as opposed to Th2 responses [294]. Perhaps the most relevant difference is animal longevity, which results in primed T cells and the formation of memory T and B cells in long-lived "antigen-experienced" primates and humans as compared to short-lived rodents, which tend to remain "antigen naïve" [290,295–297]. Tolerance strategies based on CSB, for example, are therefore very successful in rodents and less efficacious in animals with a higher proportion of memory cells [298].

Table 14.2. Comparison of rodent models to non-human primate models used in experimental transplantation research

Feature	Non-human primate	Rodent
Handling	Difficult especially larger animals	Relatively easy
Cost per animal	High	Low
Access to strains	Restricted	Widely available
Robustness	High	Low
Transgenic models	Not available	Available
Technically challenging	More	Less
Mimic human immune system	Foundations similar	Foundation and applicability
Lifespan	1–3 years	20–40 years
Physiologic readouts	Complex	Simple
Breeding	Easy	Very difficult (not done)
Availability of reagents	Limited/ non-specific	Wide variety/strain specific
Spontaneous mutations	Uncommon	Several
Useful for testing therapeutics	More applicable	Less applicable
Similarity to human anatomy	Similar	Distant
Physiology similar to humans	Similar	Distant
Nutrition	Complex regimen	Simple regimen

Transplant tolerance in small animals

Central transplant tolerance

Self-reactive T cells and B cells are eliminated in primary lymphoid organs in mice and humans, namely thymus and bone marrow [287,299,300]. While the thymus is essential for T-cell maturation and the induction of "central" tolerance to self-antigens through clonal deletion, self-reactive T cells can exit the thymus and are capable of potentially causing autoimmune disease (see Chapter 2). Immunoregulation thus necessarily includes "peripheral tolerance" mechanisms, primarily anergy [301], peripheral deletion [302], and regulatory T and B cells that can down-regulate the activity of autoreactive cells [303,304]. While the thymus' capacity for deletion of antigen-reactive T cells has been shown following intrathymic administration of islets in mice [305], intrathymic delivery of allopeptide may be limited by involution of thymus in adults. The transient presence of donor antigen may also account for the lack of graft protection observed clinically using donor cells in heart transplant patients [306].

Mixed chimerism transplant tolerance

Mixed lymphohematopoietic chimeras result from irradiated mice (and larger species), reconstituted by a mixture of T-cell depleted host and donor bone marrow. A form of central tolerance, both host and donor lymphoid and hematopoietic elements remain in these animals for the lifetime of the recipient and transplanted donor tissue is subsequently accepted without immunosuppression [307]. Mixed chimeric tolerance is robust in small animal recipients that can accept highly immunogenic skin and small-bowel grafts across extensive major and minor histocompatibility antigen barriers

[308,309]. Mechanistically, the tolerant state involves deletion of reactive cells as well as peripheral regulation mechanisms [310–314]. Requirement for pretransplant host conditioning, including sublethal irradiation, the use of myeloablative agents, and limited longevity of mixed chimerism, have limited the clinical application of this strategy except for specific patients such as those with hematological malignancies and concurrent renal failure [315]. Mixed chimerism follows T-cell-depleting monoclonal antibodies, but local thymic irradiation appears to be more durable in mice than non-human primates, in which chimerism appears to be transient [316,317]. Work in rodent models has thus focused on replacing toxic conditioning to reduce host morbidity, such as high-dose MHC mismatched bone marrow and only a single injection of anti-CD40L and cytotoxic T-lymphocyte-associated antigen 4 immunoglobulin (CTLA-4 Ig) [318]. Potentially, the use of donor-derived embryonic or mesenchymal stem cells, which possess immunoregulatory capacity, may increase the efficiency of mixed chimerism strategies [319–322]. While conceptually central tolerance is attractive, its translation into the clinic has been slow because the conditions necessary for achieving mixed hematopoietic chimerism have not yet been achieved using protocols with clinically acceptable toxicities.

Peripheral transplantation tolerance

Costimulatory molecule blockade

As self-antigen reactive cells can escape thymic deletion, additional mechanisms exist to regulate the functional capacity of T cells with potential autoreactivity that have emerged into the periphery. Peripheral tolerance is a broad term applied to naturally arising mechanisms that lead to the anergy, deletion, or regulatory cell suppression of self-reactive T cells in the periphery. Investigations using mouse transplant models have been critical in identifying mechanisms of peripheral tolerance to alloantigens, and, more importantly, developing clinically relevant strategies to exploit these mechanisms. Peripheral immune responses in mice are attenuated by "damping" receptors on T and B cells and promoted by costimulation. Damping receptors have been extensively studied in mouse transplant models and include molecules such as CTLA-4 [323], T-cell immunoglobulin mucin family (TIM) members [324], programmed cell death receptor (PD1) and its ligand PDL-1 [325,326], and on B cells, Fc, and other inhibitory (ITIM) receptors [327,328].

The blockade of the CD28-CD80/86 costimulation at the time of alloantigen recognition has been shown in experimental mouse models to be a robust and effective strategy for inducing peripheral tolerance. A second costimulatory pathway of importance is the CD40 (found on APCs)/CD40L (CD154) (found on T cells) pathway, which plays a pivotal role in the development of $CD4^+$ T-cell responses [329]. Signaling through CTLA-4 appears to be an essential pathway for graft acceptance [330]. $CD4^+CD25^+$ T regulatory cells have been shown in mice to express constitutively surface and cytoplasmic CTLA-4 (CD152) [331] and blockade of CTLA-4 with anti-CTLA-4 antibody led to acute rejection of skin allografts [332]. Early studies demonstrated that the partial inhibition of T-cell function by blocking signal 1 (anti-CD4 and anti-CD8 mAbs) or signal 2 (anti-CD40L or CTLA4Ig) induced long-term allograft survival in mice [333]. Inhibition of signaling through the CD80/CD86 (B7)-CD28 pathway using a CTLA-4 Ig fusion protein containing the extracellular domain of human CTLA-4 fused to human immunoglobulin Fc chain was shown to be effective in transplanting skin and cardiac allografts [334]. Experimental mouse models using CTLA-4 Ig were pivotal in more recent pharmacotherapeutic development targeting the B7:CD28/CTLA4 pathway by a high-affinity CTLA-4 Ig fusion protein variant termed LEA29Y (belatacept), which was FDA approved for use in clinical renal transplantation in 2011 [335–337].

There are adjunctive strategies that can enhance the effect of CSB in rodent transplant models. $CD4^+$ T cells appear to be more susceptible to CSB than $CD8^+$ T cells and in some mouse models allograft survival following costimulatory molecule inhibition is augmented by reduction or depletion of $CD8^+$ T cells [291]. In the mouse skin allograft model, treatment with anti-CD40L mAb and donor-specific transfusion of cells (DST) prolonged survival of skin grafts by inducing the deletion of alloreactive $CD8^+$ T cells [338–341]. CTLA-4Ig therapy has also been combined with an infusion of donor alloantigen or anti-CD40L antibody [342], as induced tolerant states in rodents tend to be more robust when CSB is combined with donor antigen presentation. While the use of calcineurin inhibitors can block CSB-induced tolerance under some conditions [77,343], rapamycin has been shown to enhance CSB effect and increase long-term acceptance of completely major and minor mismatched skin transplant models [77]. While CTLA-4 and CD40L are primary targets for mouse CSB transplant studies, there are additional CSB targets. In a mouse cardiac allograft model, mice deficient in the induced costimulatory molecule ICOS ($ICOS^{-/-}$) showed prolonged allograft survival [344,345]. Programmed death-1 (PD-1) and its ligands PDL-1 and PDL-2 (homologues of B7) have also been tested in mouse transplants. In a cardiac allograft model, mice treated with a PD-L1 Ig fusion protein showed prolonged allograft survival, and in some cases permanent survival [346].

T-regulatory cells

Donor-specific immunoregulatory activity exists within the lymphocyte compartment of rodent transplant recipients, and its role in transplant biology is specifically considered in Chapter 8. Suppressor or regulatory T cells are critical to the induction and maintenance of donor alloantigen unresponsiveness in vivo: this may represent an important strategy to exploit for therapeutic transplant tolerance. Peripheral regulation of immune response by lymphocytes was suggested in the 1970s, using mouse skin transplant models [347–349]. Purified splenic T lymphocytes from mice made tolerant prior to skin transplantation, transferred donor-specific unresponsiveness into antilymphocyte-treated secondary recipients, often permanently. Controversy over the existence of $CD8^+$ "suppressor cells" subsided with the emergence of strong evidence for $CD4^+$ regulatory T cells. It has been known for some time that short-term administration of blocking anti-CD4 and anti-CD8 monoclonal antibodies can produce a "dominant" form of tolerance in mouse skin allograft recipients, in that donor-specific nonresponsiveness is transferable using multiple serial transfers of lymphocytes into sequential generations of naïve recipient mice. This phenomenon is referred to as "infectious tolerance" [347,350]. However these early studies of infectious tolerance were described in mice with minor histocompatibility mismatches and infectious tolerance is less evident or absent in mice transplants with major genetic disparities.

The majority of $CD4^+$ regulatory cells (Treg) in normal individuals are so-called natural or nTreg cells which develop in the thymus. Regulatory $CD4^+$ T cells can also be induced (iTreg) by various strategies. The expression of CD25 (IL-2R alpha) expression has been used to more specifically identify and enrich subpopulations

of $CD4^+$ T cells that demonstrate powerful regulatory activity in mice [351]. $CD4^+CD25^+$ T cells capable of regulating responses to alloantigens in vivo, can be isolated from mice pretreated with donor-specific transfusion (DST) and anti-CD4 monoclonal antibody [352]. The importance of a key transcription factor in $CD4^+$ regulatory T-cell function was dramatically demonstrated using homologous mutant scurfy mice, which lack the forkhead transcription factor 3 (Foxp3). Selective ablation of $CD4^+Foxp3^+$ Treg cells resulted in "catastrophic" multisystem autoimmunity [353]. In addition to Foxp3, a number of molecules have been found in mice to be expressed by T cells with regulatory activity, including CD62, CD103, and GITR [291,351]. Alloantigen-specific $CD4^+CD25^+$ regulatory T cells are able to prevent skin graft rejection initiated by not only $CD4^+$ but also $CD8^+$ T cells [291]. Other populations of regulatory cells clearly exist, including $CD8^+$, $CD8^+CD28^+$, $TCR^+CD4^-CD8^-$ ("double negative"), and natural killer T cells. Thus $CD4^+CD25^+$ cells may only represent one subset of important regulatory cells in rodent transplant models [354,355]. Regulatory T cells in mouse models are responsive to cytokines in the microenvironment. IL-10 and TGF-β play key roles in the suppressive activity of alloantigen-specific $CD4^+CD25^+$ regulatory T cells. Tregs isolated from recipients pretreated with an anti-CD4/DST tolerizing protocol and co-transferred with naïve effector cells into T-cell deficient mice failed to prevent skin graft rejection when treated with an anti-IL-10 receptor antibody; in the absence of anti-IL-10R antibody treatment, all grafts were accepted [332]. Similarly, TGF-β1 mRNA was expressed at high levels in accepted cardiac allografts from DST-treated rats [356].

Role of the graft microenvironment in tolerance

It is increasingly evident that the donor graft has a capacity to interact or avoid recipient immune responses to allow acceptance or enhance tolerance. Antigens placed in the anterior chamber of the eye are not attacked by the immune system, demonstrating that tissues can hold back damaging immune effector systems [357]. Spontaneous acceptance of liver and kidney allografts and more recently embryoid bodies in mice without any added immunosuppression supports the existence of tissue-based protective mechanisms [134,358,359]. In co-transplantation experiments, the donor kidney was required not only to induce but also to maintain tolerance to co-transplanted heart allograft from the same donor. Interestingly, adoptive transfer of $CD25^+$ cells from heart–kidney recipients to heart-only recipients produced short-term, but not long-term, tolerance, suggesting ongoing education of Tregs in the kidney [22]. Factors, including heterogeneous genetic backgrounds, the nature of the solid organ transplanted, and concurrent ischemia or infections, which increase innate inflammation, can alter the development of tolerance. Experimental mouse models will need to address these factors to provide data relevant to the clinical transplantation.

Ischemia–reperfusion injury

Tissue ischemia is an inevitable consequence of solid organ transplantation. It is increasingly recognized that the initial injury incurred by the graft during organ procurement and implantation is a significant contributor to graft outcome.

History of cold preservation

In 1849, Loebel attempted the first perfusion of an isolated organ [360]. Then in 1895, Langendorf developed a simplified device for organ perfusion. Lindbergh and Carrel were the first to propose hypothermic preservation and later created a perfusion apparatus for this. It was not until 1964 that Belzer with Najarian, while at the University of California at San Francisco, began work on hypothermic perfusion techniques for use in the deceased donor kidney transplant program [361]. In 1969, Collins helped convert the practice of transplantation from an emergency procedure to a semielective one by developing the first effective cold storage solution using rabbit models [362,363]. This solution proved to be of great help in extending times of successful kidney preservation, but unfortunately was not suitable for prolonged periods and for hepatic tissue [360]. In early 1980, Belzer and Southard developed the University of Wisconsin (UW) solution [364]. The UW solution has seen several improvements but, despite being superior to many other solutions, was not suitable for organ storage much beyond 24 hours. Not surprisingly, cold ischemia time is the single most important predictor of delayed graft function (DGF) following kidney transplantation [365]. After transplantation into the recipient, reinstitution of blood flow (reperfusion) leads to further tissue injury through generation of free radicals that further contributes to DGF [366]. The sum of injuries resulting from "warm" and "cold" ischemia is referred to as ischemia–reperfusion injury (IRI) [367]. Although most grafts with DGF will go on to have acceptable function, the long-term survival of these grafts is significantly worse than in those with immediate function [368]. By negatively influencing graft half-life, IRI contributes to the growing problem of donor organ shortage through the number of patients requiring re-transplants. The organ shortage problem has prompted the use of expanded criteria donors; however, by their nature, ECD organs are more prone to IRI and therefore DGF [369,370]. More recently, the use of pulsatile perfusion has been demonstrated to improve renal function and is also associated with improved graft survival in human kidney allografts [371]. Thus, IRI has become a major focus of experimental transplantation research.

Cold ischemic injury

Animal models have been developed to further investigate the mechanisms and potential treatments of IRI [372–374]. For cold ischemia, the organ (e.g. kidney) is removed, flushed, and stored at low temperature under conditions similar to cold preservation for human transplantation [375]. After a variable period of time, the kidneys are then re-implanted and reperfusion ensues. Before development of the mouse transplant models and transgenic technology, cold ischemia (bowel, heart, lung, liver, and kidney) was predominantly studied in dogs, rabbits, and rats [376–378]. Recently, the mouse model has overtaken all other animals as the choice model for studies of IRI. Together, these experimental models have contributed immensely to our understanding of the mechanisms of cold ischemic injury [360,374,379,380]. In particular, animal models were instrumental to the development of organ cold preservation solutions [381]. Despite how well the cold ischemia model is representative of events during human organ transplantation, much of what we know of transplant IRI stems from studies using the warm ischemia model [367,382]. This is despite the fact that the distribution and pattern of injury may substantially differ from that of cold ischemia–reperfusion models.

Pathophysiology of IRI

IRI is a dynamic process, which involves two distinctive yet interrelated phases of injury: ischemic organ damage and inflammation-mediated reperfusion injury. Cellular injury can arise from passive

or active mechanisms. Passive mechanisms include depletion of cellular ATP/GTP [383], intracellular acidosis, and hypothermia [360,384]. The active phase of cellular injury begins with reperfusion and leads to activation of both innate and adaptive immune pathways [385,386]. A key feature of IRI is the accumulation of reactive oxygen species (ROS), believed to arise from the decoupling of the electron transport system in mitochondria as a result of IRI-mediated damage. Although multiple cellular and molecular pathways contribute and regulate tissue damage, the crosstalk between innate and adaptive immune systems plays a significant role in the pathogenesis of liver IRI. The evolving model states that the initial tissue injury from ischemia–reperfusion serves as an "adjuvant" for alloimmune response to the organ through activation of innate immune mechanisms [386].

The ischemic-injured cell faces one of two fates: either recovery from sublethal injury with regeneration the epithelium [387] or cell death by apoptosis or necrosis [388–391]. Apoptosis is a common feature of IRI [373,382]. The importance of cell death to the pathogenesis of IRI is highlighted by the fact that inhibition of apoptotic pathways at the outset of IRI ameliorated tissue damage and limited organ dysfunction [392–397]. This is perplexing because apoptosis is regarded as an immunologically silent mechanism of corpse disposal [398]. In this context, a "surge" of dead cells may overwhelm endogenous clearance mechanisms [399], enabling apoptotic cells to undergo secondary necrosis [400,401]. These necrotic cells, unlike apoptotic cells, spill their intracellular contents into the extracellular milieu and instigate a sterile inflammatory response (SIR) [402] through activation of the innate immune response [386,403,404] (see Chapter 7). This "collateral damage" inflammatory response is the key element of organ dysfunction (reviewed in [405–407]) and may be triggered in part by endogenous "danger" signals or alarmins, which are released from cells that are distressed, damaged, or dying an abnormal death in order to alert the immune system [408–410]. Danger or damage-associated molecular pattern (DAMP) molecules are normal cell or extracellular matrix components, which are released by cellular injury or action of proteases at the site of tissue damage, respectively [411]. Inflammatory signals may be transduced from injured cells and initiate tissue damage via the IL-1R-Myd88 pathway [409], or TLR4 in kidney ischemic injury [412] and renal epithelium may directly contribute by directing renal inflammatory responses.

The immune response of IRI

Innate immune cells such as NK and NKT cells are part of the early infiltrate in the mouse kidney after IRI [413]. While NKT cells lack rearranged T-cell receptors (TCR), these cells respond to lipids, glycolipids, or highly hydrophobic peptide antigen presented by the atypical MHC class I molecule, CD1d (see Chapter 2). Blockade of CD1d or depletion of NKT cells or use of mice deficient of NKT cells markedly mitigates ischemic injury in the mouse kidney [414]. In addition, NK cells may trigger syngeneic tubular epithelial cells to undergo apoptosis, and adoptive transfer of NK cells may worsen renal IRI in NK cell-, T cell-, and B cell-null mice [415]. Deletion of CD39 on NK cells, which is associated with marked decreases in phosphohydrolysis of ATP and ADP to AMP with accompanying modulation of purogenic receptors, mitigates warm ischemic injury in the liver in mice [416]. Taken together, these results demonstrate NK cells and NKT cells contribute substantially to IRI.

Animal models have been further used as critical tools to dissect the contributors of damage and injury in IRI (Table 14.3). For example, treatment of uninephrectomized rats prior to renal ischemia with anti-CD11a and anti-CD11b resulted in improved renal function and inflammatory infiltrates [417]. There is now overwhelming evidence suggesting that T cells serve as direct mediators of IRI. In CD4 and CD8 doubly deficient mice subjected to renal ischemia, there was significantly improved renal function, reduced neutrophil infiltration, and markedly decreased tubular atrophy scores compared to wild type, suggesting a protective effect of T-cell depletion on renal IRI [418]. Similarly, T-cell-deficient mice are also protected from renal [419] as well as hepatic [420] ischemic injuries, indicating the requirement for $CD4^+$ T-cell responses to mediate injury [420]. The protective effect of costimulatory blockade of the CD28/B7 pathway provides further evidence for the role of T cells in the pathogenesis of IRI [372,421].

Table 14.3. Pathways identified in ischemia–reperfusion injury using small animal models

Pathways in IRI and repair	Model	Reference
Derangements in medullary blood flow	Rat warm IRI	[480,481]
Macrophages	Mouse warm IRI; siRNA	[482]
B cells	Mouse warm IRI	[483,484]
T cells	Rat and mouse warm/cold IRI; NUDE; SCID; $Rag1^{-/-}$; adoptive transfer; $CD4^{-/-}$; $CD8^{-/-}$	[372,420,485–488]
NKT cells	Mouse warm IRI; $Jalpha18^{(-/-)}$	[489,490]
Toll-like receptors	Mouse warm IRI; $TLR4^{-/-}$	[317,412,423,491]
Neutrophils	Mouse warm IRI	[492]
ICAM-1	Mouse warm IRI; $ICAM\text{-}1^{-/-}$	[492]
Complement	Mouse warm IRI; $Cr1^{-/-}Cr2^{-/-}$	[413,483,493–496]
Selectins	Mouse warm IRI and bowel transplant; P-selectin knockout	[156,497,498]
Origin of cells replenishing dying tubular epithelial cells	Mouse warm IRI; fate-tracing; bone marrow chimera	[387,441,442]
Apoptosis	Inhibitors; knockout mice	[396,413,499,500]
Fibrosis	Mouse warm IRI; fate-tracing	[444,501,502]
Hypoxia-inducible factors	Mouse warm/cold IRI; inhibitors; knockout mice	[503–506]

Targeting IRI

As outlined in Table 14.3, the complexity of IRI and the connection of adaptive to innate immunity have made IRI a therapeutic focus beyond cold storage solutions and ex vivo machine perfusion. We have already noted the role of antiapoptotic strategies to limit IRI. Numerous studies have demonstrated a cytoprotective effect of carbon monoxide (CO) in IRI in rat liver [422,423], kidney [424–426], lung [427], and small bowel [428,429], as well as other organs and tissues (reviewed in [430]). Inflammatory cell infiltration may be mitigated by sequestering lymphocytes in secondary lymphoid organs, preventing them from entering the ischemic organ using a sphingosine 1 phosphate analog, which induces lymphopenia in mice and protects them from ischemic injury in the lung [33] and kidney [431–433]. As adenosine is a vasodilator in many tissues, selective activation of adenosine A_{2A} receptors has been shown to reduce tissue injury in the heart, liver, spinal cord, lung, and brain (reviewed in [434]). Inhibition of HMG-coA reductase has similarly been shown to ameliorate IRI in mouse kidney and with the known safety profile in man may be useful therapeutic adjunct [435]. Matrix metalloproteinases that degrade matrix molecules are also involved in propagating IRI, and following genetic deletion in the mouse have a negative impact on IRI [436]. Thus, animal studies, using genetic deletion strategies or depletion of protein or

receptor blockade demonstrate a variety of molecules that contribute to ischemic tissue injury.

One of the major limitations of targeted therapies is the potential for non-specific, off-target effects. RNA interference (RNAi) is an emerging strategy that offers high inhibitory activity, limited duration of action, minimal toxicity and precision that has been studied in IRI [437]. For example, siRNA targeting the transcription factor p53, a molecule that regulates apoptosis in renal IRI, ameliorates ischemic tissue injury in the mouse [438,439]. This strategy is already in clinical trials in man. An attractive alternative to delivering siRNA into the recipient is to add it to the organ preservation solution before transplantation.

Tissue repair after renal IRI

A remarkable feature of the kidney is its ability to repair itself after injury, but the extent of organ recovery is dependent on the extent and severity of injury as well as regulation of inflammation [440]. Tubular epithelial cell (TEC) proliferation is a hallmark of tubular repair. Shortly after the injury phase following IRI, the normally quiescent kidney tubular cells enter the cell cycle [441]. The origin of the cells repopulating the injured kidney remains controversial and the use of mouse bone marrow chimeras from green fluorescent protein transgenic mice helped establish that bone marrow-derived cells do not make a significant contribution to the restoration of the tubular epithelium after an ischemic insult [442,443]. Further, fate-tracing techniques using specific renal cell promoters and mouse IRI model have revealed that TECs are the primary source of cells that regenerated the tubular epithelium after IRI in the adult mouse kidney [387]. When IRI is mild and limited, repair pathways can restore the kidney to its normal structural and functional state. However, when the repair is more severe or is superimposed on baseline kidney abnormalities, the repair process can lead to fibrosis and chronic kidney disease. After severe injury, the proximal tubule cellular response is impaired with its proliferative response altered due to cell cycle arrest at the G2/M phase of the cell cycle, resulting in generation of profibrotic factors, including cytokines, growth factors, and matrix proteins [444,445].

The biomarker of acute kidney injury, kidney injury molecule-1 (Kim-1), a member of the immunoglobulin gene superfamily, was identified initially in a rat model of kidney IRI [446]. Subsequent studies in vitro indicate that it is a non-myeloid phosphatidylserine receptor that transforms epithelial cells into semiprofessional phagocytes [447] and is shed into the urine during injury [448]. While not considered a target for therapy, it was the use of small rodent models that was critical in the identification of its potential as a biomarker for acute kidney injury, leading to a revolution in the goal of interventional trials in man. Similarly, IL-18, produced by monocytes, has been identified as another potential therapeutic pathway but also as a biomarker of acute ischemic injury [449,450].

The limitations of existing animal models of IRI

Due to the advances in our understanding of IRI and success in rodent models, a number of strategies have been identified as potential clinical therapies but these have failed to advance meaningfully to the bedside, particularly in acute kidney injury. The lack of progress may be due to a number of issues with the models, including the fact that: (1) the pathogenesis of IRI in animal models is generally less complex than it is in humans because patients often have multiple causes of injury and multiple organs are dysfunctional; (2) IRI in humans is often in the setting of sepsis, and it has been difficult to establish good models of sepsis and IRI in animals; (3) many of the tested strategies in IRI may be applied too late because serum creatinine has been used as the biomarker for onset of acute kidney injury; and (4) therapies tested in donors are difficult to initiate in human trials due to human subjects' protection issues, for recipients as well as donor families and donor intent.

The most widely used experimental model is renal vascular pedicle or renal artery clamping for a variable length of time, with subsequent reperfusion. The rat was the model substrate but with the advent of transgenic technology there has been a shift towards using mice over the past decade. Unfortunately, this model has come under much criticism. First, the morphological findings in the animal models are not equivalent to those in the human biopsy specimen of "acute tubular necrosis" (ATN) [451]. Paradoxically, in the limited human biopsy samples that have been studied, there is limited necrosis. The appearance of ATN in the renal transplant is very similar to that of non-transplant ATN, that is limited parenchymal injury and manifest renal dysfunction [452]. The IRI is very susceptible to variability in the mouse due to the experimentalist, and even subtle variations in temperature. Because hypothermia has a protective effect, animal body temperature control is critical as the renal temperature is expected to drop upon clamping and more so if the kidney in left exposed [453]. There are also strain differences in responses in mice. Additionally, one must remember the fundamental differences between human and animal kidneys. Unlike the human, the rat kidney has a well-developed medullary outer stripe and surrounding complex vascular bundles, which include the thin descending limbs of the short Loop of Henle, versus simple vascular bundles without inclusions in human kidney. On the other hand, rodents possess unilobar and unipapillary kidneys, both in contrast to humans [454,455]. Overall, regardless of the limitations, animal models of IRI have significantly improved our understanding of kidney injury.

Xenotransplantation

With the success of allotransplantation and the ever-growing shortage of human organs, the field of xenotransplantation has seen a renewed interest. This topic is specifically covered in Chapter 12, but several aspects relate to small animal models, as xenotransplantation is, by definition, a species-specific endeavor. However, a number of hurdles still remain in this field. These include host immune response against the xenograft, the physiological incompatibility between animal tissue or organs and the human system for things such as growth factors and other protein compatibility, and the potential to transfer infectious agents from the donor to the recipient or the general population [456].

Choice of species

Although non-human primates are phylogenetically closer than other species to humans, they are not considered to be a suitable source of organs because of ethical issues, the high risk of cross-species transmission of infections to humans, difficulties in breeding, and organ size disparities [457]. For these and several practical and biological reasons, pigs are considered to be the preferred organ donor for humans. Pig organs are more comparable in size to that of humans, easily bred at large scales, and amenable to genetic modifications that could enhance their use as an organ source (reviewed in [458]). Given that pigs maintain blood glucose levels that are similar to those of humans and porcine insulin is effective

in humans, they are also considered an ideal source of islets of Langerhans for transplantation in humans. These strengths of this organism are, however, offset by the fact that the human xenogeneic immune response to pig tissues is particularly strong and presents a large immunological hurdle.

Immunological barriers to transplantation

The immunological response to xenotransplantation in humans includes both the humoral and cellular arms of the immune system. The antibody-mediated processes include hyperacute rejection (HAR) and delayed xenograft rejection (DXR), which rapidly attack vascularized organs from pigs that have been transplanted into untreated primates [456]. HAR was the first immunological barrier to xenotransplantation and is primarily mediated by so-called anti-Gal antibodies, which are IgM directed against α-linked galactose moieties Gal1α1-3Gal present on the graft endothelium [459]. This antibody is naturally produced in the recipient in the absence of clear immune challenge akin to antibodies to blood-group antigens [460]. The antigen is synthesized by the enzyme α1,3-galactosyltransferase (α1,3GT), which exists in lower mammals and New World monkeys but not in Old World monkeys, apes, and humans [461]. Thus, in phylogenetically distant, discordant combinations of donor and recipient, such as pig-to-primate, complement activation is immediate following reperfusion upon binding of anti-Gal antibodies to the graft endothelium, leading to endothelial injury and subsequently to thrombosis and necrosis of the xenograft [461]. In phylogenetically concordant combinations, such as hamster-to-rat, where anti-Gal antibodies are not present, complement likely contributes to delay rejection once antigraft antibodies are generated [456]. For this reason, pigs were developed with a targeted mutation in the α-galactosyltransferase gene (GTKO) [462,463]. The initial pig-to-primate GTKO cardiac xenografts demonstrated no hyperacute rejection with a median survival of 78 days [464,465]. Other strategies to prevent hyperacute rejection have included immunoadsorption of xenogeneic antibody by extracorporeal perfusion of the recipient using a species-specific organ such as the liver [466].

DXR is usually observed when HAR is prevented either by removal of anti-Gal antibodies or inhibition of complement activation [467]. It is characterized by infiltration of the graft by NK cells, monocytes, and granulocytes, and by endothelial cell activation with fibrin deposition and platelet aggregation [468, 469]. The key features of DXR are vascular endothelial cell activation and natural antibody (IgG anti-αGal)-mediated activation of Fc receptors on the cells mentioned above. These effector cells, upon ligation of Fc receptors, release their granule contents to mediate antibody-dependent cell cytotoxicity (ADCC). By its nature, DXR represents a key immunological barrier to successful clinical xenotransplantation.

Cell-mediated xenotransplant rejection

The xenograft is also subject to cell-mediated rejection. Xenografts can illicit potent antigen-specific T-cell responses that remain an important barrier to successful xenotransplantation [470]. As antibody responses do not seem to be largely associated with the rejection of xenogeneic islets, elucidating the cellular response in this model has extended our understanding of cellular responses focused on the antiporcine cellular responses [471]. With the addition of CSB such as CTLA4Ig or anti-CD40L, porcine xenoislet survival has improved substantially [472]. Moreover, fetal porcine islet-like cells placed under the renal capsule of nude mice demonstrate a limited and reduced inflammatory injury compared to wild-type mice [473], demonstrating the dependence of intact T cells in islet xenograft rejection. In support of this conclusion, recent studies in pig-to-non-human primates [474] and pig-to-mice [475–478] have shown improvement in xenograft survivals using CSB with anti-CD40L, or combination therapies affecting multiple pathways.

Whereas the alloimmune response targets MHC antigens, the response to a xenograft may target a wide variety of proteins and carbohydrates that differ in their molecular makeup. Akin to the allogeneic T-cell responses, the T-cell response to xenogeneic cells involves two distinct pathways: direct and indirect [471]. In the indirect pathway, xenoantigens are taken up, processed, and presented by recipient APCs in the context of the recipient MHC and does not involve any cross-species interactions with regard to signal 1 and signal 2. On the other hand, the direct pathway results from recipient CD4 and CD8 T cells interacting with donor (xenogeneic) MHC molecules on the surface of passenger (xenogeneic) APCs. This will only lead to productive T-cell activation if the interspecies molecular interactions for both signal 1 and signal 2 are compatible. In man, antiporcine responses in unprimed recipients are quite strong [479].

Prospects for xenotransplantation

Xenotransplantation remains the most viable option for increasing organ transplantation rates. Small animal models have highlighted some of the biology of these responses and demonstrated both humoral and cell mediated immunity pose formidable barriers to bringing xenotransplantation to clinical use in humans. The recent advances in pig transgenic technology and recent successes in preclinical models using non-human primates pose significant promise for entry into the clinic [458].

Summary

This chapter has highlighted the many contributions of small animal models in expanding our knowledge of transplant immunology. Key clinical challenges include late graft injury, antibody-mediated injury, and the elusive goal of tolerance. Table 14.2 has highlighted the key considerations between small and larger NHP models and their characteristic features. The opportunity to genetically manipulate mice has been one of the key features supporting their use in studies. Congenic markers have assisted in adoptive transfer experiments that have highlighted critical immune component features. The availability of reagents, including antibodies and molecular reagents, has provided a powerful complement to the genetic approaches. However, there are shortcomings in these smaller animals. The vascularized procedures are challenging technically and are limited to labs with skilled microsurgeons. Mimicking human disease in a compressed time period is difficult. The nature of the immune response is also substantially different from that of man, with a dramatically less diverse T-cell repertoire and smaller clone size, which affects not only the strength of the immune response but also its nature and variability. The role of heterologous immunity is another key consideration as housing for mice is pathogen free, while humans are constantly facing an immunological assault. However, in the continuum of our understanding, both types of models have afforded key insights into alloimmunity. As such, we should consider the small animal model as a valuable contributor to clinical therapies.

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CHAPTER 15

Large Animal Models of Transplantation

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Introduction

In 1954, a team of surgeons lead by Dr. Joseph Murray ushered in the modern era of transplantation with the successful transplantation of a kidney between identical twins [1]. One year earlier, Billingham, Brent, and Medawar published their landmark work describing the acquisition of tolerance to allogeneic cells [2]. Standing on the shoulders of these giants, over the past 60 years, clinical investigators have seen remarkable advances in surgical technique, perioperative care, and immunosuppression, allowing for steady improvement in patient outcomes [3,4]. Yet, many challenges still lie ahead. We have only just begun to achieve durable tolerance of grafts in the clinic [5–7], and thus, for the most part, we still ask our patients to trade one chronic condition for another: life-long immunosuppression. As we continue to tackle these issues for the benefit of patients, we must carefully navigate the pathway from the laboratory to the clinic. While mouse models have proven their worth countless times in aiding in the elucidation of new biological and therapeutic concepts, experiments in large animals have been and remain the best option for translation towards human trials. This chapter will provide an overview of commonly used large animal models in transplantation and the relative strengths and weaknesses of each, with the intent of demonstrating the value of large animal models in the translation from theory to therapy. This chapter serves to complement Chapters 13, 14, and 16, which address in vitro, small animal in vivo and in silico models of transplantation, respectively.

Why use large animals?

Animal experimentation has been a workhorse of medical progress since the times of Aristotle and Erasistratus, providing a rational basis for the translation of treatments and techniques to humans [8]. Indeed, the use of animals for the perfection of surgical technique dates to at least the 12th century when Ibn Zuhr (Avenzoar) performed a tracheostomy in a goat [9]. While human experimentation should not be void of novelty, it is impossible to provide the necessary and proper background so that patients may make truly informed decisions about their participation in research unless the method has previously been tested in a model with a reasonable chance of predicting at least the safety of the planned intervention. Typically, animal models have provided this foundation on which human experiments can be ethically based.

Despite the fact that animal models have often failed to be entirely predictive of a regimen's success in humans, they remain the best means for the prediction of safety. The complexity of immunity and rejection has prevented the development of any in vitro model capable of mimicking, let alone predicting, even basic immune responses. Thus, animals continue to provide the best models for advancing therapies toward clinical application. However, given the failure of animal models to predict *all* aspects of a therapy's effect in humans, experiments should be designed and interpreted with this limitation in mind.

The ethical principles guiding animal experimentation of all types, but particularly those of large animal experimentation, have become increasingly complex as modern society has recognized the rights of animals to humane treatment. The literal interpretation of Thomas Aquinas' assertion, "Animal non habet jus", translated as "Animals do not have rights", is no longer socially palatable. While his statement seems stark and uncaring at face value, more careful examination of Aquinas' humanity-centric philosophy reveals no disregard for animals' wellbeing, but rather elevates the position of humans and allows a rational justification for animal experimentation [10]. Modern views on the ethics of animal experimentation, primarily over the past half century, have led to significant regulation to ensure the proper use of animals. Specific guidance for ethical animal experimentation can be found in the *Guide for the Care and Use of Laboratory Animals* [11] and in the publications from the American Association for Laboratory Animal Care [12]. Many academic institutions and journal publishers require adherence to these guidelines. The principles of reduction, replacement, and refinement are at the heart of all ethical animal experiments. Animal models should only be used when no suitable replacement exists and all techniques should be maximally refined in terms of the animals' welfare. The number of animals used should be the minimum needed to answer the question posed in the experiment. However, no experiment is ethical if it cannot answer the question posed. Thus reducing the numbers of animals used to the point of failing the experimental objective is less ethical than a successful experiment that uses more animals.

Animal experimentation in mice offers several distinct advantages over the large animal models. Mice offer sufficient biologic similarity and easy genetic manipulation at a relatively inexpensive cost making them ideal for pathway determination and mechanistic studies. In contrast, large animal models are significantly more

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expensive and, with the exception of inbred miniature swine (see below), their genetic diversity makes definitive mechanistic studies difficult. However, the impracticalities of large animals for mechanistic studies are, at the same time, what makes them the best option for preclinical studies. The additional complexity of a large animal model adds unanticipated variables that make these experiments best suited to examine practicality, safety, and generalized efficacy. That is not to say, however, that experiments should be void of measurements to confirm consistency with overarching hypotheses. With regard to transplantation and immunology, specifically, murine models have several potential drawbacks. Laboratory mice bred in pathogen-free environments and studied at 4–8 weeks of age have largely naïve immune systems [13]. This is likely responsible for the observation that many therapies that have been rigorously studied in mice, including methods that readily induce tolerance in mice, fail in more complex animals [14,15], or even in mice exposed to pathogens [16]. Unlike all large animal species so far tested, mice do not constitutively express class II antigens on vascular endothelial cells, which may explain the greater importance of class II matching for transplant outcome in large animals than in mice [17,18]. The specific genetic background in which treatments are tested has also been shown to affect the efficacy of therapy [19]. The genetic diversity and immunologic experience of outbred large animal models helps to avoid many of these shortcomings. Thus, experiments in a large animal model, most frequently in non-human primates (NHPs), have become a de facto requirement prior to initiation of human clinical trials in transplantation [15,20].

Due to the immense complexities of the immune system, therapies may fail in translation to large animal models or, subsequently, to humans. This is rarely a failure of the model itself, but is more likely to be due to one or a few critical differences that were not initially appreciated. Differences in drug distribution or metabolism can lead to apparent failure of a therapy that may have been quite successful had appropriate dose adjustments been made. In the era of modern biologic and antibody-based therapy, it is especially worth noting that relatively minor differences in molecular structure can have profound effects on the ability of the drug to exert the desired effect. As noted previously, the diversity of immunologic experience is a major advantage to large animal models and can significantly affect therapeutic outcomes via heterologous immune interactions. The quantification and control of this experimental parameter is particularly challenging. Finally, the practicalities of caring for the animals during therapy cannot directly replicate what is available clinically. Wound care in surgical models is often difficult. Monitoring and vascular access are also challenging in conscious animal models. Innovation in cage design has allowed for indwelling catheters in NHPs; however, these cages are not always available and are not without flaws.

A final issue with large animal models is that of time, both in terms of the age of the animal and in survival outcomes. Animals are often used at a relatively young age, consistent with adolescence, for practical reasons. Evidence suggests that these young animals have a predominately naïve T-cell repertoire that will slowly mature towards a memory phenotype with age [21–24]. This more closely resembles human children of the same absolute age rather than the human adults that comprise the bulk of the clinical transplant population. With regards to indefinite graft survival in animal models, convention in mouse models has established 100 days as indicative of acceptance, while several years has been used for larger animals. While this is intuitive in terms of the shorter overall lifespan of these animals compared to humans, where grafts are expected to last a decade or longer, there are no data to support that graft survival should be interpreted proportionally to expected lifespan. Thus, it is best to refrain from interpreting data from these studies as indicative of longer-term graft survival.

Despite the challenges and drawbacks of large animal models noted above, they remain the best predictors of generalized efficacy and safety of therapies being considered for clinical translation.

Common large animal models

In transplantation, the most commonly used large animal models are dogs, pigs, and NHPs. Each species has sufficient anatomic and physiologic similarity to humans for experiments that are both technical and pharmacologic in nature. The immune responses between these species are similar, being predicated on a network of T cells responding to similarly organized major histocompatibility complexes (MHCs). Because of this, the histology and tempo of unmodified allograft rejection is similar to what is expected in humans. Despite these similarities, these models also differ from each other and from humans in ways that prevent them from fully predicting clinical outcomes.

Before discussing each animal model individually, several generalizations can be made. Drug metabolism and absorption can and often does vary widely between species, preventing the direct translation of drug-response curves and toxicities to humans or other animal models. However, when drug distribution has been appropriately adjusted, the physiologic response and efficacy of most drugs is similar between the large animal models and humans.

In human studies of transplantation, the degree of relatedness and degree of MHC matching between donors and recipients is typically known. While the relative effect on outcome of MHC matching between human transplant pairs varies with the organ being transplanted, generally, better-matched pairs have better results. The same can be said for animal models, but MHC typing in large animals is often more difficult and therefore frequently omitted. Failure to control for variable MHC disparity in animal studies potentially introduces a significant confounding factor in the interpretation of experimental results.

While a full review of MHC genetics is beyond the scope of this chapter, a basic review focusing on the differences between species is worthwhile. In depth treatment of the structure of human MHC can be found in Chapter 4. The human MHC, also known as human leukocyte antigen (HLA), is composed of over 260 genes on chromosome 6p21.3 [25]. While many of these genes are associated with immune functions, the classical genes associated with antigen presentation and pertinent to this discussion include the class I molecules HLA-A, HLA-B, and HLA-C, as well as the class II molecules HLA-DP, HLA-DQ, HLA-DR. Individuals inherit two alleles of each gene from their parents. The highly polymorphic nature of these genes results in wide diversity of HLA molecules within the population. The overall structure of the MHC locus in the three animal models presented here is similar, although there are some significant genetic differences worthy of discussion.

The canine MHC, termed dog leukocyte antigen (DLA), is well defined. Located on chromosome 12, the dog MHC contains four complete class I antigens: DLA-88, DLA-12, DLA-64, and DLA-79. The class II region contains DLA-DRB1, DLA-DQB1, and DLA-DQA each of which is polymorphic. An additional class II gene, DLA-DRA, appears to be monomorphic [26]. Since the initial structure of the canine MHC was elucidated, several additional

alleles have been discovered through large surveys of various dog breeds [27]. Two highly polymorphic satellite markers, one near each of the class I and class II loci have been found and shown to be useful in determining the inheritance of DLA haplotypes within families, allowing for the selection of littermates with specific DLA matching for transplantation studies. Because these markers do not identify specific genotypes, however, they are currently unsuitable for pairing of unrelated animals [26]. Sequencing of the entire canine genome has been achieved [28] and direct sequencing of DLA-DRB1 to confirm allelic identity has been used [29], raising hope that more complete genotyping may be possible to improve pairing for transplant studies in unrelated dogs.

A major advantage of porcine models over dogs and NHPs is the more extensive knowledge of the pig MHC, or swine leukocyte antigen (SLA), and the ability to genetically modify pigs, either through breeding or through viral transfection. Located on Chromosome 7 [25], the SLA has been well defined with three known classical class I genes, notated SLA-3, SLA-2, and SLA-1 (corresponding to SLA A, B, and C series proteins that had been previously defined by serology). The class II region has also been well defined. Similar to the DLA, SLA-DRA is monomorphic while SLA-DRB, SLA-DQA, and SLA-DQB are highly polymorphic [30–32]. Knowledge of the SLA and of its functional importance has been aided by the breeding of MHC-defined miniature swine, increasing the validity of transplantation experiments in swine substantially. The ease of breeding pigs has made genetic manipulation of swine relatively straightforward. This has been a particular boon to the field of xenotransplantation, where knockout pigs have been produced that do not express the Gal xenoantigen [33,34]. Additional genetic modifications have included the up-regulation of complement modulators [35,36]. The use of these transgenic pigs will be discussed elsewhere in this book.

Non-human primates have become the favored large animal model in most modern transplantation studies, as new biologic therapies with a high degree of receptor specificity are being tested. While monkeys cannot readily be inbred to the same extent as mice, dogs, and pigs, they cannot be considered truly outbred either. The majority of NHPs used for transplantation research are from dedicated colonies with limited interbreeding. Until recently, techniques for establishing genetic disparity of NHP have been relatively limited, consisting mostly of mixed lymphocyte reactions and analysis of the DR region either serologically or by PCR. Significant effort has been placed into understanding the structure of the rhesus macaque MHC, with the fruits of this labor now coming to bear. The rhesus MHC, located on chromosome 4, is significantly larger than the human MHC, owing to significant duplication in the class I A and B genes, known as mamu-A and mamu-B (rhesus macaques lack a homolog of HLA-C). The class II genes are also duplicated, but to a lesser extent. The duplication of these genes results in expression of up to four mamu-A genes and fourteen mamu-B genes per chromosome, dramatically increasing the complexity of heterozygous individuals [37]. Furthermore, cloning strategies have revealed a hierarchical expression of these genes rather than the codominant expression seen in humans [38], effectively rendering allele-specific DNA-based typing ineffective at predicting the expression of MHC molecules. Two recent advances have significantly improved our capability of determining relatedness in macaques. The first was the identification of several DNA microsatellite probes, allowing for the determination of autosomal and sex chromosome inheritance between generations of macaques, enabling accurate pedigrees to be drawn of macaque breeding groups [39,40]. While this method lacks allele-specific determinations of the MHC, the inheritance of complete MHC haplotypes is able to be determined. The second major advance was the development of massively parallel pyrosequencing for comprehensive analysis of MHC expression [41]. Pyrosequencing was able to confirm the extreme complexity of the macaque MHC noted above. The technique allows for determination of the complete MHC type for any animal when planning transplantation experiments. The application of these two technologies allows for experiments to be designed with knowledge of MHC disparity between animals, greatly increasing the validity of the results.

Canine models in transplantation

Dogs have held a traditional place in surgical training and the development of surgical technique. Thus, it is not surprising that dogs represented the predominant model in early transplantation research. Many of the technical issues pertaining to surgical transplantation were worked out in the canine model. Indeed, early techniques for renal [42], heart [43], liver [44,45], lung [46], intestinal [47,48], bone marrow [49], pancreas [50,51], and pancreatic islet transplant [52] were all tested in dogs. The canine model can also lay claim to the first successful use of immunosuppression in the experiments by Calne [53]. While social pressure to avoid the use of domesticated animals in research has lessened the role of the canine model, it continues to be used, with recent studies in heart [54], renal [55], pancreatic islet [56], liver [57], and intestinal transplantation [58]. Extensive use of dog models in experimental bone marrow transplantation has been and continues to be carried out by the Storb group in Seattle [59,60], who have also utilized this model in studies of organ [61] and composite tissue [62]. Its use will not be considered extensively here as dog experimentation has lessened considerably in solid organ transplant investigation, and occupies little place in the translational study of modern biologic-driven regimens.

Porcine models in transplantation

Swine have been used for many years as laboratory models for complex human disorders because of the similarity of their anatomy and physiology to those of humans. Porcine models of disease may be spontaneous, inherited, induced, or transgenic. Domestic swine—particularly miniature pigs—are used for research in the areas of physiology, pharmacology, toxicology, radiology, surgery and organ transplantation, traumatology, pathology, embryology, gastroenterology, nephrology, neonatal surgery, and cardiovascular research. Several laboratories have studied the MHC of swine [31,63–65] and there have been a number of workshops held to standardized nomenclature of the SLA system [66–68]. In addition, there are now a large number of monoclonal antibodies available for studies of swine cell surface antigens [69–73], making swine a versatile animal model with regard to characterization and manipulation of cell populations, surpassed only by mouse and human.

In addition, swine are one of the few large animal species in which breeding characteristics make genetic experiments possible. Swine have a relatively large litter size (three to ten offspring) and a short gestation time (114 days). They reach sexual maturity at approximately 6 months of age, and sows have an estrous cycle every 3 weeks (cf. dogs, which have an estrous cycle only twice per year). These breeding characteristics have made it possible for the laboratory of one of the authors (DHS) to develop MHC homozygous

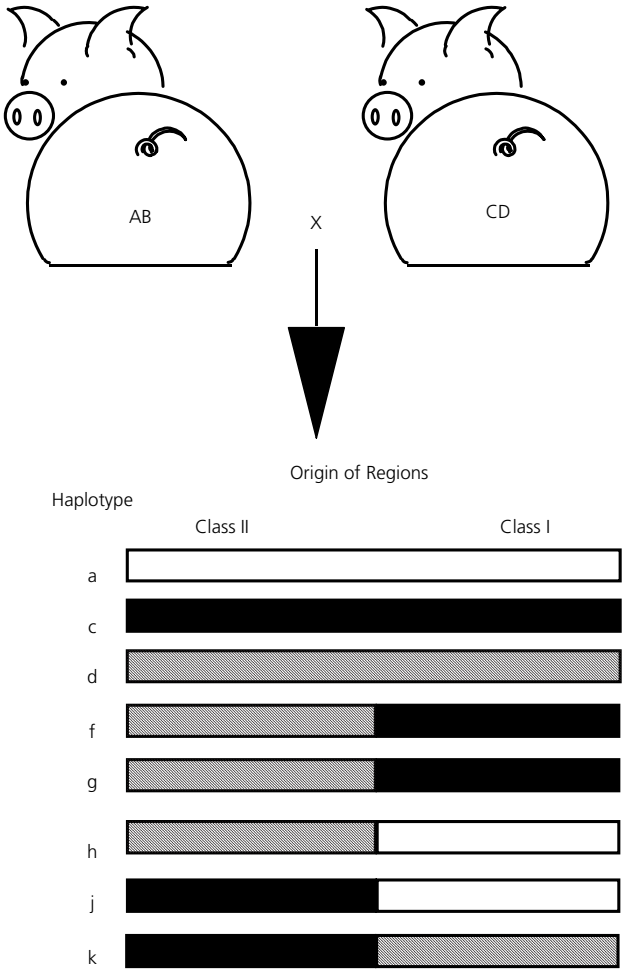


Figure 15.1. Origin of haplotypes of miniature swine.

lines of miniature swine in a relatively short time and have also made it possible to isolate new MHC recombinants, to breed them to homozygosity, and to carry out short-term backcross experiments in order to identify and study the segregation of genetic characteristics [74–76]. These miniature swine thus represent the only large animal model in which MHC genetics can be reproducibly controlled [77]. As such, they have been particularly useful in assessing the effects of selective MHC matching on rejection and/or tolerance induction.

At present, three lines bearing homozygous SLA haplotypes, SLA^{a}, SLA^{c}, SLA^{d} and five lines bearing intra-SLA recombinant haplotypes are maintained (Figure 15.1). All of these lines differ by minor histocompatibility loci, thus providing a model in which most of the transplantation combinations relevant to human transplantation can be mimicked. Thus, for example, transplants within an MHC homozygous herd simulate transplants between HLA identical siblings, while transplants between herds resemble deceased donor or non-matched sibling transplants. Likewise, transplants between pairs of heterozygotes can be chosen to resemble parent into offspring or one-haplotype mismatched sibling transplants. In addition, one subline of the SLA^{dd} animals was chosen for further inbreeding, in order to produce a fully inbred line of miniature swine. When this subline reached a coefficient of inbreeding of >94%, reciprocal skin grafts among the offspring were not rejected, defining thereby the first histocompatible strain of large animals [78]. Subsequent studies have utilized these highly inbred animals as donors for adoptive transfer of lymphoid populations, something that has previously been possible only in inbred rodents [79].

Studies of organ allograft tolerance

The partially inbred lines of miniature swine have been extremely useful in studies of organ transplant rejection and tolerance. When studies on the spontaneous induction of renal allograft tolerance were carried out in selectively MHC-mismatched combinations, all class II mismatched grafts were rejected acutely [17]. In contrast, when transplants were matched for class II and differed for one haplotype at class I (e.g. $SLA^{ag} \rightarrow SLA^{ad}$, see Figure 15.1), spontaneous tolerance induction was observed in approximately 30% of cases, with allografts being accepted long term (>100 days) [17]. The spontaneous acceptors of class II matched, one haplotype class I mismatched allografts demonstrated a significant rejection crisis during postoperative weeks 2–4, which subsided spontaneously, and normal renal function was maintained long-term. Animals that accepted class I mismatched renal allografts did not ignore the MHC antigens on the graft, but rather became actively tolerized to these antigens. Thus, during the rejection crises, cytotoxic antibodies directed against the donor class I antigens were detected in the serum of acceptor animals, but these antibodies were almost entirely of the IgM class, suggesting failure of the usual IgM to IgG switch, which characteristically occurs in animals that reject a graft. Since the switch from IgM to IgG is generally thought to depend on T-cell help [80,81], these results suggested that in the absence of sufficient T-cell help, as might occur for single haplotype class I only mismatched kidney allografts, tolerance rather than rejection had been induced. It was therefore hypothesized that rather than indicating a failure of the immune system to recognize class I, acceptance of renal grafts across class I plus minor differences might be the result of *an active immunologic process leading to tolerance*. Consistent with this hypothesis, skin grafts from the original renal donor showed markedly prolonged survival (31.7 ± 20.4 days) over similar class I-mismatched skin grafts placed on control animals (11.67 ± 2.6 d). Although prolonged, all skin grafts were eventually rejected, probably due to skin-specific antigens rather than to a break in tolerance, since there was no concomitant impairment of renal function, nor anti-class I antibody formation. In retrospect, the active process leading to tolerance in these early studies was undoubtedly the induction of regulatory T cells, a hypothesis that has subsequently been demonstrated in more recent studies in this model [82–84].

In order to increase the incidence of tolerance induction, subsequent studies utilized a short course of treatment with cyclosporine A (CyA) for renal transplants across a two-haplotype class I mismatched, class II matched barrier ($SLA^{gg} \rightarrow SLA^{dd}$, see Figure 15.1), a combination in which recipients were known to uniformly reject renal allografts rapidly without immunosuppression. Instead, long-term, specific tolerance was induced in eight of eight recipients [85]. This result has subsequently been substantiated in more than 50 additional CyA-treated animals, all of which demonstrated long-term tolerance. This transplant combination and treatment protocol have become the "standard protocol" for subsequent studies of tolerance in miniature swine, and have provided numerous insights into the mechanism of tolerance in this model [82,86–93]. Studies of heart and lung allograft tolerance have also been carried out in the miniature swine model [94–97]. Importantly, unlike the situation for transplant tolerance studies in mice, essentially all of the findings

that have been made in swine models have been able to be translated to NHP [98–101], and in some cases to the clinic [5,101–103].

NHP models in transplantation

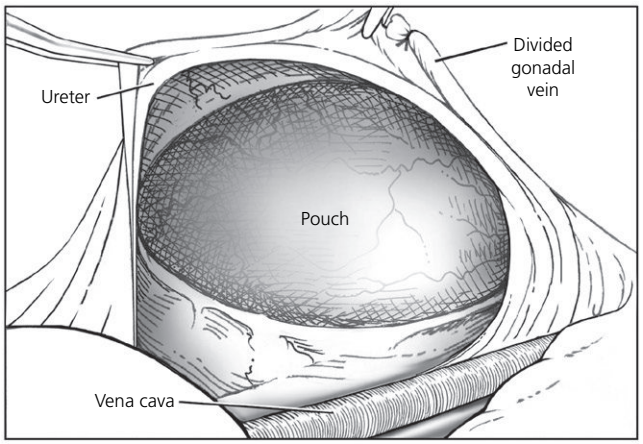
Non-human primates have emerged as the most commonly used animal group in translational transplantation research, owing to their high degree of homology with humans. This is especially true in the study of biologic agents such as monoclonal antibodies and fusion proteins with a high degree of specificity [104]. Several species of primates have been used, including baboons, macaques, and, rarely, chimpanzees. Chimpanzees have the highest degree of homology with humans, having served as donors for humans on historical occasions [105]. However, their endangered status and evolutionary stature call into question the ethicality of chimp research. Indeed, in 2011, the NIH strictly limited new studies involving chimpanzees. Baboons are commonly used in xenotransplantation studies, as their large size better accommodates porcine organs. Macaques (cynomolgus, rhesus, and pigtail) are the most widely used of the NHPs. The macaques are preferred due to their small size and relative ease of care. They exhibit sufficient homology with humans so that the majority of molecules will cross-react, with the notable exception of CD3. Macaques are now used as models in virtually all areas of transplantation.

Renal transplant models

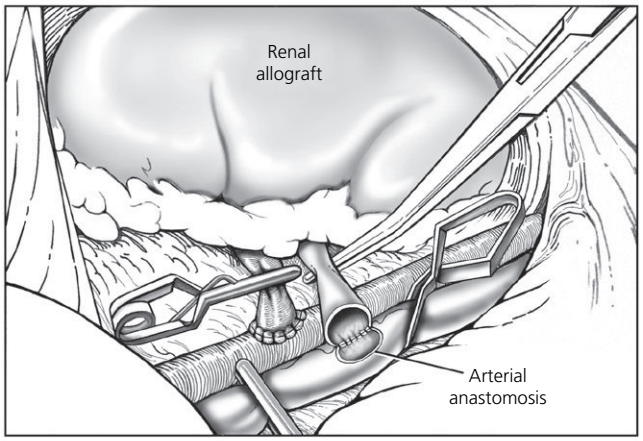
Renal transplantation in macaques is relatively straightforward, and somewhat mimics the techniques used in the pediatric population. Both donor and recipient are heparinized during excision and implantation. The donor kidney is excised, ensuring sufficient length of the vessels and ureter. While either donor kidney can be used, the left kidney is preferable due to the length of the vasculature. The donor kidney is then anastomosed to the recipient aorta and vena cava, proximal to the bifurcation, typically on the right side. A primary ureteroneocystostomy is formed, typically on the posterior aspect of the bladder after the ureter is tunneled through the retroperitoneal space (Figure 15.2). The recipient's native kidney(s) are removed following completion of the transplant in order to prevent any period of true anuria [106,107]. It is worth noting that bilateral retroperitoneal dissection is particularly stressful for the animals. If possible, preparative nephrectomy of the left kidney on a date prior to the transplant allows for the dissection to be limited to the right side on the day of transplant, which is better tolerated. Planning the experiments in a "domino" fashion, where each animal is, in turn, a donor and then a recipient, allows for use of this nephrectomy as a donation and minimizes the number of organs that go untransplanted. In general, the transplant procedure is well tolerated by the animals and there are minimal complications, usually related to the ureteral anastomosis. In a study by Song et al., 182 transplants were performed in cynomolgus macaques with an overall complication rate of 10.4%. Notably, more than two-thirds of these were related to the ureteral anastomosis, which was performed as an end-to-end ureteroureterostomy in this study [108].

Skin transplant models

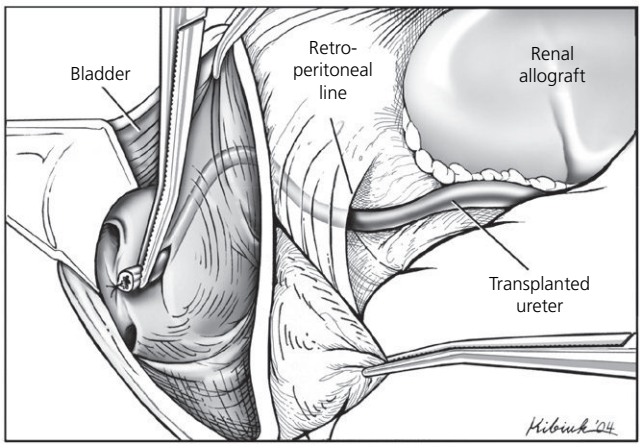
Skin transplantation in NHPs has been used to test a number of potential immunosuppressive strategies, including antilymphocyte globulin [109], adhesion molecule blockade [110], costimulation blockade via CD80 [111], and CD154 blockade [112]. Technically, the transplant varies little from skin transplants in the murine



(A)



(B)



(C)

Figure 15.2. Renal transplantation in non-human primates. (A) A retroperitoneal pouch is made by incising medial to the native ureter and dissecting laterally. (B) The vascular anastomosis is performed, starting with the vein. (C) After reperfusion of the kidney, a cystotomy is performed and a retroperitoneal tunnel is created from the bladder to the previously created pocket. The transplanted ureter is delivered through the tunnel and anchored inside the bladder.

model, except for the size of the animal. Typically, donor grafts are taken from the abdominal wall and implanted on the recipient's back. A unique aspect of skin transplantation in primates is the requirement for protection of the grafts during healing and afterwards. Soft bolsters should be used to maintain light pressure of the graft on the underlying tissue (Figure 15.3). Furthermore, the animals will be required to wear protective mesh jackets that shield the grafts from damage during grooming. Training the animals to wear the jacket comfortably requires a period of acclimatization and may take several weeks.

Islet transplant models

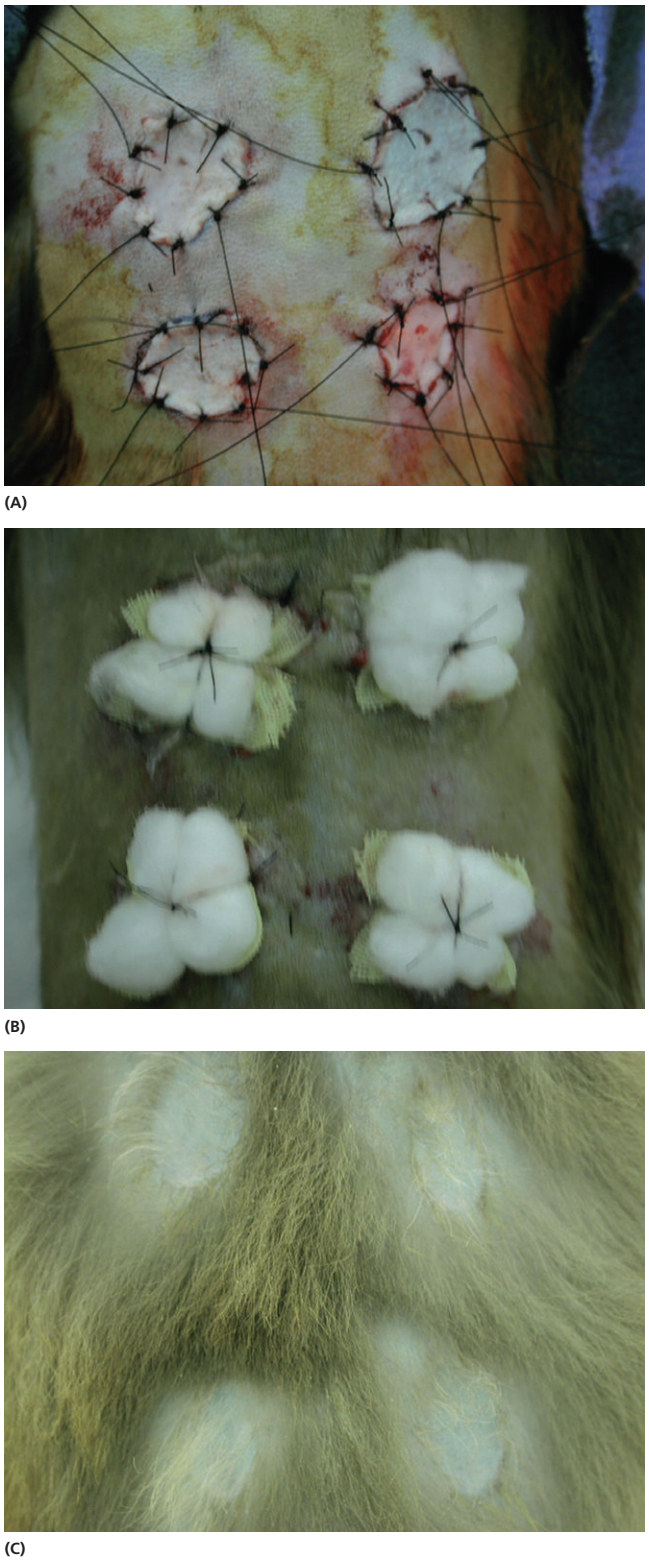
The equivalent of a non-obese diabetic (NOD) mouse strain does not exist for use in NHP models of islet transplantation. Rendering NHPs diabetic is accomplished by pancreatectomy or, more commonly, by the intravenous administration of streptozotocin. The diabetic state must be confirmed by the absence of native c-peptide production prior to transplantation. Alloislets are then procured via donor pancreatectomy and isolated in an analogous manner to the human method prior to their transplantation into a diabetic NHP recipient. The established site of islet transplantation is intrahepatic seeding by gravity-dependent infusion into the portal or mesenteric vein, although other sites such as the bone marrow, kidney capsule, peritoneal cavity, omentum, and muscle have and are being explored [113]. Daily fasting and postprandial blood sugars can be easily monitored after ear-stick training has been completed, and this also facilitates insulin injections when appropriate. The NHP model of islet transplantation has played a crucial role in the evaluation of costimulatory blockade-based regimens, including CTLA4, CD40, and CD40L (CD154) [114–120].

Heart and lung transplant models

Non-human primates are less often used as models for allogeneic heart and lung transplantation, though there are examples of both [121–123]. Allogeneic heart transplantation is usually performed using heterotopic placement of the graft in the abdomen of the animal with follow-up of contractility by palpation or ultrasound [124,125]. Implanted EKG electrodes have also been tested for use in determining graft rejection [54]. In contrast to the relatively uncommon studies of allogeneic heart or lung transplantation in NHP, primates are frequently the recipients of heart or lung grafts in studies of xenotransplantation, which will be discussed briefly below and in more detail in Chapter 12.

Vascularized composite allograft models

Recent advances in microsurgical technique and immunosuppression have made possible the transplantation of composite tissues as a functional unit. The clinical realization of such transplants has led to the development of NHP models to study the immunobiology of these grafts. Examples of composite grafts in NHP exist, such as hand transplantation in baboons [126] and transplant of the radial portion of the hand in rhesus macaques [127]; however, these grafts potentially negatively affect the function of the surviving recipient. Cendales et al. have developed a Rhesus macaque model which involves transplantation of a sensate osteocutaneous radial forearm flap containing portions of the radius, brachioradialis, medial and lateral antebrachial cutaneous nerves, palmaris longus, radial artery, cephalic vein, and the overlying skin [128] (Figure 15.4). This model preserves function for both the donor and recipient, allowing for the "domino" of donors and recipients or exchange of grafts between two individuals. A group from the University of



(A)

(B)

(C)

Figure 15.3. Examples of soft bolsters on NHP skin grafts. (A) Skin grafts have been sewn in place and long sutures have been placed to tie the bolster down. (B) Soft cotton bolsters are placed over medicated gauze and tied in place. (C) After the grafts have healed to the underlying tissue, the bolsters can be removed.

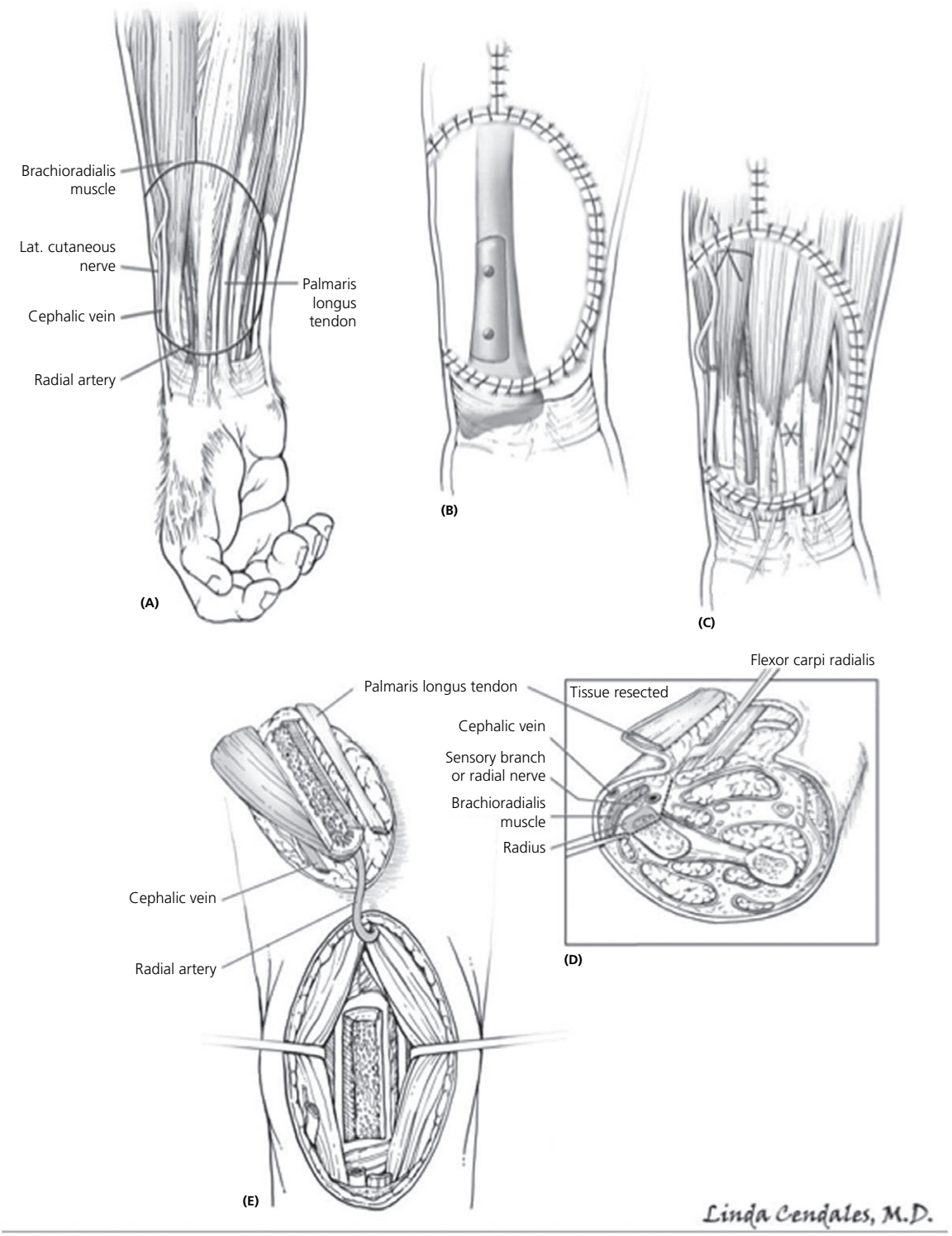


Figure 15.4. Radial forearm vascularized composite tissue allograft in a NHP. The flap contains bone (optionally), muscle, tendon, nerve, blood vessels, subcutaneous fat, and skin. (A) Anatomy of the sensate osteomyocutaneous radial forearm flap with palmarislongus tendon. (B) The osteosynthesis of the radius is performed with screws. (C) The palmarislongus tendon, brachioradialis muscle, cephalic vein, sensory branch of the radial nerve, and the proximal end of the radial artery are repaired anatomically after transplantation.(D) Cross-section view. The flap dissection begins ulnarward to include the deep fascia and the palmaris longus tendon . (E) The flap is isolated on the proximal vascular pedicle. Reproduced from [128] Cendales LC, Xu H, Bacher J, Eckhaus MA, Kleiner DE, Kirk AD. Composite tissue allotransplantation: Development of a preclinical model in nonhuman primates. Transplantation. 2005 Nov 27;80(10):1447–54, with permission from Wolters Kluwer Health.

Maryland has developed a primate model of facial transplantation in cynomolgus monkeys utilizing a portion of the madibular bone, masseter muscle, overlying skin, common carotid artery, and external and internal jugular veins [129,130].

Xenotransplant models

Specifics of xenotransplantation will be covered in depth in Chapter 12. Brief treatment of two specific topics germane to the topic will be covered below.

Swine as xenograft donors

Pigs are now considered the most likely animal to be utilized as a xenograft donor for humans, in an attempt to overcome the shortage of transplantable organs (see Chapter 12). As xenotransplantation is, by definition, a species-specific endeavor, pigs have become the prevailing donor for most clinically relevant xenotransplant experiments, as well. Lines of gal knockout pigs (GalT-KO), in which the gene for the enzyme α-1,3-galactosyltransferase has been disrupted, have been produced through genetic engineering by several laboratories [33,131–133]. These animals do not express the ubiquitous Gal epitope on their cell surface glycoproteins and their tissues and organs can thereby avoid the major problems previously caused by the high levels of natural antibodies to Gal present in the serum of Old World primates and humans [134,135]. It is hoped that this genetic modification, along with other modifications involving addition of transgenes (Figure 15.5), will eventually make the pig an ideal xenograft donor.

Miniature swine may be particularly suitable as potential xenograft donors because of their size. These animals achieve adult weights of approximately 120–140 kilograms. It would therefore be possible to obtain miniature swine of appropriate size as an organ donor for any potential human recipient, from a newborn baby to a large adult. In contrast, domestic swine reach mature weights of over 450 kilograms, clearly larger than necessary for organ donation. Using a fibroblast line derived from one of the most highly inbred lines of miniature swine (see above), the α-1,3-galactosyltransferase gene was disrupted through homologous recombination and the corresponding knockout animals (GalT-KO) were then produced by nuclear transfer [132,136]. The availability of these highly inbred GalT-KO animals has made it possible to carry out a variety of studies directed at the induction of tolerance across the pig-to-primate barrier [137–142].

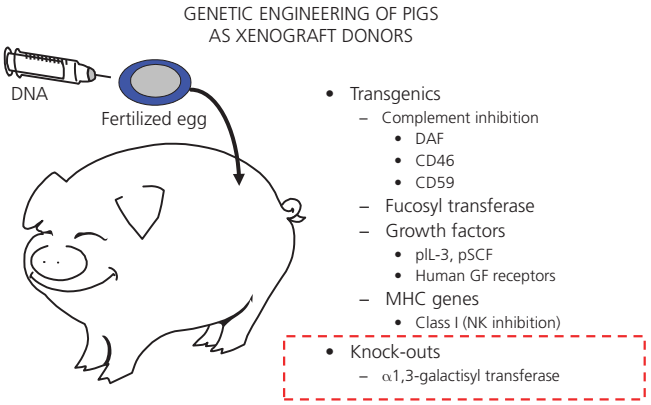


Figure 15.5. Genetic modifications of swine for use as xenograft donors.

Islet xenotransplantation

Cellularized xenografts, mainly islets, lack the endothelial component responsible for thrombotic dysregulation in vascularized xenograft models and, therefore, will likely be the first translatable platform for xenotransplantation. In large animal models these preclinical studies have been largely confined to NHP models of porcine islet xenotransplantation. Specific pathogen-free islets are primarily obtained from porcine sources due to ease of supply and similarities between human and porcine glucose homeostasis. Alternative sources include cows and tilapia. Non-human primate recipients are a robust model for translation. When compared to humans, non-diabetic cynomolgus monkeys are known to have lower levels of fasting blood glucoses and higher levels of insulin/c-peptide [143]. Some controversy exists regarding the timing of porcine islet isolation—fetal, neonatal, adult—with immature islets proving more resistant to ischemia [144]. Both neonatal and adult porcine xenoislets have been shown to be capable of producing prolonged insulin-independent normoglycemia when infused intraportally into diabetic rhesus macaques [145–148]. These feats required significant immunosuppression, including the use of CD154-specific monoclonal antibodies, although innate immunity was preserved with viral prophylaxis. As previously noted, xenotransplantation is uniquely amenable to the genetic modification of source pigs and, in the case of cellularized xenografts, ex vivo transfection. Identification of the gal epitope, Galα(1,3), Galβ(1,4),GlcNAc-R, as a major target of preformed xenoantibody has led to enhanced islet engraftment and function using gal-deficient neonatal porcine donors [148]. Further advances in the xenospecific immunity will elucidate more targets for genetic modification and likely reduce immunosuppressive requirements in the near future.

Perioperative care of non-human primates

Perioperative and follow-up care provided to NHPs can only approximate what is available for human patients. Routine animal care as well as treatment of any complications or clinical illness should be done in consultation with local veterinary staff. Careful attention should be paid to the animals' clinical status as changes in activity or appetite can represent early signs of rejection or deterioration. Recipients of renal or islet transplants and animals receiving nephrotoxic immunosuppression, such as high doses of calcineurin inhibitors, should be monitored for urine output. Hematologic studies and serum chemistries should be checked routinely in the postoperative period and with any clinical changes. Immunosuppressive medications can be delivered via number of routes. Generally, medications requiring daily dosing should be delivered by mouth or via a subcutaneous or intramuscular injection to avoid repeated sedation. Medications that are dosed less frequently can also be given via an intravenous infusion. Fluids can easily be administered intravenously or subcutaneously while animals are sedated in the case of hypovolemia, and, in some centers, special cages allowing for the use of indwelling catheters and continuous infusions in conscious animals are available. Supplemental nutrition may also be given to anorexic or malnourished animals via an orogastric tube while sedated. Many medications used clinically, such as analgesics or antibiotics, can also be used in NHP; however, dose adjustments are often required. Animals receiving immunosuppression may be at increased risk of some infections, requiring prophylactic therapy in some instances. PCR-based assays for macaque homologs of CMV and EBV have been validated.

Small molecule-based immunosuppression

Most of the commonly used small molecule immunosuppressants have been found to have some success at prolonging graft survival, and in some cases tolerance, in large animal models. As new and more specifically targeted therapies are brought forward for testing, it is important to have an appreciation of what has been accomplished with small molecule immunosuppression.

Azathioprine and mycophenolate

Calne established the utility of 6-MP and azathioprine in transplantation using the dog model [53]. The translation of azathioprine to clinical use is a major milestone in the history of transplantation, and azathioprine would be the backbone of clinical immunosuppression strategies until the development of calcineurin inhibitors. Mycophenolate mofetil, which essentially replaced azathioprine in most clinical protocols, was also shown to be effective in canine models [149,150]. As part of most clinical immunosuppression protocols, mycophenolate is still widely used and studied as part of combination therapy in large animal models.

Calcineurin inhibitors

Calcineurin inhibitors have formed the backbone of most clinical immunosuppression protocols since the introduction of cyclosporine. This class of drugs has been studied extensively in all three animal models discussed in this chapter, and has been effective in each. Following on his success with azathioprine, Calne et al. were among the first to study cyclosporine in large animal models, publishing their results first in a canine kidney model [151] and later in a porcine cardiac model [152]. Tacrolimus was also studied extensively in dogs, with evidence of efficacy promoting its development. It is worth noting, however, that due to high toxicity in the dogs studied, additional studies in NHP were required to assure its safety for translation to humans [153]. Calcineurin inhibitors are capable of preventing liver or kidney rejection in dogs when appropriately dosed, although moderate rejection is often present when not used in combination with another agent [151,153–157]. Pigs are capable of tolerating supraclinical doses of calcineurin inhibitors without overt toxicity, allowing for the development of tolerance towards kidney or liver grafts [85,158–162]. Cyclosporine has also been able to induce tolerance in a partially MHC-matched rhesus macaque model [163]. Calcineurin inhibitors remain a commonly used immunosuppressant in large animal models and frequently serves in combination therapy or as a control against which new agents can be tested.

mTOR inhibitors

The use of sirolimus in dog models has been limited to combination therapy, typically with calcineurin inhibitors, due to gastrointestinal toxicity at relatively low doses [164]. In pigs, sirolimus has been much more successful, extending graft survival to 60 days [165,166]. However, tolerance has not been achieved with sirolimus in swine models. Sirolimus monotherapy has been shown to extend renal graft survival to 20–30 days in primates with relatively few side-effects [167,168]. In both studies, serum trough levels were determined to ensure adequate drug delivery. The combination of sirolimus with tacrolimus has been shown to be synergistic [169], with few animals continuing to have graft survival after the termination of immunosuppression [170]. However, the addition of daclizumab induction therapy to a combination of tacrolimus and sirolimus has been found to have significant intestinal toxicity [171]. Sirolimus has also recently been shown to have positive effects on the suppressive activity of T regulatory cells [172] and is now being used in combination with cellular therapy with Tregs [173] or as a primer during ex vivo expansion of Tregs as the cells are prepared for use as a cell-based therapy [172]. Everolimus, a second mTOR inhibitor, is much less frequently used in large animal models. It has shown efficacy in combination with cyclosporine in a NHP lung transplant model [122] as well as with basiliximab and FTY720 in a NHP islet model [174].

FTY720

FTY720 has been found to prolong renal allograft survival either alone or synergistically with calcineurin inhibitors in both macaque [175] and canine models [176]. The effect of FTY720 on peripheral lymphocytes appears to be consistent, with both models showing a significant reduction in circulation lymphocytes. Notably, in a canine liver model FTY720 was more effective at lower doses [177]. Although the reason for this is unclear, the phenomenon appears to be species and organ specific. This study also confirmed the synergistic effects of combining FTY720 with a calcineurin inhibitor, although over-suppression leading to infection was seen with the combination of FTY720 with tacrolimus.

Sotrastaurin

The selective protein kinase C inhibitor, sotrastaurin, has been shown to prolong graft survival in a NHP renal transplant model [178]. Sotrastaurin was also found to be synergistic with a host of other immunosuppressants, including cyclosporine, everolimus, and FTY720 [178,179]. The synergistic benefits of sotrastaurin were seen even at doses below what was necessary to prolong graft survival when using sotrastaurin alone.

Tofacitinib

Tofacitinib (CP-690550) is an inhibitor of Janus kinase 3 (JAK3), which takes part in the downstream signaling pathways from a number of cytokine receptors. Naturally occurring loss-of-function mutations in the JAK3 protein result in autosomal SCID. Treatment of cynomolgus renal transplant recipients with tofacitinib prolonged graft survival to a mean of 46 days. Consistent with multiple cytokine blockade, significant decreases in circulating NK and T cells were observed [180].

PG490-88

PG490-88 is the prodrug of PG490 (triptolide), the active ingredient in an immunosuppressive Chinese herb, *Tripterygium wilfordii* Hook F. Treatment with PG490-88 alone, or in combination with tacrolimus, has been shown to prolong renal allograft survival in both dogs and cynomolgus macaques [181,182].

Costimulation

Costimulation blockade as a method of immunosuppression, and possibly tolerance induction, is based on work from the 1970s in which it was recognized that a second signal was required in addition to antigen recognition for an optimal immune response [183,184]. Further work since that time has led to the generally accepted view that multiple ancillary signals exist, which allow for fine-tuned modulation of the immune response based on a variety of stimuli [185].

While costimulation-blockade-based strategies have been able to routinely induce tolerance in murine models [186–188], it has been challenging to replicate the results in larger, more outbred

models. This is likely due to one of two phenomena where costimulation appears to be less important for immune activation. First, a high precursor frequency of alloreactive immune cells is capable of overcoming costimulation blockade by sheer numbers [189]. It is apparent that antigen recognition in the absence of costimulatory signals generates some cellular response, albeit quite inefficiently [190]. In the case of high precursor frequency, the sheer number of reactive cells makes up for this inefficiency to produce a physiologically meaningful response [191]. Second, some cell populations appear to be less dependent on costimulatory signals for activation. Memory cells, in particular, appear to be less reliant on costimulation [192]. Thus, more experienced immune systems that may have populations of alloreactive memory cells due to prior sensitization or heterologous interactions are less dependent on costimulation for activation and response to antigen.

As more attention has been paid to the role of costimulation in the immune response, increasing numbers of receptor–ligand pairs have been identified with immunomodulatory effects [193]. Furthermore, as many of these molecules fall within families of receptors, including the immunoglobulin and TNF superfamilies as well as the integrin and TIM families, there is often substantial cross-talk between specific pairs. As the individual effects of a costimulatory pathway vary, and the expression of molecules change over the course of an immune response, the application of costimulation-based therapies to transplantation has become increasingly complex. Due to the highly specific affinities of many of the costimulation blockade agents being studied, the NHP has become the large animal model of choice for these experiments [104].

CD28-B7 blockade

The CD28-B7 interaction is, by far, the best characterized of the known costimulatory pathways. CD28 on the surface of T cells interacts with B7-1 (CD80) and B7-2 (CD86) to provide costimulatory signals leading to cell activation and production of IL-2, among other effects [194–196]. Alternatively, CTLA4 on the surface of activated T cells binds the same two ligands with increased affinity and transduces inhibitory signals to the T cell, essentially exerting a breaking effect on the activated T cell [197].

Blockade of the ligands for CD28 with monoclonal antibodies has been shown to prolong renal allograft survival in macaques. Three groups have reported success with monoclonal antibodies to CD80 and CD86, 1F1 and 3D1, respectively, in either their murine or humanized forms. Kirk et al. noted a modest prolongation of renal allograft survival in rhesus macaques with either antibody alone. When the antibodies were combined, a significant prolongation of survival was seen, although tolerance was not achieved. These antibodies failed to prevent formation of a donor-specific alloantibody response [198]. In an experiment performed by the same lab, combination of 1F1 and 3D1 with blockade of CD154 failed to provide a survival advantage over anti-CD154 monotherapy; however, alloantibody formation was delayed [199]. In a cynomolgus renal transplant model, Birsan et al. combined the humanized 1F1 and 3D1 antibodies with sirolimus. The study found a prolongation of graft survival with the combination therapy over either the antibodies or sirolimus alone, but, again, tolerance was not achieved [200]. The third study, by Hausen et al., combined the humanized antibodies with either cyclosporine or steroids. Neither combination antagonized the effect of the monoclonal antibodies, and the combination of CD80/86 blockade with cyclosporine significantly prolonged graft survival above either therapy alone [201]. Ossevoort et al. studied different murine clones against CD80 and CD86, B7-24 and 1G10, respectively. In a rhesus skin graft model, the combination of B7-24 and cyclosporine provided only a modest prolongation of graft survival [202]. In a rhesus renal transplant model, the antibodies were combined with either CD40 blockade or cyclosporine [203]. The combination of CD80 and CD86 blockade prolonged graft survival. The addition of CD40 blockade had little effect on graft survival compared to CD80/86 blockade alone; however, the antimouse response was somewhat diminished. Addition of cyclosporine A to the CD80/86 blockade provided graft survival comparable to cyclosporine alone. Despite these early positive results, the company owning the rights to 1F1 and 3D1 declined to develop them further for clinical use.

Another strategy for blockade of CD28 signaling is use of a soluble form of the CTLA4 receptor, the fusion protein CTLA4-Ig. CTLA4-Ig binds to both B7 ligands, and thus blocks activating signals through the CD28 pathway and suppressive signals throughout the CTLA4 pathway. After CTLA4-Ig was shown to be effective in several rodent models [188,204,205], it has been extensively studied in NHPs. Early studies of CTLA4-Ig showed only modest prolongation of graft survival in pancreatic islet [206], renal [106], and heart transplantation (when combined with anti-CD4) [207]. Continued interest in the CD28 pathway led to the rational development of belatacept which, while only differing from CTLA4-Ig by two amino acids, had a tenfold increase in its capacity to inhibit T-cell activation in vitro [208]. Belatacept was shown to be superior to CTLA4-Ig when used as monotherapy and exhibited significant prolongation of graft survival when combined with mycophenolate and steroids or basiliximab [208]. The same group would later show efficacy of belatacept maintenance therapy when combined with sirolimus and basiliximab/anti-CD154 induction therapy [146]. Lo et al. and Lowe et al. have also shown the combination of belatacept and sirolimus to prolong kidney and islet survival, respectively, and identified this regimen as promising for clinical translation [168,209]. The success of belatacept in NHP models led to human clinical trials and its subsequent FDA approval in June 2011.

Blockade of CD28 signaling via molecules directed at B7-1 and B7-2 has the potential drawback of simultaneous blockade of co-inhibitory signals via CTLA4. Significant interest exists in development of specific blockade of CD28 that would leave CTLA4 signaling intact. A monoclonal antibody against CD28, TGN1412, was developed and found to have superagonistic qualities. Surprisingly, CD28 superagonists had been shown to improve autoimmune disease and prolong graft survival in rodent models through selective expansion of regulatory T cells [210]. A phase I clinical trial of TGN1412 met with disastrous results when all six volunteers who received the drug developed severe, life-threatening, systemic inflammatory responses [211]. The fallout from this trial led to significant and understandable concern regarding further development of anti-CD28 therapy. The National Institute for Biological Standards and Control in the UK, where the TGN1412 trial took place, produced new recommendations for experimental controls to more fully explore potential agonist or superagonist properties of antibodies [212,213]. Since the TGN1412 trial, two trials using anti-CD28 in primates have been conducted. The first utilized sc28AT, a monovalent fusion antibody. Poirier et al. showed that sc28AT in combination with tacrolimus or cyclosporine prolonged graft survival in baboon renal grafts or cynomolgus cardiac grafts, respectively [214]. The same group subsequently conducted a pharmacokinetic study in cynomolgus monkeys using FR104, a

pegylated monovalent Fab' antibody against CD28. While promising results have been shown in rodent graft-versus-host disease (GvHD) models, no transplantation experiments using FR104 in primates have yet been reported [215].

CD40-CD154 blockade

The interaction between CD40 and CD154 (CD40L) clearly plays an important role in the activation of naïve T cells [216,217]. Unlike the interactions between CD28 and B7, however, CD40-CD154 ligation likely does not play a direct role in the T-cell activation pathways. Rather, it plays a significant role in T cell-APC interaction: CD154 expressed on activated T helper cells binds with CD40 on the surface of antigen presenting cells, greatly increasing the antigen presentation and costimulatory potential of the APC and essentially licensing the APC to, in turn, activate cytotoxic $CD8^+$ effector T cells [218,219]. It also is critically involved in APC activation in response to platelet degranulation, and as such likely serves as an interface between innate and acquired immunity [220–222]. Thus, blockade of CD40-CD154 interaction may function more to interrupt activation of T cells through mollification of the initial presentation signals. This may explain why, like the experience with CTLA4-Ig, results have been more impressive in rodents than in large animals and humans [14].

In primates, monotherapy with the monoclonal antibody to CD154, hu5c8, has shown to be efficacious in multiple models including rhesus renal allografts [223,224], rhesus skin allografts [112], rhesus or baboon pancreatic islets [114,115], and, less so, rhesus heart allografts [225]. Serendipitously, hu5c8 was one of the first antibodies to be tested at the dose of 20 mg/kg rather than the historical standard of 1 mg/kg. It was later found that multiple cell types in the body express CD154, providing large quantities of available ligand to compete for binding, and in the case of platelets, possible initiation of graft rejection [221].

Hu5c8 is not the only antibody against CD154 found to have effect. The monoclonal antibody IDEC-131 has been used in NHP cardiac, skin, and renal transplant models. While the antibody had only modest effect in the cardiac model [121,226,227], efficacy was seen alone and in combination with sirolimus and DST in both the skin and renal models [228,229], even in the face of a predominately memory response [230]. Similarly, ABI793 was shown to have efficacy in both rhesus and cynomolgus renal transplant models [231,232]. Unfortunately, clinical development of anti-CD154 therapies have essentially been halted due to perceived concern regarding thromboembolic events associated with the blockade of CD154 on platelets [233].

The notable efficacy of CD154 blockade prompted continued investigation of the CD40-CD154 pathway, focusing instead on the CD40 molecule, which is not involved in hemostasis. Early rodent models met with success, leading to the rational development of Chi220, a chimeric antibody against CD40 with weak agonist properties [117]. An early study with Chi220 demonstrated moderate prolongation of median graft survival to 48.5 days from 7 days in a rhesus renal transplant model. Notably, the combination of Chi220 and CTLA4-Ig did not synergize to prolong graft survival beyond Chi220 alone in this study [234]. A subsequent study from the same group in an islet transplant model replaced CTLA4-Ig with the higher-affinity belatacept, and they reported a marked synergy with the two drugs [117]. Chi220 was found to temporarily deplete B cells in these two studies, although the 2005 report showed that B-cell depletion was not wholly responsible for the drugs effect by comparing it to the anti-CD20 agent, rituximab.

The clinical development of anti-CD40-based therapies may be hampered by their potential deleterious effects of B-cell depletion. Badell et al. described the effects of 3A8, a non-depleting monoclonal antibody directed against CD40 [119]. While maintaining its graft protective qualities in a rhesus islet transplant model, 3A8 was also found to be partially agonistic and failed to prevent alloantibody formation. Interestingly, 3A8 also failed to fully block engagement of soluble CD154, indicating that complete blockade of the CD40-CD154 interaction may not be necessary. In a separate report, the same group found the combination of 3A8 with CTLA4-Ig and sirolimus did prevent a humoral response [118]. The same combination of 3A8 with CTLA4-Ig and sirolimus was also found to allow for graft survival in a bone marrow chimerism model in rhesus monkeys [235].

A number of other anti-CD40 antibodies have been developed. 4D11, which partially depletes B cells, has been shown to prolong renal graft survival in cynomolgus monkeys [236,237]. 4D11 notably also suppressed germinal center formation in the spleen and lymph nodes, a phenomenon that had previously been noted with another, non-depleting anti-CD40 antibody, ch5D12 [238]. The chimeric antibody 2C10 was shown by Lowe et al. to bind to a different epitope than either Chi220 or 3A8 [120]. The antibody demonstrated a significant survival advantage in a rhesus islet transplant model when added to a base regimen of sirolimus and basiliximab.

Combined CD28-B7 and CD40-CD154 blockade

As seen above when discussing each costimulatory pathway individually, the combination of blockade of CD28-B7 and CD40-CD154 pathways has been studied in several settings. The initial study by Kirk et al. demonstrated a synergy between hu5c8 and CTLA4-Ig [106]. However, subsequent optimization of the blockade of these two pathways eliminated the survival advantage seen with combined therapy, although a retained benefit in delayed alloantibody formation was seen [199]. Pearson et al. studied the combination of CTLA4-Ig with either CD40 blockade with Chi220 or CD154 blockade with H106 in a rhesus renal transplant model [234]. The addition of CTLA4-Ig did not translate to a median survival benefit with either antibody, although two animals receiving CTLA4-Ig and H106 had graft survival >100 days. However, as noted above, the replacement of CTLA4-Ig with belatacept in an islet model by Adams et al. did lead to a marked synergy when both pathways were blocked [117]. Haanstra et al. combined the CD40 antibody ch5D12 with CD86 blockade and found the addition of CD86 blockade did not prolong survival beyond CD40 blockade alone [239]. Finally, Ossevoort et al. combined blockade of CD80 and CD86 with CD40 blockade and failed to show a survival advantage for the combinations; however, B-cell suppression was reported [203]. In total, the data suggest that combination of CD28-B7 blockade with CD40-CD154 blockade may not provide improved overall graft survival, but positive synergy between the two strategies does exist, particularly in terms of the humoral response.

Other costimulatory and co-inhibitory pathways

A number of additional costimulatory and co-inhibitory molecules have been discovered to play a significant role in alloimmune responses and directing the functional differentiation of T cells [240], including OX40-OX40L, PD-1-PDL-1/2, CD27-CD70, ICOS, PD-1, and the TIM family of molecules. While many of these pathways are the subjects of intense research, most have not yet made it to the level of large animal testing for use in transplantation.

Clearly, there is plenty of opportunity for further development of these interactions for future clinical use.

Adhesion molecule blockade

A wide variety of cell surface molecules mediate the adhesive interaction between cells, and several of these molecules have been found to have particular importance in the function of the immune system. In addition to their role in cell trafficking, some adhesion molecules have been found to stabilize the immune synapse and provide costimulatory signals to immune cells. This section will review two prominent adhesion molecule pairs and their effect in transplantation experiments.

LFA-1

Perhaps the best studied of these adhesion molecules is the integrin LFA-1 and its primarily ligand, ICAM-1. The interaction between LFA-1 and ICAM-1 has been shown to be important in cellular arrest of leukocytes on vessel walls, transmigration across the endothelium, and homing to secondary lymphoid organs [241–243]. Furthermore, LFA-1 is an important component of the immune synapse, stabilizing the interaction between T cells and APCs [244], and has been shown to improve antigen presentation on B cells [245]. The interaction of LFA-1 with ICAM-1 has also been found to transmit costimulatory signals directly [246,247].

The importance of LFA-1 in several areas of the immune response led to significant interest in the potential for LFA-1-based immunosuppression. Early results in numerous rodent models were promising, prompting further development [247]. In monkey models, anti-LFA-1 therapy provided prolongation of graft survival in cynomolgus skin [110] and rhesus cardiac [248] models. Badell et al. used the anti-LFA-1 antibody TS-1/22 in a rhesus pancreatic islet transplant model and showed significant prolongation of graft survival when combined with sirolimus and basiliximab or belatacept [249].

Subsequent clinical development of LFA-1 blockade regimens has been challenging. One early study failed to show success in treating acute rejection with an anti-LFA-1 antibody [250]. However, a subsequent study did find that anti-LFA-1 therapy with odulimomab was effective at preventing acute rejection, with efficacy about that of antithymocyte globulin [251]. Later clinical studies focused on efalizumab, which had already received FDA approval for use in psoriasis. Studies in kidney [252] and islet [253] transplantation yielded promising early results; however, the drug was removed from the market by the manufacturer due to development of progressive multifocal leukoencephalopathy in four psoriasis patients. Although the results with LFA-1 blockade have been promising, concerns for patient safety will be paramount in the development of any future anti-LFA-1 based regimens, and it is likely more specific agents will be required to avoid the deleterious effects of LFA-1 blockade on protective immunity.

CD2-LFA3

The interaction of CD2 with its ligand LFA-3 strengthens the interaction between T cells and APCs, but also, like LFA-1 and ICAM-1, transduces important costimulatory signals [254]. Kaplon et al. developed a soluble form of LFA-3, termed LFA3TIP, which prolonged cardiac graft survival in baboons by approximately 8 days [255]. Another early pilot study utilizing a rat antiprimate CD2 antibody, LO-CD2b, revealed significant depletion of $CD2^+$ and a very modest prolongation of renal allograft survival in baboons [256]. After a similar antibody against human CD2, LO-CD2a, showed efficacy in the prevention and reversal of acute rejection in humans [257,258], a safety and pharmacokinetic study of a humanized form of this antibody, marketed as siplizumab, was reported, although no evaluation of efficacy of the humanized form was made [259]. Weaver et al. utilized the fusion protein, alefacept (LFA3-Ig), in a rhesus model of renal transplantation [167]. The addition of alefacept to a base regimen of CTLA4-Ig, sirolimus, and DST offered significant depletion of memory cells and prolongation of graft survival. However, a subsequent study by the same group revealed that replacement of CTLA4-Ig with the higher-affinity agent, belatacept, eliminated the therapeutic gain achieved with alefacept [168]. Further experiments will be needed to determine what, if any, role CD2 blockade may play in the future.

Other adhesion molecules

As we gain better understanding of the mechanisms of immune cell homing, adhesion, and migration, it is likely that molecules designed to exploit these mechanisms will be developed and brought forward for preclinical testing. One such potential pathway already in the pipeline is the integrin VLA-4, which when blocked reduces migration into the graft parenchyma [260]. Early work in rodents indicates a synergistic effect of VLA-4 and LFA-1 blockade [261] or costimulation blockade [262]. A humanized antibody against VLA-4, natalizumab, is already approved for use in multiple sclerosis, making it an ideal candidate for translation for transplantation.

Depletional strategies

As noted earlier in this chapter, there are two major barriers to the establishment of tolerance in large animal and human models: relatively high precursor frequency and sensitization, either through previous antigen exposure or heterologous immunity. It is no surprise then that lymphocyte depletion has been seen as a method to reduce or eliminate these barriers and is increasingly seen as an adjuvant in the development of tolerance regimens. However, attention must be paid to the relative depletion of regulatory versus detrimental cell populations. Depletion of the regulatory populations may have negative effects on the graft, as will be seen in the discussion below.

Thoracic duct drainage

Perhaps the most intuitive way to deplete lymphocytes is by simply removing them from the circulation. As the majority of lymphatic fluid returns to the circulation via the thoracic duct, this provides an ideal site to remove lymphocytes from the body. In 1962, Gowans et al. showed that thoracic duct drainage was both depleting and immunosuppressive in rats [263]. Subsequent work in a canine model of renal transplantation showed modest prolongation of graft survival [264]. Several clinical studies were undertaken with somewhat mixed results; however, it was later appreciated that the time course of preoperative drainage used in the rat models was insufficient for humans. Despite relatively quick depletion of the lymphocytes, the maximal immunosuppressive effect was not seen for at least 30 days [265]. The procedure never entirely caught on, and was later fully supplanted by less-invasive and more-convenient pharmacologic therapies. In 1996, a group led by Todo and Starzl revisited thoracic duct drainage via a chyloesophageal fistula in a

dog model of renal transplantation, showing a prolongation of survival from approximately 9 to 18 days [266]. The use of internal drainage had been previously described; however, insufficient drainage had been the norm, making the procedure unreliable. One proposed advantage of esophageal drainage, however, is the introduction of alloantigens in the lymph fluid to the GI tract and subsequent induction of "oral tolerance" [267]. A 1997 study by Kinukawa et al. failed to show any advantage with the addition of thoracic duct drainage to a regimen of cyclosporine and steroids [268]. As such, thoracic duct drainage is now primarily a topic of historical interest only.

Polyclonal depletion

Polyclonal deletion of lymphocytes using antilymphocyte globulins or sera was one of the first depletional strategies developed and tested in large animal models. In addition to providing antilymphocytic antibodies, these preparations also include antibodies directed against a variety of other cell surface molecules, notably adhesion molecules. As such, they may be of particular use in cases of significant ischemic injury, such as cadaveric donors, where activated endothelium provides a rich interface for leukocyte adhesion and migration [269].

Several antithymocyte globulin preparations have been developed and tested in large animals, including dogs, swine, and NHPs [270–274]. Generalizing results of these studies is challenging at best, as the polyclonal antibodies generated against T cells from one species are likely to have differing epitope spectra in other species, if they react at all. Regardless, the results of these studies showed some efficacy, but tolerance was not seen. The use of rabbit antithymocyte globulin (ATG) has been studied in NHP and shown to prolong graft survival to approximately 30 days [275], though the dose required is significantly larger than is used in humans, likely owing to decreased specificity of the preparation [276]. The addition of total lymphoid irradiation (TLI) to ATG is capable of further prolonging survival to about 100 days [277].

The failure of ATG to induce tolerance has led to its use primarily as an induction agent followed by some form of maintenance immunosuppression. Hirshberg et al. showed that rabbit ATG followed by sirolimus monotherapy prolonged islet allograft survival in NHPs; however, the survival was limited by sirolimus toxicity [278]. Liu et al. further developed this regimen with the addition of the B-cell depleting agent, rituximab, and showed further prolongation of islet survival [279]. Interestingly, Haanstra et al. showed that depletion with ATG shortened the time to rejection and increased alloantibody formation when combined with costimulation blockade, possibly due to an observed reduction in intragraft levels of the inhibitory molecule CTLA4 and regulatory marker FoxP3 [280]. Furthermore, Pearl et al. have shown that, following depletion with ATG, repopulating cells have an enriched memory phenotype [230] with potentially lower costimulation requirements.

It is important to note that polyclonal heterologous antibody preparations are, by definition, species-specific reagents; they are also highly variable with regard to their specificity. As such, their behavior in one species is unlikely to encompass the same breadth of effect in another, and it is unreasonable to anticipate that an agent raised against one species (for example RATG raised in rabbits against humans) will behave comparably when used in another species (e.g. rhesus). At present, there are no polyclonal agents that can be used in both in humans and in an animal model with similar effects; all require alterations of dose, result in variability in the spectrum of depletional and blocking effects, and have substantially altered duration of effect. As such, there is no scientifically valid large animal model for polyclonal agent use.

Monoclonal antibody preparations

The goal of monoclonal antibody-based depletion is to eliminate or coat reactive T cells and prevent them from carrying out their effector functions while minimizing the pan-immunosuppressive effects of less specific polyclonal depletion. Most preclinical large animal studies involving monoclonal antibodies are carried out in NHPs due to the need for highly conserved, cross-reactive epitopes on the target molecules. However, this is not exclusively the case. Watson et al. successfully developed monoclonal antibodies against canine CD4, CD8, and Thy-1 and showed they prolonged renal graft survival, particularly in combination [281].

The conjugation of CD3 and CD4 antibodies to idarubicin has allowed for the establishment of operational tolerance in half of kidney grafts when combined with TLI (itself a potent depletion therapy) in a baboon model [282]. Interestingly, when the conjugates were given for 2 weeks post-transplant rather than as a single pretransplant dose, no animals developed tolerance, potentially indicating a role for regulatory cells during this early post-transplant period. The use of the CD4 conjugate alone provided only modest prolongation of graft survival [283]. Additionally, previous studies with another non-depleting CD4 antibody, OKT4A, had shown only modest prolongation of graft survival [284–286], perhaps indicating the importance of depletion in the establishment of tolerance.

Broad depletion of CD45-positive cells is being tested as a conditioning strategy in hematopoietic transplantation and has shown some efficacy in dogs [287]. The selective depletion of CD45RB-positive cells has also been shown to be effective at prolonging solid organ graft survival in rodent models [288]. Chen et al. subsequently showed this strategy to be efficacious in a cynomolgus model of renal transplantation [289], with modest prolongation of survival as monotherapy as well as synergy with tacrolimus.

While the agents discussed thus far have focused on T-cell depletion primarily, depletion of B cells has also been shown to have positive effects in transplantation. The anti-CD20 monoclonal antibody, rituximab, has been found to promote graft survival in islet [279] and cardiac [290] NHP models when combined with other immunosuppressants. However, there is some concern regarding deleterious effects on protective immunity with rituximab, as a study of NHP renal transplantation failed to show a survival benefit when rituximab was added to a regimen of ATG, sirolimus, and donor bone marrow infusion, partially attributed to a high rate of SV40 infection in the animals treated [291].

It is worth noting that several of the monoclonal agents discussed earlier in this chapter in the context of costimulation and adhesion blockade may also exert some of their effect via depletion. Indeed, the anti-CD2 fusion protein, alefacept, and the anti-CD40 antibody, Chi220, both have some depleting effect [117,168]. Additionally, there are several depletion strategies being tested in rodents that have not yet transitioned to large animal models. The depletion based on the activation marker LAG-3 has shown promise in rats [292], and has been shown to prevent delayed-type hypersensitivity responses in baboons [293]. However, a pilot study of anti-LAG3 therapy using a different clone showed minimal effect in a rhesus renal transplant model (unpublished data).

Immunotoxin

Perhaps the most successful depletional agent in NHPs has been immunotoxin (IT), a fusion of a modified diphtheria toxin (CRM9) with the FN18 monoclonal antibody directed against primate CD3 [294,295]. Groups led by Knechtle and Thomas have independently shown pretransplant depletion with IT promotes tolerance in primate renal transplant models [107,296,297], with a modest increase in efficacy when paired with donor bone marrow infusion [297]. Thomas has also shown efficacy of IT in concordant islet xenotransplantation [298]. Furthermore, IT has also been shown capable of reversing rejection in a rhesus renal transplant model [299].

Perhaps surprisingly, treatment with IT at the time of transplant has been less effective in promoting tolerance. The treatment regimen prolongs graft survival and prevents cellular rejection; however, alloantibody-driven graft damage and rejection occur due to an intact antidonor IgG response [300]. This model has been pushed forward as a model of chronic allograft rejection due to similarities seen in the histology of primate grafts with clinical samples [301] and represents a rare, reproducible animal model of chronic rejection. Jonker et al. studied the effect of IT when given post-transplant and found a modest prolongation of survival that synergized with conventional immunosuppression such as cyclosporine and sirolimus [302]. This regimen was also notable for a high level of toxicity and infectious complications.

Concerns about the chemically linked FN18-CRM9 IT, particularly related to production yield and linkage heterogeneity, led to the development of a recombinant anti-CD3 immunotoxin [295,303,304]. Subsequently, Page et al. developed a model of chronic, antibody-mediated rejection in rhesus renal transplant recipients using the recombinant IT and tacrolimus [303]. Their model demonstrated development of de novo alloantibodies in 100% of animals treated with the regimen and represents a reproducible, modern model of antibody-mediated rejection.

Immunotoxin was also developed for use in porcine models [305]. However, significant neurotoxicity, not seen in the primate studies, limited the applicability of IT in the pig model [306]. The excellent results seen in the primate models prompted further development, and a new porcine immunotoxin utilizing a different construct was shown to lack neurotoxicity in swine while maintaining T-cell depletion, albeit not as efficiently as its historic counterpart [307].

The success of depletional strategies in large animal models has led to several human trials. Unfortunately, immunotoxin is not directly translatable to the clinic, owing to differences in the CD3 molecule between humans and primates. However, the anti-CD52 antibody, alemtuzumab, produces a similarly impressive depletion of T cells in humans and has been shown to prolong allograft survival [308,309], although tolerance has remained elusive [310].

Chimerism

Chimerism refers to the coexistence of genetically distinct donor and recipient cells within the recipient. This differs from mosaicism in that mosaicism refers to genetically different cell populations arising from a single zygote. While it can be said that all transplant recipients are chimeras due to the presence of the graft, the use of the term "chimerism" within transplantation almost exclusively refers to hematopoietic chimerism due to the unique effectiveness of these cells to influence the immune response. Chimerism-based strategies hope to extend the mechanisms of self-tolerance to the graft by introducing a replenishing source of donor APCs [311].

It can be said that the study of immune tolerance began with chimerism. In 1945, Owen described red cell chimerism in free-martin cattle. In this case, a placental vascular anastomosis in utero allows for the exchange of hematopoietic progenitors and development of life-long chimerism [312]. The group led by Medawar would subsequently show these cattle capable of accepting skin grafts from only their twin [313]. Continued research would reveal chimerism to be a potent strategy for tolerance induction in mice [2,314] and even capable of tolerance induction in humans [5,315–318].

Hematopoietic chimerism can be further classified by the relative contribution of donor cells to the population of mature, circulating cells. Full chimerism and microchimerism refer to the states of approximately 100% of circulating cells being of donor origin and $<1\%$ (i.e. detectable only by exquisitely sensitive techniques, such as PCR) of circulating cells of donor origin, respectively. Mixed chimerism refers to the intermediate state of detectable (i.e. $>1\%$) but incomplete (i.e. $<100\%$) of circulating cells originating from the donor. Infusion of hematopoietic stem cells, either from bone marrow or mobilized into the peripheral blood, into an unconditioned recipient leads to rapid rejection of the cells, failure to engraft, and resultant microchimerism, if any. However, conditioning of the recipient to allow for engraftment requires more intensively ablative regimens, sometimes with severe toxicity. Given the relative safety and efficacy of post-transplant immunosuppression, this has been a major barrier to widespread clinical translation of chimerism-based strategies, and significant effort has been placed into improving these regimens [311].

Microchimerism

The establishment of microchimerism by donor-specific unfractionated marrow infusions without preconditioning has been used in attempts to improve graft survival in a number of large animal and human studies [319–323]. However, it has been shown that likely all transplant recipients are microchimeric to some degree [324], and clearly not all recipients accept their grafts. The question has therefore been raised whether such chimerism in the presence of immunosuppression is biologically meaningful or whether it merely indicates that sufficient immunosuppression to maintain a transplanted organ is also sufficient to prevent rejection of some "microchimeric" cells [325]. Indeed, many studies have failed to show a benefit based solely on microchimerism alone [326–333]. It seems reasonable that the microchimeric state allows for presentation of donor antigen in secondary lymphoid organs, but at a level that is not tolerogenic in its own right. However, such antigen availability may have been of benefit when paired with other therapies, such as is seen when pairing costimulation blockade with donor-specific transfusion [334]. The studies of Thomas et al. [322]. provided evidence for the presence of veto cells as a subpopulation of marrow cells. These cells were shown to abrogate antidonor cytotoxic responses and represent one possible mechanism of peripheral tolerance induced by bone marrow infusion.

Full chimerism

Full chimerism is the goal of bone marrow transplantation for hematologic malignancy, where eradication of the cancerous cells is necessary. Such full chimerism is generally only achievable without problematic GvHD between HLA identical siblings. However, this full chimerism has been shown to carry with it

tolerance of subsequent kidney transplants from the same donors [315,335,336]. Additionally, it has been shown in large animal models, including miniature swine [337] and dogs [338,339], that full marrow replacement following lethal total body irradiation leads to acceptance of kidney grafts from donors that are MHC-identical to the original marrow donor. However, full chimerism does have some less desirable characteristics in the search for tolerance induction [314]. Compared to mixed chimerism, the achievement of full chimerism requires more intense conditioning and increases the risk of GvHD. Additionally, full chimeras often exhibit immune incompetence due to MHC disparity between positive-selecting cells in the thymus and peripheral APCs [340]. Thus, regimens designed to achieve full chimerism should be approached cautiously, especially when considering transplants across MHC barriers.

Mixed chimerism

It has been generally postulated that macrochimerism is necessary for the development of durable tolerance in animal models. Interestingly, rodent experimentation has demonstrated stable chimerism is required for durable central tolerance [341,342], while in larger animals and humans, only transient macrochimerism appears to be necessary [316,343,344]. Similarly, states of durable chimerism (although not of all hematopoietic lineages) have been established in NHPs without conferring tolerance on a renal allograft [345]. Thus, multilineage chimerism may be required for tolerance induction [346]. Nevertheless, these results point out that the fundamental relationship between chimerism and allograft tolerance remains incompletely understood.

Studies of mixed chimerism in the dog model have been led by Storb et al. His group has been able to establish mixed chimerism in their canine model using a regimen of sublethal total-body irradiation and a short course of CyA with or without mycophenolate mofetil [61,344,347,348]. These animals accepted kidney grafts from their donors [61,344,348]; however, some degree of tissue-specificity was observed, as not all animals subsequently accepted skin grafts from the same donor [61]. Storb and his colleagues were able to refine their method, showing that reduction of the total-body irradiation was possible by addition of CTLA4-Ig and DST [349] or by limiting the field to the lymphatic chains [350]. Together, these studies imply the value of conditioning irradiation lies in immunosuppression of the recipient and that the donor marrow is capable of creating its own physical space in the marrow cavity. Tillson et al. reported similar results [351]; however their study is notable in two ways: long-term survival of renal grafts was observed in both DLA-identical and DLA-haploidentical dogs, but skin grafts were only accepted in the DLA-identical pairs; secondly, animals receiving the conditioning regimen without the bone marrow infusion also accepted their kidney grafts, raising questions on whether or not chimerism was required for tolerance induction with that preparative regimen.

Mixed chimerism has also been successfully established in a miniature swine model using a combination of T-cell depletion with or without irradiation [87,352]. These animals subsequently accepted skin grafts from their donors, but not third parties. Interestingly, these animals were found to have a high risk of developing PTLD associated with a porcine gammaherpesvirus [353]. Further analysis showed that increasing degree of MHC mismatch and increased T-cell depletion were risk factors for development of PTLD in this model [354] and that the incidence of PTLD could be decreased substantially by including B-cell depletion in the preparative regimen [355]. Tolerance of kidney, heart and vascularized composite tissue allografts have all been achieved through mixed chimerism in miniature swine models [97,356,357].

The Boston group has utilized a similar regimen in cynomolgus macaques with successful establishment of transient mixed chimerism and acceptance of renal grafts following irradiation, bone marrow infusion, T-cell depletion, splenectomy at the time of transplant, and a short course of CyA post-transplant [99,100]. This regimen was also capable of preventing development of donor-specific antibodies in most of the animals tested [343], but it is worth noting that some animals did have antidonor activity on biopsies taken following the cessation of CyA therapy [99]. Similar to the porcine experiments, these studies also demonstrated development of PTLD, likely related to LCV [358]. Kawai et al. also studied this regimen in cardiac transplantation; however, full tolerance could not be established and grafts were ultimately lost to chronic cellular and humoral rejection [359]. The addition of CD154 blockade with 5C8 allowed for the omission of splenectomy from the protocol; however, long-term tolerance was not achieved uniformly as chronic rejection developed in some animals [360]. It is notable that the regimens developed by Kawai et al. were capable of inducing tolerance in most monkeys even across full MHC barriers without GvHD [99,100,360] and that these regimens have been the basis for the first successful human trial for tolerance induction through mixed chimerism in HLA mismatched kidney transplantation [5].

A mixed chimerism model has also been developed in rhesus macaques [361]. This regimen is based on busulfan rather than irradiation, and is followed by induction with basiliximab and continued immunosuppression with belatacept, sirolimus, and the anti-CD154 agent H106. This model is notable for its high levels and persistence of chimerism achieved (~80% lasting a median of 145 days). However, rejection of the donor marrow was found to be rapid following the cessation of immunosuppression [362], and viral infections occurred at a high rate following bone marrow transplant [361]. The same group sought to determine if CD40 blockade would provide the same benefit. A regimen consisting of busulfan, basiliximab, CTLA4-Ig, sirolimus, and the anti-CD40 agent 3A8 was found to promote engraftment and mixed chimerism. Similarly to the previous protocol, however, graft rejection and loss of chimerism followed discontinuation of the immunosuppressive drugs [235]. Neither of these models has been tested to determine their effect on the rejection of solid organ transplants following establishment of chimerism.

Tolerance based on induction of mixed chimerism is one of the few strategies that has been successfully translated from mice to large animals, and, as noted above, selectively in humans. However, the required conditioning regimens remain significantly more toxic than standard immunosuppressive therapies, so that this approach remains experimental at this point. Furthermore, safety concerns involving viral infection and PTLD that have been raised by the primate studies will also have to be considered. Despite these drawbacks, the strategy remains very promising and further research efforts into chimerism-based approaches to tolerance are definitely warranted.

Summary

In this chapter, we have reviewed the major large animal models for solid organ transplantation, discussing both the technical issues associated with working with these animals, and also a number of strategies to prolong graft survival with, in some cases,

establishment of long-lived tolerance of a graft. While a full discussion of all the available literature on the subject would be prohibitively long, we have focused on those approaches likely to provide meaningful advances in understanding of the immune response and improvements in clinical outcomes. Some of the therapies discussed have already been brought forward to clinical reality; others have been tested in humans with less impressive results. Clearly the reasons for differing outcomes are many, some of which we can account for, while we may not yet even be cognizant of others. Despite these issues, large animals remain the best models of the human immune response. Any ethical human trial must be predicated on at least some animal data, and large animal experiments provide the most relevant test bed for exploration of strategies to improve the outcomes for our patients.

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CHAPTER 16

Advancing Transplantation In Silico: Studying Global Gene Expression Using Functional Genomics for Transplantation Research

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Introduction

Our working definition of functional genomics comprises four general disciplines and their closely linked technologies: global gene expression profiling, tandem mass spectrometry proteomics, genetics, and epigenetics. However, we will only discuss the first discipline and applications of global gene expression profiling to transplantation research in this chapter. We use the term "functional" to communicate a focus on using these different genomic disciplines as a suite of tools to understand the biology of health and disease at a "molecular" level. The reader is no doubt aware that the continued proliferation of references to "-omics" and new fields like microbiomics and metabolomics are worthy of the name and their own chapters. But we also share the growing concerns in the field that the routine overuse of this term serves no valuable purpose.

In first principles, we organize our approach to functional genomics in biological terms (e.g. how complex systems like cells and tissues work at the molecular level). We think in terms of advancing from mechanistic knowledge of basic cell biology to understanding how a state of health transitions to disease at the cellular and organismal levels. The chapter is meant to complement Chapters 13, 14, and 15, which deal with the in vitro, small animal, and large animal model systems used in transplant research. This is a bottom-up approach. Applied to clinical medicine, we believe that functional genomics illuminates how diseases start, manifest, and progress. The course of any disease in an individual patient is actually determined by a combination of multiple factors, including genetics, environment, personal history, and our choices of therapy. For example, transplant rejection is triggered by an immune-mediated process with multiple balancing mechanisms but the outcome is impacted by the genetics of both recipient and donor, recipient age and immune history, the choice and timing of immunosuppressive therapies, the individual's genetically programmed and epigenetically conditioned responses to tissue injury, and the ability to heal or compensate. Therefore, by definition, bottom-up functional genomics creates a very complex view of biology and medicine. This is a challenge to a reductionist view where mechanisms of disease are defined by much clearer and simpler rules.

We believe functional genomic knowledge will contribute to creating the next generation of diagnostics and therapies. We also believe that personalized medicine will be part of our future and that it will be transformative, despite our concern that this future has been prematurely over-hyped. The rationale for this confidence is that the current sum total of all the biological information evolved by our ancestors is contained in the DNA of our genome. From this huge human knowledge base the information is constantly called upon, like a huge living search engine, to create, differentiate, maintain, protect, and heal. In our view, functional genomics applies to every step in this flow of genomic information, from reading the DNA, to splicing RNA, to translating and modifying the proteins, as well as the entire complex of regulatory and communication pathways controlling all these systems. When functional genomics matures as a fully integrated discipline and we can routinely follow the complete details of this information super-flow, there will be no place for the causes of disease to hide from our detection or our understanding. Whether we get there in our lifetimes is much less certain.

Functional genomics in practice

We will review the current technologies available for global gene expression profiling in transplantation. These are just tools and we will explain the basic principles of each. We will also discuss their limitations in the context of experimental designs and data interpretation. The reader can then ask what more they need to know to successfully apply any or all of these tools to whatever scientific or clinical challenge inspires them.

Gene expression profiling as a concept

Genomic information flow starts with the activation-dependent transcription of DNA into mRNA in the nucleus. This level of information flow is studied by gene expression profiling. The first product of gene transcription is called pre-mRNA, an RNA transcript that still contains all the introns and exons encoded in the DNA. A large multiprotein complex called the spliceosome is then assembled, which is comprised of over 100 different proteins that

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are required to splice out the introns of the pre-mRNA [1,2]. Assembly of the spliceosome is regulated by sequence differences marking intron–exon junctions, by specific sequences in the exons, and by energy-dependent phosphorylation of various proteins in the complex. Essentially, all pre-mRNA transcripts require **splicing** into mRNA before export to the cytoplasm where protein can be made. One of the great mysteries at the present time is how cells determine the "when" and "where" of **alternative splicing** such that specific exons in the middle of a pre-mRNA transcript are included or removed from the mRNA under specific conditions [3]. We have shown that immune-mediated human T- and B-cell activation produced as much differential alternative splicing as up-regulated mRNA transcript expression [4]. We pointed out that most of the current approaches to measuring differential gene expression were completely ignoring the potential impact of this alternative splicing.

After splicing or alternative splicing at specific exon–intron junctions, the mature mRNA transcripts are exported in another energy-dependent manner to the cytoplasm for entry into the ribosomal machinery and protein translation. Translation is regulated at multiple steps, including entry and assembly of ribosomes, the delivery of amino acids for protein synthesis, and pathways that regulate mRNA transcript half-life such as the miRNAs bound to the RISC complex [5]. Importantly, protein synthesis is also regulated by the mTOR complexes that are the targets of the immunosuppressive drugs in the rapamycin class [6]. The entire process is remarkably complex. Thus, it is important to give a first reality check for the reader here: gene expression profiling of the mature mRNA produced by this process is very revealing but it is still only a limited view of the true information flow and the many regulated steps involved in cell activation. New methods are allowing us to add global profiling of alternative splicing and that is a great advance. But we all need to keep a firm grasp on what each piece of new knowledge contributes to our understanding of a profoundly complex process and avoid the temptation and the pressure to shape exaggerated hypotheses about the mechanistic significance.

The value of gene expression profiling starts whenever we want to compare one or more states (resting vs. activated, early vs. late) or phenotypes (rejecting vs. tolerant, viral infection vs. acute rejection) and study what genes are activated in individual cells or whole tissues to direct protein synthesis and influence function. Gene profiling done in a targeted fashion can be used to follow whole networks of molecules already known to be important, such as inflammation, apoptosis, cancer transformation, cell signaling, cell proliferation, and so on. A growing number of very good commercial, pathway-based sets of quantitative PCR assays are available for these purposes and should be considered as options. However, the rapidly falling costs of doing global gene expression profiling on microarrays or by deep RNA sequencing allows such comparisons of gene expression changes to be done without any bias to known networks of function. Global profiling allows for discovery and yet takes full advantage of everything known about functional pathways in the literature. Another common application of microarrays has been biomarker discovery [7,8]. Here the objective is to identify a signature of mRNA changes that consistently reflect a given state or patient phenotype (acute or chronic rejection, liver transplant regeneration vs. dysfunction) [9–12].

A decade of genomics literature in transplantation has already created many different sets of published gene expression profiles from mouse models to human patients. These data include biopsies of normal and rejecting kidneys, livers, hearts and lungs, profiles of many different immune and inflammatory cell types in tissue cultures, small animal models and patients, profiles of blood cell subsets (Treg, B cells, macrophages), and profiles of peripheral blood cells from operationally tolerant kidney and liver transplant patients. Therefore, a good start before getting into any gene profiling project is to check out what has already been done and deposited in the public domain databanks. For this purpose two good starting places are the Gene Expression Omnibus (GEO), the NIH National Library of Medicine gene expression database (http://www.ncbi.nlm.nih.gov/geo/), and the Array Express Archive maintained by the European Bioinformatics Institute (http://www.ebi.ac.uk/arrayexpress/). Another good data source is individual papers in the specific area of interest, as almost all journals now demand that gene expression data created by the research being described is provided to the reader in detailed supplemental tables unless already deposited in one of these public databases mentioned above. These datasets are powerful tools, especially when used correctly by skilled bioinformaticians, and they should be fully exploited.

A note of caution is also important. There are submission guidelines called MIAME (Minimum Information About a Microarray Experiment) that help standardize what terms are used to describe the experiment. However, there are no quality filters for datasets in these public domains. They vary dramatically in quality, technology platforms, methods, and by experimental designs in many critical details that are not described and rarely apparent from review of the MIAME metadata. The fact that they represent thousands, even millions, of data points does not mean that the rules of basic experimental design and rigorous laboratory conduct are suddenly suspended. Moreover, the same obligations on investigators remain for any conclusions reached by mining these datasets to validate claimed results with orthogonal approaches and validate mechanistic claims experimentally and under strictly controlled conditions. Thus, much of what is done and claimed using different bioinformatics tools to "mine" such databases is greatly exaggerated in our opinion. But in fairness, there are experts in the bioinformatics field that would strongly disagree with our view. They believe that they have changed the rules of experimental science with new statistical approaches designed to combine and mine these data troves successfully. Our view is that the tools alone cannot make the furniture for a carpenter but the proper use of tools is very useful. Time will sort these claims out but we would not recommend buying an expensive chair until you are sure that you are comfortable sitting on it.

Gene expression profiling by microarrays

Global gene expression profiling can readily be done with commercial microarrays (Table 16.1). Microarrays start by printing or

Table 16.1. Commercial microarrays

Affymetrix
3′ IVT arrays: HG U133 Plus 2.0
Exon probe-based arrays: Human Gene ST Array
Whole exome-based arrays: Human Exon ST Array
Agilent
Gene-based arrays: SurePrint G3 Human Gene Expression Array
Whole exome-based arrays: SurePrint G3 Exon Array
Illumina
Bead-based profiling: HumanHT-12 v4 Expression Bead Chip

synthesizing in situ complementary DNA probes onto a physical surface like a glass or plastic slide [13,14]. Each individual spot on the array contains several million probes which are all identical and designed to be specific for one mRNA transcript. Thus, you can profile as many different RNA transcripts as you can make unique probes, identify and record the results of hybridization at separate spots. Current technologies can readily print probe spots for the entire human genome on the surface of a chip that can fit in the bottom of a 96-well microtiter plate. Similarly, putting the probes on the surfaces of beads can also create expression arrays (e.g. Illumina). The principles are essentially the same though the geometry and mechanics of the instruments are somewhat different. The results with all these technologies are highly reproducible as well as reliable.

To visualize and quantify mRNA expression the RNA transcripts from total RNA preparations of a given sample are most commonly reverse transcribed to generate the complementary cDNA. In most oligonucleotide arrays, in vitro transcription of the cDNA is employed to obtain biotin-labeled cRNA. These cRNA molecules are then hybridized to the oligonucleotide probes on the microarray. After hybridization, the microarray is stained with a fluorescent molecule that binds to biotin and is finally washed. After washing off unbound transcripts and then using UV light excitation, the amount of fluorescent signal released from each spot on the array is recorded, and this is proportional to the amount of labeled mRNA transcript for each gene present.

By providing the user a numerical, signal-based metric for relative gene expression, the differences in expression can now be normalized. We have used many normalization tools over the years but currently do all our normalization using the rank-based Quantile Normalization with RMA [15]. However, there is one additional step before proceeding to sample comparisons. We have found that microarrays have a tendency to amplify apparent differential expression changes for genes detected at very low signal levels. This is because at low signals the processes of normalization and subsequent ANOVA-based class comparisons (see next) tend to select out a class of data points with less technical signal variation and exaggerate the significance. If you have hundreds of arrays per group this is not an issue but very few studies reach such numbers to avoid this limitation. Therefore, typically in our laboratory we perform one additional signal filter step after normalization by removing any low normalized signals, which is usually below the normalized background signal intensity across all microarrays in a particular experiment. Though this is data dependent, the values are usually a log2 signal in the range of 5.64 to 6.64, which corresponds to a natural log10 scale signal of 50 to 100 using Affymetrix microarray data. However, while the principle remains the same, it is advised that the user determine the background threshold based on the array type used for the experiment before removing low-expressed genes as described above.

Next, we take all the results from the multiple individual samples in two or more different experimental groups (e.g. classes) and do **class comparisons**. For example, compare the profiles of biopsies taken from 50 different patients with acute rejection to 50 control transplants with excellent function. These gene differences by class of sample are called **differential expression**. The original data are delivered as an output file with a numerical value or signal for each gene that is expressed above the normalized background of the chip. Based on the direction of the class comparison chosen (e.g. rejection compared to controls), a positive change is designated as "up-regulated" and a negative change as "down-regulated." Differential expression is often referred to and expressed as a "fold-change" so that we can think in terms of a given gene being up- or down-regulated so many fold with rejection and so on.

The list of differentially expressed genes can then be mapped to functional biological pathways using different tools to gain insights into the mechanisms driving acute rejection in our example. The reader should just remember that these mapping tools are only as good as the current knowledge for a gene as there are clearly many things genes do that remain to be discovered. So just because one researcher looking at a gene's function in the brain of a mouse publishes that it is linked to signaling via a growth factor receptor doesn't mean that its function in an activated B cell is the same, though it is likely that it is a signaling factor of some sort. A new generation of mapping tools takes a very different approach. They find patterns of coordinate expression in complex data sets that suggest groups of genes function in some kind of associated network. There could be a dozen different discrete pathways defined by function contained within such a network but the point is to identify the fact that all the linked genes in the network, thus all the functional pathways, change in a predictable way with a given situation. So for example, acute rejection could cause signaling via the T-cell receptor and a costimulatory pathway that activates a number of cytokine genes that then up-regulate and signal through their cognate receptors and trigger cell proliferation inducing functional pathways and also inhibit apoptosis. These related events would all be represented in a single network using these new tools. Cytoscape is an excellent example of this new generation of network mapping tools and is available for anyone as open source software (http://www.cytoscape.org). It is too early to claim that you can go from gene expression data to such detailed understanding of linked functional pathways at this time but the use of such tools should be part of any discovery project.

Differential expression data can also be used to create biomarker signatures that might diagnose the presence of rejection in a biopsy or inform the user on some characteristic like severity or acuity. **Class prediction** refers to another set of tools that take the differentially expressed gene lists created with one set of samples to predict the classifications of a new set of samples chosen from different but similar subjects, for example acute rejection versus controls. This is how we validate such gene lists and how biomarkers for clinical diagnosis can be developed and the diagnostic results in terms of positive and negative predictive values (the balance between false positive and false negative classification of each sample in a cohort to the correct diagnosis like acute rejection) in Receiver Operating Curves (ROC). As already noted, having thousands of data points does not suspend the rules of experimental design. You still need to carefully design experiments with proper controls and appropriate numbers of samples in each group and then repeat and validate any results.

Experimental designs: power, replication, batching, and validations

Statistical power is always necessary to consider before trusting conclusions drawn from global expression profiling on microarrays. The tools now used for class comparisons and class predictions can provide lists of highly statistically significant gene candidates. But if there are not enough samples in the study set then the statistical power of these candidate gene calls can be very low and that is often not explained in publications focused on discovery. As a

general guide based on years of calculating statistical power for array-based experiments, we have found that 20 samples per group is sufficient for equal or greater than 85% power to detect fold-change differences of 1.5 or greater in gene expression experiments. Nonetheless, even with appropriate statistical power in the first round, you have to replicate the experiment at least a second time with an independent sample cohort of similar size in a clinical sample profiling design or a second series of biological replications of the same experimental design in the case of cell culture or animal model-based experiments.

However, there are alternative approaches to consider for specific objectives. For example, hypothesis generation in a well-defined experimental system can start with a small number of microarrays as a first discovery step; for example, six arrays per group is about right usually. You still have to replicate once to validate the results but you are still at only 12 arrays per group. Then, based on the confirmed list of high-value candidates, proceed to orthogonal mechanistic approaches to validate your results like knocking down a few key signal molecules that you are predicting are critical for the response and measuring downstream gene changes by qPCR or protein phosphorylation, or global impacts on cell proliferation or some other cell function assay.

Batching refers to the critically important step of how you group samples and prepare them for the actual profiling. Every time you have a different technician prepare the total RNA or any of the many steps in the labeling protocol, you can introduce a type of technical bias we call "batch effect." Every time you change the kit type or kit lot for labeling or buy a new set of arrays and every different day that the experiments are done, can add batch effects. So we take great pains to organize experiments so that each part is done by the same technician in the exact same way. We always randomize samples so that individual samples from different experimental groups and controls are mixed evenly across days, arrays, and reagents. We also include at least three replicates of mRNA pools on each day (e.g. activated T cells) that are treated exactly like all the other samples but where any data variability has to be introduced by the technical procedure. While there are statistical tools that can be used to remove batch effects in silica, these work in a manner that necessarily reduce the number of genes you can study as well as the dynamic ranges of the signals for comparisons thereby sacrificing potentially important biological detail. We strongly believe that good experimental design is the best way to minimize batch effects and emphasize the biological differences that are the whole point of the work.

Finally, we should comment on validation as this frequently comes up in the review of papers and grants. First, there is no point in technical replicates for the current generation of microarrays since their excellent inter- and intraplatform reproducibility has been well established [16] and these are now just a waste of money and effort. Biological replicates are what should be done. Second, there is really no point in demanding qPCR replication of significant differential gene expression results at this point if the work is done properly as discussed above. No one would demand that anyone do a microarray to confirm results produced by qPCR because we "trust" the results of qPCR as we should now "trust" the results of microarrays if they are done properly. And if microarray profiling is not done properly, then demanding qPCR is not enough to save the results. So if validation is necessary, then the best approach is orthogonal by a different approach and technology. For example, if the claim by microarrays is that a given signal pathway is critical to an experimental system, then knocking down or overexpressing the pathway, creating a reporter construct, demonstrating the downstream impacts, and so on would be of more value, especially in the mechanistic sense. If the conclusion relates to a clinical outcome like acute rejection then consulting the existing literature including animal models, and considering doing mass spectrometry proteomics on parallel clinical samples or testing a hypothesis in a cell-based or animal model of the clinical outcome, is warranted (e.g. T-cell activation or proteomic profiling of acute rejection biopsies).

Gene profiling by deep RNA sequencing (RNAseq)

The latest technology for gene expression profiling is deep RNA sequencing. These instruments function by detecting and reading out the sequences of mRNA or miRNA transcripts fixed by adaptors to the surfaces of slides, beads, or electronic sensors. The details of these technologies are complex and beyond the scope of the present chapter [17]. But the basic principle is massively parallel sequencing of the individual mRNA transcripts obtained from the target cells or tissues. Thus, instead of simply detecting the amount of a given mRNA sequence hybridized to a specific complementary probe on the microarrays, discussed above, deep RNA sequencing reads out the entire sequence of the captured transcript by adding one base at a time, cycle after cycle, and detecting that event as a signal generated by fluorescence or release of a charged molecule like hydrogen. The massive scale is accomplished by capturing millions of transcripts on the same surface and sequencing them all in parallel in every cycle.

The relative number of mRNA transcripts is determined based on the number of reads obtained for each mRNA in a pool of RNA where the determined sequence aligns or maps to that specific gene. This is very different from labeling the whole mRNA transcript at the start, hybridizing it to complementary oligonucleotide probes, and reading out the total amount of fluorescent signal detected at a given spot on a microarray. On the other hand, it is the nature of evolving technologies to be different. So the first question is whether there is any significant difference in the actual value of the data obtained by either technology. Once either technology accurately provides the numerical metrics for the relative number of mRNA transcripts in a pool of total RNA, the value in terms of understanding the biological significance of the transcriptional information revealed is the same. The fact that deep sequencing is the latest technology doesn't make it magic or impart magical value to your data. The key is to understand the limitations and the advantages and then use both these technologies strategically.

A common metric used to compare deep sequencing technologies is "read length" and it is literally the maximal length of sequence that a platform can read in a single string of reads (Table 16.2).

Table 16.2. Deep sequencing technologies

Next generation deep sequencing (short read)
Illumina HiSeq 2000
SOLiD 5500xi
Next generation deep sequencing (long read)
Roche 454 FLX
Pacific Biosciences
"Personalized" deep sequencing platforms
IonTorrent
Illumina MiSeq
Roche 454 Junior

The current "short read" instruments can read up to 250 bp but can also do this in both directions of the gene in the same run, called paired-end reading. The current "long read" instruments can read up to 750 bp, though several manufacturers claim they will hit 1000 bp reads, and much longer in the near future. While this all sounds good in the descriptive literature, the fact is that single direction reads of 50–100 bp long are completely sufficient for doing gene expression profiling by deep RNA sequencing. Longer reads and paired-end reads are not necessary and just increase costs. However, if your intention is to also profile alternative splicing of mRNA and identify differential exon usage, then paired-end 100–150 bp reads are highly desirable, if not necessary.

Nonetheless, most of the commercial hype regarding achieving greater and greater read lengths, and more and more sequencing throughput, is directed to DNA sequencing and genetics. The commercial competition is being the first to offer the complete sequencing of a human genome that meets a standard for accuracy which is "medical grade" for only $1000 or less, a cost point that is commonly assumed will suddenly usher in the promised era of personalized medicine. We look forward to this technological achievement and recognize that it will lower the threshold for clinical sequencing of whole genomes in various patient populations. More data in a new field is a good step. But simply having more data available, obtained at the rate of 3.5 billion bases for every human genome sequenced, does not equate with suddenly understanding the biological functions and implications for health and disease that successfully delivering true personalized medicine will require. Until these remarkably complex challenges to functional genomics are addressed and the information contained in the genomic code is understood in terms of biology, health, and disease, the claims of the transformative impact of simple sequencing success are just more hype.

Another key metric for deep RNA sequencing is "read depth." Data generated by deep sequencing of RNA reveals the number of reads detected for any given sequence by matching all the read results from a given run to the known sequence of the human genome. As a rough guide for successful RNAseq, we aim to hit about 75–80% successful alignment of all reads obtained to the human genome. The objective of RNAseq-based gene expression analysis is to simply detect the presence and quantify the relative number of specific mRNA transcripts in the total RNA pool. Thus, approximately 200 million reads in one Illumina HiSeq2000 lane with 80% alignment gives about 160 million reads of genomic sequence. If you started with an RNA library created by putting sequencing adaptors on only mRNA, you can count every read (e.g. 160 million) that maps to the coding sequence of the known human genes as individual events. It is generally agreed that a minimum of ten reads for any transcript is sufficient to have statistical confidence that the transcript is present. Thus, if you want to measure the relative expression of the same transcript or miRNA in two different samples, the difference in the number of reads detected between two conditions (e.g. resting vs. activated, control vs. rejection) is all that is necessary to detect and quantify differential gene expression.

However, if you also want to compare multiple different mRNA transcripts to each other in the same experiment, then you have to "normalize" the data first. Normalization is critical because every mRNA is a different length. Thus, the probability of finding 100 reads for a gene transcript that is 5000 bp long is significantly greater than finding 100 reads for an mRNA that is only 1000 bp long. Normalization of deep RNA sequencing data to individual gene lengths produces the term FPKM (Fragments Per Kilobase of exon per Million fragments Mapped) [18]. This is important to understand because that is the way you should be shown your final results if you want to go beyond simply identifying the presence of a given gene transcript and compare its changes to transcription of other genes that might create functional pathways or regulate your gene of interest.

In fact, once normalized gene detection values (FPKM) are established, all the standard tools for analyzing gene expression profiling on microarrays can use the deep RNA sequencing expression data interchangeably. This fact is critical in practical terms because the last decade has given us a very mature pipeline, with multiple validated tools for analyzing and graphically displaying global gene expression data obtained from microarrays without having to depend on bioinformatics experts to analyze your data. That these can also be used with the data generated by deep RNA sequencing is a great deal for all of us.

With that clear as a starting point, there are a number of things that deep RNA sequencing does now that are major advances over microarrays. In fact, these are opening up whole new areas for functional genomics research. For example, given the proven efficacy of multiplex barcoding to successfully sequence up to a dozen different samples in one lane of a flow cell, we now routinely do miRNA profiling by deep sequencing. The cost calculation and unbiased scope of the data is significantly better than any of the currently available commercial miRNA arrays. We are now taking advantage of deep miRNA sequencing to go further and profile both the mature miRNAs and the precursor miRNAs (pre-mIRs) in parallel. Thus, initially an miRNA gene is transcribed to create the primary miRNA (pri-mIR) in the nucleus and this transcript is spliced by Drosha and DGCR8 (e.g. Pasha) at a specific stem loop structure that these splicing proteins are evolved to recognize. The pre-miRs are then transported to the cytoplasm by an energy-dependent step in complex to another protein called Exportin 5. Once in the cytoplasm another RNA splicing complex is formed by DICER and Argonaut to create the mature miRNA and transport it to the target mRNA transcript. All of these steps are regulated but very little is known about any of it. Deep sequencing has created a novel opportunity to advance these studies. Another example is the crossover between RNA expression profiling and genetic variation studies. Because deep sequencing also reveals actual sequence, it can be used to discover the presence of genetic variations in coding transcripts (e.g. exon sequences). In this application the technology is orthogonal to genetic variation studies done by deep sequencing of DNA after exome capture (e.g. whole exome sequencing). However, the reader should note that the experimental designs including the depth of sequencing required and the analysis pipelines for this kind of variant detection are very different from those typically applied to gene expression profiling. Finally, another really fascinating new direction for the field is the analysis of long noncoding RNA (lncRNA). It is now clear from the exciting insights being generated by the ENCODE consortium (Encyclopedia Of DNA Elements; funded by the National Human Genome Research Institute of the NIH) that RNA is produced by many intronic, non-coding regions in the human genome as well as by transcription of certain genes where the antisense strand of DNA is also transcribed but does not code for known proteins [19]. The roles played by lncRNA and the rules for how lncRNA regulate gene expression and cell function in response to activation, stress, and other signals remain to be discovered.

Alternative splicing by deep RNA sequencing and current challenges

We believe that the study of alternative splicing is one of the next big discovery areas in transplantation biology because such splicing is a powerful creator of genomic diversity, changing the cell's proteome in dramatic ways [20]. Moreover, cell activation results in significant changes in the alternative splicing profile of cells that are independent of the changes in the levels of mRNA transcription measured by just expression profiling.

Our experience to date is that data obtained with paired-end read deep RNA sequencing is greatly superior to the data obtained with the latest generation of microarrays for alternative splicing. But, in our experience, alternative splicing requires paired-end reads, typically 100 × 100 in length, as well as greater depth of sequencing (e.g. less samples per lane) and a much more complex bioinformatic workflow to detect differential exon usage, all of which significantly increase the costs and time required to complete a run. Thus, cost-wise, it is currently more cost-effective to do alternative splicing profiling with the commercial microarray offered by Affymetrix (GeneChip Human Exon 1.0 ST Array) that has probes for every known exon in the current build of the human genome. The problem comes with deciding on the quality of the results generated. In our experience with both this array and deep sequencing, we found that up to 25% of the splicing events detected by the array could not be validated by sequencing. That still leaves 75% detected and these comparisons are complex enough, as discussed next, that it is important to keep a very open mind about both strategies at this time as used properly each has value. It is also important to remind the reader that microarrays are also evolving. Newer generations of splicing junction arrays have been promised by Affymetrix that have new probes custom designed, based on growing knowledge bases of alternative splicing and exon–intron junction boundaries, which might narrow or eliminate the advantage of paired-end RNA deep sequencing in the future. Thus, the reader should always consult with the local technical genomic technology experts for the most up-to-date information before making these technology and experimental design decisions.

But alternative splicing also presents two serious challenges at this time. First, it is difficult to detect differential exon usage because, unfortunately, the reality is not simple as a given gene having only one possible alternative form that coexists in some simple 50–50 mix. Most genes have multiple alternative transcripts (often six or more), they can exist at any level in a mix, and at any given time under any given condition there might be multiple alternative transcripts (i.e. isoforms) for each known gene profiled present together in the total RNA pool. And if you want to drive yourself even crazier, different cells in a pool of cells could have different sets of transcript isoforms for the same gene and that kind of detail is totally lost until we get to single-cell gene expression profiling (see later in the chapter). So the first point is that getting into alternative splicing requires a major commitment of technical effort and resources.

The second challenge of alternative splicing data is understanding what it means once you have detected it. A little background on the current state of functional genomics is informative and necessary here to explain this challenge properly. The field is now comfortable with the notion that if you properly normalize and detect significant differential expression of an mRNA transcript for a given protein coding gene that the protein is present. There are still some diehards that like to repeat the scientific canard that "there could be transcripts without proteins." The reality is that differences in detection technologies, depth of coverage, and dynamic ranges for current mRNA profiling and proteomic technologies explain why it is difficult to detect every known protein predicted by a global mRNA expression profile. In fact, when really deep proteomic coverage is done, it is possible to detect much of the cell's protein-coding transcriptome in protein [21]. So raising this concern over a transcript sounds critically scientific but it demands author's wasting precious resources and efforts doing parallel proteomics that ultimately are very superficial efforts (e.g. a Western blot or non-quantitative immunohistochemistry) and do little to advance the original insight. Honestly, if you have a documented coding mRNA transcript in a well-designed experiment that is validated by proper and conservative use of the latest generation of informatics tools for data analysis, there is protein.

However, the deal is completely different for alternative splicing. First, many alternative splicing events can lead to truncation of a transcript and prevent protein translation. But more importantly, there is just precious little information about what any given alternative splicing change does to a protein's function and almost nothing regarding its role in the complicated molecular pathways of a cell. For example, we can demonstrate conclusively that a given exon is spliced out of 25% of the transcripts in a particular cell population or tissue and observe that this exon contains an adaptor protein binding sequence or a tyrosine that is phosphorylated by activation. But at the current time, we can only guess at what these major structural changes mean to the cell's response in a given situation like immune activation of a memory CD4 T cell. So the second point is that discovering alternative splicing is only the first step and not particularly useful until we also understand what actually happens next in true functional and molecular terms. What is not in doubt, however, is that alternative splicing can create significant changes in a protein's function, location in the cell, ability to complex with other critical partners in a pathway, or recognition of cognate ligands and even regulate the half-life of the mRNA transcript. Figuring all this out for transplantation immunobiology and integrating these discoveries with existing knowledge is a remarkable scientific opportunity created by these new genomic technologies.

But it is instructive to add a note of caution too. These challenges for alternative splicing are a perfect example of one of the pitfalls in functional genomics research; whenever a new capability is realized by new technology there is a temptation to run out and just do a lot of profiles without taking into consideration the complexity of the endeavor in biological terms. However, with properly designed experiments and expectations, discovery profiling with new technology can be absolutely justified as a necessary first step; for example, to demonstrate the complex relationship between gene transcription and alternative splicing changes with T- and B-cell activation that we reported several years ago [4]. What is the point of gearing up to study alternative splicing in some comprehensive way without first using these new profiling technologies to establish at least the reasonable possibility that it is relevant to the biology we want to advance? But once such a proof of concept is established, the next series of experiments must be justified by the extent that they create new knowledge and in the case of alternative splicing that is no minor challenge. As long as we are clear about the objectives and relative value of any given set of experiments, deal properly with the limitations, and don't confuse using amazing new technology with magical knowledge, then this is how scientific work naturally evolves.

Comparison of microarrays to RNA deep sequencing

The obvious question at this point is which technology, deep sequencing or microarrays, should be used for gene expression profiling. The answer is "it depends."

The sensitivity for RNA deep sequencing is dependent on read depth. Thus, some experts originally concluded that with really deep sequencing many transcripts were identified that were never detected by microarrays. This was the beginning of the current view that microarrays are "on the way out" and that RNA deep sequencing revealed a whole new level of transcriptional activity. Ironically, this view also supported another incorrect view that RNA transcriptional profiling requires very deep sequencing depth so that these "hidden" transcripts can be detected and that deep depth is prohibitively expensive. The observation of low-level transcript detection by very deep RNA sequencing is absolutely true but the "devil" is hidden in the details. When you sequence well-characterized cell systems like cancer cell lines deeply enough it turns out that most of the low-level gene transcription detected may actually be only transcriptional noise and these often do not map to biologically significant pathways linked to the conditions being studied [22–24]. In fact, that is not surprising as we have known for many years that all genomic systems are "leaky" and that led us to the hypothesis of the "noisy transcriptome." We recently did extremely deep RNA sequencing on activated human T cells to demonstrate that much of this low-level transcription is indeed "noise" in functional terms and represents genes located in close proximity to highly transcribed genes and within their transcriptional insulator boundaries of the latter explaining the "leak" (manuscript submitted). We also demonstrated that most of these noisy transcripts are not detected by very deep proteomic coverage. Moreover, with the recent development of new directional sequencing methods, it was also realized that some of this low-level transcription is coming from antisense strands of coding genes. The putatively regulatory lncRNA transcripts discussed earlier are an example of what used to be thought was actual coding transcription. One practical impact of these insights is that very deep depth RNA sequencing coverage is not required for global gene expression profiling and suddenly the cost/value equation for arrays versus deep sequencing is changed dramatically.

At the present time, if your objective is to do simple global gene expression profiling, it actually matters very little whether you choose microarrays or deep RNA sequencing. This conclusion is based on a comparison study we recently completed for the NIH Clinical Trials in Organ Transplantation consortium (manuscript submitted). The details of the experimental workflows are very different but the resulting RNA profiling data is essentially the same. And contrary to the hype recently, deep RNA sequencing is not significantly more sensitive, more specific, or more reproducible than the latest generation of commercial microarrays. Of course, we cannot change the natural bias that a new technology with such potential must be magically powered or that being "cutting edge" for new grant applications demands the use of deep sequencing. But the simple truth is that the latest generation of commercial DNA microarrays is very powerful, easy to use, extremely cost effective, highly reproducible, can achieve fully automated throughputs of a 100 arrays in 2 days, and available to essentially every academic institution either locally in Cores or in commercial laboratories in the community as a service. Moreover, a cost comparison at Scripps of global RNA expression microarrays (Affymetrix ST 1.1^{ST} vs. deep RNA sequencing [Illumina HiSeq2000 with NuGEN library preparations and multiplexing]) reveals a per sample cost including labor of about $225 for microarrays and about $400/sample for deep sequencing.

On the other hand, it is also true that the depth of sequencing can be controlled during the design of a deep RNA sequencing experiment. Thus, it is possible to increase the effective sensitivity in order to detect transcripts that exist at very low copy number. This situation could be very important in profiling complex mixtures of cells, like a transplant biopsy, in which the effector cells under study represent only a very small fraction of the total cells being analyzed.

But, as we have discussed in detail above, once you want more than simple gene expression profiling, the situation is very different. Deep sequencing has huge advantages for the study of miRNAs, alternative splicing, genetic variations, lncRNA, and discovery of new RNA forms, as in ongoing projects in the ENCODE consortium. It is also not surprising that it is all evolving rapidly. Recently, a number of commercial automated instruments have been introduced that can produce the sequencing libraries for deep RNA sequencing and these have considerable promise to greatly lower the barriers, time, and labor costs. Another rapidly evolving area of technology for deep sequencing is represented by the IonTorrent instrument offered by Life Technologies, which uses a novel solid-state sequence detection protocol and can shorten the time of a sequencing run to less than a day from the current week required (http://www.iontorrent.com). And not to be out-competed, Illumina has introduced the latest version of TruSeq chemistry for sequencing on their MiSeq instruments, which accomplishes nearly equivalent shortening of the run time (http://www.illumina.com/systems/miseq.ilmn) and increases the read lengths to 250 bases (which can be run in a paired-end format to yield 500 bases of sequence per read). As one example of why this is important, such a significant increase in paired-end read coverage now allows sequencing of an entire VDJ region, and thus the ability to profile the recombinations in B-cell antibody production driven by antigen exposure after transplantation or blood transfusions.

Thus, before making a decision, you should consider the best options for your experimental questions with your functional genomics experts and the technology experts in your local Core Laboratory. A final thing to remember is that the coolest approach or technology is of no value unless you have access to technical expertise with real experience in performing these assays, designing experiments, and interpreting the results properly. Just buying instruments is not enough. Ultimately the considerations should be available expertise, workflow complexity, total costs, time and resources for experimental conduct, and resources available for data analysis.

Next-generation qPCR—digital and single-cell qPCR

As often happens during the rapid evolution of new technologies, new discoveries reveal new opportunities for older technologies to evolve and fill specific niches or requirements. In the case of microarrays and deep sequencing, the cycle of technology development has also turned back to create a new generation of quantitative PCR (qPCR) technologies. Thus, these new technologies should also be

part of the considerations for experimental design in mRNA transcription profiling. Profiling RNA transcripts using microarrays or deep sequencing has great utility for identifying relative changes in levels between different conditions or clinical states. And the obvious advantage is that they enable unbiased, global expression profiling and even discovery of new RNA forms by sequencing. But there are also several limitations to these technologies that can still be addressed better by qPCR.

First, arrays and sequencing are not strictly quantitative as qPCR can be, despite the claims that RNA deep sequencing is a form of "digital expression" data. If properly normalized, deep RNA sequencing data can give highly accurate comparisons of changes in a single transcript across many different conditions but more caution is necessary before assuming that comparisons of expression across thousands of genes is equally digital and precise. For example, we took a laboratory synthesized set of 400 miRNAs that were pooled at the same concentrations and used this as the "positive" control to benchmark a new multiplexing deep sequencing protocol we developed [25]. Instead of getting close to the same number of reads for all 400 miRNAs, as would be predicted for a "digital expression" technology, there was over a log difference in the number of reads obtained for each. We are still not certain what causes this surprising result.

Second, microarrays are based on capturing labeled mRNA by hybridization to complementary DNA or RNA probes and this is variable from sequence to sequence, creating data variability and the potential for selecting bad or low-performing probe sets. The good news is that RNA deep sequencing libraries are less subject to this limitation. On the other hand, RNASeq library preparation has multiple enzymatic and amplification steps that are vulnerable to sequence context-specific bias. Third, a qPCR can be done in less than an hour now, while most array-based and sequencing methods still require at least 1 or 2 days to complete and with much higher analysis costs. This makes qPCR a much more cost effective approach if you know the genes you wish to profile and this is particularly effective when applied to functional pathway-focused qPCR panels (e.g. Tregs, inflammation, apoptosis, NFκB signaling). Fourth, sensitivity for RNA transcript detection based on hybridization capture strategies on microarrays will always be significantly lower than sensitivity based on qPCR detection. This limitation is not an issue for deep RNA sequencing. However, if it is true that with deep enough sequencing that you can detect almost any transcript even if it has no biological function, one wonders how many times a study has depended on very high cycle PCR to detect transcripts as proof that a given pathway or gene was involved in some experimental event.

The first challenge in this new genomics era was to make qPCR technologies that could handle at least hundreds and even thousands of genes in a single run. One early example was qPCR instrumentation developed by Applied Biosystems to break the 96-well barrier and create 384-well qPCR plates (TaqMan Low Density Arrays; TLDA). These reactions could be done in as little as 1 microliter volumes to save significantly on reagent costs and time. We often use this technology to validate gene expression results obtained with microarrays for up to 100 gene candidates in a single run and more recently have tested it for validating alternative splicing results. However, the latest generation of ABI qPCR technology provides the OpenArray system that can generate over 30 000 data points in a single run and do up to a thousand genes easily.

The qPCR technology developed by Wafergen creates a chip that can run 5184 different qPCR reactions at once to enable profiling of a 1000 genes in quadruplicate. This technology is also done in nanoliter volumes, with significant potential for automation to high-throughput performance. Using a different platform but with similar objectives, Luminex has designed a strategy for high-throughput, multiplex, bead-based qPCR using their fluorescent bead technology. The advantage of this technology is that they claim they can multiplex up to 500 different qPCR assays in a single bead-filled well and easily run several hundred or more different clinical patient samples a day through their bead analysis instrument. The capabilities represented by these technologies will be critically important as the field of transcript profiling for biomarkers is translated into clinical diagnostics where cost, labor, and time become paramount. The reader should expect continued development in this area with new technologies and new companies.

Another new technology platform for qPCR, developed and implemented by Fluidigm as the BioMark system, takes advantage of microfluidics engineering to create a platform capable of doing 9216 qPCR reactions in nanoliter volumes in a single run with only several hours time. This system uses the ABI TaqMan technology for qPCR with nearly identical sensitivity and reproducibility. The first major advantage is the dramatic increase in the number of reactions done in a single run. In a standard configuration, the platform can do 96 different genes on 96 different samples (suitable for clinical biomarker profiling, for example) or 9216 genes on one sample or any combination of genes and samples possible with a 96×96 reaction well format. In fact, the microfluidic-engineered design creates the second major advantage, the exciting application of this new qPCR technology to do single-cell gene expression. This is enabled because the microfluidic chips in the BioMark instrument have multiple parallel sample sites that can be selectively piped to multiple reaction sites. Thus, using their 96×96 chip design, a researcher can profile 96 single cells for 96 genes that are measured on every individual cell in one run. This single-cell approach has started to reveal how diverse every different cell can be in a complex cell mixture. That is clearly critical for cancer cell mixtures where the behavior of a single malignant cell can change the entire outcome.

How single-cell profiling will inform the mechanisms of transplantation biology and immunology remains to be discovered. Thus, a key question is whether our biology is better represented by analysis of single cells or analysis of a whole population of cells in one big mixture. As already noted above, it is only when these technologies are available and investigators are willing to make the investments in time and work effort to develop these technologies for transplantation that we can even begin to ask these critical questions.

Non-PCR transcript detection and digital PCR

While we recognize the remarkable power and utility of PCR-based gene detection, PCR also has a number of limitations. First, the performance of PCR is significantly influenced by the target sequence just like the DNA hybridization approaches used for DNA microarrays and we suspect also the stepwise base pairing required for deep RNA sequencing. For every perfect PCR primer set, there are also sets that function less efficiently for the same target gene at target sequences just a few hundred bases in either direction or even when there are just a few base pair changes in

the sequence for the primer binding sites between two individuals due to inherited or acquired genetic differences. While we can almost always optimize PCR performance for a single reaction, the challenge is very different when we want to do hundreds or thousands of different PCR reactions at once and thus must do every reaction with the same reaction conditions. Second, one of the prices paid by PCR for high sensitivity is a higher degree of variation from reaction to reaction in the current detection technologies that are often accentuated by using log scales. This is why qPCR reactions are typically done in quadruplicates and the results used to create mean expression data. Third, the more cycles of PCR done, the greater the risk of differences in the underlying target sequences causing amplification biases. Thus, truly quantitative PCR-based approaches require parallel controls comprised of purified target sequences (typically cDNA clones of the gene) and serial dilutions of precisely determined concentrations for each gene. This need for gene-by-gene controls greatly complicates and limits the scope of any strategy to do absolutely quantitative, high-throughput, multigene qPCR profiling. In fact, streamlining of these challenges has not been created by any of the "next generation" qPCR platforms discussed above.

One solution to these limitations of PCR is offered by the Affymetrix Panomics Quantigene system where there is no PCR amplification required to detect as few as 200 total mRNA molecules in a single well. In this technology, gene-specific capture probes are first bound to the bottom of an assay well or to the surface of a Luminex bead. The sample is added to each well or to a mixture of many beads and hybridized to the capture probes overnight. In the next steps, a second set of probes binding the captured RNA is followed by branched chain DNA probes with many molecules of enzyme that when exposed to a soluble substrate creates the colorimetric or fluorometric quantifications. If it is not already obvious, this approach is essentially an ELISA assay using RNA probes instead of antibodies and it targets RNA transcripts instead of proteins. This technology is very useful for profiling several hundred genes in a single run, it is highly quantitative and can be implemented in both 96-well and Luminex bead formats. The one potential drawback for clinical diagnostic application is that it takes an overnight hybridization step and thus adds about a day in time to the current technologies used for qPCR. It is also presently not cost competitive with simple qPCR platforms.

Another solution to PCR amplification bias is a new generation of digital PCR technologies using emulsion PCR. In simple terms this involves creating tiny oil droplets and diluting the input total RNA target sample so that approximately one mRNA transcript is enclosed in each droplet. In effect, you have created a separate universe containing a single RNA target and can take the reaction to its maximum of 40 or more PCR cycles without any competition or any of the bias created by all the relative differences in efficiency between primer pairs for different gene sequences.

New technologies for digital PCR are using microfluidic engineering to create instruments capable of doing thousands of emulsion PCR reactions in a single run using microdroplets or a combination of droplets with tiny beads. For example, RainDance Technologies developed the RainDrop digital PCR system that can automate the creation of 10 million picoliter emulsion PCR droplets for eight different samples at a time and then detect the amplicons of 96 samples in a single run on a separate instrument. This platform is technically capable of using different fluorophores to do multiplexing so the platform could potentially allow profiling of several hundred genes in one sample or ten genes in multiple samples at once. Another emulsion-based PCR instrumentation is BioRad's QX100 Droplet Digital PCR, which has similar workflow capabilities to the RainDrop system though creates around 20 000 drops per sample. Statistically, BioRad maintains that 20 000 drops is more than enough to accurately produce digital data that captures the differential expression being interrogated and this is probably true for many situations. However, one potential advantage of the RainDrop system is that with so many drops to analyze, that optimization of the initial RNA input concentrations is not necessary and that might greatly improve the workflow.

It is too early to predict where digital PCR capability will fall in the current spectrum of using microarrays, RNA deep sequencing, and high-throughput quantitative PCR technologies. However, none of the other gene expression platforms are truly quantitative in their present forms. The key point is that if this new digital PCR technology fully delivers on its "digital" promise, then we will have the ability to absolutely quantify mRNA transcript numbers and their changes down to single cells with different experimental manipulations or disease states for thousands of genes in a single run. That is something to watch for with interest.

Technology and experimental designs

Ultimately, functional genomics and systems insights must be pursued and then validated with multiple technologies and the results from these multiple technologies can be integrated and conclusions tested mechanistically to achieve our objectives. But these technologies individually are complicated and all are evolving rapidly. The consequences are that the technology requires tremendous expertise to use properly. The first point for this section is that just giving a set of samples to a core laboratory with an order to run a particular technology and send back a spreadsheet of final results is simply not going to work. Scientists must be engaged enough to really understand the technology chosen and the data created, even if ultimately a set of technology experts and their core technicians still run the samples.

The fact that these genomic technology platforms are almost entirely driven, from instruments to kits, by the commercial life sciences companies adds yet another layer of hype, confusion, costs, and competition. If you read the advertisements, you can change your approach a dozen times a year and each time find "guarantees" of how wonderful the new data will be. But it is simply not true and these changes can dramatically undermine the value of your data, particularly if you need to compare experiments done in stages where data may be generated over a year or more. Thus, the second point is that when you create a strategy to address a scientific objective using functional genomics, you must commit to all the separate pieces of the experimental plan, not just the choice of the main instrument platform. The way the sample materials are collected, stored, purified, amplified, and labeled, even the manufacturers of the kits chosen are absolutely as critical to data integrity as the chip chosen. For example, we used to purify total RNA in a certain way that we all believed was necessary to "optimize" hybridization to DNA microarrays. We were very happy until one day we decided that we now wanted to also study miRNA and found out that using this technique was filtering away all the miRNA making our entire sample archive useless for this purpose!

Another point is that integrating data obtained by very different technologies requires integrating data obtained using very different

physical rules. For example, let us say we want to conclude something as basic as that when an RNA transcript was up-regulated that the up-regulated translation of a corresponding protein followed. The answer requires integrating hybridization-based DNA capture of a labeled RNA transcript on DNA microarray probes with ionization energy-mediated emission spectra created by mass spectrometry proteomics of peptides. Thus, we need specialists in bioinformatics to develop computational methods for successfully integrating the results from multiple technology platforms that can successfully find the intersections of very different kinds of data. What this means in practice is that real progress will take time, significant resources, and teams of scientists with the detailed domain knowledge to use these tools correctly. Frankly, all the technology has become the "900-pound gorilla" in the field. You can't ignore it; you have to deal with it. But it is definitely making an already remarkably complicated scientific challenge harder. Human nature is often to reject this kind of challenge and argue that the "gorilla" is not really important, "it is just a stupid animal" or technology is a distraction from "real science" or "I just hire professionals to run machines." This is not going to work for functional genomics any more than pretending that you just hire transplant surgeons to do the surgery. In the process of designing a strategy and interpreting the results, you must accept at least some "ownership" of the technologies you choose because they are complicated and changing constantly, and this is not a minor challenge.

Demonstrating the value proposition

If the complexity of functional genomics, both biology and technology, constitutes the first challenge, the second challenge is demonstrating the value proposition of the work. These compelling new views of biology beg the question of how functional genomics can be applied to change the practice of medicine. A powerful value proposition is that basic and translational scientists will be guided by functional genomics to the next generation of mechanistic insights into the biology of the cell or organism. These basic insights will come from many model systems as well as human subjects. Advancing a mechanistic understanding of biology in health and disease is critical and sufficient alone to justify the work. Good science is good medicine. But our ultimate success as a field will be judged in terms of the value proposition we create for patients, their families, the health care delivery system, and our Society. All our work is directly or indirectly funded by these expectations. There are many ways to deliver value from science but ultimately value must be delivered.

For example, let us say you decide that you are going to understand the molecular basis of chronic rejection mediated by B cells. We would all agree that this is a very worthy objective. Now consider the responsibility. No one starts off taking on such a huge task in one step. This is the kind of effort that rolls out in discrete stages, needs refinement, and is funded and published in stages. The facts that functional genomics is powerful, biology is complex, and the clinical challenge you choose is critical to medicine does not mean you can simply profile a bunch of biopsies or blood samples and have any useful answer or deliver any value proposition. In the design of any new functional genomics strategy, ask first what you expect to learn and how that work will contribute to a real calculation of value. Make this analysis with the help of experts in the technology or technologies you will use. Then you can design and power your studies to be successful.

Summary

We believe that functional genomics will drive discovery. To have value, this discovery must be informed by a pragmatic understanding of our clinical discipline of transplantation. If properly done, this will guide biomarker development in transplantation and both direct applications to practice and hypothesis-driven mechanistic studies about the nature of transplantation immunology, rejection, and tolerance. But we must stay firmly grounded in reality. The complexity of cell biology and immunology revealed by functional genomics today will require huge investments in research, the training of a new generation of clinical and basic investigators, and a reinvention of our current approach to experimental design before we really are the "masters of the universe."

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CHAPTER 17

Current Pathways for Immune Manipulation

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Introduction

Organ transplantation has evolved into a successful standard therapy through progressive definition of the mechanisms of immune-mediated rejection and responsive development of therapeutic agents to counter alloimmunity. While all commonly used agents impair physiologic immune processes and are associated with off-target side-effects, they can be used successfully providing the practitioner has an understanding of their actions and can thus anticipate the adverse issues that may arise. Similarly, their adverse effects can be minimized through combination therapies that pair mechanistically complementary agents. This chapter will provide an overview of the mechanisms of action of the major immunosuppressants in common clinical use. It complements Chapters 98 and 101, which detail the clinical preparation specifically indicated or used off-label for transplantation, respectively. Pathways being exploited for agents still under investigation are covered in Chapter 18.

Steroids

Corticosteroids (CS), the product of the adrenal cortex, have broad immunosuppressive and anti-inflammatory effects, and were one of the first classes of drugs used to suppress immune responses [1]. Glucocorticoids (GC), representing both natural and synthetic steroids that activate gluconeogenesis, include prednisone and methylprednisolone, and are widely used as part of the immunosuppressive regimen to suppress transplant organ rejection. Despite their effectiveness in preventing acute rejection, they also lead to morbidity and probably mortality [2]. In particular, GC have various adverse effects on cardiovascular diseases, bone metabolism, and increased risk of infection [3]. As a result, starting in the late 1990s, GC doses have often been reduced, withdrawn, or discontinued in many induction and maintenance transplant immunosuppression protocols [4].

Structural and biochemical pathways

Prednisone is the most widely used GC and is metabolized in the liver, primarily to prednisolone, its major active metabolite. As shown in Figure 17.1, unbound steroids passively diffuse through the cell membrane into the cell, and corticosteroid-binding globulin (CBG)-bound steroid can also enter cells via receptor-mediated transport or endocytosis [1,5–7]. In the cytosol, steroids bind to and activate glucocorticoid receptors (GR), and then the receptor dimers interact with glucocorticoid response elements in various gene promoter sequences to regulate immune gene expression [1,5]. GC suppress multiple inflammatory pathways. They induce and activate annexin I, a molecule that interacts with and inhibits cytosolic phospholipase A2α, to block release of other proinflammatory molecules. GC also induce and activate MAPK phosphatase 1, and consequently inhibit the MAPK signal cascade-mediated inflammatory responses. GC directly interfere with NF-κB signal pathways to suppress expression of inflammatory cytokines, chemokines, cell adhesion molecules, complement factors, and other inflammatory mediators [5]. As a result, GC preferentially block proinflammatory Th1 and perhaps Th17 cytokines, promote Th2 cytokine production [8], and maintain or increase interleukin-10 (IL-10) production to benefit transplant graft survival [9,10].

Cellular targets

The mechanisms and cellular targets of GC are highly variable and remain incompletely understood. They induce apoptosis of T lymphocytes [11] and down regulate gene expression of cytokines and adhesion molecules in a variety of cell types as discussed above [12,13]. In addition to genomic actions (transactivation and transrepression) mediated by the cytosolic GR, GC also suppress immune responses by non-genomic mechanisms, including a direct interaction of the GR with cytosolic signaling molecules, the intercalation of glucocorticoids into the plasma membrane, and the activation of endothelial nitric oxide (NO) synthase [14–16]. GC anti-inflammatory properties include suppression of prostaglandin synthesis, stabilization of lysosomal membranes, reduction of histamine and bradykinin release, suppression of dendritic cell antigen presentation, and suppression of CD4 T-cell proliferation, trafficking, and activation [17,18].

Adaptive immunity

GC suppress antigen presentation by dendritic cells (DC), decrease circulating $CD4^+$ T cells, and suppress IL-1 production and lymphocyte activation [17,18]. Higher doses of GC inhibit B-cell

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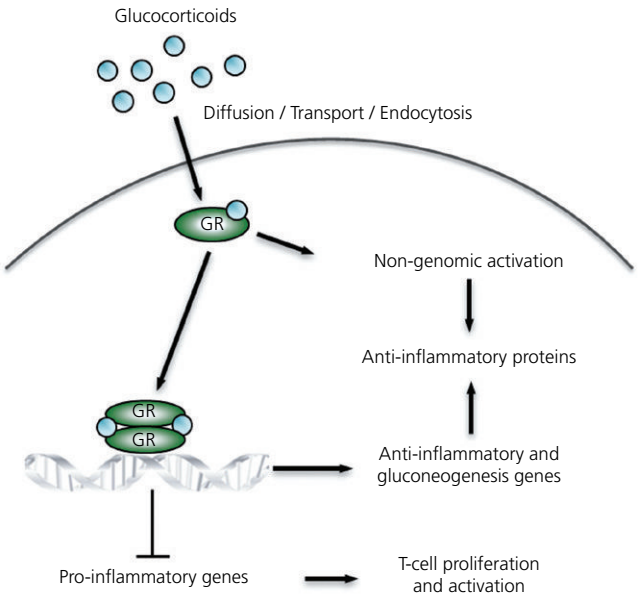

Figure 17.1. Mechanism of action of glucocorticoids. Unbound steroids passively diffuse through the cell membrane into the cell, and corticosteroid binding globulin (CBG)-bound steroids can also enter cells via receptor-mediated transport or endocytosis. In the cytosol, steroids bind to glucocorticoid receptors (GR), then the activated receptor dimer translocates into the nucleus, where it binds to glucocorticoid response elements (GRE) in the promoter sequence of the target genes, resulting in transactivation of gene expression. The activated receptor also interacts with specific transcription factors (such as AP-1 and NF-κB) and prevents the transcription of target genes. The net result is decreased proinflammatory cytokine production, lymphocyte proliferation, and changes in cellular trafficking.

production of immunoglobulins [19,20]. Thus, GC suppress diverse aspects of adaptive immunity.

Innate immunity

GC reduce neutrophil trafficking from blood vessels, and reduce their adherence to vascular endothelium and bactericidal activity [19]. GC limit chemotaxis, phagocytosis, and the release of cytokines such as TNF-α and IL-1 from macrophages and other antigen-presenting cells, and repress the expression of inflammatory mediators such as prostaglandins, leukotrienes, and platelet-activating factor [19]. Thus, GC also inhibit a vast and diverse array of innate immune mechanisms and effectors.

Infection

As noted above, GC suppress both adaptive and innate immunity, and thus are associated with an increased risk of infections. Acceleration of hepatitis C virus (HCV) infection is a major concern with GC [21]. Tapered GC usage and steroid-free regimens reduce the incidence of fibrosis related to HCV reinfection, cytomegalovirus (CMV) infection, and HCV recurrence [22–26].

Wound healing

Macrophages are essential for wound healing. GC inhibit circulating monocytes, the influx of macrophages, growth factor and cytokine production, and phagocytosis [27–30], all of which adversely affect wound healing. GC also diminish wound healing by delaying re-epithelialization, decreasing the fibroblast response, slowing capillary proliferation, and inhibiting collagen synthesis and wound maturation [27,29,31].

Table 17.1. Common complications of glucocorticoids

Organ/system	Complications
Endocrine and metabolic	Adrenal suppression, hyperglycemia, water retention, growth failure in children
Hematopoietic	Leukocytosis
Immune	Bacterial, fungal and viral infections, delayed wound healing, suppression of type IV hypersensitivity
Gastrointestinal	Gastric irritation, peptic ulcer
Cardiovascular	Hypertension, myocardial infarction, and cerebrovascular disease
Skin	Dermal thinning, skin fragility, ecchymosis, violaceous striae
Musculoskeletal	Osteoporosis, avascular necrosis, myopathy
Ophthalmic	Cataracts, glaucoma
CNS/psychiatric	Sleep disturbances, insomnia, euphoria, depression, mania, psychosis, obsessive behaviors, pseudotumor cerebri
Reproductive	Impotence, menstrual irregularity
Morphologic	Moon facies, dorsocervical hump, truncal obesity

Metabolism

GC are associated with many adverse effects (Table 17.1) related to their primary activity of inducing multiple processes related to gluconeogenesis. GC may lead to Cushingoid changes, hyperglycemia, decrease in bone density, avascular necrosis, cataracts, glaucoma, dermal thinning, skin fragility, ecchymosis, violaceous striae, hypertension, water retention, adrenal insufficiency, and secondary cardiovascular complications including myocardial infarction and cerebrovascular disease [20].

Calcineurin inhibitors

Introduction

Calcineurin inhibitors (CNIs) have been the mainstay of maintenance immunosuppression in transplant medicine since the introduction of cyclosporine A (CsA) [32–34]. CNIs work by suppressing activation of T cells and the transcription of cytokines by blocking the interaction of calcineurin with its substrate, the nuclear factor of activated T cells (NFAT) [35–37]. Clinically, CNIs are well tolerated; however, they may be associated with nephrotoxicity and islet toxicity [38–40]. Additionally, they have been associated with wound complications, particularly when used in combination with mTOR inhibitors [41]. For these reasons, many groups have recently focused on limiting the role for CNIs in the transplant population [42–44]. This section of the text will focus on the structure, function, and pharmacology of these frequently utilized drugs.

Structural and biochemical pathways

An understanding of calcineurin and its role in immunomodulation is a requisite for an understanding of CNIs. Calcineurin, a protein phosphatase, is calmodulin and calcium dependent [45,46]. It is a heterodimer that contains an active domain in addition to an autoinhibitory domain, and mutant calcineurins without this autoinhibitory domain are persistently active [45,46]. T cells are

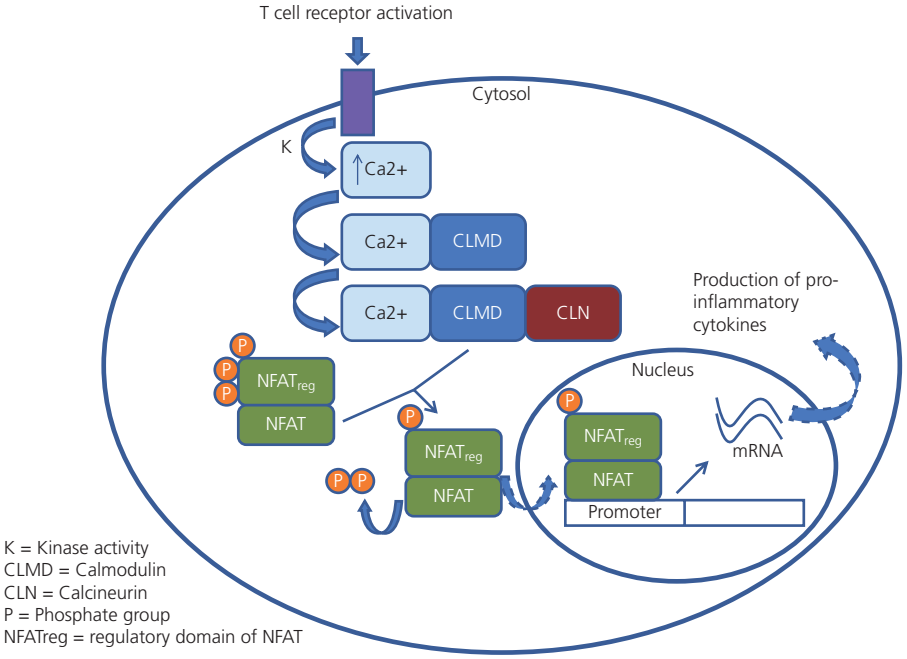

Figure 17.2. T cells are activated by cell surface receptors, leading to increases in intracellular levels of calcium. Calcium–calmodulin activates the phosphatase activity of calcineurin, which activates NFAT by cleaving PO_4 from the regulatory domain of nuclear factor of activated T cells (NFAT). NFAT is then translocated from the cytosol to the nucleus, where the dephosphorylated, and now nuclear, NFAT acts as a transcription factor for various immunologically important genes.

activated by cell surface receptors, leading to increases in intracellular levels of calcium. Calcium–calmodulin–calcineurin signaling then activates NFAT [47]. The NFAT family, which is comprised of at least five members, is a group of transcription factors that induce the genes encoding cytokines and chemokines associated with inflammation (Figure 17.2) [47,48]. Mice deficient in just two of these family members (NFAT1 and NFAT2) are essentially devoid of the ability to produce cytokines [48]. Calcium activates the phosphatase activity of calcineurin, which cleaves PO_4 from the regulatory domain of NFAT. This sets in motion a cascade of events during which a nuclear export signal binds Crm1 (also called nuclear export receptor), leading to the translocation of NFAT into the nucleus, wherein the dephosphorylated, and now nuclear, NFAT acts as a transcription factor for various immunologically important genes [48].

Tacrolimus (also called FK506) and CsA bind to different intracellular targets called immunophilins. Specifically, CsA binds cyclophilins and tacroimus binds FK-binding proteins (FKBPs) [35]. Despite these differences, both exert their immunosuppressive effect through inhibition of the calcineurin–calmodulin moiety by forming drug-dependent complexes [35,49].

Cellular targets

Most notably, CNIs act to suppress the proliferation and activation of T cells. This effect is exploited by transplant physicians trying to quell antidonor T-cell responses. CNIs, specifically tacrolimus, inhibit the ability of T-cell-dependent cytokine production, including IL-2, 4, 5, TNF-α and INF-γ, which are required signals for T-cell help and T-cell proliferation [50–52]. Interestingly, the CNI effect on T cells may be specific for the alloreactive population. Additionally, CNIs may potentiate T regulatory cell function, particularly in autoimmune skin disorders, although this point has been controversial [53–55]. CNIs affect both mature and immature T cells and are capable of inhibiting the development of not only $CD4^+$ but also $CD8^+$ single-positive lymphocytes from double-positive precursors during positive selection [56], likely enhancing their immunosuppressive efficacy.

The most obvious way in which CNIs affect B cells is through inhibition of T-cell help and immunoglobulin class switching [50,57]. Thus, CNI effects on B cells are largely indirect. In an in vitro system, in which either tacrolimus or CsA was co-cultured with T cells and B cells, immunoglobulin production was subsequently reduced. However, the same was not true when T cells were removed from the co-culture and B cells alone were co-cultured with tacrolimus and CsA. In the latter case, tacrolimus failed to suppress immunoglobulin production. CsA only modestly reduced immunoglobulin production [50]. Other reports suggest that CsA may indeed have direct effects on B cells. Cyclophilin B, an intracellular target of CsA, is involved in protein folding and may be inhibited by CsA [58]. In B cells, this inhibition leads to dose- and time-dependent decreases in IgG biosynthesis [59]. In the same study, CsA had no effect on transcription of IgG; rather, the reduction in biosynthesis was mediated by endoplasmic reticulum stress [59].

Neutrophil function is also affected by CNIs. These effects have been studied in parallel with angiotensin II in an in vitro model of vascular injury. Because angiotensin II up-regulates activity and synthesis of calcineurin, and is associated with neutrophil activation in the endothelium, investigators hypothesized that CsA might decrease neutrophil activation by inhibiting upstream

events. Experimentally, CsA decreased activation of angiotensin II-stimulated neutrophils by inhibiting both the MAPK and ERK pathways [60]. In the same system, CsA was shown to inhibit the translocation of Rac2, a molecular switch thought to lead to release of proinflammatory reactive oxygen species [61], from the cytosol of human neutrophils to the nucleus [62]. Impaired translocation was a downstream effect of inhibition of angiotensin II, a known stimulus of Rac2. Thus, CsA interferes with angiotensin II-stimulated neutrophils, a process which is critically dependent on translocation of Rac2 [60].

CNIs also reduce cytokine production, including INF-γ, from T cells. Because INF-γ is a powerful stimulus of macrophages, CNI downstream effects include the suppression of monocytes and macrophages. The direct effect that CNIs have on monocytes and macrophages may be limited [52]. In a rodent study of spontaneous ulcerative colitis, investigators noted that tacrolimus inhibited mucosal T-cell activation rather than mucosal monocyte and macrophage activation, and that macrophage activation was lessened by the addition of CNIs [52].

Effect on parenchymal cells

CNIs have well-known toxicity to endothelial cells [63,64], a pathology thought to underlie cardiac allograft vasculopathy. When bovine aortic endothelial cells were cultured with CsA, time- and dose-related cellular damage (and eventual cytolysis), characterized by increased levels of eluted lactate dehydrogenase, prostacyclin, and thromboxane [64], was observed. These deleterious effects of CsA on endothelial cells were likely due to alterations in nitric oxide (NO) and endothelin-1 (ET-1) regulation, which subsequently impair vascular vasodilatation. Interestingly, when investigators evaluated rapamycin, which is not known to lead to allograft vasculopathy, the same alterations in NO and/or ET-1 were not observed [65]. Because NO and ET-1 endothelial dysregulation led to the production of inflammatory cytokines, it is hypothesized that suppression of this system through the endothelial growth factor may abrogate calcineurin-related vasculopathy [66]. Indeed, in an in vitro model using human endothelial cells, investigators observed a significant decrement in CsA and tacrolimus-induced cell adhesion when cells were concomitantly cultured with endothelial growth factor 7, and thus concluded that targeting these pathways may be beneficial for cardiac transplant [66].

CNIs are known to be nephrotoxic [67,68]. CNIs have been shown to decrease both tubular and glomerular function [69], and that this decrease may be due to aquaporin gene down regulation and decreased protein expression [69,70]. In addition to their effects on urinary production and collection, both CsA and tacrolimus have been shown to be mitogenic for renal mesangial cells, likely through an extracellular-signal-regulated kinase pathway (ERK) [71]. Results have also implicated both endoplasmic reticulum stress and up-regulation of CD147, which is associated with renal fibrosis, in the development of CNI-induced nephrotoxicity [58].

It has long been reported that CNIs inhibit islet function [72]. CNI-related decreases in islet function are partially due to the involvement of calcineurin in insulin release [73]. When beta cells were cultured with tacrolimus and CsA at supratherapeutic levels both drugs led to a 48–69% decrease in basal insulin production in the steady state. In addition, tacrolimus was noted to have an acute effect at supratherapeutic levels, dropping insulin release by 61–87%. The diabetogenic effects of tacrolimus and CsA are due not only to calcineurin inhibition, but also to the islet-specific down-regulation of the transcription factor SREBP-1c, which is known to control beta cell function. In addition, both drugs are cytotoxic and lead to islet apoptosis [72].

Functional pathways of immunity (adaptive response, ischemia reperfusion injury, infection)

The ability of CNIs to inhibit T-cell activation, proliferation, and downstream activation of macrophages underlies the beneficial antirejection effects observed clinically, but portend potential morbidity manifested by inhibition of the adaptive and innate immune responses to pathogens. In toxicity studies comparing CsA with FK506, CsA was associated with a higher risk of overall infection, and the highest risk of opportunistic infection, in a dose-dependent manner [74]. Conversely, CNIs may have other immunologic effects, unrelated to transplantation, such as improvement in focal and global ischemia, as well as improvement in ischemia–reperfusion injury [75,76]. Improvements in ischemia are thought to be related to the ability of CNI to inhibit nitric oxide synthase [75], preventing oxidative stress, and subsequent prevention of DNA damage and lipid peroxidation [75]. Based on these and other data, a randomized controlled trial was performed in which pretransplant donor livers were perfused with tacrolimus at 20 ng/mL and compared with grafts perfused without tacrolimus [77]. Investigators found no difference in graft outcomes, but did observe decreases in ischemia–reperfusion injury at the molecular level [77].

Non-immune functions (metabolism, wounds/healing, tumors/proliferation)

CNIs have been associated with poor wound healing, neurotoxicity, nephrotoxicity, hypercholesterolemia, hypertriglyceridemia, and cholestasis [67,74,78]. With regard to wound healing, CsA is known reduce fibroblast-derived growth factors, specifically activin-β. CsA has little or no effect on fibroblast TGF-β or integrin-1, despite its other immunologic effects [79]. Post-transplantation hyperlipidemia (PTHL) is a well-described finding. While steroids have a well-known association with PTHL, the role that CNIs play is less clear [80]. Interestingly, CsA (but not tacrolimus) has been shown to increase total cholesterol as well as low-density lipoproteins (LDL), with a peak occurring 1 month following transplantation [81]. Interestingly, there was no concomitant increase in high-density lipoproteins (HDL) at the same time point [81]. Other groups that have observed similar results have shown that the improvement in lipid levels with tacrolimus over that of CsA may be due to alterations in LDL oxidation [82]. Neurologic complications are well documented after introduction of CNIs. These effects may be the result of: (1) binding of CNIs to intracellular substrates used for other physiologic processes, (2) vasoconstriction, (3) endothelin production, and/or (4) production of excessive reactive oxygen species [83].

Malignancy is also a risk of immunosuppression [84]. The most common malignancies following renal transplantation are skin cancer and non-Hodgkin's lymphoma [84]. Skin cancers affect approximately 7% of patients following kidney transplantation after 3 years; additionally, approximately 7% of patients also develop non-skin cancers, of which non-Hodgkin's lymphoma is the most common [84]. Interestingly, although tacrolimus is more efficacious in preventing rejection, it does not appear to lead more frequently to malignancies [85]. Although it is convenient to attribute the increased risk of malignancy following transplantation to

immunosuppression, some data also suggest that the rate of malignancy amongst kidney transplant waitlist patients is higher [86]. Additionally, the types of cancer that patients with end-stage renal disease (ESRD) develop is similar to the pattern observed in post-transplantation patients [86]. The neoplastic risk of ESRD may be a result of genotoxic uremic toxins and possibly increases in oxidative stress [87]. The malignant potential of CNIs may be related to regulation of the nuclear factor of kappa B cells (NF-κB), which is known to modulate growth factors and apoptotic mechanisms leading to cancer [88,89].

mTOR inhibitors

The profound effects of rapamycin on proliferation in a variety of cell types led to elucidation of the mammalian (or mechanistic) target of rapamycin (mTOR). mTOR is a serine/threonine protein kinase that acts as an integral component of two distinct signaling complexes (mTORC1 and mTORC2) involved in multiple pathways regulating cellular metabolism, activation, and proliferation [90–94]. Currently available inhibitors of mTOR (mTORi), including rapamycin (sirolimus) and everolimus, block mTOR activation, inclusion in the mTORC1 complex, and subsequent kinase activity, preventing the conversion from catabolic to anabolic metabolism [90,95]. In addition, there is evidence that mTORi are capable of blocking mTORC2 signaling in some settings, and that this blockade can further impact cell function [96]. Overall, current pharmacologic mTORi demonstrate broad suppression of cell proliferation, particularly proliferation induced by exposure to growth factors [90–94] (Figure 17.3).

While mTOR is a ubiquitous cellular signaling component, the significant increases in cellular metabolic demands associated with immunity results in a preferential reliance of immune cells on mTOR activation for immune cell survival and function. As a consequence, mTOR inhibitors exhibit significant immunosuppressive capabilities, particularly when combined with drugs targeted to alternate pathways of immunosuppression, such as mycophenolate mofetil [97,98]. However, recent studies have demonstrated that inhibition of mTOR can also result in enhanced production of inflammatory cytokines by cells of the innate immune system and enhanced adaptive immune memory. These contradictory results suggest that mTOR has multiple complex roles in immunity.

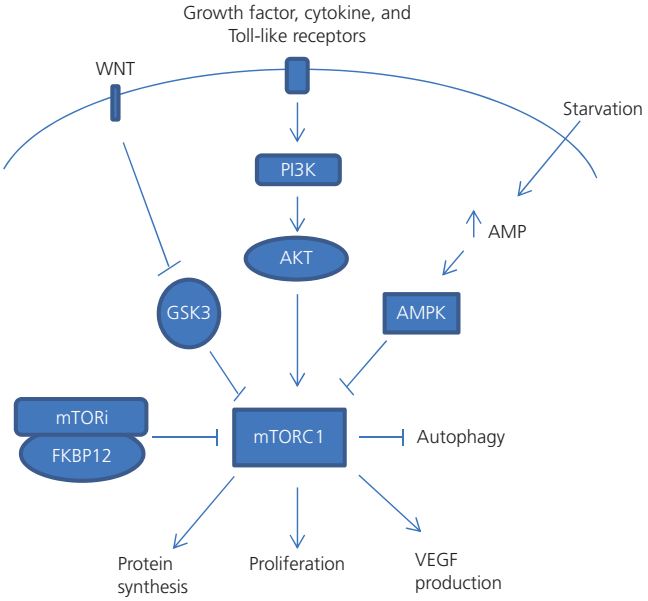

Figure 17.3. Activators and inhibitors of the mTOR signaling pathway. Activation of the mTORC1 signaling complex results in cell growth through enhanced protein synthesis and cellular proliferation and inhibition of autophagy. Simultaneously, mTORC1 induces production of other factors, including VEGF, that allow tissue remodeling and repair. Complexes of mTORi and the intracellular protein FKBP12 inhibit mTORC1 function. Endogenous regulators such as glycogen synthase kinase 3 (GSK-3) and adenosine monophosphate activated kinase (AMPK) can also block mTORC1 activity. Signaling through WNT receptors inhibits GSK3 activity while AMPK activity is only activated by increased AMP concentrations associated with energy depletion. Activation of mTORC1 can occur through AKT activation via phosphoinositide 3-kinase (PI3K) signaling activated by receptor recognition of growth factors, cytokines, and Toll-like receptor ligands (such as lipopolysaccharide).

Cellular targets

T cells are a major participant in graft rejection. The capacity of mTORi to decrease proliferation of T cells is well recognized and thought to play a vital role in mTORi-mediated immunosuppression. Inhibition of T-cell proliferation is associated with cell cycle arrest in the G1 phase and with a severe reduction in protein synthesis. In addition, antigenic stimulation of T cells in the presence of mTORi has been shown to result in hyporesponsiveness to subsequent stimulation, termed anergy [99,100]. Finally, new data suggest that mTORi can affect T-cell trafficking, mediating immunosuppression through sequestration of T cells within lymphoid tissue [101].

The mTORi-mediated induction of anergy is linked to an increase in the induction and/or survival of $FOXP3^+$ regulatory T cells (Tregs) [102–109]. There is evidence for a positive effect of mTORi on both de novo induction of FOXP3 expression and regulatory function in T cells, and preferential survival of established regulatory T-cell populations [102,107,110,111]. In the latter case, there are suggestions that Treg may utilize alternate signaling pathways for modulation of metabolism [110]. Overall, this suggests that mTORi change the balance of effector to suppressor T cells in favor of suppression.

Interestingly, mTORi treatment can also increase memory formation following antigen-specific stimulation of T cells. Memory enhancement has been shown clearly in multiple systems, and is thought to account for the enhanced accumulation of memory phenotype cells after induced lymphopenia in transplantation [109,112–115].

Like T cells, B lymphocytes appear to rely on mTOR signals for development and generation of effector functions. Interestingly, studies have demonstrated that both inhibition of mTOR signaling [116] and constitutive mTOR signaling [117] can negatively impact B-cell development and activity. These findings suggest that under normal circumstances, carefully controlled mTOR signaling is vital to B-cell function. However, many aspects of the mTORi–B-cell interaction in immunosuppression remain unexplored.

In addition to the significant role of mTOR in adaptive immunity, it also appears to play vital roles in inflammatory processes within innate immune cells. Inhibition of mTOR has been shown to decrease the number of circulating neutrophils and inhibit

neutrophil GM-CSF associated tissue migration [118]. For antigen-presenting cells such as DC and monocytes/ macrophages, the effects of mTOR inhibitors are complex. Studies that examined the effects of mTOR inhibition on antigen presentation by DC have suggested that mTOR activation is a necessary component of DC maturation and subsequent activation of T cells. DC treated with rapamycin demonstrated a reduced capacity to increase expression of costimulatory molecules in the presence of inflammatory signals normally associated with DC maturation, including GM-CSF, IL-1, and IL-4. This reduced capacity for DC maturation following mTORi treatment has been suggested to be involved in the production of Tregs.

In contrast, treatment with mTORi has also been shown to increase DC and monocyte production of inflammatory cytokines, including TNF-α, IL-6, IL-12, and IL-23, while blocking production of the anti-inflammatory cytokine IL-10 [119]. Further, mTORi treatment has been reported to inhibit the immunosuppressive effects of corticosteroids [120]. Thus, mTORi can have both anti- and proinflammatory effects on DCs and monocytes [121,122].

Finally, parenchymal cells are a vital component of inflammation and they are also modulated by treatment with mTORi [123–125]. Indeed, mTORi appears to strongly regulate the capacity of tissue cells to participate in inflammation with significant effects on tissue destruction, remodeling, and healing. Specifically, mTORi has been shown to decrease the responsiveness of fibroblasts to wound-associated growth factors, preventing cell replacement and wound closure [126,127]. This inhibition is accompanied by a reduction in VEGF production at the wound, with a concomitant loss in vascular tissue recovery [128].

Adaptive immunity

The effects of mTORi on individual components of the immune system outlined above predict the complex effects observed on adaptive immune responses. As previously mentioned, mTORi has significant immunosuppressive effects on lymphocyte-mediated graft rejection, while enhancing the development of CD8 T-cell memory [109,112–115]. This finding is true for both antigen-specific T-cell responses and memory phenotype T cells generated by homeostatic expansion [113], both of which can occur in a transplantation setting. Overall, it is clear that mTORi contributes to general immunosuppression and as such acts in conjunction with other immunosuppressants to increase susceptibility to infection, while also lowering the risk of infection with specific pathogens (see the infection section below).

Ischemia–reperfusion inflammatory responses

Several groups have demonstrated that mTOR signaling is critical for the protective effects of ischemic preconditioning and/or insulin on ischemia–reperfusion injury (IRI). As such, it might be expected that mTORi treatment could exacerbate IRI and enhance the inflammatory events associated with IRI. However, there is also some evidence to suggest that mTORi may act to inhibit IRI [129]. Further work will be required to determine the exact interplay between mTORi treatment and IRI.

Infection

Interestingly, the contributions of mTORi to development of CD8 T-cell memory appears to ameliorate transplant immunosuppression associated development of post-transplant lymphoproliferative disease (PTLD) [130,131] and cytomegalovirus infections [132]. Thus, while mTORi-mediated immunosuppression, like other immunosuppressive drugs, may play a role in allowing opportunistic infections, its inclusion in an immunosuppression regimen clearly decreases the susceptibility of the patient to specific pathogens.

Metabolism

As a vital component of cellular metabolism, it is not surprising that mTORi can have significant pathological effects on metabolism and homeostasis. Administration of sirolimus produces dose-dependent dyslipidemia, and can contribute to cardiovascular morbidity [133]. There is strong evidence that mTORi treatment increases the incidence of post-transplant diabetes [134,135]. Studies in preclinical models suggest that the diabetogenic effect of mTORi is mediated both through reduced insulin sensitivity and disruption of beta cell function and/or survival [135].

Wounds/healing

The powerful antiproliferative effects of mTORi are clearly seen in the wound healing process. Treatment with sirolimus increases surgical wound complications by interfering with several aspects of the healing process [125,136–139].

Tumors/proliferation

mTOR is a vital component in angiogenesis and growth factor-mediated proliferation. These functions appear to contribute to the association of mTORi with a significant decrease in tumor incidence associated with transplantation [140,141]. Further, there is evidence to suggest that mTORi-induced CD8 T-cell memory enhancement can result in immunological control of tumors in some settings. mTORi are now being investigated for use in the treatment of a variety of different tumor types.

Purine analogues

Purine analogues are chemicals mimicking the structure of essential building blocks of DNA and RNA. By impeding synthesis of DNA, RNA, and other nucleotides, as well as protein, they inhibit cell proliferation and immune responses. Azathioprine (AZA), and 6-mercaptopurine (6-MP) are purine analogues that are widely used as immunosuppressants and anti-inflammatory reagents for preventing organ transplant rejection. 6-MP and AZA were introduced clinically by Sir Roy Calne, the British pioneer in transplantation. Dr Thomas Starzl introduced the concept of using AZA plus steroids in transplantation. AZA is a prodrug of 6-MP with less toxicity, and after oral ingestion, AZA is metabolized into the active 6-MP. Other related compounds in the purine pathway are mycophenolate mofetil (MMF) and mycophenolic acid (MPA), with MPA being the active metabolite of MMF. Through inhibiting inosine monophosphate dehydrogenase (IMPDH), and thus repressing de novo synthesis of guanosine nucleotides [142], MMF inhibits cell proliferation. While other cell types can use the salvage pathway for guanosine nucleotide synthesis, lymphocytes depend on IMPDH for de novo synthesis, therefore MMF is a more specific and potent drug than AZA in suppressing purine synthesis. As a result, MMF has substituted AZA in many transplant centers in recent years.

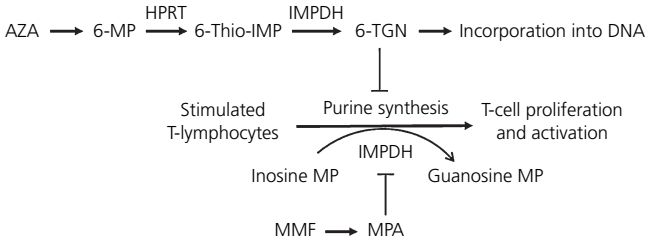

Figure 17.4. Mechanism of action of AZA, 6-MP, and MMF. AZA, Azathioprine; 6-MP, 6-mercaptopurine; HPRT, hypoxanthine phosphoribosyl transferase; 6-Thio-IMP, 6-thioinosine 5′-monophosphate; IMPDH, inosine monophosphate dehydrogenase; 6-TGN; 6-thioguanine nucleotide; MMF, mycophenolatemofetil; MPA, mycophenolic acid.

Structural and biochemical pathways

As shown in Figure 17.4, after AZA is absorbed, it is metabolized into its active form 6-MP, which is further metabolized into 6-thioguanine nucleotide (6-TGN) through 6-thioinosine 5′-monophosphate (6-Thio-IMP). 6-TGN can incorporate into DNA and RNA and act as an antagonist to the endogenous purines that are essential for the S phase of the cell cycle, and thus directly block cell proliferation. Studies of 6-MP in murine leukemia cells showed that cell death was consistent with a mechanism caused by DNA damage due to incorporation of 6-TGN into DNA. 6-TGN can also inhibit endogenous purine synthesis, alter the synthesis and function of RNA and DNA, and, as a result, prevent cellular and especially lymphocyte proliferation. For MMF, it is metabolized into its active form of MPA. MPA acts as a non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), consequently suppressing de novo synthesis of guanosine nucleotides [142] to specifically target lymphocyte proliferation (Figure 17.4).

Cellular targets

After transplantation, host immune cells will respond to alloantigen, proliferate, and mount immune responses to reject and destroy transplant organs. The main target for AZA and MMF is cell proliferation, thus preventing the clonal expansion of lymphocytes in the induction phase of the immune response, and suppressing both cellular and humoral immune responses. AZA/6-MP converts CD28 costimulation signals into apoptotic signals. Specifically, AZA/6-MP generated 6-thio-GTP binds to the GTP-binding protein Rac, thus blocking Rac1 activation, which prevents the activation of Rac1-regulated genes such as MEK, NF-κB, and Bcl-xl, and ultimately leads to a mitochondrial pathway of apoptosis. It has also reported that 6-TG blocks GTPase activation via blockade of Vav activity on Rac proteins, interfering with the interaction between T cells and antigen-presenting cells (APCs), and thus blocking immune activation [143].

The interactions between 6-MP and DC differentiation and activation were recently examined. It was shown that 6-MP inhibits DC differentiation from CD14 precursors and reduces LPS-induced CD83 expression [144]. Since CD83 is a marker of DC maturation and facilitates interaction between APCs and T cells, influences MHC class II turnover on the DC cell surface, and determines $CD8^+$ T-cell survival during immune responses [145–147], down-regulation of CD83 by 6-MP results in impaired allogeneic and antigen specific T-cell proliferation [144,148]. 6-MP reduces DC CCR7 expression, which is critical for lymph node homing. Together these reports all suggest that AZA suppresses immune responses also by modulating DC maturation and activation [144].

MMF exerts antiproliferative effects, especially on T lymphocytes by IMPDH. Two isoforms of IMPDH have been identified, and activated T lymphocytes strongly express type II, which has fivefold higher affinity than IMPDH I for MPA. MPA is able to increase apoptosis from 12% to 82–92% in cultured MOLT-4 cells [149], and accelerates the elimination of superantigen-reactive T cells by triggering apoptosis in both mice and humans [150,151]. MPA inhibits IMPDH and endogenous purine synthesis, depleting GTP, and consequently interfering with the transfer of mannose and fucose to lymphocyte glycoproteins [142], components of adhesion molecules for lymphocytes. MPA decreases attachment of human T lymphocytes to human endothelial cells, and treatment of both T cells and endothelial cells with MPA results in further decreases in adhesion, indicating that MPA affects immune responses through endothelial cells [152]. MMF significantly reduces ICAM-1 expression, LFA expression, and LFA^+ cell infiltration in various transplant and inflammation models [153–157], supporting a mechanism of MMF-mediated immune suppression through inhibition of adhesion molecule expression and immune cell migration.

MMF reduces CD40 and CD86 expression, and LPS-induced IL-12 production, leading to the decreased ability of murine DC to stimulate allogeneic T cells [158]. In human monocyte-derived DC cultures, MMF reduces the number of immature DC, induces apoptosis, down regulates costimulatory and adhesion molecules such as CD40, CD54, CD80, and CD86, lowers production of TNF-α, IL-10, IL-12, and IL-18, and inhibits stimulation of alloreactive T cells [159]. Together, these results indicate that MMF suppresses immune responses by affecting DC differentiation and activation.

MPA lowers GTP levels in monocytes [142], decreasing mannosylation of glycoproteins and monocyte attachment to endothelium [160,161]. Similar to the proapoptotic effects in lymphocytes, MMF induced apoptosis in THP-1 and U937 monocyte cell lines, and prevents glomerular macrophage infiltration in streptozotocin-induced diabetes [149,162]. Together, these results indicate that MMF can directly attenuate monocyte and macrophage responses.

Adaptive immunity

As noted above, purine analogues and related drugs directly inhibit lymphocyte proliferation, and therefore suppress induction phases of adaptive immunity via both humoral and cellular immune responses. These compounds also affect APCs, particularly a variety of DC functions, and thus interfere with interactions between lymphocytes and APCs, to suppress adaptive immune responses.

Innate immunity

AZA and MMF inhibit DC differentiation, adhesion molecule expression, maturation, and cytokine expression to suppress both innate and adaptive immune responses. In addition, MMF directly induces monocyte and macrophage apoptosis as well as inhibiting their migration and infiltration to allograft and inflammatory sites, further indicating their suppressive activities on innate immune responses.

Infection

The unique ability of MPA to specifically target T and B lymphocytes in transplant immunity [163,164] also increases the risk of infectious complications [165]. It has been reported that MMF leads to better allograft survival than AZA, but the rates of rehospitalization for infection was significant higher in patients who received MMF [166]. CMV infection is the most common infectious complication associated with MMF usage [167–169], and may be due to decreased levels of serum anti-CMV IgM in patients who received MMF compared to AZA [170,171]. BK virus infection has also been implicated in immune suppressive regimens that included MMF [172–174], as well as varicella and fungal infection [175,176]. On the other hand, there are reports showing possible antimicrobial activities of MMF, including against Dengue virus, hepatitis C virus, hepatitis B virus, pneumocystis, West Nile virus, and yellow fever virus [177].

Metabolism

Typical adverse effects of MMF for transplant patients are gastrointestinal symptoms, including diarrhea, nausea, vomiting, and abdominal cramps [178,179], possibly due to enterocyte toxicity and/or immunosuppression caused low-grade enteric infections [178,180]. MMF has also been associated with: hematologic side-effects including leucopenia, anemia, thrombocytopenia, and dysplasia [181–184]; genitourinary adverse effects including urgency, frequency, dysuria, and sterile pyuria; and other side-effects such as fever, myalgias, headache, and insomnia, as well as cardiovascular symptoms including peripheral edema and hypertension [180,185,186].

Monoclonal biologics

Biologic medicines are produced using biological processes involving recombinant DNA technology rather than being chemically synthesized. Biologics are designed to selectively target a key immune pathway without the side-effects associated with some of the pharmaceutical drugs [187,188]. This section will focus on monoclonal biologic therapies for prevention and treatment of transplant rejection. Kohler and Milstein won the Nobel Prize for their 1975 publication describing the creation of antibodies of predetermined specificity, now referred to as monoclonal antibodies [189]. Monoclonal antibodies have been instrumental in revolutionizing transplantation and cancer therapies and are key members of a class of drugs known as biologics or biologic medicines.

Basiliximab and daclizumab: anti-IL-2R antibodies

Structural and biochemical pathways

The receptor for IL-2, IL-2R, is comprised of three subunits: alpha, beta, and gamma, or CD25, CD122, and CD132, respectively [190]. Basiliximab (Simulect) and daclizumab (Zenapax) are both potent monoclonal antibodies directed at CD25 and are potent antagonists of IL-2. They are used to prevent transplant allograft rejection [191]. Basiliximab and the IL-2R have been studied and modeled. Basiliximab, a human–mouse chimeric antibody, and daclizumab, a humanized murine antibody, both bind the IL-2α chain (basiliximab specifically binds the IL-2Ra ectodomain), markedly limiting the immune response [190,191]. Daclizumab was withdrawn from the market in 2009 for financial reasons but figures prominently in the literature over the last 15 years. IL-2 is both a product of T cells and a signal involved in clonal T-cell expansion [192]. In addition, IL-2 signaling up-regulates expression of CD25 in a positive feedback loop that leads to rapid activation and proliferation of T cells [191]. In vivo, basiliximab decreases $CD25^+$ T cells for nearly 6 weeks [193]. Because $CD25^+$ is present on both activated $CD4^+$ cells in addition to suppressive Tregs, investigators hypothesized that this agent may compromise Treg-suppressive mechanisms [193]. In human patients, although numbers of $CD4^+/CD25^+/FoxP3^+$ cells decrease after the drug's administration, the number of $CD4^+/CD25^-/FoxP3^+$ cells has been shown to increase [193]. Authors of a study evaluating T-cell subsets after basiliximab therapy concluded that although the drug decreased Treg numbers, the decrease was not associated with functional consequences, and that the drug may not inhibit their tolerogenic potential [193]. Other groups have shown that basiliximab does not change the T effector cell to T regulatory cell ratios, in contrast to rabbit ATG which is thought to bolster Treg populations [194].

Rituximab: anti-CD20 antibody

Structural and biochemical pathways

CD20 (initially referred to as B1) is expressed on a large percentage of mature B cells, but not on early B-cell precursors or active antibody secreting cells and plasma cells [195,196]. Rituximab, a humanized chimeric anti-CD20 agent, was originally approved to treat relapsed indolent lymphoma [197,198]. Rituximab is now used to eliminate B cells to control the alloantibody response. Rituximab eliminates B cells through both complement-mediated cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). Rituximab-induced ADCC is mediated by ligation of its Fc receptor [199]. For CDC, rituximab directly C1q binds, initiating the complement cascade [199]. Interestingly, steroid therapies such as dexamethasone have been shown to enhance CDC, but not ADCC [199]. Rituximab is also thought to be proapoptotic for B cells, likely due to down-regulation of IL-10 via the MAPK pathway [200]. Because plasma cells and highly mature antibody secreting cells express little or no CD20, the efficacy of rituximab in preventing or treating alloantibody responses and humoral rejection is likely very limited.

Alemtuzumab: anti-CD52 antibody

Structural and biochemical pathways

Alemtuzumab (Campath) is directed at CD52, a glycoprotein found on the surface of T cells, B cells, macrophages, monocytes, and NK cells. CD52 is not found on erythrocytes or hematopoietic stem cells [201]. The drug was originally approved to treat chronic lymphocytic leukemia, but was adopted by the transplant community as an induction therapy that causes widespread and rapid leukocyte depletion, which might lead to calcineurin inhibitor and steroid minimization, and a tolerogenic immunologic state [202,203]. In a multicenter trial alemtuzumab was associated with fewer episodes of acute rejection after 3 years, but had a worse malignancy profile [204]. Further, the drug yields more sustained lymphodepletion than other induction agents, such as equine antithymocyte globulin, so that it may take 1 to 2 years for patients to regain normal peripheral white blood cells counts and distribution of the various leukocyte subsets.

Muromonab: anti-CD3 antibody

Structural and biochemical pathways

Muromonab, also known as OKT-3, is a murine monoclonal IgG2a antibody [205]. The target of OKT-3 is CD3, a component of the

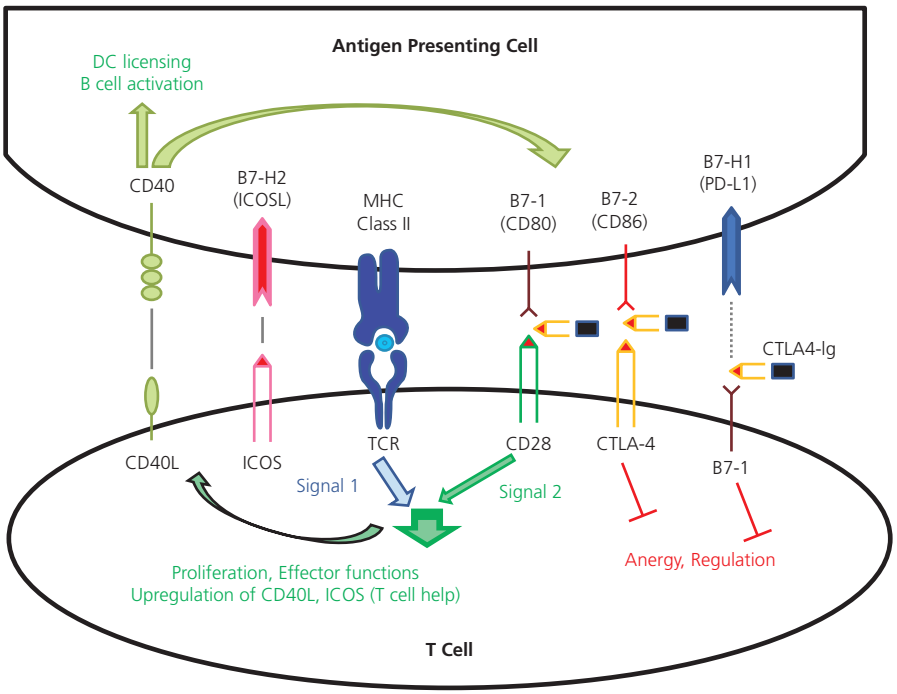

Figure 17.5. Mechanism of action of belatacept. The CD28-B7 counter-receptor group consists of two B7 ligands (CD80 and CD86) and two T-cell receptors (CD28 and CTLA-4). Both B7 ligands can bind to both CD28 and CTLA-4. If a T cell receives an antigen-specific T-cell receptor (TCR)-mediated signal (Signal 1), then B7-CD28 interactions generate a costimulatory signal (Signal 2) that promotes full T-cell activation, including up-regulation of additional costimulatory molecules like CD40L and ICOS, and reciprocal dendritic cell (DC) and B-cell activation (through T-cell help). In contrast, B7-CTLA-4 and B7-1-PD-L1 interactions appear to inhibit immune responses by inducing T-cell anergy or regulation. Belatacept is a recombinant fusion protein composed of the extracellular domain of CTLA-4 and the human IgG1 Fc domain. It binds to B7 ligands with a high affinity and thereby prevents interactions between B7 and other receptors. The end result is a status of immunosuppression with decreased T, B, and dendritic cell activation.

antigen specific T-cell receptor complexes, found on the surface of mature T cells as well as medullary thymocytes [205]. OKT-3 was the first monoclonal antibody ever approved for human use. It was removed from the market several years ago as it has been superseded by other agents, but remains an important basis in the historic transplant literature and our notions for current use of antibody reagents. Whereas other induction agents may act to reduce T-cell counts for days or weeks, CD3 counts begin to rise only 48 hours after administration of OKT-3 has ended [205]. Like rituximab, OKT-3 acts through CDC and ADCC, but it also exerts its effects via activation-induced cell death (AICD) and opsonization [206]. OKT-3 is also thought to modulate the T-cell receptor off the cell surface, such that it can no longer interact with APCs [206]. OKT-3 may also induce a long-lasting state of clonal anergy, simulating immunodeficiency [206]. As for many other antibody reagents, cross-linking of T-cell surface receptors can cause rapid and massive T-cell activation with release of cytokines, inducing clinically the cytokine storm characterized by fever, tachycardia, hypotension, vascular leak, and multiple end-organ dysfunction [207].

Belatacept

Concept and structure

Whereas biologic drugs used so far in transplantation are monoclonal antibodies, belatacept belongs to a new structural class of biologics: it represents a recombinant fusion protein corresponding to the soluble form of a receptor expressed at the surface of T-cells linked to an immunoglobulin frame [208]. Moreover, belatacept is the only biologic targeting a specific immune pathway (costimulation) currently used clinically.

Rejection of major histocompatibility complex (MHC)-disparate organ transplants is orchestrated and mediated by T lymphocytes. Costimulation can be defined as a second signal necessary for optimal T-cell activation delivered jointly to T cells along with a first signal mediated by engagement of the T-cell receptor (TCR) with the peptide–MHC complex on antigen presenting cells (APCs) (Figure 17.5). In vitro, signaling through the TCR alone (signal 1) in the absence of adequate costimulation (signal 2) fails to generate T-cell responses, and results in the induction of T-cell anergy or hyporesponsiveness [209,210]. These findings suggested a novel way to prevent pathogenic immune responses in autoimmunity and transplantation [187,211–213], an area that attracted much attention in the last decade. Many of the costimulatory molecules thought to be essential for T-cell activation have now been identified [214]. Costimulation blockade in various transplant models has now confirmed that costimulatory signals shape the outcome of an immune response and are potent immunomodulators of alloimmunity [215–222].

Elucidation of the now best-characterized costimulation receptor (CD28) in the 1990s led to the development of belatacept. Cytotoxic T lymphocyte antigen (CTLA)-4 is a structural homologue of

the T-cell costimulatory receptor CD28 that binds the same B7 ligands with a higher affinity. A recombinant fusion protein composed of the extracellular domain of CTLA-4 and the human IgG1 Fc domain (CTLA4-Ig) was designed to specifically bind B7 ligands and thus block CD28-B7 interactions. This blockade was shown to act as a potent immunosuppressive molecule in various animal models [213,215–220]. A first clinical formulation of CTLA4-Ig (abatacept) was developed with a mutated Fc domain for the treatment of rheumatoid arthritis and psoriasis. Belatacept (LEA29Y) was developed later as a second-generation higher affinity mutant for transplantation clinical trials [208]. In 2011, the US Food and Drug Administration (FDA) approved belatacept (Nulojix™) for the prevention of acute rejection in adult patients with a kidney transplant.

Cellular targets

The direct molecular targets of belatacept, B7-1 (CD80) and B7-2 (CD86), are weakly expressed by specific hematopoietic cells (DC, B cells, macrophages) and are up-regulated by signals that activate these cells to mature into antigen-presenting cells (APCs) (CD40, TLR agonists, inflammatory mediators). B7-1 and B7-2 represent the ligands for the costimulatory receptor CD28 constitutively expressed on essentially all murine and most human peripheral T cells.

The CD28/B7 pathway is pivotal for the activation of T cells [213]. The CD28 T-cell receptor provides a signal that synergizes with T-cell antigen receptor (TCR) stimulation in activating T cells to proliferate and secrete cytokines [223–225] (Figure 17.5). T-cell expansion is stimulated through increased transcription and mRNA stability of IL-2, and increased expression of the antiapoptotic protein Bcl-X_L. As a result, interruption of this pathway leads to an inhibition of T-cell proliferation and, under some circumstances, induces either antigen-specific hyporesponsiveness, anergy, or cell death [209,226]. CD28 signals also regulate T helper cell differentiation [227] and migration [228,229] through up-regulation of cytokines and chemokines. CD28 signals also enhance the expression of other costimulatory molecules like CD154 and ICOS, also involved in T-cell activation. Accordingly, CTLA4-Ig was shown to inhibit T-cell proliferation, activation, and differentiation in multiple in vitro and in vivo experimental models [230–233].

Different subsets of T cells exhibit varying degrees of susceptibility to CTLA4-Ig. For many years it was believed that memory T cells do not require CD28/B7-derived costimulation for recall responses [234], based on previous studies showing that memory CD4 T cells are fully activated by B7-deficient APC in vitro [235,236] and that $CD28^{-/-}$ mice are not impaired in the generation or recall of memory CD8 T-cell responses to a viral infection [237]. However, recent evidence has challenged this concept and shown that both $CD4^+$ and $CD8^+$ murine memory T cells require CD28 costimulation for maximal expansion and pathogen clearance [238–240], although some but not all T-cell functions are regulated by CD28/B7 interactions [241]. Clinical efficacy of CTLA4-Ig in treating rheumatoid arthritis [242] and psoriasis [243], diseases known to be driven by memory CD4 T cells [212], suggest that the CD28/B7 pathway may also regulate human memory CD4 T-cell-mediated responses. However, subpopulations of human and primate effector/ memory T cells that do not express CD28 have been described [244–249] and are therefore expected to be non-susceptible to CTLA4-Ig [250,251]. In addition, B7 blockade with CTLA4-Ig inhibits both positive (CD28/B7) and negative (CTLA-4/B7 and PD-L1/B7) immunomodulatory signals, which could result in a complex effect of CTLA4-Ig on effector and memory T-cell populations [234,252–258]. Besides naïve and memory T cells, CD28 and CTLA-4 receptors are also expressed by natural and induced/ adaptive Tregs [259], B7/CD28 costimulation is essential for the homeostasis of Tregs [260,261], and CTLA-4 regulates the potency of natural Tregs [262]. CTLA4-Ig can therefore alter the steady-state level and quality of Tregs [263–265], with potential implications regarding its effects on endogenous Tregs in patients receiving long-term therapy.

Although CD28 has been almost entirely characterized as a prototypic T lymphocyte receptor, it is also expressed on the surface of plasma cells (PCs) [266,267] but its expression is specifically repressed in B cells [268]. Therefore, whereas the effect of CTLA4-Ig on modulating humoral responses in various models [219,220,269] has been attributed to inhibition of helper T-cell costimulation and germinal center formation, a B-lineage-intrinsic role for CD28 in controlling the differentiation or function of plasma cells beyond germinal center formation is theoretically possible [268].

Treatment of macrophages with CTLA4-Ig in vitro was shown to down-regulate cytokine production [269,270] and in vivo CTLA4-Ig decreased macrophage-associated genes in rat renal allografts [219]. In addition, CTLA-4-Ig was shown to bind B7 molecules expressed on DC and activate a pathway of tryptophan catabolism resulting in the induction of indeolamine 2,3-dioxygenase (IDO), an intracellular enzyme that breaks down tryptophan and can lead to indirect inhibition of lymphocyte activation and T-cell death [271,272]. However, whether clinical versions of CTLA4-Ig (abatacept and belatacept) retain this intrinsic property despite a mutated Fc domain is unclear.

Adaptive immunity

As expected from its role in modulating T-cell activation and cognate reciprocal T-B and T-APC costimulatory signals, CTLA4-Ig/belatacept potently repress adaptive pathogenic immune responses in autoimmunity and transplantation [230–233].

Ischemia–reperfusion injury and inflammatory responses

Blockade of T-cell CD28-B7 costimulation with CTLA4-Ig significant decreased T-cell and macrophage infiltration and activation in a rat model of kidney IRI [273]. Moreover, B7-1 has been implicated in the regulation of inflammation in innate immunity in murine sepsis, a process mediated by macrophage/ neutrophil contact dependent on the B7-associated NF-κB repressor IRAK-M [274]. These findings raise the possibility of a direct effect of belatacept on innate immune responses.

Infection

As for other immunosuppressive therapies, belatacept is associated with an increased risk of opportunistic infections. In clinical trials of kidney transplantation, the overall incidence of infections in belatacept-treated patients was similar to that in the cyclosporine control group, with a predilection of tuberculosis and herpes infections with belatacept.

Metabolism

In a phase III multicenter clinical trial in renal transplantation, patients treated with belatacept exhibited fewer associated non-immune toxicities (nephrotoxicity, hyperlipidemia, cardiovascular

events) as compared to the control group, which received standard cyclosporine-based immunosuppression. In addition, patients who received belatacept had lower blood pressure levels compared to those on cyclosporine [275–277].

Tumors/proliferation

Similar to cyclosporine, belatacept is associated with an increased risk of tumors and malignancies due to the systemic immunosuppression. In addition, belatacept-treated patients have an increased risk for developing post-transplant lymphoproliferative disorder (PTLD), predominantly involving the central nervous system. Consequently, belatacept is contraindicated in transplant recipients who are Epstein–Barr virus (EBV) seronegative or with unknown EBV status. No direct inhibitory effect is expected on tumor proliferation, except if a specific tumor expresses B7.

Antithymocyte globulins

Antilymphocyte sera, particularly antithymocyte serum, have a long and successful history as transplant immunosuppressants [278–280]. Currently, two types of antithymocyte-γ globulin (ATG) products, produced in rabbits (rATG, clinically available as Thymoglobulin®) or horses (eATG, clinically available as ATGAM®) are utilized in both induction regimens and in treatment of acute graft rejection. Both ATG are purified polyclonal immunoglobulin mixtures containing mostly IgG isotype antibodies recovered from animals immunized with human thymocytes. Direct comparisons of rATG and eATG demonstrated increased efficacy of rATG in the induction and maintenance of lymphopenia and in preventing acute rejection of renal grafts [281].

Cellular targets

T lymphocytes are the major cellular target of ATG. The molecules recognized by ATG are extremely varied. ATG-targeted molecules include T-cell-specific targets, such as CD3, CD28, CD152, and the T-cell receptor, as well as molecules largely confined to T-cell populations, including CD2, CD4, CD8, and CD25, and more broadly expressed proteins such as CD11a, CD45, and HLA class I and II.

ATG recognition of T cells has multiple consequences [282–288]. Depletion of T cells is rapid and is thought to occur through several different mechanisms, including complement-mediated lysis [285], receptor-mediated apoptosis [282], and antibody-dependent cell-mediated cytotoxicity [287]. Further, ATG has been shown to negatively regulate T-cell proliferation through interaction with CD25 [289]. ATG has been also been shown to modulate T-cell trafficking, apparently as a result of specific binding to T-cell adhesion molecules or chemotactic receptors [283,290]. ATG has also been shown to enhance the development and/or accumulation of Tregs [291–294]. These cells in turn can negatively modulate effector T-cells function by a variety of mechanisms [295].

While T cells are the intended target for ATG, it is clear that other cells types are also modulated by ATG treatment. Both NK and B lymphocytes can be depleted by ATG [296,297]. NK cells that are not depleted demonstrate increases in activation markers and production of IFN-γ [298], but also demonstrate reduced capacity for antibody-dependent cell-mediated cytotoxicity [299].

In addition to its effects on lymphocytes, ATG also can modulate cells of the innate immune system, particularly DC and neutrophils. ATG treatment can result in brief activation of neutrophils followed by neutropenia [300]. In DC, ATG can block antigen uptake and maturation in response to inflammatory signals, and induce apoptosis [301].

Adaptive immunity

ATG can deplete $CD4^+$ and $CD8^+$ T cells, B cells, and plasma cells, and the DC that initiate adaptive immunity. Further, cells that survive exposure to ATG can still be functionally inhibited by antibody recognition of molecules involved in antigen presentation and recognition, B-cell help, trafficking, and effector function. These activities of ATG result in greatly diminished effector lymphocyte responses. Together these combined activities of ATG on leukocytes make it a profound inhibitor of adaptive immunity [278,279].

Ischemia–reperfusion inflammatory responses

There is some evidence to suggest that ATG inhibits IRI, presumably by inhibiting the trafficking and functional activity of innate immune cells involved in IRI responses [290]. Treatment with ATG in a primate model revealed significant reduction of endothelial adhesion molecules. This correlated with a reduction in expression of proinflammatory cytokines, suggesting that inhibition of leukocyte trafficking reduced inflammation associated with ischemia–reperfusion.

Infection

Treatment with ATG has been shown to increase susceptibility to opportunistic infections, particularly cytomegalovirus (CMV) [302–305]. However, other groups have reported that ATG treatment increased risk of CMV infection to the same extent as another method of induction, suggesting that it is general immunosuppression and not an inherent effect of ATG that drives increased risk [306]. Another study demonstrated that the increased CMV infection risk was abolished in the presence of CMV prophylactic treatments [307].

Metabolism

ATG treatment has not been associated with adverse metabolic effects, but rather has been utilized in corticosteroid reduction regimens aimed at preventing adverse metabolic complications [308,309].

Tumors/proliferation

Multiple studies have examined the effects of ATG on development of malignancy, particularly PTLD, with contradictory outcomes [310–312]. While the individual contribution of ATG to malignancy is unclear, it seems that the overall state of immunosuppression generated by combined immunosuppressants can increase the risk of malignancy [311]. Thus, ATG, like other drugs used in induction immunosuppression, can increase the rate of PTLD and other malignancies [310,311].

Intravenous immunoglobulin (IVIG)

Living donations have increased the availability of organs, but patients are increasingly found with existing sensitization to alloantigens, manifest by antibodies with broad specificity against HLA antigens (panel reactive alloantibodies). In addition, the role of alloantibodies developed de novo post-transplant in antibody-mediated rejection (AMR) has recently emerged as a major barrier to the success of kidney [313,314], as well as heart and pancreas,

transplantation [315]. Unfortunately, current immunosuppressive therapies do not efficiently target these antibody-mediated immune mechanisms. Intravenous immunoglobulin (IVIG) has demonstrated great clinical success in a number of antibody-mediated autoimmune diseases and chronic inflammatory disorders [316–318] and recently became part of the clinical armamentarium to desensitize transplant patients exposed to alloantigens prior to transplant, and to treat antibody-mediated rejection [319]. Although well documented, the efficacy of IVIG in this context is still controversial [320–322] and its mechanisms of action not well understood. IVIG has also been successfully used in the treatment of ABO-incompatible transplants [323]. Moreover, IVIG was widely used as therapeutic or prophylactic therapy for viral infections in bone marrow transplant patients before the development of cytomegalovirus-negative blood products and ganciclovir [324], and is still occasionally utilized against infections after bone marrow [325] or organ [326–328] transplantation.

Concept and structure

IVIG mostly (95%) contains the pooled immunoglobulin G (IgG) fraction extracted from the plasma of blood donors, with only trace amounts of immunoglobulin A (IgA) or immunoglobulin M (IgM). It is manufactured as a sterile, purified product. Despite its efficacy, the fact that IVIG is composed of the pooled IgG fractions from 5000 to 50 000 donors limits potential availability, increases cost, and each product (batch) is associated with its own characteristics that may affect tolerability and efficacy.

Although the use of IVIG has gained increasing popularity from the treatment of humoral immunodeficiencies to immunomodulation in autoimmune, inflammatory, and transplantation conditions, our understanding of its mechanisms of action are still incomplete [329–331]. Some of its mechanisms of action have been attributed to the presence of natural or anti-idiotypic antibodies [332,333]. IVIG directly binds to and "neutralizes" pathogens and various target cells and molecules, and blocks complement deposition. In addition to these antigen-binding or Fab-mediated mechanisms, recent evidence identified a key role for the Fc portion of immunoglobulins and the role of IVIG-mediated Fc multimerization in mediating immune modulation [334–337]. These Fab- and Fc-dependent mechanisms are probably not mutually exclusive and are likely variably involved in different pathologic conditions.

Cellular targets

IVIG can directly bind to various cell types by interacting with Fcγ receptors (FcγRs). Four different classes of FcγRs have been identified in mice (FcγRI, FcγRII, FcγRIII, FcγRIV) whereas orthologous proteins in humans and primate are divided into three large classes (FcγRI, FcγRII, FcγRIII) with additional variants within each family. Various hematopoietic cells, including monocytes, macrophages, DC, neutrophils, mast cells, NK, and NKT cells, express one or more FcγRs, but FcγRs are typically absent in T cells. Most FcγRs transmit activating signals involved in cellular immune functions such as phagocytosis and antibody-dependent cytotoxicity (ADCC). In contrast, FcγRIIB as the sole FcγR bearing an inhibitory ITIM motif, acts as a suppressor of cellular immune responses, and is associated with immune tolerance [338–340]. B cells only express FcγRIIB, and both their activation and survival [341,342] are modulated by FcγR signaling, findings consistent with the therapeutic effect of IVIG on humoral rejection. IVIG may also accelerate the clearance of pathologic IgG antibodies through binding to the neonatal Fc receptor (FcRn) on endothelial cells [343,344].

Adaptive immunity

Clinical use of IVIG targets the progression (B cells, plasma cells) and product (antibody) of humoral adaptive immune responses. Moreover, the presence of FcγR on various hematopoietic lineages as well as in subsets of Tregs [345] and suppressor cells suggest additional potential effects of IVIG in immune homeostasis [346] and tolerance.

Ischemia–reperfusion injury and inflammatory responses

Amplification of IRI and inflammation mediated by alloantibody is likely to be indirectly decreased by IVIG-based therapies. Additionally, direct effects of IVIG on FcγR-bearing innate cells are expected, based on anti-inflammatory properties of IVIG in other diseases [333].

Infection

IVIG was an established therapy for the treatment or prophylaxis of viral infections in bone marrow transplant patients [324], and is still occasionally administered against infections in bone marrow [325] and organ [326–328] transplantation. Desensitization with IVIG and rituximab did not increase the infection risk in kidney transplant patients [347]. However, the human origin of IVIG has potential unknown infectious risks.

Metabolism

A small but significant IVIG-related thrombosis rate was observed in patients treated for AMR, which was prevented by a slower rate of infusion combined with antiplatelet and anticoagulation [348]. Studies on IVIG in atherosclerosis suggest beneficial effects of IVIG on metabolism [349].

Tumors/proliferation

Natural antibodies found in IVIG preparations were shown to suppress tumor growth [350]. However, immune-modulation mediated by FcγR inhibitory signals may contribute to tumor development by supporting immunosuppression.

Summary

Modern transplantation remains dependent on numerous classes of immune modifying drugs, which can be group by general mechanism and structure into glucocorticoids, calcineurin inhibitors, mTOR inhibitors, purine analogues, and monoclonal and polyclonal protein biologics. Used cognizant of their effects and side-effects, these drugs have enabled the practice of transplantation. The reader will find reference to the clinical use of these drugs throughout this text, and is encouraged to consider the mechanisms described in this chapter when formulating clinical strategies to prevent and treat rejection.

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CHAPTER 18

Future Pathways for Immune Manipulation

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Introduction

Calcineurin inhibitor (CNI)-based regimens, while largely responsible for decreasing acute rejection rates and increasing graft survival at 1 year have failed to improve attrition rates beyond 1 year. Long-term renal allograft survival has remained unchanged since 1995 [1]. Furthermore, CNIs are associated with multiple systemic side-effects, including nephrotoxicity, as well as metabolic derangements, including hypertension, hyperlipidemia, and glucose intolerance. The metabolic derangements of CNIs likely contribute to the significant amount of cardiovascular disease seen in the transplant population, the leading cause of death with a functioning graft [2–6]. In addition, while effective at inhibiting acute T-cell mediated alloresponses, CNIs seem less effective, especially when used in minimization regimens, at targeting a chronic alloantibody response, now thought to play a significant role in late allograft loss [7–9]. This evidence suggesting the prominent role of alloantibodies and chronic humoral rejection in late allograft loss has sparked interest in the pursuit of novel B-cell therapeutic targets, including blockade of the CD40–CD154 costimulatory pathway with antibodies to CD40, as well as B-cell-activating factors (BAFF) and a proliferation-inducing ligand (APRIL), both tumor-necrosis factor family ligands which act as antiapoptotic factors critical for maturation of the B-cell lineage [10]. Still, T cells are thought to remain central to graft rejection. Landmark studies identifying that signal 1, delivered through the T cell receptor (TCR) interacting with antigen in the context of MHC, is insufficient to trigger productive T-cell responses, has spawned significant interest in costimulatory molecules. Costimulatory signals are now known to be responsible for signal 2, a signal required to drive survival, clonal expansion, and differentiation of activated T cells [11,12]. The best characterized costimulatory pathways under active investigation as therapeutic targets in transplantation thus far include the CD28/B7 and the CD40/CD154 pathways. LFA-1/ICAM and the CD2/LFA-3 pathways are also of interest in transplant immunology [13,14]. Monoclonal antibodies and fusion proteins developed specifically to target some of these pathways have shown promise in providing similar efficacy to CNI-based regimens in preventing rejection, with fewer side-effects. However, the complexity of costimulation, including the existence of negative costimulatory signals (i.e. CTLA-4) which inhibits T-cell activation, as well as the variable expression of these molecules both on different cell subsets and at different times points of immune activation, have made clinical trials with biologics challenging [15]. Finally, small molecules, including tofacitinib, a Janus kinase 2/3 inhibitor, have demonstrated efficacy in animal models in improving graft survival and Phase IIa and IIb clinical trials in humans have shown similar efficacy, albeit with more immunosuppressive-related complications including infections and post-transplantation lymphoproliferative disease (PTLD) [16,17].

This chapter will focus on prominent pathways currently being targeted in transplantation, including intracellular pathways, as well as B- and T-cell surface receptors and ligands. Emphasis will be given to the general mechanisms and novel targets that might be exploited for pharmacologic immune manipulation in the future. Ultimately, the goal of therapeutics in transplantation is to achieve a robust immunologic tolerance to the allograft while maintaining an intact immune system for surveillance of other antigens. This chapter will focus on therapeutics aimed at achieving this goal, and is complemented by Chapter 17, which focuses on those pathways exploited in common clinical practice.

Costimulatory pathways in transplantation

Costimulation pathways have long been viewed as important targets for the control of adaptive immune responses. While the recent approval of belatacept heralds the beginning of the therapeutic manipulation of costimulation pathways to combat rejection, it is generally held that their true promise has only begun to be realized, and that belatacept's use will be refined and followed by numerous other compounds. This section uses the development of belatacept as a launch point to outline how other costimulation pathways are likely to be explored in transplantation.

CD28/CTLA-4/B7 pathway

T cells play a central role in the initiation and regulation of the adaptive immune response and require two collaborative signals for activation [18]. Signal 1 is delivered through the T-cell receptor (TCR) interacting with the MHC and antigenic peptide complex on antigen-presenting cells (APC). Signal 2—the costimulatory signal—is provided by engagement of T-cell surface receptors with their ligands on the APC. In the absence of a concomitant signal 2, naïve T cells become anergic, precluding the development of an effector $CD4^+$ T-cell subset and clonal expansion of activated cytotoxic T cells (CD8 T cells). The best-characterized positive

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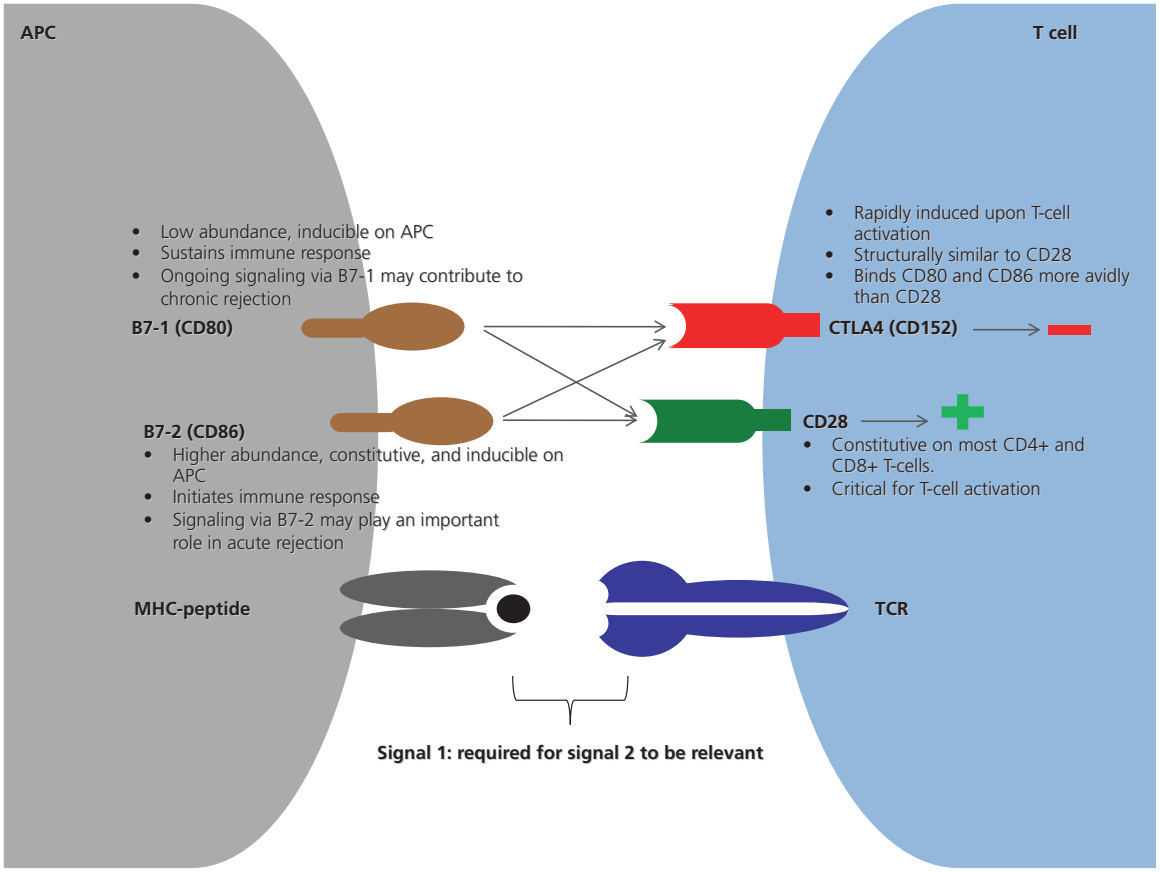

Figure 18.1. Signaling via B7-1/2 and CD28 plays a critical role in T-cell activation. Shown are the relationships between ligands B7-1 and B7-2, and their receptors CD28 and CTLA4. Note that engagement between the T-cell receptor (TCR) and MHC (signal 1) is required before the costimulatory signals are relevant. APC, antigen-presenting cell.

costimulatory pathway is the CD28/B7 pathway [13]. CD 28 is a homodimeric transmembrane protein constitutively expressed on all naïve $CD4^+$ and $CD8^+$ T cells [19] and has two known ligands, B7-1 (CD80), which is inducibly expressed, and B7-2 (CD86), which is constitutively expressed on the surface of APCs [19]. Engagement of CD28 by B7 ligands in the presence of TCR stimulation induces T-cell clonal expansion, cytokine production (including IL-2 and IFN-γ), and enhanced T-cell survival (Figure 18.1) [13,14]. Interestingly, as an example of the complexity of costimulatory signaling, activated T cells up-regulate cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), a molecule that is structurally similar to CD28 but with higher affinity for ligands B7-1 and B7-2. CTLA-4 binding provides a negative costimulatory signal, down-regulating the T-cell response and providing a negative feedback loop. CTLA-4 is also critical for the function of $FoxP3^+$ T regulatory cells, cells instrumental in promoting tolerance [20,21].

Various biologics have been developed to target the CD28/CTLA-4/B7 pathway, most notably the recombinant fusion protein CTLA-4–Ig (abatacept), which consists of an extracellular domain of CTLA-4 and the Fc portion of IgG1. The development of CTLA-4–Ig was based on the known higher affinity/avidity of CTLA-4 for ligands B7-1 and B7-2 and its ability to bind B7-1 and B7-2 in lieu of CD28, thereby blocking positive costimulatory signals [22]. While in rodent transplant models CTLA-4–Ig prolonged allograft survival, this effect was not observed in non-human primates (NHP) [23,24]. This finding led to the discovery that CTLA-4–Ig's inhibition of B7-2 (CD86) is less potent than its inhibition of B7-1 (CD80) and its inhibition of the CD28/B7 pathway is therefore suboptimal for transplantation [22,25,26]. As a result, belatacept (previously referred to as LEA29Y), a second-generation CTLA-4–Ig fusion protein (with two amino acid substitutions in the binding sites) with higher affinity for B7-2, was developed and was found to prolong renal allograft survival in NHP when used in combination with mycophenolate mofetil and steroids (Figure 18.2) [27]. Additionally, belatacept inhibited the development of antidonor antibodies [27].

A Phase II multicenter clinical trial designed to demonstrate the non-inferiority of belatacept (using two dosing regimens) to cyclosporine (CsA) in preventing rejection revealed comparable rejection rates and higher glomerular filtration rate (GFR) and less chronic allograft nephropathy (CAN) in both belatacept groups at 1 year [28]. Given these encouraging results, two Phase III trials, "A Phase III Study of Belatacept-based Immunosuppression Regimens versus Cyclosporine in Renal Transplant Recipients" (BENEFIT) and "A Phase III Study of Belatacept Versus Cyclosporine in Kidney Transplants from Extended Criteria Donors" (BENEFIT-EXT), were designed to assess whether treatment with belatacept was equivalent or superior to cyclosporine with respect to acute rejection rates, GFR, and patient and graft survival at 1 year, in

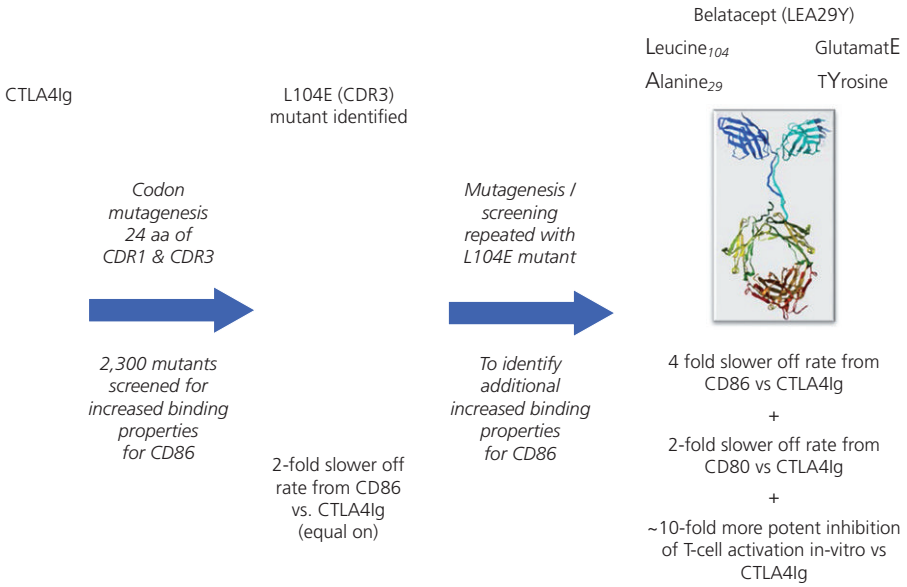

Figure 18.2. Belatacept (LEA29Y): rationally designed for transplantation.

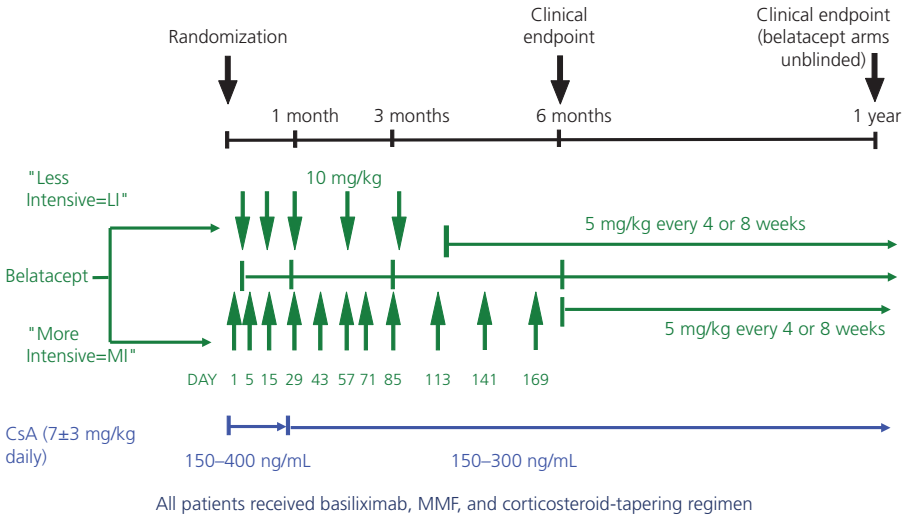

Figure 18.3. The immunosuppressive regimens used in the BENEFIT and BENEFIT-EXT trials.

recipients of living or standard deceased donor kidneys and extended criteria deceased donor kidneys, respectively [29,30]. Figure 18.3 depicts the treatment regimens employed in both studies. Results of the BENEFIT trial revealed equivalent patient/graft survival to cyclosporine at 1 year in both the more intensive (MI) and less intensive (LI) belatacept groups (95% MI; 97% LI; and 93% CsA) [29]. Renal function in both belatacept groups was superior to cyclosporine at 1 year (mean GFR 65 mL/min/1.73 m^2 MI; 63.4 mL/min/1.73 m^2 LI; 50.4 mL/min/1.73 m^2 cyclosporine, $P < 0.0001$ for MI and LI vs. CsA). Follow-up at 3 years showed that the difference in eGFR continued to widen between the belatacept and cyclosporine treated patients.

The presence of biopsy-proven CAN at 1 year protocol biopsies was lower in the belatacept groups compared to CSA (18% MI; 24% LI; 32% CSA). Further, cardiovascular outcomes, including blood pressure and lipids, were better in the belatacept groups (133/79 MI, *P* value vs. CsA 0.001; 131/79 LI, *P* value vs. CsA 0.0273; 139/82 CsA). The incidence of new-onset diabetes after transplantation (NODAT) was not statistically significant between the three groups.

Acute rejection rates, however, were higher in the belatacept groups (22% MI; 17% LI; 7% CsA) and more patients in the belatacept groups had Banff Grade IIB rejections compared to those in the cyclosporine group [29]. Further, an evaluation of safety endpoints, including malignancy and infections, revealed no difference between groups with respect to urinary tract infections, CMV infection, or BKV infection. Importantly, the incidence of PTLD at 1 year was 1, 2, and 1 patients in the MI, LI, and CsA groups, respectively; however, after month 12, two additional patients in the MI group developed CNS PTLD. Patients who developed PTLD had risk factors for its development, including serologic negativity for the Epstein–Barr virus (EBVIgG$^-$). In the BENEFIT-EXT trial, the incidence of PTLD was also increased in the belatacept groups (1 MI; 2 LI; 0 CsA at 12 months, then 2 MI; 3 LI; 0 CsA after month 12); 3/5 were negative for EBV IgG as well [30]. Otherwise, the findings of the BENEFIT-EXT trial were comparable to the BENEFIT trial, with the exception of equivalent rejection episodes between the three groups (17.9% MI; 17.7% LI; 14.1% CsA), equivalent incidence of CAN (45% MI; 46% LI; 52% CsA) and increased NODAT at 1 year in the cyclosporine group (2% MI; 7% LI; 11% CsA $P = 0.0308$ MI vs. CsA) [30]. The lower intensity regimen of belatacept, which had a better safety profile, was FDA approved as an antirejection therapy for EBV-positive patients in renal transplantation in June of 2011.

The histologic severity and increased incidence of rejection in the belatacept groups illustrates the complexity involved in costimulation blockade. Several hypotheses to explain this phenomenon include the notion that belatacept's blockade of B7-1 and B7-2 on APCs also inhibits the binding of CTLA-4, a known negative costimulatory signal as well as a molecule critical for the function of FOXP3$^+$ regulatory T cells [21,31]. Furthermore, memory T cells, a major hurdle to allograft tolerance, are less dependent on the CD28/B7-1/B7-2 for stimulation and have therefore been implicated as key mediators of costimulation blockade-resistant rejection [32–35].

A logical approach would be to develop a biologic that specifically targets CD28. This would theoretically allow for more CTLA-4/B7-1/B7-2 ligation and therefore promote allograft tolerance. Several attempts at humanized anti-CD28 monoclonal antibodies have failed [36,37]. One study investigating a superagonist, TGN1412, which promoted T regulatory cell expansion in preclinical studies, resulted in cytokine storm and multiorgan failure in six volunteers [37]. However, a chimeric human/primate monovalent fusion protein named Sc28AT, which competes with B7-1 and B72 for binding to CD28 and lacks superagonist activity, has been studied in non-human primates. In this study they found that monotherapy with Sc28AT significantly prolonged renal allograft survival and the combined administration of Sc28AT with tacrolimus further prolonged allograft survival [38]. In addition, there was an increase in peripheral CD4$^+$ T regulatory cells with Sc28AT and on renal allograft biopsies there was a predominance of FoxP3$^+$ CTLA-4$^+$ Tregs with a paucity of CD20$^+$ B cells, and reduced expression of inflammatory cytokines [38]. It is likely that clinical studies with Sc28AT, or related proteins, are forthcoming in the transplant population.

CD40/CD40L(CD154) pathway

Another costimulatory pathway of interest in transplantation is the CD40/CD154 pathway. CD40 is constitutively expressed on APCs, including B cells and dendritic cells (DCs), and, in an inflammatory milieu, its expression is up-regulated by endothelial cells and fibroblasts. Further, its own expression is up-regulated upon activation [39–41]. CD154, the only ligand identified for CD40, is expressed on activated T cells, NK cells, eosinophils, and platelets [42]. Binding of CD40 to CD154 promotes activation and maturation of dendritic cells, activates B cells, and promotes immunoglobulin class switching. Further, downstream signaling of CD40 increases DC expression of MHC complexes and costimulatory molecules (i.e. CD80 and CD86), and promotes the secretion of inflammatory cytokines including TNF and IL-12 (Figure 18.4) [41,43].

CD40's ligand, CD154, was the first target of monoclonal antibodies aimed at inhibiting the CD40–CD154 pathway. Targeting the ligand tends to be safer than targeting the receptor, since binding the receptor has the potential to promote agonistic signaling. In addition, in comparison to CD40, CD154 has more limited expression. Early trials of monoclonal antibodies targeting CD154 were encouraging in mouse and non-human primate models, mostly when used in combination with other therapies including CTLA-4–Ig, donor specific transfusion, and rapamycin [23,44–47]. In both Phase I trials using a humanized mAb to CD154 (Hu5C8) for lupus patients and kidney transplantation, as well as subsequent studies with non-human primates, an unexpectedly high rate of thromboembolic events occurred, now thought to be related to the expression of CD154 on platelets and the role of CD154 in clot stabilization [48,49]. Further development of Hu5C8 as well as other anti-CD154 antibodies has been suspended. However, if efforts to manipulate the Fc portion of anti-CD154 mAb to prevent its adhesion to platelets are successful, clinical trials will likely resume. In the interim, interest in blockade of this pathway has now shifted to the development of anti-CD40 monoclonal antibodies. A chimeric anti-CD40 mAb (Chi220) prolonged islet allograft survival in NHP in combination with belatacept and prolonged renal allograft survival in combination with anti-CD86 antibodies in another NHP model [23 45,50,51]. Other novel antibodies targeting CD40 in the pipeline are: OM11-62MF, fully human (Novartis, Basel, Switzerland) currently being studied in patients with rituximab-refractory lymphoma; PG102, deimmunized chimeric (PanGenetics, Utrecht, Netherlands), a recently terminated (due to poor recruitment) study in patients with psoriatic arthritis; and, finally, ASKP1240, a fully human antibody (Astellas Pharma, Deerfield, IL and the only antibody being tested in the transplant population. Following the safe completion of a Phase Ia trial in healthy volunteers, Astellas has completed a Phase Ib, randomized, double-blind, parallel-group placebo-controlled pharmacokinetic, pharmacodynamic safety and tolerability study using ASKP1240 in de novo kidney transplantation in centers across the United States (http://clinicaltrials.gov/ct2/show/NCT01279538) [52].

Memory T cells and Ox40–Ox40 L/LFA-3/CD2 pathways

Combined blockade of the CD28/B7 and CD40/CD154 pathways in an attempt to promote allograft tolerance and prolong allograft survival in experimental transplant models has shown success [23,45]. However, this combination is not always effective in preventing allograft rejection [53,54], and a new phenomenon, coined "costimulation blockade-resistant rejection" has emerged, with the effector memory T cell implicated as the main culprit. Effector memory T cells are less dependent on CD28 costimulation for activation [55,56] and, even in the absence of costimulation blockade, have been identified as a barrier to transplant tolerance [57–59]. While calcineurin inhibitors are effective at inhibiting the

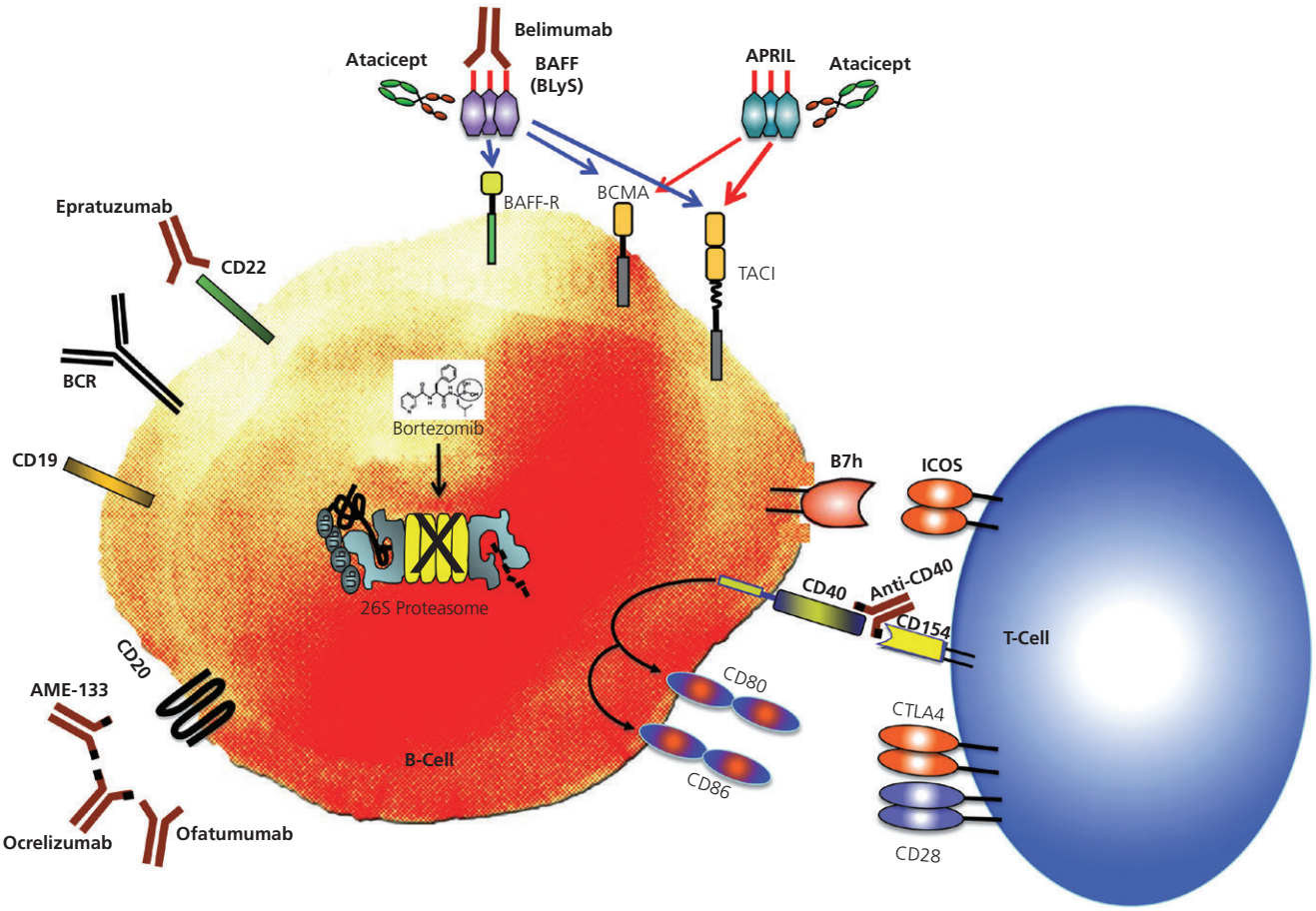

Figure 18.4. The CD40-CD40L (CD154) pathway. Reproduced from [52] Webber A, Hirose R, Vincenti F. Novel strategies in immunosuppression: issues in perspective. Transplantation 2011; 91:1057–1064, with permission from Wolters Kluwer Health.

de novo generation of $CD8^+$ memory T cells and the function of a pre-existing memory T cell repertoire, CNI-associated toxicities have prompted a search for alternatives [60].

OX40 (CD134) is expressed on activated T cells, and, when bound to its ligand (OX40L), which is expressed on dendritic cells, B cells, activated endothelial cells, and macrophages, promotes effector T-cell activation, as well as the humoral immune response through B-cell proliferation [61,62]. Conversely, OX40/OX40L ligation on T regulatory cells is an inhibitory (negative costimulatory) signal and results in the down-regulation of FoxP3 and therefore the loss of suppressor Treg cell function [63]. But perhaps most important is the OX40/OX40L role in $CD4^+$ memory T cell generation. OX40/OX40L interactions are imperative for the creation of a robust $CD4^+$ memory pool [64,65].

In a CD28/CD154 double-knockout mouse model to study skin allograft rejection, a vigorous rejection occurred largely dependent on OX40 costimulation [34]. In this study, neither treatment with mCTLA-4–Ig and anti-CD154 combined nor anti-OX40L alone prevented rejection in a wild-type mouse, but the mice treated with the combination of mCTLA-4–Ig, anti-CD154, and anti-OX40L experienced long-term skin allograft survival [34]. Similarly, long-term cardiac allograft survival was realized using a combination of CTLA-4–Ig and anti-OX40L in a rat heart transplant model [66]. The OX40/OX40L pathway is therefore a desirable target for future manipulation in solid organ transplantation.

While the OX40–OX40L costimulation pathway is vital for the proliferation and survival of $CD4^+$ memory T cells, the CD2–LFA-3 pathway is critical for $CD8^+$ effector memory T-cells function. CD2, a cell surface protein present on activated and memory T cells, acts as both an adhesion as well as a costimulatory molecule when bound to its ligand, LFA-3 (CD58). Alefacept (human LFA-3–IgG1 fusion protein, Amevive, Biogen, Cambridge, Mass) is a fusion protein FDA approved as an immunomodulatory therapy for psoriasis [67]. Interestingly, psoriatic plaques are characterized by infiltrating effector memory T cells and alefacept has been shown to preferentially reduce circulating effector memory T cells as well as effector memory T cells localized to psoriatic plaques in patients [68,69]. In response to the findings of increased acute rejection rates in patients treated with belatacept in the BENEFIT trial [29], a study aimed at better characterizing human effector memory T cells implicated in costimulation blockade resistant-rejection using flow cytometry was initiated. This study found that effector memory T cells, compared to other memory T-cell subsets, expressed the most CD2 [70]. Further, they found that alloresponsive $CD8^+CD2^{hi}CD28^-$ T cells produced the most cytokines and cytotoxic molecules (IFN-γ, TNF, and IL-2 granzyme B, CD107a) of all alloresponsive T-cell subsets, and that these cells are belatacept resistant. They theorized that these cells may have been responsible for the higher rejection rates seen in the BENEFIT trial [29]. The addition of alefacept in vitro inhibited belatacept-resistant

alloresponsive proliferating $CD8^+$ T cells [70]. The same group had previously demonstrated that, when used in combination with CTLA-4–Ig (abatacept) and sirolimus, alefacept significantly prolonged renal allograft survival in NHP [71].

Two trials were conducted (in the United States and Europe) comparing Alefacept administered initially i.v. and then subcutaneously for 3 months with a variety of tacrolimus-based regimens [72,73]. The alefacept-treated patients had either similar or higher rejection rates based on the tacrolimus dosing and the use of mycophenolate acid [72,73]. However, there were no safety signals and, as shown in the psoriasis studies, memory T cells in circulation were depleted. Astellas has decided to discontinue development of alefacept for renal transplantation. While an intriguing combination, a clinical trial combining CTLA-4–Ig and alefacept, previously shown to prolong allograft survival and deplete effector memory T cells in NHP [71], is unlikely to be performed.

LFA-1/ICAM pathway

Leukocyte function associated antigen-1 (LFA-1) is a beta integrin heterodimer and is composed of a unique alpha chain (CD11a) and a common beta chain (CD18) [74]. LFA-1 acts as a direct costimulatory molecule [75] but is also an integral component in stabilizing the immune interface between the TCR and MHC complex [76]. In addition, it is a cell adhesion molecule central to lymphocyte trafficking, primarily by interacting with intracellular adhesion molecule-1 (ICAM-1) [74,75]. It is expressed on T cells, B cells, and, like CD2, is more highly expressed on memory T cells [77,78].

Multiple studies using monoclonal antibodies to block the LFA-1/ICAM pathway in kidney transplantation have been performed [79–83]. Efalizumab is a humanized anti-LFA-1 monoclonal antibody, which had been FDA approved for the treatment of psoriasis [84–86]. A Phase I/II open-label multicenter trial using efalizumab in renal transplantation enrolled 38 patients and divided the patients into low-dose efalizumab (0.5 mg/kg) or high-dose efalizumab (2 mg/kg) at weekly subcutaneous doses in addition to maintenance therapy with either full-dose cyclosporine, mycophenolate mofetil, and steroids or half-dose cyclosporine, sirolimus, and prednisone [83]. While patient and allograft survival at 6 months were excellent, 8% of patients developed PTLD, albeit all of them treated with 2 mg/kg efalizumab and full-dose cyclosporine [83]. Eight patients with type 1 diabetes underwent islet cell transplantation and received induction with antithymocyte globulin followed by maintenance with efalizumab (0.5 mg/kg) plus sirolimus or mycophenolate. All patients achieved insulin independence and there were no reported adverse events related to efalizumab, and, interestingly, a marked increase in circulating $CD4^+$ $FoxP3^+$ T regulatory cells was demonstrated [87]. Similarly, another study using efalizumab for islet cell transplantation in diabetic patients showed that all patients treated with the efalizumab-based regimen achieved insulin-independence after a single islet cell infusion [88]. In April 2009, efalizumab was voluntarily withdrawn from the United States market due to safety concerns specifically related to progressive multifocal leukoencephalopathy (PML) seen in patients treated for psoriasis (www.fda.gov/Safety/MedWatch/default.htm). However, in light of the recent surge of interest in costimulation blockade-resistant rejection, a study, capitalizing on the known increased expression of LFA-1 on effector memory T cells, showed that TS-1/22, a mouse antihuman CD11a mAb, when combined with basiliximab and sirolimus or belatacept, prolonged pancreatic islet allograft survival in NHP [89]. Future clinical trials in transplantation will likely investigate the effect of combinations of biologics, targeting both costimulatory pathways integral to naïve T cell activation (i.e. with belatacept, anti-CD40) as well as pathways vital to the proliferation and activation of memory T cells (i.e. alefacept, anti-LFA-1, anti-OX40), and other pathways critical to the alloresponse, including complement and B-cell proliferation pathways. In a study using a mouse model of heart transplants, the combination of CTLA-4–Ig with a monoclonal antibody to the complement factor C5 (for both the donor and the recipient) showed significant prolongation of graft survival [90]. These combinations have shown great promise in experimental transplantation [34,70,71,90,91], but in human models may be limited by their potential for over-immunosuppression.

Future therapeutic targets of B cell and antibody-mediated injury

With recent data supporting the notion that chronic antidonor antibody mediated injury may be the primary pathology responsible for late allograft loss, therapeutic targets aimed at the B cell and plasma cell have been under active investigation in transplantation [7,8,92,93]. Current therapies used in desensitization protocols as well as for the treatment of acute and chronic antibody mediated rejection (AMR) include plasmaphersis, IVIG, and a chimeric anti-CD20 monoclonal antibody, rituximab [94–98]. Newer therapies with anecdotal success in AMR and desensitization include bortezomib and eculizumab. Bortezomib, a proteasome inhibitor, targets metabolically active, rapidly dividing cells by selectively inhibiting the 26s proteasome and inhibiting the transcriptional activator nuclear factor kappa B, thereby inducing apoptosis. It is FDA approved for the treatment of plasma cell dyscrasias, and by targeting the normal plasma cell in transplantation, has shown promise in reducing donor-specific antibodies and combating AMR in several case series, albeit limited by its neurotoxicity [99–104]. Eculizumab, a humanized anti-C5 antibody FDA approved for complement-associated diseases, including paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome (both recurrent and de novo post-transplant) [105–108], has been shown to decrease the incidence of AMR and transplant glomerulopathy in desensitization protocols [109] as well as improve outcomes in the treatment of AMR post-transplantation [110]. More agents targeting the complement cascade as a means to curtail complement-dependent alloantibody-mediated injury are likely to surface in the next few years; however, rigorous clinical trials will be required for bortezomib and eculizumab to better define their efficacy and safety.

It is important to note that B cells participate in the alloimmune response on many levels. They are clearly an integral component of antibody-mediated injury, but they are also effective antigen-presenting cells, and are thus crucial to the activation of T cells [111]. Thus agents targeting the B cell itself may become valuable therapies in promoting more enduring graft acceptance (Figure 18.5). New B-cell depleting antibodies in the biopharma pipeline include monoclonal anti-CD20 antibodies atumumab, ocrelizumab, veltuzumumab, and the B-cell depleting anti-CD22 antibody, epratuzumab, which is currently under investigation in a Phase IIb trial for lupus (www.clinicaltrials.gov) [112,113]. Belimumab and atacicept are novel B-cell therapeutics, mainly studied in the treatment of autoimmune diseases and blood-borne malignancies, with unique targets. Belimumab is a fully human monoclonal antibody that binds to and inhibits the action of soluble B-lymphocyte

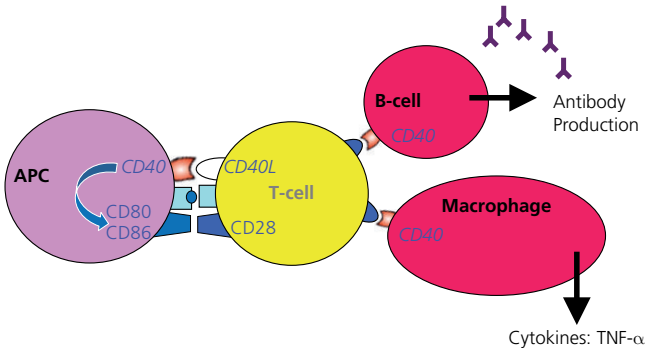

Figure 18.5. Novel B-cell targets.

stimulator (BLys, also known as B-cell activating factors BAFF). BLys is a tumor necrosis-factor family ligand that acts as a B-cell survival factor when bound to its receptors BAFF-R, B-cell maturation protein (BCMA), and the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) [10]. Belimumab has been efficacious in the treatment of systemic lupus erythematosus and has recently received FDA approval for this indication [113–115]. The University of Pennsylvania is investigating belimumab as a desensitizing agent for highly sensitized renal transplant recipients in a 1-year, Phase II study (www.clinicaltrials.gov).

Atacicept is a fusion protein composed of an extracellular domain of TACI, fused to the constant region of human IgG1. It interferes in the binding of a proliferation-inducing ligand (APRIL) to its targets BCMA and TACI, and thereby impedes B-cell stimulation and proliferation. Importantly, APRIL is required for later stages of B-cell differentiation and plasma cell survival [10]. A Phase II study of atacicept plus mycophenolate in patients with lupus nephritis was halted early due to an increase in infectious complications [113,116]. Further studies in lupus patients are ongoing. At present, there are no clinical studies investigating the role of atacicept in transplantation, but given its proven potency in reducing plasma cells and immunoglobulin levels, its future role in desensitization protocols and treatment of antibody-mediated rejection seems promising.

IL-6

The cytokine IL-6, traditionally considered a regulator of acute phase inflammatory responses, is emerging as a critical promoter of TH17 cells and inhibitor of T regulatory cells [117]. The relevance of the effects of IL-6 in autoimmune disease is well established both experimentally and clinically with the first anti-IL-6 therapy Tocilizumab (Genetech), a humanized anti-IL-6 receptor antibody, already approved for rheumatoid arthritis. The two other biologics targeting IL-6 are being developed for autoimmune disease by Bristol-Myers and Regeneron. Experimentally the effect of anti-IL-6 therapy in transplantation is most pronounced in combination with costimulatory blockade [118]. At present single center trials are underway with Tocilizumab at Cedar Sinai as a desensitization therapy to improve transplant rates (clinicaltrials.gov # NCT01594424) and at UCSF to treat inflammation and borderline rejection (clincialtrials.gov). If successful these efforts may be a catalyst for industry to consider extending their clinical development to organ transplantation.

Small molecules

JAK3/protein kinase C

Cytokine receptors that utilize the common gamma chain (i.e. receptors for T- and B-cell growth factors IL-2, 4,7, 9, 15, and 21) all require the cytoplasmic tyrosine kinase Janus kinase 3 (JAK3) for signaling. Cytokine signaling through JAK3 is critical for lymphocyte activation and differentiation and is the third and final signal (after MHC-TCR engagement and costimulation signals) necessary for T-cell activation [119,120]. In fact, illustrating its potent role in lymphocyte biology, mutations in the common gamma chain and/or JAK3 result in a severe combined immunodeficiency (SCID) phenotype [121]. JAK3's expression (as opposed to JAK1/2 expression) is predominantly limited to lymphocytes and natural killer cells, thereby making it a critical component of the alloimmune response and an appealing target to prevent allograft rejection [122]. The small molecule JAK3 inhibitor tofacitinib (originally named CP-690,550) has shown efficacy in the treatment of human autoimmune disorders [123,124] and interest in its role as an agent in transplantation followed, especially in light of its potential for selectively inhibiting T effector cell function while preserving the suppressive activity of $CD4^+$ $FOXP3^+$ T regulatory cells [125].

After successful use of tofacitinib in mice and NHP models of transplantation [126–129], a Phase I trial using JAK3 inhibition in renal allograft recipients was performed [130]. In this dose-escalation study, the co-administration of tofacitinib at different dosing regimens with mycophenolate mofetil in 28 stable renal transplant patients at least 12 months out from transplant revealed an acceptable safety and tolerability profile with doses ranging from 5 mg twice daily to 30 mg twice daily [130]. A Phase IIa, multicenter, randomized, open-label pilot study comparing two dosing regimens of tofacitinib (15 mg p.o. b.i.d. and 30 mg p.o. b.i.d.) compared with tacrolimus in de novo kidney transplant recipients induced with anti-IL2 receptor antibodies and maintained on MPA and steroids found the incidences of biopsy-proven acute rejection (BPAR) at 6 months to be 5.3% in the 15 mg twice daily group (CP15), 21.1% in the 30 mg twice daily group (CP30) and 4.8% in the tacrolimus group [16]. Estimated GFRs at 6 and 12 months were similar across all treatment groups but there was a higher incidence of BK virus nephropathy (20%) and CMV disease (21.1%) in the CP30 group compared to the tacrolimus group (0%). In terms of metabolic parameters, there was no difference in the incidence of NODAT; however, dyslipidemias were more common in the two groups treated with tofacitinib, with all cholesterol parameters increased by up to 34% and 44% in the CP15 versus CP30 groups respectively [16].

Data from the Phase IIb study has been published [131]. In this study, 322 de novo kidney transplant patients were randomized to one of three groups: tofacitinib (CP) dosing starting at 15 mg p.o. b.i.d. in groups 1 and 2, but the group 1 dose was reduced to 10 mg b.i.d. after month 6 and group 2 dose was reduced to 10 mg b.i.d. after month 3, versus cyclosporine [132,133]. All patients received induction with anti-IL2R antibodies and were maintained on mycophenoloic acid and steroids. Both tofacitinib groups demonstrated non-inferiority in 6 and 12 month biopsy-proven acute rejection rates, with statistically significantly higher estimated GFRs at 12 months (64.6 and 64.7 mL/min CP1 and CP2 versus 53.9 mL/min cyclosporine). The incidence of NODAT was reduced in both tofacitinib groups compared with cyclosporine; however, immunosuppressive–related complications, including infection (i.e. CMV) and malignancy, were higher in both tofacitinib

group. Alarmingly, five patients in the tofacitinib groups developed PTLD. No one in the cyclosporine arm developed PTLD [132,133]. Thus tofacitinib's role in transplantation is limited by the need to elucidate the optimal dosing regimen required to prevent rejection without precipitating adverse consequences from over-immunosuppression.

Protein kinase C

Finally, sotrastaurin, a novel small molecule targeting protein kinase C isoforms PKCθ and PKCα, represents yet another example of an agent studied in transplantation that targets calcineurin-independent pathways. Protein kinase C is central to antigen receptor signal transduction pathways in lymphocytes, and PKCθ and PKCα are isotypes crucial for IL-2 and IFN-γ production, respectively [134]. After showing prolonged renal allograft survival in murine and NHP models [135–138], and its utility and safety in the treatment of psoriasis patients in a proof-of-concept study [139], oral sotrastaurin was studied in a Phase II investigation evaluating its safety and efficacy in de novo renal transplant recipients [140]. In this study 216 patients were randomized to one of three groups: sotrastaurin 200 mg twice daily + tacrolimus standard exposure (SET), sotrastaurin 200 mg twice daily + tacrolimus reduced exposure (RET), versus control tacrolimus + MPA. Sotrastaurin recipients meeting conversion criteria were converted to a CNI-free regimen at month 3. All patients received induction therapy with anti-IL-2R antibodies and were maintained on steroids [140]. Prior to CNI withdrawal in the sotrastaurin groups, the primary efficacy endpoint (composite of BPAR, graft loss, and death) were equal amongst the groups; however, by the end of the study (after withdrawal of CNI in the sotrastaurin groups), the primary efficacy end point was reached in 7.8% of the control group, but 44.8% and 34.1% of patients randomized to the sotrastaurin + SET and sotrastaurin + RET, respectively. Conversely, the incidence of adverse events, including GI, cardiac, and infectious, were equal amongst the groups [140].

A more recent study randomizing de novo renal transplant patients to either a tacrolimus-based regimen or a sotrastaurin based regimen (at 300 mg twice daily dose) plus MPA and steroids (after anti-IL2R induction) had similar findings. Namely, despite improved eGFR in the sotrastaurin group at 3 months (59 ml/min vs. 49.5 mL/min), the primary efficacy endpoint (composite BPAR, graft loss, or death) was reached by statistically significantly more patients in the sotrastaurin arm than controls (25.7% vs. 2%, respectively), and the study was terminated early. Again, adverse events were similar between the two groups [141].

Summary

An editorial written by H. U. Meier-Kriesche and B. Kaplan entitled *The Search for CNI-Free Immunosuppression: No Free Lunch* aptly paraphrases the frustration involved in the ongoing search for novel targets in transplantation [142]. JAK3 inhibitors, while equally as efficacious at preventing allograft rejection as CNI's, do so at the expense of precipitating more adverse events. Conversely, PKC inhibitors, with similar incidences of infection and malignancies, fail to provide similar efficacy in endpoints including biopsy-proven acute rejection and allograft survival rates. Agents targeting costimulatory pathways appear remarkably efficacious against naïve immune responses, but less suited to control memory responses, leading to a combined phenotype of efficacy and adverse event that is largely dependent on the recipient's prior immune history. Nonetheless, it is clear that the future of transplantation and its quest for CNI-free immunosuppressive regimens is underway. Whether using combinations of novel biologics, optimizing the therapeutic window of small molecules, or some combination thereof will ultimately provide robust immunologic tolerance to the allograft while maintaining an intact immune system to other antigens, has yet to be elucidated.

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SECTION 3

Organ Procurement

CHAPTER 19

Administration of Organ Procurement and Allocation

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Introduction

Ethically sound and technically successful organ transplantation is logistically complex. It requires substantial attention to the best practices for identifying potential donors, obtaining donation authorization, determining donor medical suitability, coordinating the recovery and distribution of all donated organs, and maintaining methods for fiscal sustainability of the transplant process. Although historically, dedicated transplant clinicians based at the few centers with active transplant programs handled these functions, over the past four decades these needs have increasingly been codified in federal and state law and exercised by specific Organ Procurement Organizations (OPOs). This chapter examines the role of OPOs in organ recovery and allocation—how they are organized and operate, the legal and institutional framework within which they operate, and their integration into the general fabric of clinical transplantation. The chapter initially lays out the basis for OPOs, and the state and federal statutory frameworks governing their conduct of organ procurement and distribution. It then walks the reader through the process, noting the important interactions required to facilitate a successful organ placement.

This chapter focuses on the US' experience, and does so not as a matter of exclusivity, but rather to provide a means for a systematic survey of the numerous functions served by OPOs. The authors are cognizant of the fact that this is a prominent, but not exclusive, way to manage organ acquisition and distribution. The chapter closes with a brief discussion of the organizational models used in selected countries outside the US, with specific reference to the model originating from Spain and used in many countries outside the US. This section provides contrast and international perspective. An exhaustive account of all the practiced approaches is beyond the scope of this chapter.

What is an Organ Procurement Organization?

In the US, OPOs play "a crucial role in ensuring that an immensely valuable, but scarce resource—transplantable human organs—becomes available to seriously ill patients who are on a waiting list for an organ transplant" [1]. They are the entities designated by the federal government to be responsible for promoting organ donation, for identifying potential organ donors, and for recovering and distributing the recovered organs for transplantation into all patients who require them. OPOs are the stewards of the nation's donation process.

OPOs were created by the National Organ Transplant Act (NOTA), passed by the US Congress in 1984 [2]. The NOTA established a national system of OPOs to recover and distribute donated organs from deceased donors. The system and the OPOs exist today in substantially the same form as initially established by the NOTA. Additional discussions of national organization and regulation of transplant activities can be found in Chapter 128.

Each OPO is responsible for a defined geographic area, i.e. a "donation service area" (DSA). DSAs are not uniform. They vary in area, from approximately 3600 square miles to >866 000 square miles; in population numbers, from 1.3 million to 19.1 million people; in population diversity (ethnic, racial, urban, suburban, rural); and in number of hospitals served, from 12 to approximately 220 acute care hospitals in a single DSA. The country is divided into 58 DSAs, which are operated by 58 OPOs—hospitals or universities operate seven, and the others are serviced by independent entities E. Eidbo, Association of Organ Procurement Organizations, personal communication, February 19, 2013). The OPOs function as the foundation level of a national system of recovery and allocation that is coordinated through the Organ Procurement and Transplantation Network (OPTN).

The NOTA provided two sources of federal government funding: (1) Medicare reimbursement, under the authority of the Social Security Act (SSA), as the principal source of funding for kidney procurement, and (2) Health Resources and Service Administration (HRSA) grants for OPOs [3]. Participation in the Medicare reimbursement system requires that OPOs file cost reports, which are audited and adjusted by Medicare on a retrospective basis. In addition, Medicare regulates the amount that each OPO may charge a transplant hospital for a kidney [4].

OPOs are required to have arrangements to be paid for the acquisition of all organs. Payments from transplant centers for organ acquisition charges (OACs) are the single largest source of OPO funding. Each OPO develops its own OACs, based on its cost accounting data, which include direct acquisition expense

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(including inpatient hospital services, physician services, laboratory testing, transportation, and other medical expenses), clinical staffing, information technology, administration, and the expenses related to mandated activities in community/public education and education for hospital and medical professionals.

Many OPOs also provide tissue donation services, including cornea donation, in their DSAs. In these tissue efforts, OPOs must comply with Food and Drug Administration (FDA) rules, in addition to Centers for Medicare and Medicaid Services (CMS) certification requirements for their organ procurement status.

The NOTA and the SSA place OPOs under the general supervision of the Secretary of Health and Human Services (HHS). They operate under regulations issued by the HHS CMS [5], and under the policies and procedures of the OPTN [6]. OPTN itself operates under the general supervision of the HHS Health Resources and Services Administration (HRSA) [7]. OPOs are subject to both federal and state law.

State law framework

Between 1967, when the world's first successful heart transplant was performed, and the passage of the NOTA in 1984, transplantation developed into an effective mainstream medical treatment. By 1978, the science was sufficiently defined that the federal government began reimbursing, through Medicare, the transplantation costs associated with end-stage renal disease. The need for transplantable organs of all types grew rapidly. Then, as now, the numbers of organs available for transplant greatly exceeded the numbers of desperately ill patients waiting for them.

Transplantation's emergence as an accepted therapy was facilitated by developments in state law that gave clear legal authority for the strides being made in science and medicine. The National Conference of Commissioners on Uniform State Laws (NCCUSL) [8] proposed two laws: the Uniform Anatomical Gift Act of 1968 (AGA) and the Uniform Determination of Death Act of 1981. When adopted into state law, these laws provided the legal foundation for the expanded availability of transplantation as a medical treatment. [The NCCUSL (now called the Uniform Law Commission) is a non-profit entity supported by the states. Through a broad-based collaborative process, it develops and recommends the adoption of uniform statutes on matters of national importance that by law are the responsibilities of the states. NCCUSL describes its function as bringing "clarity and stability to critical areas of state statutory law."] These state laws are the legal foundation on which OPOs, and numerous other aspects of transplantation, rely today.

The Uniform Determination of Death Act (UDDA)

The Uniform Determination of Death Act (UDDA) was issued by the NCCUSL in 1981 [9]. It recognized a new medical and scientific consensus that death could be determined based on neurologic criteria (brain death), in addition to the traditional cessation of circulatory function (discussed in detail in Chapters 21 and 22, respectively). This expert view had no precedent in the common law, which in the absence of specific precedent, acted as an impediment to organ recovery and transplantation. UDDA proposed that states enact a statute to permit death to be determined based on the cessation of neurologic function. UDDA was adopted by 38 states; but all states came to enact laws that recognized death by neurologic criteria. Based on this new clarity in the law, donors whose deaths were determined on neurologic criteria rapidly became the principal source of donated organs.

The Uniform Anatomical Gift Act of 1968 and its successors [10]

The AGA was introduced in 1968 to clarify uncertainties in common law that emerged with the new transplantation science. It was adopted rapidly in all 50 states and the District of Columbia.

It laid out, for the first time, a statutory right to give, receive, and use an anatomical gift from a deceased donor. The AGA recognized that individuals and their families had, effective at death, a right to donate organs, eyes, and tissues for transplantation, therapy, research, and education. It established some essential rules about who could recover anatomical gifts, to whom those gifts could be given, and how those gifts could be used. It gave immunity from legal liability to those who acted in good faith to comply with the AGA, thereby alleviating a significant concern for all participants in the developing donation and transplantation process [11].

NCCUSL emphasized the importance of the AGA in encouraging people to donate and in building a national legal foundation for organ donation and transplantation. It stated: "Both the common law and the present statutory picture is one of confusion, diversity and inadequacy . . . [T]he Act will provide a useful and uniform legal environment throughout the country for this new frontier of modern medicine" [12].

The AGA evolved through two major revisions in 1987 and 2006 that were made necessary by emerging federal law, by uncertainties left by prior AGA versions, and by the still developing science of transplantation. The 1987 revision, following federal law, clearly established the basic rule that it is unlawful to "sell, procure, transfer or facilitate the transfer for valuable consideration . . . of any part that could be the subject of an anatomical gift" (section 10) and specified that "valuable consideration" did not include "reasonable payments" for removing, processing, preserving, quality control, storage transportation, and implantation of a donated part. The Revised Uniform Anatomical Gift Act (RUAGA), issued by NCCUSL in 2006, brought state and federal law into harmony for the first time. It also restored the state law uniformity that the first AGA sought and that states (and NCCUSL) were unable to maintain in the face of developing medical science and changes in federal law.

The RUAGA expands and simplifies the methods by which individuals can make an anatomical gift; it now includes all the methods that recent experience has shown to be most helpful in encouraging donation, e.g. designation on a drivers permit, registration in a donor registry, having a healthcare agent, or any signed record, including a will or advance directive. It clarifies who has priority to make an anatomical gift. The AGA recognized the right of the individual to make an anatomical gift; however, OPOs, hospitals, families, doctors, other medical professionals, and the community at large routinely acceded to the wishes of bereaved family members—often at the expense of a decision made by the deceased individual. The RUAGA addressed this issue squarely. If a person has made a decision to donate, or to refuse to donate, the RUAGA bars everyone else from changing that decision [13]. The bar applies to everyone; however, because of their front-line role in the recovery process, OPOs have a primary responsibility for honoring the donor's wishes and ensuring that others do as well. The RUAGA is now the law in 46 states, and even where it is not, the law requires that the deceased individual's decision be respected.

Reflecting the donation and transplantation practices of the time, the 1968 AGA and its 1987 revision both permitted hospitals, surgeons, and physicians to receive donated organs and tissues

for transplantation and therapy. The 2006 RUAGA, however, authorized donation to a named person for transplantation and therapy if the named person is the recipient of the donation, or is an eye or tissue bank. All other donations for transplantation or therapy must be received by the appropriate procurement organization (i.e. eye, tissue, or organ). Donations for research and education can still be made to named institutions [14].

The RUAGA also spells out a list of OPO rights and duties. When a hospital refers a potential donor to the OPO (as required by federal law), the OPO must make a reasonable search of department of motor vehicles records and donor registries to see if the referred individual is registered as a donor, and must make a reasonable search for any other person authorized to make an anatomical gift. It authorizes the OPO to make any reasonably necessary examination, including an examination of all medical and dental records, to determine the medical suitability of a part that could be an anatomical gift, and further provides that while this examination is being conducted, "measures necessary to ensure the medical suitability of the part may not be withdrawn unless the hospital or procurement organization knows that the individual expressed a contrary intent." After the donor's death, the RUAGA authorizes the OPO, among others, to conduct any reasonable examination necessary to ensure the medical suitability of the body or part for its intended purpose [15].

When a patient dies in a hospital, state law determines, among other things, whether the patient is dead, and whether the patient or another person authorized by law made an anatomical gift that can be recovered if medically suitable.

Federal law framework

Prior to the NOTA in 1984, the US did not have an organized or coherent national response to the task of recovering and distributing organs. The system developed in an ad-hoc manner, responding to local and regional circumstances and the events of the time. The state laws described above were an outgrowth of this process. Organ procurement agencies (OPAs) began operating in the 1960s and their functions were a natural outgrowth of the expanding availability of transplantation. By 1984, approximately 90 OPAs operated in the US. Fifty were hospital based and 40 were separately incorporated legal entities serving multiple hospitals and regional areas [16].

The congressional hearings and public discussion that preceded passage of the NOTA focused attention on the persistent and growing gap between organ supply and the inability of the existing process to resolve the issue. A national response was seen as a necessary part of the answer, and the NOTA sought to address the shortage by creating a national system that would encourage donation and ensure recovered organs were distributed and used in an equitable manner [17].The NOTA established the basic institutional framework for organ recovery, distribution, and use in the US; it created a Task Force on Organ Transplantation that was charged with responsibility for conducting a broad ranging inquiry into organ donation and transplantation in the US, and with making recommendations on how the system could be improved (see Chapter 137).

Institutions and system created by the NOTA

Prohibition of the sale of organs

The Act declared it unlawful to "knowingly acquire, receive, or otherwise transfer any human organ for valuable consideration for use in human transplantation" [18].It also provided that: "valuable consideration" did not include "reasonable payments" associated with the "removal, transportation, implantation, processing, preservation, quality control, and storage of a human organ or the expenses of travel, housing, and lost wages incurred by the donor of a human organ in connection with the donation of the organ" [18].

Creation of OPOs

The Act organized a nationwide network of OPOs to take on functions previously exercised in an ad-hoc manner by the 90 OPAs. The NOTA prescribed the structure, governance, and responsibilities of these OPOs. It also required the OPO to have an advisory board to make recommendations to its regular board about all matters pertaining to organ procurement and allocation. To assure the independence of this advice, the NOTA prescribed that the members of this advisory body should represent all parties with an interest in organ recovery and procurement, e.g. transplant hospitals and surgeons, members of the public, donor families, hospital administrators, eye and tissue banks, and individuals with skill knowledge and experience in neurology and histocompatibility.

The NOTA required OPOs to be members of the newly formed OPTN, the entity created for the purpose of coordinating OPOs at the national level and operating a national list of people requiring transplants. The role of the OPTN is discussed more fully below.

The NOTA and subsequent amendments set in place a mechanism that mandated co-operation with all other participants in the donation process, e.g. donor hospitals, transplant hospitals, and tissue and eye banks, although it left it to the affected parties to work out the content of that co-operation. These requirements are incorporated, in expanded form, in federal regulations administered by CMS [19].

Creation of the OPTN

The OPTN was established to: develop and maintain a computerized organ transplant waiting list, now called UNET; to facilitate organ matching and placement; to develop consensus-based policies and procedures for organ recovery, allocation, and transportation; to collect and manage scientific data on the organ transplantation process; to provide data to the government and others, including the Scientific Registry of Transplant Recipients (SRTR; see Chapter 133 for additional detail regarding this registry), for use in the effort to improve solid organ allocation and transplantation; and to provide professional and public education about donation and transplantation, the activities of the OPTN, and the critical need for donation [20] (see Chapter 137).

The OPTN is operated by a private, non-profit contractor, the United Network for Organ Sharing (UNOS). The UNOS operates and maintains UNET on behalf of the OPTN. UNET is the computer system used to receive donor and recipient information and facilitate organ allocation and distribution. Transplant hospitals are responsible for placing data on the UNET waitlist program, and keeping current the name and pertinent medical information of patients in need of transplants. UNET maintains different lists for different organ systems. OPOs use the DonorNet module of UNET to enter similar information for donors. They then use this program to match the donor with possible recipients listed on the UNET waitlist program.

OPOs are required to be members of the OPTN. The OPTN requires its members to comply with its policies and procedures

[21]. The HHS requires OPOs to comply with the OPTN "rules and requirements," which are OPTN pronouncements that the HHS has determined should have the force of law [22]. Like CMS, the OPTN also measures the effectiveness of OPO performance.

Creation of the SRTR

The SRTR was established by the NOTA to be a repository of accurate information about all organ transplants performed in the US [23]. OPOs among others are required to submit specified information to it on a regular basis [24]. The data collected in the registry support ongoing evaluations of the status of solid organ transplantation in the US.

The SRTR also designs and implements scientific studies using the accumulated data to better understand the performance of the US donation and transplantation system. It disseminates its information and analyses to the transplant community (transplant programs, OPOs, policy makers, transplant professionals, transplant recipients, organ donors and donor families, and the general public). It also gives qualified researchers access to data to assist their independent efforts to study various aspects of solid organ transplantation.

The US organ transplantation system employs evidence-based allocation policy development through collaborative efforts between the OPOs, transplant community, the SRTR, and the OPTN. Policy making is the OPTN's responsibility; the SRTR, however, plays an important role by performing data analyses to provide policy makers with the information necessary to make informed decisions.

SRTR is administered for the HHS by the Chronic Disease Research Group of the Minneapolis Medical Research Foundation [25].

NOTA requires OPOs to be effective

Since the inception of the national system, one goal has remained constant: that of closing the gap between available organs and the people awaiting transplantation. In 1984, the NOTA envisaged that participants in the donation and transplantation process would make continuous efforts to improve their performance. Changes in the law after that time have aimed to strengthen that initial and perpetually relevant idea.

The Organ Procurement Organization Certification Act of 2000, P.L. 106-505 [26], required CMS to develop and apply standards to measure OPO performance. The Act was designed to rectify uncertainties created by the performance measurements then in use. In 2006, CMS issued new performance standards in response to this legislation [27]. These regulations specified that OPOs (other than those in non-contiguous states) must meet all three of the following outcome measures to retain their certification to operate as an OPO [27]:

- The OPO's donation rate of eligible donors as a percentage of eligible deaths must be no more than 1.5 standard deviations below the mean national donation rate of eligible donors, averaged over the 4 years of the OPO's recertification cycle. Both the numerator and denominator of an individual OPO's donation rate ratio are adjusted by adding 1 for each donation after cardiac death and for each donor over the age of 70 years.
- The observed donation rate is not significantly lower than the expected rate for 18 or more months of the 36 months of data used for recertification purposes.
- At least two of the three following yield measures are no more than 1 standard deviation below the national mean, averaged over the 4 years of the recertification cycle:
  - the number of organs transplanted per standard criteria donor, including pancreata used for islet cell transplantation;
  - the number of organs transplanted per expanded criteria donor, including pancreata used for islet cell transplantation; and
  - the number of organs used for research per donor, including pancreata used for islet cell research.

Failure to meet all outcome measures resulted in automatic decertification. Within the OPO community, these outcome measures were criticized for failing to provide any useful insight into OPO performance. OPOs requested CMS to revise the regulation. In July 2013, CMS acknowledged OPO concerns and proposed several regulatory revisions [28]. In particular, CMS determined that an OPO would retain its certification if it satisfied two, instead of three, of the outcome measures. Despite continuing OPO concerns about the validity and utility of the outcome measures that were articulated again during the rulemaking proceeding, the proposed rule became final in December 2013 [29].

CMS performance evaluation

At this time (January 2014), the 2006 regulation, as amended in December 2013 to require compliance with two of the three outcome measures, is the standard by which OPO performance is measured. OPOs are required to comply with that regulation in order to be certified by CMS to operate as an OPO.

CMS contemplates that an OPO that fails to meet these outcome measures will lose its certification to operate as an OPO. Once an OPO is decertified, its DSAs could then be taken over by any other entity that submits an application to CMS and is approved as meeting the requirements to be an OPO [30].

As noted above, OPOs are also required by law to comply with OPTN policies and procedures and rules and requirements. The OPTN uses a different methodology to determine whether an OPO is effective at its job.

OPTN performance evaluation

The OPTN uses a performance methodology developed by the SRTR using a set of statistical models and based on actual donor information collected in the registry. These models take into account various donor characteristics that are not under the control of the OPOs, in an attempt to risk adjust for differences in the types of donors managed by each OPO. A separate risk adjustment model is used for each of six organs: heart, intestine, kidney, liver, lung, and pancreas. The models were based on donors from whom at least one organ was recovered for the purpose of transplant (not necessarily transplanted), and "yield" is defined as an organ that was eventually transplanted. Expected yields are given for kidneys, lungs, hearts, livers, pancreata, and intestines. For hearts, livers, pancreata, lungs, and intestines, the expected yield will range from 0 to 1. For example, if the expected liver yield is 0.7, one would expect a similar donor to yield a transplanted liver seven out of 10 times. The lung yield is modeled as the probability of yielding at least one lung for transplant; therefore, expected lung yield ranges from 0 to 1 rather than 0 to 2. For kidneys, the expected yield will range from 0 to 2. The total expected organ yield is then calculated as the sum of the organ-specific expected yields and ranges from 0 to 7. On an organ-specific basis, the models predict

how many organs would have been expected to be recovered and transplanted based on the national experience for donors with similar characteristics.

In July 2012, the OPTN adopted SRTR's expected organ yield methodology to monitor OPO performance. The performance requirement is contained in the OPTN Bylaw, Appendix B (Membership Requirements for Organ Procurement Organizations), at Section B.2 [31]. This section also specifies that the OPTN Membership and Professional Standards Committee (MPSC) will evaluate all OPOs to determine if the difference in observed and expected organ yield can be explained by some unique aspect of the DSA or OPO in question.

The MPSC is required to review all OPOs whose observed organ yield rates fall below the expected rates by more than a specified threshold. The absolute value of the relevant parameters in the formula may be different for different organs. It is anticipated that the values will be reviewed and modified periodically, as the OPTN Board agrees.

The initial criteria used to identify a lower than expected organ yield, for all organs as well as each organ type, are:

- >10 fewer observed organs per 100 donors than expected yield (Observed per 100 donors – Expected per 100 donors $=< -10$);
- a ratio of observed-to-expected yield of <0.90;
- a two-sided P value of <0.05.

An MPSC review is triggered when an OPO meets all three criteria. If an OPO's organ yield rate cannot be explained by donor mix or some other unique clinical aspect of the OPO or DSA in question, the OPO member, in co-operation with the MPSC, is required to implement a performance improvement plan. Failure to do so is a violation of OPTN obligations.

Participants in the organ donation and transplantation process have engaged in voluntary efforts, outside the formal legal and regulatory framework, to improve performance by identifying "best practices," and by using evaluation methods that more accurately reflect the effectiveness of OPO efforts. The most far-reaching and potentially transformative of these efforts was the Organ Donation Breakthrough Collaborative (ODBC or Collaborative).

Organ Donation Breakthrough Collaborative

The ODBC started in 2003 as an initiative of the Secretary of HHS conducted under the auspices of the HRSA's Healthcare Systems Bureau (HSB), Division of Transplantation (DoT). Its aim was to dramatically increase the availability of transplantable organs and provide best practices for organ recovery and placement. The HRSA worked in partnership with the Institute for Healthcare Improvement (IHI), Quality Reality Checks, Inc. (QRC) and teams of OPOs and their hospital and transplant center partners from across the country to create change that could lead to breakthrough transformations in the system for organ donation.

The initial focus of the effort was national in scope and aimed to improve organ donation conversion rates (the conversion rate is the ratio of the number of actual donations occurring at the hospital as compared to the number of eligible donors) in hospitals with more than eight eligible donors annually. The methodology used was built on the Model for Improvement, a nationally recognized and highly effective method of performance improvement used by the IHI.

During the first two cycles of the ODBC conducted in 2003–2005, the HRSA and an expert faculty, comprised of healthcare professionals from high performing organizations across the country, helped each participating organization try to achieve goals, set by HRSA, to:

- notify OPOs of all deaths or imminent deaths in a timely manner;
- increase the donation authorization rates in participating hospitals by 30%;
- increase the donor conversion rates to 75% or greater;
- notify OPOs of all pending decisions to withdraw mechanical support from patients with non-survivable conditions;
- increase to 100% the number of families who receive organ donation counseling from a trained and effective donation request team;
- respond to all death notifications in <90 min.

The goal of the ODBC was to identify best practices and create systems based on those practices that would assure, within each DSA nationwide, accurate and timely donor referral, screening, consent, organ recovery, and placement.

In 2006, during the fourth cycle of the ODBC, the HRSA introduced "organs transplanted per donor" (OTPD) as another way to assess OPO, transplant program, and donor hospital performance. The OTPD is the ratio of the number of all organs transplanted to the number of actual organ donors. The HRSA established goal is to achieve an average OTPD rate in excess of 3.75 organs transplanted from each donor.

In 2007, the ODBC pushed its national learning strategy to the regional level to provide a local, more accessible platform for ongoing improvement efforts and to complement the ongoing national learning sessions. The purpose was to sustain cross-DSA learning opportunities in pursuit of national performance goals; to make cross-DSA learning opportunities accessible to more OPOs, and hospital and transplant center representatives; and to create local and regional ODBC leadership opportunities that would enhance individual professional development. This strategy proved only minimally successful and was discontinued as a major point of focus in 2012. A broader approach involving all partners in every DSA seems to be the most effective means of implementing best practice and evaluating outcomes.

The HRSA established outcomes goals for each DSA are:

- 75% conversion rate;
- 10% of all donors to be donation after circulatory death (DCD) donors;
- 3.75 OTPD;
- 20% increase in volume of deceased donor organs transplanted.

The ODBC produced breakthrough increases in both organ donation and transplantation. To ensure that this progress continues, the work of the ODBC has been institutionalized in the "Donation and Transplantation Community of Practice (DTCP)," a grouping that includes all the various disciplines and professionals involved in the donation and transplantation process nationwide. The DTCP is organized and coordinated by the Organ Donation and Transplantation Alliance (the "Alliance") with funding provided by an HRSA grant. The Alliance is a 501(c)(3) non-profit, independent organization incorporated in 2006 for the purpose of ensuring a continued national commitment to increasing organ availability and eliminating deaths on transplant waitlists. The Alliance provides a permanent structure for seeking lasting cultural change within the organizations and individuals whose cultural and institutional behavior impact organ donation and transplantation. The Alliance performs this task by continuing specific activities focused on meeting and achieving the national organ donation and transplantation goals established by the HRSA. The Alliance, in

partnership with the HRSA, oversees and organizes meetings, subject-focused task forces, and learning opportunities for the DCTP. Experience with the DTCP and the OBDC shows that the goal of 35 000 organs transplanted per year will be achieved only by creating a donation and transplantation system open to learning and systematically engaged in monitoring, assessing, and improving its own performance [32].

How does the system work?: step-by-step from recovery through allocation

The overarching theme of this section is that the system works best when all participants, be they individuals (doctors, nurses, hospital executives and administrators, and OPO staff) or institutions, understand the importance of organ donation, subscribe to organ donation and recovery as a mission in its own right and not just an afterthought, know their roles in the process in advance, and co-operate to implement the shared mission. At each step, co-operation and timely communication and coordination between all the parties involved are essential. For a more detailed discussion about effective donation approaches, see [33,34].

The organ donation and recovery process applies only to those deaths that occur in hospitals. Some 2.46 million people died in the US during 2010. [35,36]. Of those who die each year, approximately 30% (or 738 000) die in hospital. The deaths most likely to be suitable for donation are those that occur in the hospital emergency department (ED) or intensive care unit (ICU). Living donations have emerged as a significant source of donated organs. The OPTN has adopted some policies and procedures applicable to such donations [35], but the system described here addresses only donation after death.

Notice to OPO of all deaths or imminent deaths

The hospital and the OPO are required by law to ensure that every family is informed of its organ donation options [37], and to ensure that this right is not compromised, they are required to have an agreement to notify the OPO of every death or imminent death in the hospital. The OPO is required to co-operate with the hospital and the hospital's chosen eye and tissue banks to ensure that tissue banks and eye banks receive timely notice of possible eye and tissue donors [38]. Hospitals may elect to make separate referral calls to the tissue and eye banks. Most often, however, the hospital makes only one call to the OPO or the OPO's referral call center. The OPO then coordinates with the eye and tissue banks, or in still other instances, performs the eye and tissue recoveries under agreement with the hospital or with the tissue and eye banks. These regulatory obligations seek to ensure that the maximum number of eyes and tissues are recovered, as well as the maximum number of organs. They also seek to ensure a coordinated approach to families, so that families are not subject to the distress of multiple donation requests from different organizations.

Since the adoption of the UDDA and other state laws recognizing brain death, most donors have donated after cessation of neurologic function—so-called DND donors. More recently, improvements in medical science have resulted in increased attention again being paid to DCD (also known as circulatory death). All OPOs are required to have a policy on DCD recoveries whether or not it performs them [39]. If an OPO does perform DCD recoveries, its agreements with hospitals are required to address DCD recoveries, although the law does not require that hospitals engage in such recoveries [40]. Currently, most OPOs do perform DCD recoveries.

Table 19.1. Imminent death clinical triggers for hospital referral calls used by Washington Regional Transplant Community (WRTC)

Hospital shall notify WRTC of:

1 Every ventilated patient who meets one or more of the following criteria:
- Glasgow coma score of 5 or less without continuous sedation
- Brain death testing being considered or pursued
- Patient being made do not resuscitate (DNR) or family considering comfort care measures
- Life-sustaining therapy is to be withheld
- Family initiates a conversation about donation

Note: these triggers are in addition to required notification for non-ventilated patient deaths

2 Every patient death, within 1 h of death

Reproduced with permission of WRTC.

A patient who has serious, irrecoverable neurologic injury, but has not clinically progressed to neurologic death, is a candidate for DCD recovery. The decision to withdraw life-sustaining medical treatment will have been made by advance medical directive or a legal surrogate. When the medical intervention (the ventilator) is stopped, and circulatory and respiratory functions cease, the patient is then declared dead following a defined period of asystole, ventricular fibrillation, or pulseless electrical activity. To be a viable organ donor, the patient must be declared dead within a limited time frame following the removal of the ventilator. The time limits may vary by OPO protocol, but generally all are within a period of 60–90 min. The recovery of organs then is carried out rapidly to ensure organ viability.

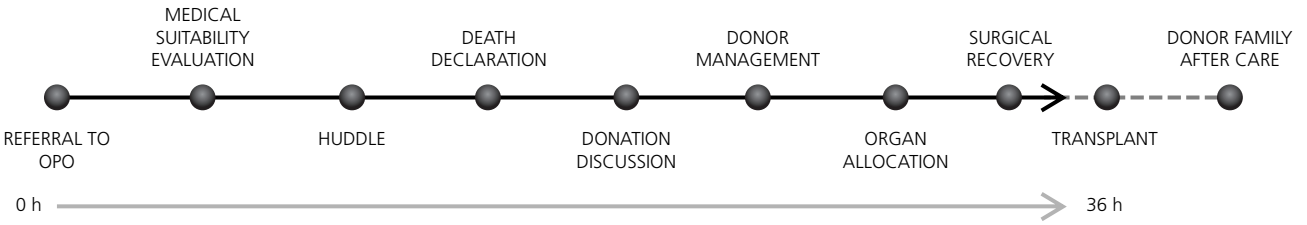
Clinical triggers for the referral call

The OPO and the hospital agree in advance on the clinical criteria that trigger the referral call. There is currently no single set of standard clinical triggers that all OPOs and hospitals use. The particular triggers used may depend on the preferences and capabilities of the OPO, and the hospital concerned, e.g. some hospitals do not perform DCD recoveries. Table 19.1 shows an illustrative set of the clinical triggers used by one OPO within its DSA.

In general, a referral made >1 h after a death occurs is untimely. Early notification of imminent death is the best practice. The clinical triggers define when death is considered "imminent." They intend that the hospital will not remove ventilator support or make any treatment decisions that foreclose the possibility of donation until the OPO has been notified. This will help to ensure that the OPO has adequate opportunity to perform its statutory responsibility to identify potential donors and ensure that every family knows of the option to donate.

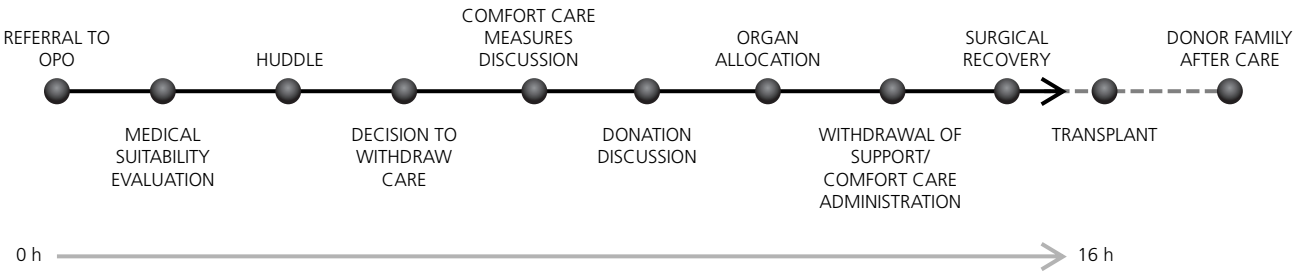
Donor identification and evaluation

The OPO is open 24/7 to receive these referral calls and to begin the process of identifying potential donors. Figures 19.1 and 19.2 illustrate the donor identification, recovery, and allocation timelines with respect to DND and DCD donors that are initiated by the referral call made on the death or imminent death of each and every one of the individuals who dies in hospital. Upon receipt of the call, the OPO has the legal right to review the patient's medical record and at this point, begins to perform its initial screening to determine the medical suitability of the potential donor. This initial screening may be performed over the phone or by OPO staff on site at the hospital.



REFERRAL TO OPO	➢	30–45 min obtaining referral information; 1–1.5 h arrival of OPO at hospital
MEDICAL SUITABILITY EVALUATION	➢	30 min chart review to determine medical suitability
HUDDLE	➢	5 min (may take 15 min–1.5 h to get all parties available for huddle)
DEATH DECLARATION	➢	4–24 h for brain death determination (may take several additional hours if brain death is not definitively diagnosed and additional testing is required)
DONATION DISCUSSION	➢	1–3 h for donation discussion with family/LNOK (includes 1–2 h discussion with family/LNOK for review of past medical/social history)
DONOR MANAGEMENT	➢	8–10 h for serologies and preliminary tissue typing results; 2–12 h awaiting special procedures (echocardiogram, bronchoscopy, special labs, etc.)
ORGAN ALLOCATION	➢	5 h (liver, kidney) and 24 h (thoracic) for organ-specific allocation to primary recipients and required back-up recipients
SURGICAL RECOVERY	➢	4–8 h for surgical recovery of organs (delay in OR availability may increase ICU time)
DONOR FAMILY AFTER CARE	➢	2-year program, including grief seminars, widowhood support group, donor family retreat, annual donor family gathering, facilitate communication between donor families and recipients

Figure 19.1. Optimal timeline for do not resuscitate (DND) donor identification, recovery, and allocation process initiated at the death or imminent death of all persons who die in hospital. Timeline used by Washington Regional Transplant Community (WRTC). (Reproduced with permission of WRTC.) OPO, Organ Procurement Organization; LNOK, legal next of kin; OR, operating room; ICU, intensive care unit.



REFERRAL TO OPO	➢	30–45 min obtaining referral information; 1–1.5 h arrival of OPO at hospital
MEDICAL SUITABILITY EVALUATION	➢	30 min chart review to determine medical suitability
HUDDLE	➢	5 min (may take 15 min–1.5 h to get all parties available for huddle
DONATION DISCUSSION	➢	1–3 h for donation discussion with family/LNOK (includes 1–2 h discussion with family/LNOK for review of past medical/social history)
ORGAN ALLOCATION	➢	2–12 h organ specific allocation to primary recipients and required back-up recipients
SURGICAL RECOVERY	➢	2–4 h surgical recovery of organs (delay in OR availability may increase ICU time)
DONOR FAMILY AFTER CARE	➢	2 year program, including grief seminars, widowhood support group, donor family retreat, annual donor family gathering, facilitate communication between donor families and recipients

Figure 19.2. Optimal timeline for donation after circulatory death (DCD) donor identification, recovery, and allocation process initiated at the death or imminent death of all persons who die in hospital. Timeline used by Washington Regional Transplant Community (WRTC). (Reproduced with permission of WRTC.) OPO, Organ Procurement Organization; LNOK, legal next of kin; OR, operating room; ICU, intensive care unit.

The OPO also proceeds to check state Department of Motor Vehicle (DMV) records and donor registries to determine if the patient has made an anatomical gift.

Role of the family in making an anatomical gift and obtaining authorization

If the patient is registered with the DMV or a donor registry as a donor (or has made any other document of gift), no other permission is required for the donation to be effective, and the OPO, hospital, physicians, and the donor family are required to uphold the patient's decision. If the patient is not a donor, federal law requires that every family be offered the option to donate or to decline. The OPO is responsible for locating a reasonably available decision maker, usually the patient's next-of-kin, to whom the request to donate must be made. An approach to the family, whether made for authorization or to inform the family of the patient's own decision, is made only after consultation between the OPO and hospital staff, and is conducted by staff who are trained in making such approaches. Federal law requires the approach to be made with discretion and sensitivity to the circumstances, views, and beliefs of the potential donor family. Federal law as well details the types of information required to be provided to the family [41].

Based on studies and experience, it has been the practice to approach the family about donation after it is informed of, and had time to accept, the patient's terminal prognosis. More recently, this premise has been re-examined. New evidence indicates that families are more likely to respond favorably to a donation request when the request is seamlessly integrated into an end-of-life care process that respects the grief of family members, and that communicates with them openly and often about dying and appropriate end-of-life care options [42]. The newfound importance of DCD donors as a source of available organs has increased the importance of addressing end-of-life care in a holistic way that continues after the recovery has been performed (Figures 19.1 and 19.2).

DCD recoveries are extremely time sensitive and optimally require that some prerecovery treatment be administered to the donor to facilitate the donation. Regardless of whether the patient made a donation decision or left that decision to surrogates, the administration of any prerecovery treatment requires assistance from the family before death is declared [43]. OPOs and hospitals are examining how best to make this approach so that families do not lose the option to donate by not knowing about this until organ viability is compromised and donation for transplantation is no longer an option.

Death declaration

The patient's physician declares death in accordance with state law. The OPO confirms that the declaration of death satisfies the requirements of the law. To protect the integrity of the donation process and to assuage the concern of donors and their families that the need for organs may dictate end-of-life decision-making, state and federal laws prohibit the patient's physician and the physician who determined the patient's death from taking any part in the recovery or transplantation process. Once death is declared, or the family agrees, as in the case of the DCD recovery discussed above, the OPO proceeds with the remaining tests and examinations necessary to determine whether the potential donor is medically suitable to be a donor. Federal regulation and OPTN policies and procedures spell out tests that are required, who must perform the tests and in what time frame, and the information about the potential donor that must be gathered, e.g. the medical and behavioral history (risk assessment). The OPO is responsible for donor evaluation and management [44].

Organ allocation

All organs from a deceased donor are required to be recovered and distributed through the national system created by the NOTA. All patients who require a transplant must be listed on the national waitlist. No patient can receive a deceased donor organ outside this process, except in the rare possibility, permitted by the RUAGA, of a donor designating a specific individual to receive the donation and the named individual is a match.

When the OPO determines that an individual is medically suitable to be a donor and authorization for donation is confirmed, the OPO enters the donor's information, including at a minimum, blood type and tissue type, in the OPTN's national organ allocation program, DonorNet, so that the computer algorithms appropriate to the particular organ can be run to identify potential recipients [45]. The computer match run yields a list, or lists, of potential recipients in rank order, depending on the organs available and based on screening criteria provided by the particular transplant center. The OPO then offers the available organ(s) to the transplant center or centers that listed the potential recipient(s). The transplant center has 1 h to accept or express interest in the offer. The OPO is primarily responsible for making the organ offer; the transplant center, however, has ultimate responsibility for deciding in any particular instance whether an offered organ is transplanted into a patient. The OPTN encourages the OPO to make back-up offers to ensure against possible organ wastage.

Recovery

The OPO is responsible for coordinating the recovery, a task that includes making arrangements with the donor hospital for access to the operating room and the necessary staff support, arranging for the recovery teams, and subsequently for packaging and arranging to transport the organs to their intended destinations. Organ recovery may be performed by any physician or technician qualified to perform it. The OPO is required to maintain credentialing records on all physicians or other practitioners who regularly recover organs within the OPO's DSA. It also is required to ensure anyone who performs a recovery in its donor hospitals, including members of recovery teams from outside the DSA, is qualified and trained. The OPO medical director, who is required to be a physician, is responsible for implementing the OPO's protocols on donor evaluation and management and organ recovery and placement. The medical director is as well responsible by law for supervising the clinical management of potential donors, and for assistance in managing a donor case when the surgeon on-call is not available [46].

Record keeping

The OPO is responsible as well for obtaining and maintaining records of recovery activities in its DSA, including obtaining out of DSA recovery teams records pertaining to the organs recovered by those teams. The records kept by the OPOs are used by the OPTN (as discussed above) for OPO evaluation purposes and by SRTR for research purposes.

Quality assurance obligations

In 1984, the NOTA required OPOs to perform annual evaluations of their own effectiveness in acquiring all available organs. Since then, in response to the chronic shortage of donated deceased donor organs, these requirements have become considerably more specific. CMS regulations require the OPO to have a formal "data driven" quality assessment and performance improvement program. The program must cover all areas of OPO activity and the OPO must act on and monitor any identified deficiencies. At least monthly, the OPO must conduct death record reviews in the hospitals from which it receives referrals to identify missed or mishandled donation possibilities. CMS also requires the OPO to have written policies and procedures for identifying and correcting any adverse events that occur in the course of its work [47].

The Association of Organ Procurement Organizations (AOPO) has developed a peer-reviewed accreditation program that requires accredited OPOs to implement organizational and ethical standards that in many respects are more detailed and rigorous than those required by CMS. Fifty-three of the nation's 58 OPOs are accredited by AOPO and another three are in the process of becoming accredited. AOPO conducts accreditation reviews every 3 years. The OPO's ability to meet AOPO and CMS quality standards is a principal focus of these reviews.

These programs provide the means by which OPOs can gain a detailed understanding of their own operations, which is an essential prerequisite for the required self-improvement efforts that have marked US efforts to close the gap between the organs needed for transplant and those available.

In addition to these explicit quality assurance activities, the OPOs are subject to regular audits conducted by CMS and the OPTN as part of their oversight responsibility. A survey team from the OPTN/UNOS conducts ongoing and periodic reviews and evaluations of each member OPO for compliance with OPTN rules and policies. OPOs are surveyed every 3 years by the OPTN. The OPTN will also conduct special reviews of an OPO if there is reason to believe that the OPO may not be in compliance with these rules and policies, or may be acting in a manner that jeopardizes patient safety.

International organ donation models

All donation activities operate under some form of national authority for donation and transplantation. A solid legal framework that provides the authority for donation, defines an organizational structure, establishes policies for organ allocation, and prescribes a financing model is critical to the procurement system.

The organizational structure and staffing of organ procurement functions vary globally with each nation responding to its unique challenges, resources, and political and legal frameworks. While there is no accepted international model, most donation systems across Europe generally follow either the independent OPO-based model of the US or the frequently referenced Spanish model [48].

OPOs as independent non-profit companies are organized to serve a defined geographic area and operate outside of donor hospitals or transplant centers. In contrast, the Spanish model utilizes organ procurement personnel who are located within the donor hospital (Figure 19.3). Referred to as "transplant procurement management (TPM) coordinators", the inhospital staff are hospital employees who carry a part-time dedication to organ procurement. Most transplant coordinators are ICU doctors or anesthesiologists who are responsible for identification and evaluation of donors, donor management, and family authorization. Registered nurses also serve in this role. Each hospital has dedicated staff and the transplant coordinators typically do not travel to donor hospitals outside of their home institution. Onsite familiarity with potential donor patients, through a dual role of treating physician and procurement professional, allows close monitoring of potential donations, but requires clear separation of duties.

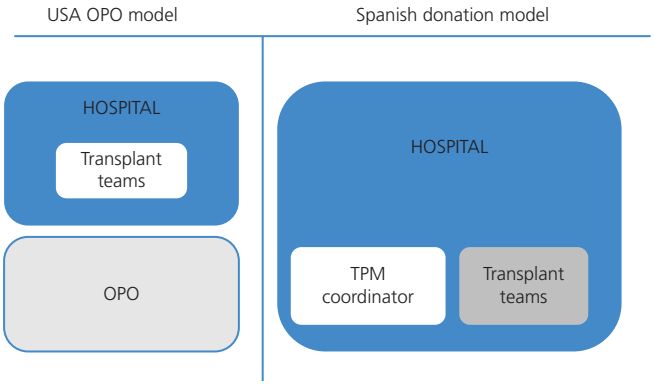


Figure 19.3. Contrast between the US and Spanish organizational models. Spain utilizes organ procurement personnel located within the donor hospital. (Reproduced from Vidal MM, et al. Transplant Coordination Manual, 2001; Transplant Procurement Management – TPM: A transplant coordination model to procure donors for transplantation, by permission of TPM – Les Heures, Universitat de Barcelona.) OPO, Organ Procurement Organization; TPM, transplant procurement management.

While OPOs in many cases have implemented inhouse donor coordinators, this is significantly different from the Spanish transplant coordinator model. OPO inhouse coordinators are employed by the OPO, and do not have shared accountability for patient care and donation. The professional profile is also markedly different, with most OPO coordinators having clinical backgrounds in nursing, respiratory therapy, or a related field. In contrast, in Spain and several other European countries, physician model is primarily used.

Many countries have adopted key components of both the US and the Spanish model, including the utilization of hospital-based, part-time transplant coordinators. Some countries, such as Germany, have created a national organ procurement system, the DSO, which is based on the OPO structure of centralized oversight and independent/regional organ procurement teams. Changes over time tend to incorporate elements of both models, with the German DSO, for example, introducing a pilot hospital-based donor program [49].

Responsibility for organ allocation and distribution also varies significantly between countries. Eurotransplant, a non-profit organization, is responsible for organ allocation and distribution for seven European nations. Other countries such as Switzerland have centralized organ-sharing offices located outside of the OPO activities [50]; and in some cases the local organ procurement team is responsible for determining organ allocation.

With perhaps the exception of Eurotransplant centers, organ allocation policies are much less complex than those adopted by UNOS/OPTN.

While there is significant variability in structure, authority, and operating scope of donation programs across Europe, all programs remain focused on the shared goal of providing organs for transplantation. The professionalization of procurement organizations and sharing of international best practices will lead to global improvements in donation.

Summary

The US organ procurement and allocation system operates on a nationwide basis with the goal of making the best and most equitable use of a scarce resource, donated deceased donor organs. The system was started in response to the shortage of transplantable donated organs and it continues to be driven by that persistent scarcity.

OPOs are the most visible public face of donation in the US. They are responsible, each within their geographic service area, for educating the public about organ donation; for persuading members of the public to use driver license and donor registries to become organ donors; and for interacting with the families of all potential donors who die in hospital, to obtain family authorization for donation if necessary and to provide them with information if not, but in any event to provide them with bereavement support.

Within the hospital setting, the OPO is responsible for determining whether a potential donor is medically suitable to donate. It manages the deceased donor until the recovery takes place, and arranges all aspects of the organ recovery, including coordinating and overseeing the medical teams that perform the actual work of removing the organs from the deceased donor. It locates potential recipients through UNET, extends offers to the transplant center that listed the potential recipient, and, upon acceptance of an offer, arranges transport to the pertinent transplant center for transplant into the listed patient.

OPOs are regulated by CMS and the OPTN, and are subject to audit inspections by both to review all aspects of their operations, and their performance in identifying donors and recovering organs is subject to measurement and frequent review. Most OPOs are also accredited by the AOPO and subject to its standards and audit requirements. The OPOs are held accountable for their performance and are responsible for identifying and implementing improvements.

The gap between the number of organs available and those needed for transplant, which impelled the passage of the NOTA in 1984, has not been closed. The number of organs available in any given DSA is a function of population size and location (e.g. rural, urban, or suburban), as well as other demographic characteristics, such as age, race, ethnicity, religion, and incidence of public health issues (HIV, hypertension, diabetes, heart disease). The organ donation and transplantation community and its regulators have focused considerable time and effort on identifying and understanding the societal impediments to donation, and the cultural and structural obstacles to organ donation and transplantation that exist within the community itself. To the extent that these factors are within the control of OPOs and hospitals to influence, they can be best addressed by closer co-operation and coordination between all the institutions and individuals involved in the donation and transplantation process. The ultimate effectiveness of the system depends on all the parties, the hospitals, doctors, nurses, administrators, and their OPO counterparts, working together over the long haul to make organ donation a valued societal priority.

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CHAPTER 20

Deceased Donor Management

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Introduction

Organ transplantation remains the only life-saving option for an increasing number of patients with a variety of medical conditions that lead to failure of vital organs and death. Advances in the transplantation process and the prevention and management of post-transplant complications have resulted in markedly decreased morbidity and mortality in recipients of transplanted organs. This has increased the spectrum of disease states where transplantation is considered an appropriate treatment. This in turn has led to an increase in demand for organs for transplantation with resultant increases in the number of patients waiting for life-saving organs.

The opportunity for organ donation occurs either as a voluntary gift from a living donor or at the time of declaration of death in the appropriate setting, i.e. deceased organ donation. Living donation is discussed specifically for kidney and liver transplantation in Chapters 23 and 24, respectively. In the situation where deceased organ donation is possible, the event of death must occur in such a way that the transplantable organs do not suffer significant injury. Deceased donors of organs are declared dead either by neurological criteria (DNC; formerly called "brain dead donors"), donation after circulatory determination of death [DCDD; formerly called donation after cardiac death (DCD)], or non-heart beating donors (NHBD). Additional specifics regarding DCDD donation can be found in Chapter 22.

DNC donors represent the majority of donors utilized in the US and Europe, and provide the largest potential pool of organs annually for transplantation. DCD accounts for ~12% of the annual deceased donors in the US, with fewer organs transplanted per donor. According to recent data from the Organ Procurement and Transplantation Network (OPTN), the transplantation needs are far from being met. At the end of 2010 there were more than 120 000 patients awaiting organ transplantation, and only 22 000 patients receiving a transplant, with 11 000 patients dying on the waiting list or becoming too ill to be transplanted in 2010 [1]. Therefore, despite a steady increase of patients in need of organ transplantation, the number of organs available for transplantation has increased minimally, while the imbalance between organ availability and need continues to widen.

Over the past decade concerted efforts to make more Americans aware of the critical need for organs for donation led to modest increases in donation, with the numbers of organ donors annually increasing from ~5000 per year to ~8000 per year; however, this has plateaued over the last 5 years. In addition, attempts to increase organ utilization per donor have not significantly changed the average number of organs transplanted from each donor, which remains at around three organs per donor. This is due in part to the changing characteristics of donors that represent higher risk for organ failure, such as older age, diabetes, overweight, hypertension, and causes of death that represent concomitant medical conditions. Thus, a major challenge is how to maximize the number of organs available for transplantation by impacting the numbers of viable organs recovered and successfully transplanted from each donor, while improving the post-transplantation functionality of each organ to maximally benefit each recipient.

There is little in the way of published science on the medical management of the organ donor and what is available is often "best practice" approaches, small observational studies, or retrospective reviews. In most situations, donor management decisions post declaration of death is the responsibility of the Organ Procurement Organization (OPO) for the region in which the donor is located. There has been a recent trend of OPOs to involve intensive care physicians to assist in the management of the deceased donor, which may arguably present some of the most challenging physiological issues they may face. This practice has met with some success [2].

The critical care management of the deceased organ donor can at its simplest be broken into two phases: one prior to pronouncement, the pre-OPO management; and one after declaration of death occurs (OPO management). However, it is much more complex as optimal management represents a continuum, all based on accepted critical care principles. Initially, care of the critically ill intensive care unit (ICU) patient includes supporting cardiac output and maintaining oxygen delivery, essential to improving the potential for survival from the devastating injuries/illnesses that threaten recovery. Additionally, if the patient succumbs to the underlying process and fulfills neurological criteria for brain death, this hemodynamic support increases the likelihood of successful donation of organs should the patient go on to become an organ donor.

The timely recognition of the ICU patient who cannot survive their devastating illness or traumatic process, and subsequent continued support of their cardiopulmonary system, is essential to minimize the insults that may potentially compromise function and

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use of organs. During the course of the ICU management team care of the critically ill patient, when likelihood of futility of care is increasing, it is imperative that the individual's wishes about end-of-life care are clarified. In the situation where progression to death by neurological criteria is imminent, intentions regarding organ donation should be determined – in the case of first person consent this may be made known through donor registries or through other legal documents, or if this information is absent, the next of kin should be approached regarding willingness to pursue donation of their family member. Family refusal to donate remains a significant impediment to organ donation. Studies have shown that this can be minimized with appropriate interactions between the ICU team and the family that facilitate understanding and communication with the family throughout the patient's ICU stay.

The correct recognition and timely declaration of death by neurological criteria is also critical. Delay in diagnosis or non-recognition of evolving death by neurological criteria is another concern that puts organ donation at risk. However, educating healthcare professionals about clinical features and diagnosis of brain death can minimize this.

Even after the diagnosis of DNC, loss of a potential donor can occur in spite of implementation of OPO management protocols. Organ donors unfortunately show such heterogeneity in their clinical presentations that care of these patients is challenging for the healthcare professionals. DNC triggers numerous physiological changes associated with hemodynamic instability and end-organ damage. Up to 25% of potential donors are lost due to clinical deterioration after the development of DNC [3]. Therefore, appropriate management of potential donors is essential to prevent organ damage and loss [4,5]. With increasing understanding of the physiology of DNC and the ability to mitigate the potential physiological changes with appropriate critical care management strategies, significant increases in the number of transplantable organs as well as improved post-transplantation organ function in the recipient may be achieved [6]. Physiological changes begin with the herniation process and, if left untreated, may lead to rapid deterioration and cardiovascular collapse [7]. These changes are associated with profound dysregulation of hormones, massive cytokine release, and numerous electrolyte abnormalities, which may be blunted, moderated, or even reversed with a number of management interventions. Significant efforts have been made to maintain or improve organ function following DNC and have successfully increased the number of donors for those who need transplantation [3,6,8].

In this sense, a standardized approach with a focus on multiorgan resuscitation with maintenance or improvement of oxygen delivery (DO_2) to transplantable organs would be a useful tool in guiding a systematic and comprehensive approach to overall donor management. This philosophy necessitates an aggressive approach to interventions in the donors, from the declaration of brain death until the operating room and completion of the organ retrieval and preservation processes.

Diagnosis of brain death

Diagnosis of death by neurological criteria

Declaration of death by neurological criteria is a clinical diagnosis made following a devastating neurological injury leading to a comatose state confirmed by serial neurological examinations showing persistent loss of all reflex activities mediated by both brain and brainstem activity [9,10]. The final event in the progression to brain death is the development of catastrophic intracranial pressure (ICP) elevation, leading to loss of brainstem function due to ischemia, infarction, hemorrhage, or herniation. A variety of brain pathology is known to cause elevations of ICP. In the early process of evolving brain death, a rapid expansion of supratentorial lesion or progressive brain edema causes a loss of upper brain function, resulting typically in decreased level of consciousness. Further progression of brain injury causes transtentorial herniation with infratentorial compartment compression.

Brain death is defined as a clinical condition characterized by the total and irreversible loss of function of the whole brain, including the cerebral cortex and brainstem, as set out in the published guidelines in adult patients, first in 1981 [11] with recent updates [12]. Key is that brain death is a clinical diagnosis. It can be made without confirmatory testing if one can establish the etiology, eliminate reversible causes of coma, and conduct a full neurological examination including an apnea test. The diagnosis requires demonstration of the absence of both cortical and brainstem activity as well as the irreversibility of this state. In neurological examinations, the diagnosis of brain death requires documentation of coma or unresponsiveness, loss of brainstem reflexes, and apnea. Prior to initiating testing for DNC, it is necessary to exclude reversible medical conditions that can confuse the clinical assessment including:

- severe imbalance of electrolyte, acid base, and endocrine function;
- drug intoxication and poisoning;
- use of sedatives or neuromuscular blockades;
- hypotension [may suppress electroencephalography (EEG) activity]; and
- hypothermia (core temperature $<36\,^{\circ}C$, pediatric revision requires $>35\,^{\circ}C$).

Once the absence of these exclusions is confirmed, testing of brainstem reflexes is performed with cranial nerve examinations. In DNC, patients show fixed and midpoint or dilated pupils with no response to light, no corneal reflex, no oculocephalic reflex (Doll's eye phenomenon), no oculovestibular reflex (tonic deviation of eyes toward cold stimulus), no gag reflex or cough to tracheal suctioning, and absence of facial muscle movement to noxious stimuli (spinally mediated reflexes with peripheral stimulation may be noted) [12]. Once the absence of brainstem reflexes has been confirmed, apnea testing is then conducted to verify the loss of brainstem function. Certain conditions must be met before an apnea test is performed to avoid cardiac dysrhythmias and systemic hypotension that may occur during the test. These are the prerequisites to performance of the apnea testing:

- normotension;
- normothermia;
- euvolemia;
- eucapnea; and
- absence of hypoxemia.

The procedure for apnea or hypercapnea testing is shown in Table 20.1. The apnea test is confirmed to be unresponsive by the absence of any signs of respiratory effort; a $Paco_2$ of >60 mmHg; or >20 mmHg above baseline for chronic CO_2 retainers. Because apnea testing can induce further clinical deterioration, such as hypotension, severe cardiac arrhythmias, and elevated ICP, it should be performed last in the clinical assessment of brain death. When apnea testing is inconclusive or cannot be done safely, ancillary tests should be considered [9,12].

In the clinical determination of brain death, serial observations further confirm the irreversibility of brain damage. A repeat evaluation of cardinal findings in brain death is recommended. Most

Table 20.1. Apnea test

1. Pre-oxygenate with 100% oxygen for 10–15 min with SaO_2 >95%
2. Reduce ventilator rate to allow for normal PCO_2 (40–45 range) prior to beginning the apnea test:
   - If the patient has chronic CO_2 retention, then the goal is pH 7.35–7.40 prior to beginning the apnea test
3. Obtain initial arterial blood gas (ABG)
4. Remove from ventilator – place on either t-piece with flow by oxygen or with small non-occluding tubing (either cut cannula or feeding tube) with O_2 at 4–6 L flow:
   - Leaving the patient on the mechanical ventilator may result in autocycling of the vent, which may be confused with patient respiratory effort
5. Remove any warming blanket or obstructing material, so chest rise may be observed, and monitor for any signs of respiratory effort
6. Continue to monitor as long as patient remains hemodynamically stable (<20% decrease in blood pressure or heart rate), and O_2 saturations remain >90%. At the 10-min mark, draw a blood gas and replace patient on mechanical ventilator at settings prior to apnea testing while awaiting results of ABG. If at any point these criteria are not met, immediately draw ABG and replace patient on prior ventilator settings while awaiting the results
7. If the $PaCO_2$ rises to >60 Torr and by >20 Torr above baseline without respiratory effort, then this is considered a failed hypercapnea test and is consistent with declared dead by neurological criteria (DNC)
8. If the $PaCO_2$ failed to incrementally rise and the patient was hemodynamically stable at the time the gas was drawn, the test may be repeated. The baseline ABG should be reattained, the test should be redone for an incrementally longer period of time, at least 12 min, and the ABG should be repeated. (repeat steps 6–8 but for 12 min or longer if the patient is hemodynamically stable). If the test is inconclusive and cannot be done due to patient instability, then an ancillary test should be performed to aid in determination of DNC

experts recommend an arbitrary interval of 6 h between initial and repeat observations for clinical determination of brain death in adults.

Special consideration for declaration of brain death in infants and children

The guidelines for the determination of brain death in infants and children were initially published in 1987 [13], with a recent update on the subject [14]. In this update, a few significant changes from the prior recommendations for pediatric determination of DNC have been made.

The clinical exam remains the cornerstone of diagnosis, with waiting periods between serial exams; however, the age ranges have been changed [14].

1. Infants over the age of 37 weeks' gestation to 30 days of life may be evaluated for DNC if they are >24 h old and more than 24 h after receiving cardiopulmonary resuscitation. For this age range the recommendation is that two complete neurological examinations, by two separate physicians, occurring at least 24 h apart and both consistent with DNC be required prior to declaration of death.
2. For pediatric patients aged 31 days to 18 years the time interval is decreased to 12 h, but other stipulations are unchanged.
3. The use of confirmatory testing for instances where the complete examination cannot be completed or to decrease the waiting period.

Ancillary testing for determination of brain death

Ancillary testing for brain death is not mandatory in most situations, but it is necessary for declaring brain death in patients in whom the results of specific components of clinical testing cannot be reliably evaluated (Table 20.2). Choice of testing depends on the discretion of physicians who perform brain death diagnosis. Neurophysiological technology and neurodiagnostic testing are the gold standards for confirmatory testing.

Table 20.2. Clinical conditions that need confirmatory testing to determine brain death

- Coma of undetermined etiology
- Incomplete brainstem reflex testing
- Incomplete apnea testing
- Toxic drug levels
- Children younger than 1 year old
- Required by institutional policy

Cerebral angiography

Selective four-vessel angiography is a neurodiagnostic test performed by a neuroradiologist. An angiographic finding consistent with brain death is absent cerebral blood flow above the entry of the carotid bifurcation or circle of Willis, where the external carotid circulation is patent. From a technical standpoint, the contrast medium should be injected under high pressure in both anterior and posterior circulations. However, the presence of cerebral perfusion does not mean that brain death has not occurred [10].

Electroencephalography

EEG can be used to determine brain death. In patients with brain death, EEG should demonstrate a lack of reactivity to intense somatosensory or audiovisual stimuli over a period of 30 min [9,15]. It should be noted that EEG findings are affected by high barbiturate dosing [16].

Transcranial Doppler ultrasonography

Transcranial Doppler ultrasonography may show abnormalities such as: absent diastolic or reverberating flow; systolic flow only or diastolic reversal flow; and small systolic peaks in early systole. Blood flow velocities may be influenced by marked changes in $PaCO_2$, hematocrit, and cardiac output [17]. A complete absence of flow may not be a reliable finding owing to inadequate transcranial windows for insonation. Ten per cent of patients may not have temporal insonation windows.

Cerebral scintigraphy

Cerebral scintigraphy (technetium nuclear medicine scan) is another alternative to determine brain death. In brain death, the scintigraphy shows no uptake of the radioisotope (hollow skull phenomenon). The isotope should be injected within 30 min after its reconstitution and a static images should be obtained at several time points; immediately, between 30 and 60 min later, and at 2 h.

Somatosensory and brainstem auditory evoked potential

This can be performed at the bedside with a portable instrument that provides bilateral stimulation of median nerves. In patients with brain death, no responses are observed to tests for somatosensory and brainstem auditory evoked potentials. Both types of tests are less sensitive that the other confirmatory tests.

Physiology of brain death

Severe brain injury or disease induces primary damage to the brain that causes secondary cerebral ischemia. As this catastrophic injury progresses to brain death, a cascade of extensive, progressive, and

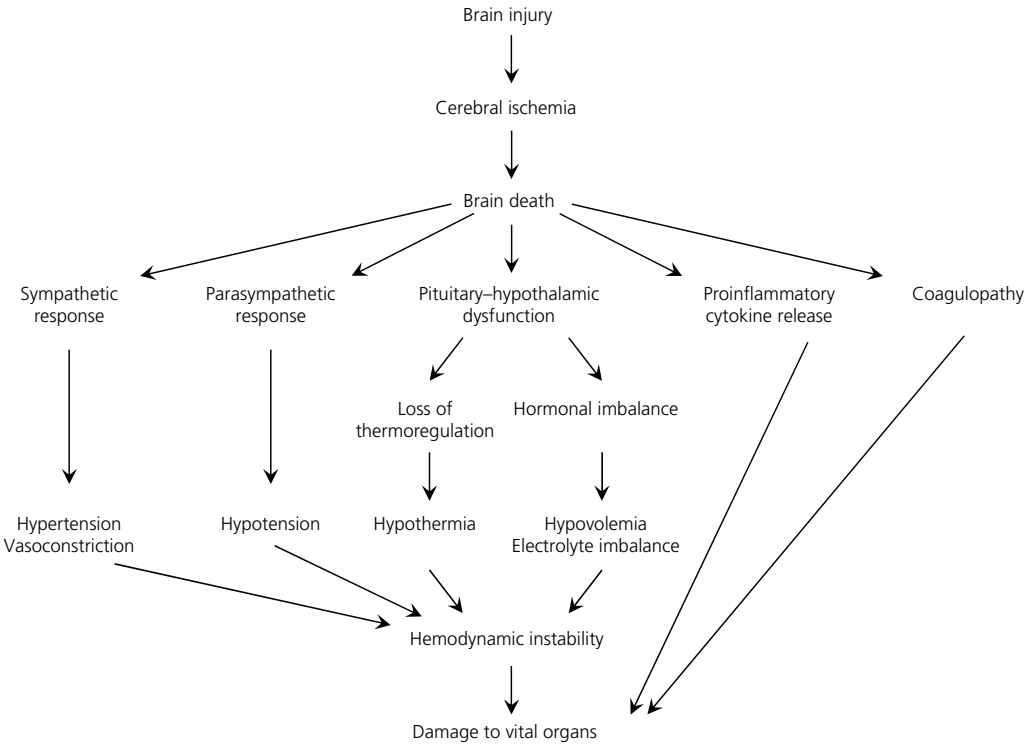

Figure 20.1. Pathways by which brain death evokes organ injury.

Table 20.3. Progressive cerebrospinal ischemia

Brain structures damaged	Physiological correlations
Cerebrum	↓ heart rate, ↓ cardiac output, ↓ blood pressure
Pons	↓ heart rate, ↑ blood pressure, irregular breathing
Medulla	↑ heart rate, ↑ blood pressure, impaired thermoregulation
Spinal cord	↓ heart rate, ↓ cardiac output, ↓ blood pressure

irreversible changes is activated, leading to a variety of physiological changes, including sympathetic and parasympathetic responses, pituitary–hypothalamic dysfunction, proinflammatory cytokine release, and coagulopathy. These changes have been shown to result in a variety of complications that may jeopardize vital organ function (Figure 20.1). Not all these changes are seen in every potential donor, but the incidence and severity of the complications depend upon the etiology and time course of brain death. The risk of complications increases with time after the onset of brain death. These complications are directly and indirectly associated with significant alterations in vital organ function.

Physiological changes related to brain anatomy

The development of brain death is accompanied with coning of the brain structures in a predictable rostrocaudal pattern (Table 20.3). When the cerebrum is involved, vagal activation occurs, leading to suppression of cardiovascular systems. This typically coincides with destruction of the hypothalamus and the pituitary gland, causing subsequent temperature and hormonal dysregulation. Pontine ischemia is associated with Cushing's reflex leading to mixed vagal and sympathetic outflow [18]. Medullary ischemia occurs in the early phase of brainstem death and is characterized by sympathetic or autonomic storm [19]. Ischemia of vagal and cardiomotor nuclei results in unopposed catecholamine surge. This causes intense systemic and coronary artery vasoconstriction, tachycardia, hypertension, and further elevation of ICP. This hypersympathetic state can lead to systemic organ and tissue ischemia. Elevated systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) result in increased pulmonary capillary bed pressure and endothelial damage. Ischemia to the respiratory center in the lower medulla results in apnea. When brainstem ischemia is completely developed, a progressive loss of spinal sympathetic pathways and total sympathetic denervation can occur. Global loss of sympathetic vascular tone and profound reduction in SVR leads to cardiovascular collapse [20]. Due to the combination of the loss of sympathetic activation and vasopressin deficiency caused by pituitary ischemia, a majority of potential donors fails to maintain hemodynamic stability [4]. Furthermore, ischemia of the pituitary and hypothalamic regulatory system can induce the loss of various homeostatic control mechanisms.

Cardiovascular changes

Brain death is associated with profound physiological changes that result in cardiovascular instability and widespread organ damage [4,5]. As previously described, the hemodynamic instability after brain death occurs in two serial phases. The first phase results from the physiological sequelae of mobilization of catecholamine stores. In an animal study, brainstem death causes marked increases in circulating dopamine, epinephrine, and norepinephrine levels, by 800%, 700%, and 100%, respectively [21]. Similar phenomena have also described in human donors who experience brain death [22]. This sympathetic storm causes intense vasoconstriction leading to profound hypertension and tachycardia. Although this causes a

secondary increase in myocardial oxygen demand, there is no increase in myocardial oxygen supply because the catecholamine storm also induces coronary vasoconstriction. This imbalance of oxygen demand and supply may induce early cardiac dysfunction and may cause direct injury to the myocardium in brain dead donors [23]. Myocardial damage in brain death is characterized by myocytolysis, subendocardial hemorrhage, edema, and interstitial inflammatory changes [19]. Contraction band necrosis, one of the common pathological changes in myocardial injury, is observed in 90% of patients following brain death [24]. The catecholamine storm may induce significant increases in intracellular calcium, resulting in impaired adenosine triphosphate (ATP) production and increased free radical formation, which cause further tissue damage. A significant rise in systemic vascular resistance increases afterload and results in acute cardiac failure in potential donors. Acute pulmonary edema may develop because of raised hydrostatic pressure and increased capillary permeability [25–27].

The initial phase of sympathetic outflow is followed by autonomic collapse with the loss of sympathetic tone and significant reduction of systemic vascular resistance associated with depletion of catecholamine stores rather than cardiac damage. Clinically, this is characterized by profound hypotension and bradycardia [3,18]. The uncontrolled vasodilation accelerates heat loss and hypothermia. The loss of vascular tone results in marked functional hypovolemia due to tremendous increase in vascular capacity and fluid volume shifts. Acute electrocardiographic abnormalities are common in brain death, including atrial and ventricular arrhythmias, conduction abnormalities, and changes in ST segment further compromising hemodynamic status.

Aggressive treatments for brain edema such as diuretics and mannitol cause further volume depletion and exacerbate hemodynamic instability. Insensible fluid loss due to diabetes insipidus also contributes to hemodynamic instability. Volume loss due to diabetes insipidus can exceed 2 L/h, leading to rapid total body water depletion. Close monitoring of urine output is crucial to assess hemodynamics of potential donors.

Pulmonary changes

Pulmonary dysfunction is common after severe brain injury [28]. Subsequent brain death is frequently complicated by pneumonia, aspiration, and neurogenic pulmonary edema. In trauma patients, pulmonary contusion and pneumothorax are common and result in severe respiratory dysfunction. The catecholamine release related to brain death also causes direct cellular injury to the lungs [5]. Severe vasoconstriction induces increased pressure in the pulmonary capillary bed and endothelial damage. Inflammatory response causes directly an increase in pulmonary capillary permeability. Volume overload after fluid resuscitation increases the risk of pulmonary edema. Patients who receive blood transfusions are also at risk for transplant related acute lung injury (TRALI). These changes make successful organ procurement challenging and negatively affect transplant outcomes [4,29].

Endocrine changes

With the onset of brain death, a number of endocrine changes occurs and may precipitate metabolic and hemodynamic instability [27]. Brain death disrupts the hypothalamic–pituitary axis, leading to serum hormone depletion. Dysfunction of hypothalamic and pituitary systems induces loss of thermoregulation, leading to either hyperthermia or hypothermia. Posterior pituitary function is commonly lost, resulting in low or undetectable levels of vasopressin in up to 90% of brain death organ donors [21,30]. This commonly causes central diabetes insipidus, which is clinically characterized by polyuria, leading to severe hypovolemia, hyperosmolality, and hypernatremia.

With dysfunction of the anterior pituitary gland, TSH, triiodothyroxine (T3), thyroxine (T4), adrenocorticotropic hormone (ACTH), and growth hormone (GH) are commonly depleted. Consequent hypothyroidism and adrenal insufficiency may lead to loss of the mitochondrial ability to regenerate ATP, resulting in functional organ instability [31]. Reduced levels of thyroid hormones may therefore contribute to deterioration in cardiac function and general organ perfusion in some donors. Rapid decline in free T3 is seen in brain death due to impaired thyroid stimulating hormone (TSH) secretion and peripheral conversion from T4 to T3 [31]. The lack of T3 results in a shift to anaerobic metabolism and acidosis.

Significant decrease in cortisol levels may occur after brain death, resulting in impaired stress response. In association with T3 depletion, this change may negatively affect the cardiovascular system. The decrease in cortisol levels may be associated with a decreased release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland [21]. Exogenous corticosteroids contribute to stabilization of organ function.

Hyperglycemia is a common endocrine imbalance. Decrease in intracellular glucose concentration is common and results in a cellular energy deficit, which is unfavorable to aerobic metabolism and induces acidosis. Steroid administration and increased catecholamine levels may further adversely affect hyperglycemia. High-dose insulin infusion is often required to prevent the development of a hyperosmolar state, a severe osmotic diuresis, and profound hypovolemia.

Inflammatory responses

Brain death is associated with various systemic inflammatory responses. Increased levels of inflammatory cytokines are associated with poor outcomes after organ transplantation [32–35]. This response is mutually related to activation of the immune, coagulation, and complement systems [36,37]. Brain death is known to trigger the production of various cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6. The mechanisms of cytokine production in brain death are not fully understood, but there several pathways are reported. Ischemic brain tissue is known to release inflammatory cytokines into the systemic circulation [38]. Brain death initiates an inflammatory state, which may up-regulate adhesion molecule expression on damaged endothelial cells, resulting in adherence of recipient T lymphocytes and macrophages and further inflammatory cytokine release upon graft reperfusion after organ transplantation [35]. The inflammatory response is maintained or amplified by the physiological changes related to brain death [34,39]. This affects all vital organs and is associated with significant organ damage in the donor, and subsequent poor function and increased risk of graft loss in the recipient with elevated levels of proinflammatory cytokines and up-regulation of receptors in target organs.

Coagulopathy

Abnormalities in coagulation are commonly seen in brain death. Because of the pathophysiological changes, brain death is associated with an increased risk of disseminated intravascular coagulation (DIC) [40]. There are two main explanations for the etiology: first, release of thromboplastin, fibrinogen, and tissue plasminogen from damaged brain tissue; and second, dilution of circulating

clotting factors due to large volume fluid resuscitation [3,41]. Because hypothermia exacerbates coagulopathy, management includes the use of warmed intravenous fluids, warming blankets, and heating inspired gases in order to maintain normothermic body core temperature. It has been controversial whether disseminated intravascular coagulation (DIC) in the donor compromises donor organ viability. Theoretically, DIC may adversely affect the graft quality due to the deposition of fibrin and microthrombi in the graft organ, and many transplant centers consider the presence of microthrombi a relative contraindication to organ donation. In contrast, other studies have shown that both thoracic and abdominal organs from such donors can be transplanted without compromising recipient outcomes [21,42,43].

Management for organ donation

Before the diagnosis of brain death, all medical efforts are directed at caring for the critically injured patient, maintaining adequate cardiac output to support all vital functions, including preserving potential residual brain function and maximizing any reversible injuries. Once futility of care is determined and declaration of death by neurological criteria is imminent, the focus of care is switched to maintaining and optimizing organ function to allow for the opportunity for organ donation.

Once the declaration of death is confirmed and consent for organ donation is confirmed, unnecessary delays should be avoided because the risk of organ dysfunction progressively increases with time. If the physiological changes related to brain death are untreated or under-treated, donors may deteriorate and undergo cardiac arrest within a relatively short time frame. Appreciating the importance of donor management and coordination for timely procurement has to be shared institutionally and individually. Donor management requires a multidisciplinary collaborative approach between the OPO and clinical care team. Organ donors should be monitored in an ICU setting to provide vigilant care. Close hemodynamic monitoring and aggressive resuscitation are essential to optimize organ quality and maximize potential for organs to be recovered for transplantation [6]. The application of a standardized donor management has significantly increased the number of organs transplanted without compromising post-transplant outcomes [6,44].

Achieving and maintaining stability in the donor enhances the opportunity to maximize organ function, resulting in increased placement and retrieval rates. Organs that appear unusable at the time of declaration of DNC may have functional improvement with aggressive donor management and thus become transplantable. Although in the past, the duration of time from declaration of brain death to recovery of organs for transplantation had been seen as an adverse factor for the outcome of transplanted organs [45]; more recent evidence suggests a period of good donor management can moderate this effect and, indeed, lead to improved outcomes [46,47]. In contrast, failure to maintain stability of the donor is associated with high rates of donor loss, fewer transplantable organs, and impaired organ function after transplantation.

UNOS Critical Pathway for the Organ Donor

Since the early era of transplantation, it was known that failure to restore normal physiology led to deterioration and cardiac arrest in potential organ donors. Goals were therefore set and standard critical care protocols were applied, with the intent to achieve better organ quality for transplantation. However, up to 25% of potential donors lost adequate perfusion to their vital organs, making them unsuitable for donation [7].

In addition to intensive medical care, OPO personnel have to obtain or confirm consent for organ donation as well as provide emotional support to the donor family. In response to these challenges, a guideline aiming to enhance donor management has developed by the UNOS [48]. This guideline, "Critical Pathway for the Organ Donor," facilitates management and delivery of quality care, which has been widely used and found to have beneficial effects in increasing the number of organs available for transplantation [6]. This pathway is designed to provide the information necessary to evaluate the functional status of donor organs, including heart, lungs, liver, kidneys, and pancreas, and to determine the management steps necessary to improve and optimize the performance of each organ. This also promotes communication between professional medical staff in the care of organ donors.

Elements of the Critical Pathway

The Critical Pathway has five distinct but partially overlapping phases. Each phase has subsections that may be used by professional medical staffs caring for organ donors as a guide to conduct thorough evaluation and management.

Phase I (Referral)

When a patient with a severe brain injury is identified as a potential organ donor, critical care staff should initiate the Critical Pathway. This initial step establishes contact between the hospital and the local OPO, and initiates referral before the potential organ donor becomes brain dead. Unless this step is initiated, the likelihood for organ donation will be diminished. In this phase, the organ procurement coordinator (OPC) performs a quick assessment of the potential donor to determine suitability for organ donation.

Phase II (Declaration of brain death and acquisition of consent)

Once the potential donor is confirmed brain dead, the patient's choice about organ donation is evaluated through the statewide/local donor registry (usually through the motor vehicle registry or on the driver's license) and the family is provided with this information or, if no first person consent is found, the family is approached about the option of organ donation. If the patient is not on the donor registry and the family is approached prematurely or by inexperienced requestors, they may opt against donation because of the difficulty of the situation. Therefore, the family should be offered the option of donation only after they have been given enough time to understand that death of their loved one has occurred [49,50]. If the family decline donation, the pathway is terminated at this phase and postmortem care should be initiated. If the patient is on a first person consent donor registry or the family consent to donation, donor management becomes a priority. First person consent supersedes a family declining the option of donation in many states and OPOs in the US.

Phase III (Donor evaluation)

After consent for organ donation is obtained or confirmed through a donor registry, donor evaluation and management protocols are initiated. The OPC obtains all the necessary history and the results of diagnostic tests. The cause of death, length of hospital stay before the development of brain death, estimated or witnessed down time (duration of cardiopulmonary arrest), and duration of cardiopulmonary resuscitation, thoracic or abdominal trauma, and vital signs are crucial pieces of information. Past

medical history needs to be obtained, including the history of hypertension, diabetes, coronary artery disease, pulmonary disease, liver disease, kidney disease, malignancy, infection, and history of blood clot. If old medical charts or a donor's primary care physician is available, more useful information can be collected. Surgical history is important information for procurement surgeons. History of smoking and alcohol abuse should be obtained as well. If the donor has a history of having been incarcerated, having used intravenous drugs or having multiple sexual partners, the potential donor may be considered to be high risk for transmission of human immunodeficiency virus (HIV) and hepatitis viruses. A thorough physical examination is also important to rule out any findings that may affect the donation process, including presence of tattoos, needle track marks, or suspicious lesions or masses. Laboratory tests include complete blood count, serum chemistries including liver function tests (LFTs), arterial blood gases, coagulation profile, infectious disease serology testing, and tissue typing for cross-match are performed. Cultures of blood, urine, and sputum for bacteria and fungi are sent to the labs to screen for infection. In addition to a chest X-ray, electrocardiography (ECG), echocardiography, and arterial blood gas, indications for cardiac catheterization and bronchoscopy will need to be determined with thoracic transplant teams. Completing these tests in a timely fashion is important to promote expeditious organ placement and recovery.

Phase IV (Donor management)

At the same time that the donor is being evaluated, optimal management also needs to be started to achieve hemodynamic stability, adequate oxygenation, and optimal fluid and electrolyte balance. Placement of an arterial line and a central venous line are necessary for aggressive respiratory and hemodynamic monitoring, and management of the cardiopulmonary status of the donor. Occasionally, minimally invasive hemodynamic monitoring or pulmonary artery catheters are considered helpful as well. The duration of this phase of care will depend on the status of the donor going into this phase of care, the stability of the donor, and discussion of potential organs thought to be viable for transplantation in each donor. The current trend is for longer donor management times of up to 48–72 h to maximize improvement in organ function prior to transplantation.

Phase V (Organ recovery)

After potential recipients are identified, the donor is transferred to the operating room where the organs are recovered. The clinical focus is shifted from donor management to organ preservation. All organ recovery teams should resolve potentially conflicting priorities prior to the recovery procedure.

Crystal City Meeting

Recognition of the donation crisis by the federal government led to a consensus meeting in March 2001 in Crystal City, VA, USA to develop initial acceptable guidelines that would improve the recovery and transplantation of organs from deceased donors. Five work groups were assembled that focused upon maximizing the use of hearts, lungs, livers, and kidneys, and increased use of organs from deceased donors with a history of malignancy or positive serology testing for hepatitis C or B virus. At this meeting the multidisciplinary teams outlined some management options and made treatment recommendations to improve utility of their individual organs or organs from the higher risk donors described above.

Table 20.4. Critical pathway for the organ donor (UNOS)

1. Early echocardiogram for all donors:
   - Insert pulmonary artery catheter to monitor patient management (particularly important in patients with an ejection fraction (EF) of <45% or on high-dose inotropes)
2. Fluid and electrolytes:
   - Maintain Na^+ <150 mEq/L
   - Maintain K^+ >4.0 mEq/L
   - Correct acidosis with sodium bicarbonate and mild-to-moderate hyperventilation (P_{CO_2} 30–35 mmHg)
3. Ventilation:
   - Maintain tidal volume 10–15 mL/kg
   - Keep peak airway pressure <30 mmHg
   - Maintain mild respiratory alkalosis (P_{CO_2} 30–35 mmHg)
4. Hormonal resuscitation:
   - Triiodothyronine
   - Arginine
   - Vasopressin
   - Methylprednisolone
   - Insulin

The heart work group made a recommendation to improve the evaluation and utilization of potential cardiac donors [41,51]. According to its recommendations, a new donor management algorithm was incorporated into the UNOS Critical Pathway (Table 20.4). After conventional management to adjust volume status, acid–base balance, oxygenation, anemia, and doses of inotropic agents, an echocardiogram is performed to assess ejection fraction and rule out structural abnormalities. If the ejection fraction is <45%, hormonal resuscitation is initiated, aiming to stabilize and improve cardiac function:

- T3: 4-μg bolus followed by 3 μg/h continuous infusion;
- arginine vasopressin: 1 U bolus followed by 0.5–4.0 U/h drip (titrate to an SVR of 800–1200 dyn·s/cm^5);
- methylprednisolone: 15-mg/kg bolus (repeat every 24 h as needed); and
- use of an insulin drip: at a minimum rate of 1 U/h, titrate blood glucose to 120–180 mg/dL.

A pulmonary artery catheter is also placed to allow for aggressive monitoring and management of the cardiopulmonary status. This is initiated simultaneously with hormonal resuscitation and continued for a minimum of 2 h. Adjustment of fluids, inotropic agents, and vasopressors every 15 min should be based on serial hemodynamic measurements to minimize the use of α-agonists to meet the following target criteria: mean arterial pressure >60 mmHg; central venous pressure 4–12 mmHg; pulmonary capillary wedge pressure 8–12 mmHg, SVR 800–1200 dyn·s/cm^5[5]; cardiac index >2.4 L/min/m^2 [2]; and dopamine or dobutamine 10 μg/kg/min. The use of hormonal replacement has been recommended as an integral component of donor management [4,6,41,51]. The hormonal replacement abrogates certain physiological changes related to brain death, resulting in cardiovascular stabilization and better organ perfusion. This effect has been associated with a 22.5% increase in organs transplanted per donor [6].

Fluid and electrolyte management

There are specific problems associated with the frequent development of central diabetes insipidus, which should be suspected if a patient develops inappropriate diuresis with rising plasma sodium and osmolality. Diabetes insipidus needs to be aggressively treated because it is closely linked with hemodynamic instability in organ donors [52]. Hypotonic intravenous fluid may be required to restore normal sodium levels but in the face of untreated diabetes insipidus

is rarely successful. Thus, euvolemia, normonatremia, and normal osmolality require a multipronged approach to correcting the underlying pathology (pituitary failure) and replacing volume deficits with free water. The use of large volumes of D5W may cause secondary elevation of blood glucose levels, which should also be treated with insulin, preferably as an insulin drip. Hypernatremia has been associated in some studies with poor organ function after transplantation [53], but it may in fact be a marker of poor overall donor management. A recent study showed, however, that hypernatremia in brain dead donors did not affect transplanted organ quality or graft survival [53].

Donor management is commonly complicated due to conflicting priorities of different specialties that have a different perception of optimal intravascular fluid balance amongst and within thoracic and abdominal organ teams. Generally, lung surgeons and pancreas transplant surgeons prefer to minimize intravenous fluid to minimize lung and pancreas edema. By restricting fluid administration, edema or congestion of these organs are thought to be minimized. In contrast, optimal fluid management for hearts and kidneys is diametrically opposite. By aggressively hydrating donors, kidneys are well perfused to maintain brisk urine output and cardiac output can be optimized. Because multiple recovery teams work concurrently in organ management and subsequent multiorgan recoveries, the importance of communication between different teams and OPO coordinators must be emphasized.

Inflammatory responses

Administration of methylprednisolone to the donor in an attempt to reduce inflammation has been shown to be beneficial in randomized studies of lung and liver transplants, and is commonly given as soon as a plan for donation is confirmed [54–56]. In a recent observational evaluation of organ donors, higher IL-6 levels were correlated with worsening subsequent organ outcomes in recipients [36]. In addition, the same group performed a recent pilot study to explore the feasibility of using a hemoadsorption device to remove inflammatory cytokines in brain dead donors [57]. This device was able to achieve modest reduction in plasma cytokine levels; however, no data are available on clinical transplant outcomes.

Coagulation

Coagulopathy may be related to donor pathology or release of activating substances from the injured brain. Treatment is required if there is active bleeding and correction of a markedly abnormal coagulation profile should be considered before a retrieval procedure. Point-of-care coagulation testing, including thromboelastography, may aid treatment decisions. Transfusion should be considered if necessary as for any ICU patient, except if TRALI develops.

Intraoperative management during organ recovery

A multiorgan donation operation generally involves a laparotomy extended by sternotomy, even if thoracic organs are not to be retrieved. As with any major body cavity surgery, there is potential for significant blood loss and hypothermia. Marked cardiovascular instability can occur during organ retrieval and vasoactive drug infusions are likely to be in progress. The goal of perioperative organ donor management is to maintain stability during the organ recovery period to allow unhurried removal of optimized organs. Surgical safety checks (appropriately modified) should be carried out.

Intraoperative active warming should be continued, as the duration of retrieval may be extended if there are any delays. Intravenous fluids should be warmed and blood and blood products should be immediately available if required. Cardiovascular changes mediated by spinal reflexes may be induced as a result of surgical manipulation and may require treatment with vasoactive drugs. Since brainstem death has occurred, the administration of anesthesia is unnecessary. Some retrieval teams administer volatile anesthetic drugs during retrieval for the control of hypertension as they may have beneficial preconditioning effects on retrieved organs, although there is no evidence for this. Coordination of drug administration, particularly heparin, is important. Retrieval teams should be skilled in abdominal and thoracic perfusion techniques.

Assessment of donor quality

With the disparity between the need for organs for life-saving transplants and the limited numbers of organ donors, the general premise that most OPOs have adopted in evaluating a potential organ donor is an "every organ every time approach." The goal is to make available to transplant surgeons and their patients all possible organs for transplantation from all individuals who choose to give this gift. Unfortunately, not all organs from individuals who desire to help others by being organ donors at the time of their death are in fact suitable.

It is the responsibility of the OPO to review the available information on each potential donor to assess each potential donor's quality and determine which organs from each specific donor are potentially transplantable. The OPO begins with a thorough review of the potential donors past medical and social history. Assessment of donor quality is generally based on donor age, size [especially body mass index (BMI)], general medical history, organ specific medical history, smoking history, with attention to malignancies, infections, and high risk behaviors, including substance abuse [alcohol or drugs (intravenous, inhalational, oral, etc.)], multiple sexual partners, incarceration, etc. The cause of death, length of hospitalization, hemodynamic status (blood pressure, heart rate, inotropic support), pulmonary function, hepatic function, urine output, glucose level, and acid–base balance are also important in the analysis of donor quality. Increasing age, high BMI, history of co-morbidities, prolonged ischemic time, and hemodynamic instability have all been known to increase the risk of organ dysfunction and poor survival after transplantation.

It is important to remember that the only current absolute contraindication for organ donation in the US at this time is a proven diagnosis of HIV in a potential donor. This is currently federal law, but is being re-evaluated in light of a recent article published from South Africa showing that HIV-positive donors may donate to HIV-positive recipients with success of these organs post transplantation [58]. Other potential contraindications to donation are disseminated malignancies, ongoing treatment of cancers, non-identified causes of meningoencephalitis, and severe multiorgan dysfunction. Nevertheless, these should be evaluated on a case-by-case basis and should not result in automatic turndown as potential donors.

Next, the OPO will evaluate the circumstances of the cause of death of each donor and again assess the potential impact of the catastrophic event leading to death. One consideration is anoxia time and whether cardiopulmonary resuscitation (CPR) was performed, as well as the duration of the CPR until return of

spontaneous circulation. Also important is functionality of the organ systems between the time of catastrophic injury and the deterioration and pronouncement of death by neurological criteria. Any respiratory, cardiovascular, renal, hepatic, and pancreatic dysfunction may negatively impact potential for recovery and transplantation of organs. One mechanism of tracking these variables and evaluating the impact of the ICU management predeath is to utilize "donor management goals (DMGs)." DMGs are agreed parameters that are used to direct the management of an organ donor. These parameters vary by program/OPO/region, but all are based on targeting normal physiological endpoints in terms of cardiopulmonary function while minimizing additional support (fewer inotropic agents), maintaining normal or near-normal chemistries and pH, and evaluating adequate perfusion of end organs. These DMGs have been evaluated and meeting more of these DMGs appears to correlate with increased numbers of organs recovered and transplanted from donors [59,60]. A proposed usage of the DMGs is the establishment of a uniform objective DMG list for all organ donors to allow study of the variations in management to achieve the physiological parameters outlined in the DMGs, as well as to determine superior management outcomes. Another proposal is to evaluate DMGs at varying times through the donor management time period and serially assess the percentage of DMGs completed. There is some evidence that if the donor meets a high percentage of the DMGs prior to the OPO management of the donor, the potential for more organs transplanted per donor will be increased. This may be used as a guide for pre-OPO management strategies prior to OPO involvement. Accurate and detailed accounts of problems during the donor management period, the period after DNC is determined, and prior to recovery of organs for transplantation should be chronicled, as there are a host of potential problems that may be identified during this management period of the potential donor.

Once all the information has been gathered and collated, and any issues identified, these are made available electronically to the transplant centers for evaluation and review for suitable recipients among those listed by their center through the OPTN computer network. Each specific center has developed its own independent criteria by which recipient suitability is evaluated and determined. Ultimately, it is the transplant surgeon and their patient who must make the final decision based on the combination of the condition of the recipient and the anticipated donor organ quality. Included in the donor organ quality assessment is the potential risk(s) of the donor based on the information available and the anticipated cold ischemic time that lead to a final determination of acceptance of each organ for transplantation. The assessment of organ suitability from the perspective of the recipient surgeon can be found in Chapter 53.

Heart

As previously discussed, there are a multitude of factors affecting the potential quality of the donated heart. Most transplant programs have a maximum donor age criteria of between 60 and 65 years, with some room for exception with extremely fit donors above this age range. Other exclusion factors include prior cardiac pathology, prior heart surgeries, and suspicion of primary cardiac etiology behind the acute event that resulted in the donor's death (myocardial infarction, primary arrhythmia, myocarditis, cardiomyopathy, etc.). If the catastrophic event caused the donor to lose spontaneous circulation, then the amount of time without perfusion is a significant concern, as well as the time of CPR and time to return of spontaneous circulation. The usage of inotropic or vasopressor agents and the dosages needed to maintain hemodynamic stability during the predonor care and following declaration may affect the heart transplant potential.

We have previously commented on the physiological changes that are associated with death by neurological criteria; these may lead to temporary or permanent cardiac dysfunction, cardiac arrhythmias, and/or decreased myocardial DO_2. Assessment of the potential for heart donation hinges upon donor management through the catecholamine storm, subsequent hemodynamic collapse, and until normal physiological cardiac output (CO)/cardiac index (CI) with normal SVR can be established. If the donor can be supported through this cascade without compromising heart function, the post-traumatized heart may be reassessed to determine the likelihood of successful transplantation. Based on data previously quoted, current practice is for most OPOs to institute thyroid replacement therapy in all potential heart donors, as well as in many donors who are not potential heart donors, to stabilize the hemodynamics and allow the reduction or weaning of inotrope infusions. Decisions as to T3 or T4 replacement, the dosing of each, and whether delivery is intermittent or continuous are based on expert opinion and local practice. As with most aspects of organ donor management, little randomized data exist. Our practice locally is to initiate levothyroxine (synthetic T4) as a continuous infusion, beginning at 10 μg/h and titrating to a maximum of 30 μg/h as long as the hemodynamic effect is tolerated without adverse effects – the most common being tachycardia and hypertension. In addition, routine doses of corticosteroids are given and continued until organs are recovered. Again, objective data are limited and best practices vary as to specific medication choice, i.e. glucocorticoid and/or mineralocorticoid, doses used, and continuous versus intermittent infusion.

In the evaluation of the heart for transplant potential it was common practice to obtain an echocardiogram at the time of initiation of OPO management; this practice has shifted to postponing echo evaluation until after weaning catecholamine infusions to a minimum and preferably completely. In addition to assessing adequate functionality, commonly by ejection fraction, ventricular wall thickness and motion dynamics must demonstrate near-normal function. In older donors, a cardiac catheterization is often necessary to determine if coronary artery disease renders the heart unsuitable or if it can be utilized after back table coronary artery bypass grafting.

The use of pulmonary artery catheters has declined in donor management, as it has in general ICU management, due to the lack of demonstrable benefit, recognition of risks associated with insertion and maintaining the catheter, and the lack of qualified personnel and equipment in most hospitals. It has been replaced by minimally invasive hemodynamic monitors that for the most part utilize pulse contour to measure either stroke volume variability or pulse pressure variability as a marker of hemodynamic status. Some OPOs have utilized tracheal Doppler flow probes with success in guiding their donor management.

Lungs

Available data on lung acceptability for transplantation focus primarily on the PaO_2-to-FiO_2 (P/F) ratio being >300–350 on minimal mechanical ventilator support. As with the heart, factors affecting lung transplantability include age (<60 years old), historical information, cause of death, treatment of the donor in the pre-OPO phase, and treatment during the OPO phase. When the catastrophic

event was traumatic or associated with CPR and vomiting, concerns include the extent of the contusion injury, development of pneumothoraces, and the possibility of aspiration of gastric contents, all of which impact lung transplantation potential. In a donor with a history of moderate-to-severe asthma, pulmonary artery hypertension, or emphysema, transplantation of the lungs is unlikely. A smoking history of more than 20 pack years or history of marijuana use for a significant period of time decreases the potential of lung donation as well.

The duration of intubation and extent of mechanical ventilation, as well as placement of a tracheostomy tube due to long-term mechanical ventilation needs, are other risk factors for non-transplantation as they introduce the possibility of infection and ventilator-induced injury, e.g. barotrauma. The cascade of catecholamines, hormones, and mediators released during the herniation process may cause direct cellular injury to the lung tissue. Increased transthoracic pressures cause pulmonary capillary bed and endothelial injury, resulting in an acute respiratory distress syndrome (ARDS) event that leads to increased lung dysfunction and ongoing inflammatory cascade. With cessation of neurological function, loss of spontaneous respirations, loss of cough, and loss of spontaneous movement occur, all of which result in mucus plugging, atelectasis, and derecruitment of alveoli. Additionally, "neurogenic pulmonary edema" develops, leading to further water accumulation in the lungs and further worsening of the gas exchange. Also, the need for aggressive fluid resuscitation to support hemodynamics and renal function during the phase of vascular collapse, will worsen lung water status.

Early chest X-ray is helpful in identifying potential issues in the lungs, both easily and without having to move the patient. Initiation of chest physiotherapy – with the vest or bed – and albuterol to enhance mucociliary clearance are helpful in reversing some mucus plugging. N-acetylcysteine may assist as a mucolytic agent as well. The use of systemic corticosteroid treatment has also been shown to improve lung function, along with better assessment and control of donor volume status to accomplish an isovolemic state.

Alveolar recruitment strategies with the mechanical ventilator include the San Antonio Lung Transplant (SALT) protocol [61], a low tidal volume (TV), high positive end-expiratory pressure (PEEP) recruitment approach, and an alternative approach using a severely inverted inspiratory-to-expiratory (I:E) pressure ratio controlled ventilation – BiVent or APRV-like modes [62]. Recalling that the neurologically deceased donor produces little CO_2 and so needs decreased minute ventilation, there is little downside to this approach. Both strategies of donor management have been shown to increase the likelihood of successful recruitment and subsequent lung recovery and transplantation (Lebovitz et al., personal communication). An argument may be made in most donor management cases for early bronchoscopy, but this is particularly true in cases of excessive mucus plugging or aspiration of gastric contents to relieve the obstruction and improve the VQ matching, leading to improved P/F ratio.

While chest X-ray is a good initial screen to look at lung fields, computed tomography (CT) scan of the lungs may assist in the case of a smoker with no past history of emphysema to rule out the likelihood of this condition in the early OPO donor management phase. If evidence of emphysematous changes are noted, the lungs are not suitable for transplantation and a less aggressive ventilation strategy may be utilized.

All donors need be thought of as potential lung donors. Addressing the numerous factors described above is among the highest priorities for the OPO early in the immediate post-OPO management phase, as it focuses the goals of management on improving the DO_2 and the pulmonary dynamics, while minimizing the potential for barotrauma and oxygen toxicity and improving the fluid status of the lungs and the patient. Support of DO_2 is essential to maintain viability of all transplantable organs. If the P/F ratio can be raised above the 350 range with a low peak ventilator pressures and a PEEP of 5 cmH_2O, and if the chest X-ray is cleared with little concern for an ongoing pneumonic process at the end of the OPO donor management phase, the likelihood of successful lung recovery and transplantation is high.

New on the horizon is a process for recovering lungs that do not appear to be able to be transplanted and supporting these lungs with an ex-vivo apparatus that allows them to be reconditioned. This apparatus has been used and studies published that demonstrate that marginal lungs (i.e. that would not have previously been used for transplantation) can be recovered, reconditioned, and transplanted. [63]. The Food and Drug Administration (FDA) is currently engaging some US transplantation programs in the evaluation of these product/processes for use in the US.

Liver

The liver is one of the organs most resilient to a lack of adequate DO_2 in the donor. In addition, the age of the donor is less of a factor for post-transplantation functionality. Nevertheless, there are a number of unique issues to be evaluated when liver transplant potential is being considered. BMI of the donor is an important factor to take into account. Donors with BMIs of >30 are more likely to have significantly fatty livers, which some surgeons will not consider for transplantation. Other factors include alcohol ingestion history – duration and quantity. A long history of alcohol abuse results in a fibrotic and dysfunctional liver that is not transplantable. The presence or absence of viral hepatitis in the donor is also significant in the assessment of donor liver quality. The presence of past indicators of hepatitis B infection or evidence of active hepatitis C serologies makes it more challenging to identify the correct recipient for such a liver transplant. Additional testing may be indicated if it is uncertain if there is an active infection or if the serologies are explained by prior immunization and other factors. It is critical therefore, to have the necessary knowledge to interpret the results of serological testing in order to place these livers into the most appropriate recipient. Occasionally, it is helpful to perform a bedside needle biopsy of the liver of a donor who has a number of risk factors for poor liver transplant potential.

The issue of elevated serum sodium in the donor leading to poor outcome of these livers post transplantation is controversial. While the literature is divided, and a recent study from Indiana appears to debunk this myth [53], the elevation of sodium in fact reflects that the volume status of the donor is inadequate, which may be a major factor in determining liver function post transplantation.

Kidneys

Kidneys are the organs in by far the highest demand for transplantation in the US and around the world. However, age and other fairly common underlying medical diseases may limit their desirability as donor kidneys.

When a patient is treated in the ICU for catastrophic brain injury with hyperosmolar therapy, fluid limitation, and vasoconstrictors, in order to decrease intracranial pressures and support cerebral perfusion pressure, the result may be inadequate perfusion of the renal parenchyma. As the patient continues to deteriorate

neurologically, these treatments are further pushed, worsening potential injury to other vital organs. Once care is determined to be futile and the patient is evaluated for DNC, these treatments may or may not be stopped by the primary care team prior to consent for donation and the OPO orders beginning. After DNC is declared, during the pre- and post-donor management periods, the kidneys are at significant risk for developing worsening acute tubular necrosis. This is due to the rapid fluid shifting and the variability in CO/CI of potential donors as the catecholamine surge and subsequent hemodynamic collapse occurs. Additionally, the loss of the hypothalamic/pituitary access, resulting in development of diabetes insipidus, may additionally cause severe intravascular volume depletion and exacerbate hemodynamic instability.

Organ donors are at risk for renal failure from the prehospital events they suffered (e.g. trauma, hypotension, toxins such as ingestion of ethylene glycol), hospital events (e.g. sepsis, nephrotoxic agents such as intravenous pyelogram dye or antibiotics), or the treatment regimen they underwent (e.g. fluid restriction). Catastrophic events such as crush injury, drug overdoses (especially cocaine, heroin, amphetamines, and PCP), seizures, severe hypoxia, and trauma, can lead to rhabdomyolysis causing renal injury from myoglobin deposition. Donors are at risk for multiorgan system failure with DIC and microthrombi formation in the kidneys as well. Procedures requiring intravenous contrast in deceased donors like cardiac catheterization may cause "contrast-induced nephropathy."

Thus, aggressive adjunctive therapies directed at maintaining isovolemia, enhancing urine output, and providing protection from toxic effects might have some utility. In a recent study, kidneys from donors receiving low dose dopamine during the OPO management phase had improved renal function post transplantation in recipients [64].

The serum BUN, creatinine, and hourly urine output are monitored to give the best indication of the likelihood of kidney donation by a deceased donor. In addition, renal biopsies and ex-vivo pumping – shown to improve renal function post transplantation – can be helpful adjuncts in determining suitability for donation and success after transplantation.

Pancreas and intestines

Both the pancreata and intestines in a potential donor are extremely fragile organs and many stressors in the potential donor can lead to untransplantability of these organs. They are particularly susceptible to hypoxic ischemic insults with autolysis, sloughing of their mucosal lining, and ischemia.

The best indicators of potential for transplantation of the pancreas are a normal amylase, lipase, and hemoglobin $A1_c$. More data need to be gathered to determine if there are indicators for the intestines that are helpful in predicting likely successful transplantation of these organs.

Infectious disease risks

Inherent in transplantation is the potential for transmission of disease from the donor to the recipient. This can be the result either of a pre-existing disease process in the donor or an acute process associated with the current hospital illness/injury. Steps need to be taken in evaluation of the potential donor, treatment of the donor during the acute hospital phase and including the donor management phase, and the post-transplantation treatment of the transplant recipient. Detailed discussions of donor-derived infections are found in Chapter 92.

Evaluation

Initial evaluation of the donor per the current UNOS Policy 2.2.4.1 requires non-hemodiluted donor serological testing for HIV 1 and 2, HepBsAg and HepBcAb, Hep C, cytomegalovirus (CMV), Epstein-Barr virus (EBV), Venereal Disease Research Laboratory (VDRL) or rapid plasma reagin (RPR), as well as acute bacterial cultures of blood, urine, and for lung donation, bronchoscopy/sputum Gram stain and culture. As previously discussed, positive serology testing for HIV currently disqualifies a potential donor in the US. Other positive findings from these screening tests may influence the potential transplantation of specific organs from seropositive donors or influence the post-transplantation treatment of the recipient of these organs.

Nucleic acid testing

There is recent controversy amongst experts in donation/transplantation regarding additional testing using the Nucleic Acid Test Systems (NAT) to screen potential donors for HIV, hepatitis C, and hepatitis B viruses. This newer technology allows for small amounts of DNA/RNA to be detected by a process of massive copying of the gene fragments. The advantage of NAT testing is that the "window period" – the period between infection of an individual and time to detection – may be shortened by NAT testing.

Proponents of NAT testing of all potential donors (as is currently done in the blood banking industry) state that mandatory NAT testing may allow for detection of positive individuals prior to serological conversion, thus further decreasing the likelihood of unanticipated transmission. In addition, a survey of organ procurement organization clinical directors suggested that the incorporation of NAT testing for Hep C and HIV may improve "high-risk donor" organ utilization [65].

This decrease in the "window period" is different for each of the viruses tested. For HIV, standard serology testing will give a positive result 17–22 days following infection; enhanced serology (combined antibody–antigen) testing decreases this to 7–16 days, with NAT decreasing it to 5–6 days. For Hep B, standard serology testing can give a positive result in 35–44 days following infection; no enhanced testing is available, but NAT decreases this time to 20–22 days. Most significantly, the average window for Hep C can be reduced from ~70 days for standard serology testing, to 40–50 days with enhanced serology testing, and to 3–5 days when NAT is used [66–72].

Opponents remain concerned about the potential adverse effect of NAT in terms of lost donors and organs available to transplant due to false-positive testing, which is known to occur with inexperienced/infrequent use of this testing, and inability to obtain testing results prior to transplantation (time limitations on testing sites/travel of serum to sites, etc.) [73]. A recent expert consensus conference concluded that, "there is insufficient evidence to recommend universal prospective screening of organ donors for HIV, HCV and HBV using current NAT platforms." Further study of viral screening modalities may reduce disease transmission risk without excessive donor loss [74]. Since the publication of these recommendations, the Centers for Disease Control and Prevention (CDC) has distributed and is continuing to revise new guidelines for testing of all prospective organ donors, both living and deceased. Recently, the public comment period on its proposed revised guidelines generated a significant outcry from the transplant community and the recommendation to have all parties meet further to balance the opposing viewpoints. This revision may have impact on testing of donors in the future.

Antibiotic use in donors

The use of antibiotics in donors depends on a number of conditions and situations, but should adhere to standard medical and surgical guidelines. Traditionally, relatively young, trauma victims are rarely chronically ill and usually devoid of infectious risk complications – prophylaxis is often accomplished with the use of a first-generation cephalosporin. However, donors with more complex medical conditions and underlying co-morbidities, or those who have been hospitalized for a long period of time, may have acquired antibiotic-resistant organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), and have other risk factors for infection associated with hospital care of the critically ill patient. The OPO must decide if antibiotics are needed or helpful in the ongoing management of these patients as potential donors. Antibiotics with known specificity should be targeted based on site-specific cultures, e.g. cerebrospinal fluid, respiratory, urinary, blood, or catheter sites. In many donors, the approach of using broad-spectrum antibiotic coverage during the OPO management phase reflects the short time available for preparing for donation. One concern is the use of antibiotics that may cause acute organ dysfunction and decrease the likelihood of successful transplantation of organs. Recent publications have commented on the potential of acute renal dysfunction/failure in critically ill patients treated with piperacillin–tazobactam. Another concern is that this acute respiratory failure may be worsened with co-incidental usage of vancomycin [75]. In general, unless there are specific organisms that require more toxic antibiotics, a fourth-generation cephalosporin with extended Gram-negative coverage is recommended. If Gram-positive cocci thought to be staphlococci species are identified in the donor process, vancomycin may be added with close monitoring of renal function and vancomycin serum concentrations in the event of any identified issues. Conversion to a less toxic antibiotic should be accomplished as soon as sensitivities are obtained.

Summary

There is a critical shortage of organs available for life-saving transplantation, and the numbers of deceased donors have remained static for the last 5 years. The responsibility to maximize the numbers of organs available for transplantation is shared by the hospital team that has cared for the patient prior to declaration of DNC (physicians, nurses, respiratory therapists, etc.) and the OPO team (requestors, bedside management professionals, etc.). Protocol management of critically ill patients who transition to potential donors aids in the ability to reach target goals and to normalize physiological functions, and thereby to increase the likelihood of successful donation and subsequent transplantation.

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CHAPTER 21

Techniques for Organ Procurement after Brain Death

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Introduction

To date, brain dead donors represent the largest source of organs utilized for transplantation. During the last decade, organs procured from brain dead donors have increased each year in the US, peaking at approximately 28 000 organs in 2007. Since then, however, numbers for brain dead donors have slightly declined. At the same time, an increasing availability of organs from donors after circulatory death has been observed; more than 10% of all available organs are currently procured from donors after circulatory death [1,2].

Brain death criteria were established by a committee at Harvard Medical School in the late 1960s and define an irreversible loss of brain and brainstem activity caused by extensive and irreversible central nervous system damage subsequent to trauma, hemorrhage, or infarction [3]. Clinical criteria defining brain death include coma with cerebral unresponsiveness, apnea, absence of brainstem reflexes, and persistence of these conditions for 6–24 h. Although confirmatory tests are not mandatory, additional testing, such as electroencephalography, transcranial Doppler sonography, somatosensory and brainstem auditory evoked potentials, cerebral blood flow and magnetic resonance imaging studies, may be necessary to determine the diagnosis. The presence of sedative drugs, hypothermia, shock, or other potentially reversible conditions that may depress brain function must be excluded prior to confirming the diagnosis [4].

Brain death leads to considerable systemic changes, including the so-called autonomic storm, arterial hypo- and hypertension, massive coagulopathy, and abnormalities in electrolyte balance [5]. This chapter details the technical methods for organ procurement from brain dead donors, with particular attention to the consequences of brain death for these techniques. Organ procurement is a surgical procedure that proceeds in the face of, at times, catastrophic instability, necessitating speed and precision in its conduct. A general understanding of the decedent's abnormal physiology is important to allow the surgeon to assess the conduct of the case and coordinate compensatory plans with the procurement team. Specific details regarding the management of brain dead donors prior to procurement are given in Chapter 20 and the techniques specific to the procurement of organs from non-heart beating donors after circulatory death are featured in Chapter 22.

Profound effect of brain death on the cardiovascular system

The profound increase in intracranial pressure linked to brain death causes ischemia from the cerebrum to the spinal cord (Figure 21.1). This results in herniation of the medulla oblongata, followed by vagal motor neuron ischemia, culminating in systemic hypertension and tachycardia. The observed peripheral ischemia is a consequence of a sympathetic overload causing increased vascular resistance and subsequent hypertension [6].

Despite an increased coronary blood flow, cardiac perfusion remains poor, leading to global myocardial ischemia and subsequent structural changes, such as interstitial hemorrhage, diffusely scattered subendocardial necrosis, and mononuclear cell infiltration. Following brain death, the "catecholamine storm" remodels the myocardium structurally in ways similar to the changes observed after the administration of high doses of catecholamines [7,8].

Large animal experiments have shown that a cardiac sympathectomy can limit local catecholamine effects and structural remodeling subsequent to brain death. The magnitude of the cerebral insult and the level of intracranial pressure is often proportional to the extent of released catecholamines [9].

Following the initial hypertensive period, peripheral vascular resistance declines, leading to hypotension mainly due to spinal cord ischemia. Cardiac and peripheral organ hypoperfusion are the consequence of a combined effect of hypotension, vasodilatation, hypovolemia, impaired vascular autoregulation, and compromised microcirculation subsequent to impaired endothelial-dependent vasodilatation [8,10].

Judicious management of donor cardiac output, peripheral vascular resistance, and perfusion pressure determine the quality of organs from brain dead donors. Fluid resuscitation, inotropic support, and hormonal substitution are usually used for stabilization. Coagulopathy following brain death leads to fibrin deposition

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

and organ damage due to free hemoglobin. Strategies pre-empting the consequences of those events include aggressive fluid replacement, hormonal replacement with the administration of platelets, clotting factors, and early organ procurement. Whether delayed procurement and stabilization of the donor help to ameliorate some of the neurohumoral consequences of brain death remains controversial [5] (Figure 21.1).

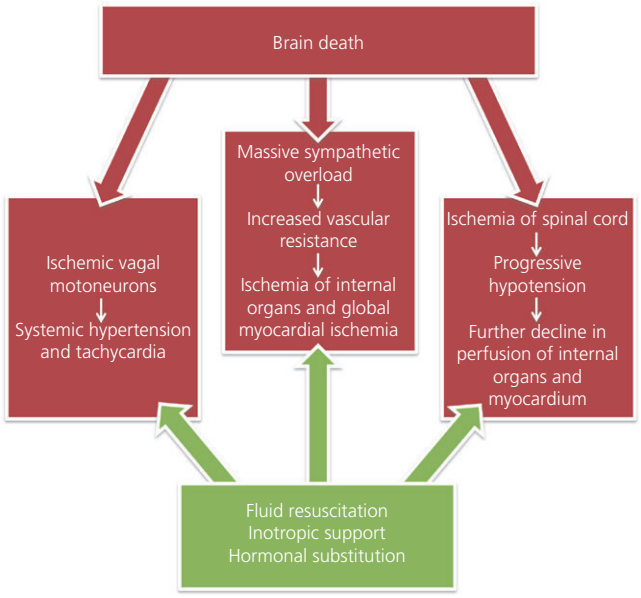


Figure 21.1. Sequelae of brain death on the cardiovascular system and possible treatment strategies.

Endocrine changes as a consequence of brain death (Figure 21.2)

The autonomic storm following brain death leads to a dramatic increase in plasma catecholamine levels. Moreover, brain death is associated with the so-called anterior and posterior pituitary failure, leading to dysfunction of the hypothalamic–pituitary axis. In animal models, brain death is linked to a dramatic decrease in plasma levels of thyroid hormones, cortisone, and insulin. Although some clinical reports have described comparable findings, most authors describe normal levels of cortisone and insulin following brain death [11,12].

In the presence of varying degrees of necrosis, hemorrhage, and edema of the pituitary gland, thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), and human growth hormone (hGH) are mostly within normal limits. However, subnormal concentrations of thyroid hormones have been observed and substitution of T4 has been shown to prevent hemodynamic and metabolic alterations [13,14].

The posterior pituitary function is impaired in almost all patients after brain death, resulting in diabetes insipidus with subsequent hypovolemia and massive electrolyte imbalances. Lack of vasopressin aggravates the peripheral vasodilatation, which is furthermore promoted by an impaired thermal regulation linked to brain death [15].

Morphological changes in donor organs after brain death

Brain death has profound effects on organ morphology and functional properties, influencing not only immediate graft function but also long-term graft outcome. Systemic and local inflammatory consequences following brain death have been explored extensively.

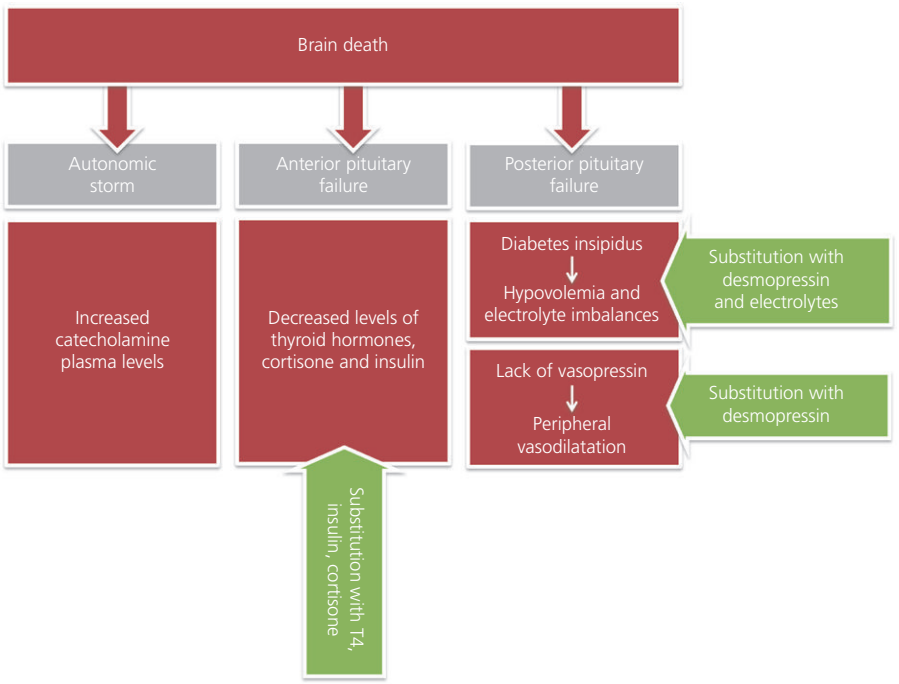


Figure 21.2. Neurohumoral impact of brain death and therapeutic options.

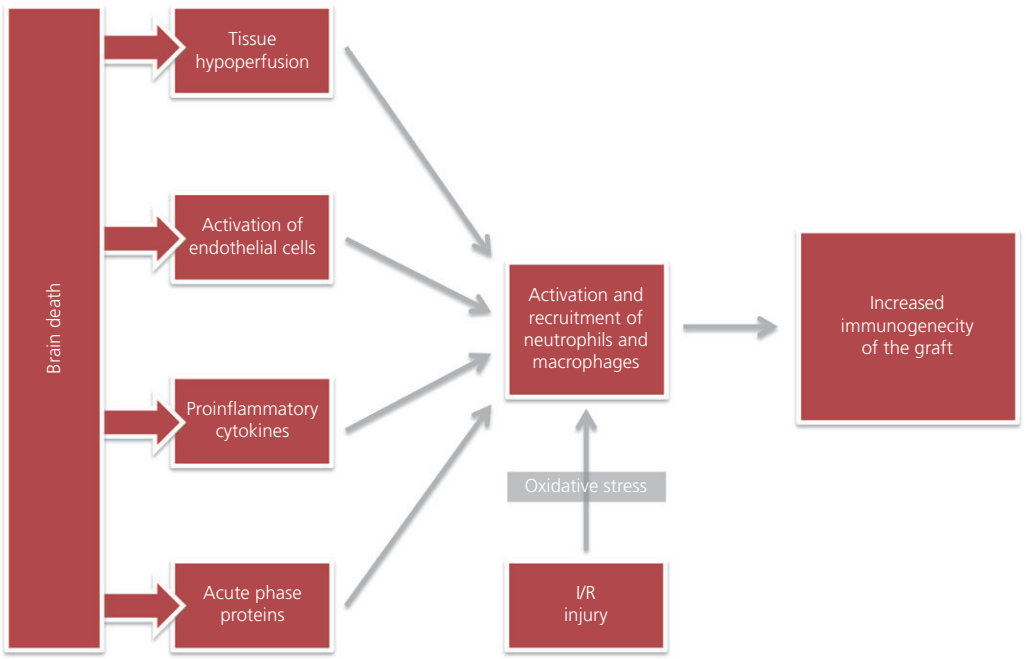


Figure 21.3. Pathophysiology of increased graft immunogenicity following brain death. I/R, ischemia/reperfusion.

Of note, most of those studies have thus far been descriptive and basic mechanisms remain unclear.

It has been shown that the explosive increase in intracranial pressure is linked to an increase in proinflammatory cytokines (TNF-α, IFN-γ, IL-6), chemokines, and adhesion molecules (ICAM, VCAM, LFA-1). Increased cellular infiltrates were found in kidneys and hearts after brain death, potentially augmenting the immunogenicity of donor organs and leading to an intensified alloimmune response. Putative modes of actions include (Figure 21.3):

1 a direct release of inflammatory cytokines from the ischemic brain and brainstem after brain death;
2 a secondary release from circulating lymphocytes and macrophages as a consequence of the catecholamine storm;
3 endothelial cell activation;
4 a bacterial translocation as a consequence of gut ischemia [5,16].

As the sympathetic storm following brain death reduces coronary perfusion, structural changes are comparable to those observed after cardiac ischemia. Moreover, norepinephrine is released from sympathetic nerve endings located in the epicardium. The combination of local and systemic effects causes an imbalance between oxygen supply and consumption. As a consequence, a predominantly anaerobic metabolism is observed, resulting in an accumulation of lactate within the myocardium. Morphological hallmarks of hearts from deceased donors include subendocardial necrosis and petechial hemorrhage with loss of desmin and interstitial mononuclear cell infiltration. However, these changes occur even in well-perfused hearts and as a thoracic sympathectomy is protective, local effects of catecholamine seem to be critical [17].

Following the onset of brain death and subsequent to hemodynamic imbalances, kidney perfusion is compromised. Impaired perfusion and proinflammatory changes are the major cause of kidney damage seen in grafts from brain-dead donors. Typical histomorphological changes in kidneys from brain-dead donors prior to organ procurement include a degeneration of the proximal tubular lining, endothelial proliferation, periglomerulitis, leukocyte infiltration, and interstitial collagen depositions. Moreover, brain death leads to an alteration of kidney viability as the sodium-to-potassium ratio is dramatically reduced in kidneys from brain-dead donors [18,19].

Reports on the effects of brain death on the liver are limited. The liver seems to be less affected by brain death, potentially related to the organ's relative resistance to compromised perfusion. Morphological characteristics include central and periportal fibrosis, diffuse cell necrosis, fatty metamorphosis, and mononuclear cell infiltrates. Clinically, donor treatment with steroids has been shown to reduce proinflammatory cytokines in the liver, thus improving graft function [6,20].

The lung is especially vulnerable to damage subsequent to brain death, resulting in structural injuries and pulmonary edema. Direct toxic effects of catecholamines, elevated left atrial pressure, systemic hypertension, pulmonary vasoconstriction, and pulmonary endothelial damage all ultimately lead to an increased permeability of pulmonary capillaries. Moreover, volume overload subsequent to volume resuscitation may increase the risk for pulmonary edema compromising gas exchange [21].

Technical considerations of organ procurement in heart beating donors

General preparation and positioning of the donor

Prior to the procurement procedure, the responsible surgeon needs to verify that the donor's blood group has been determined, compatibility with the recipient's blood group has been confirmed, the donor's history has been reviewed, and the brain death protocol has been completed.

Successful organ recovery requires close coordination between all surgical teams participating in the organ procurement process. The primary donor surgeon should introduce the procurement team to the operating room staff and briefly review the steps of the procedure with all participating teams so that recovery can

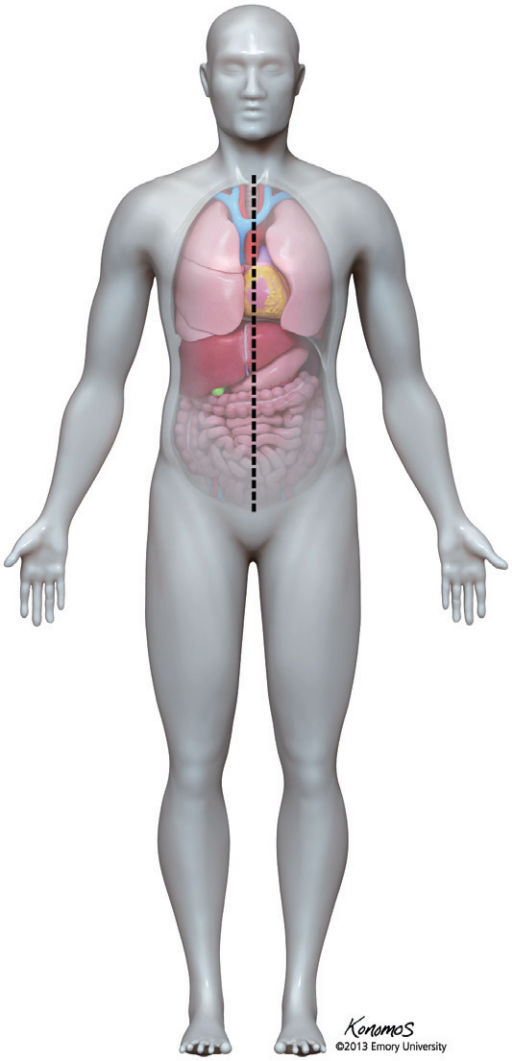


Figure 21.4. The opening exposure for a multivisceral organ procurement procedure.

proceed in a synchronized and expeditious manner. Cardiothoracic organs (heart and lung) are removed first as they are the most susceptible to I/R injury, followed by small bowel, liver, pancreas, and kidneys. The liver and pancreas are recovered en bloc or, alternatively, they may be procured separately. The donor is positioned supine with both arms by the side. Hair is shaved, the surgical site is prepped, and the donor is covered with sterile drapes. For best access, a long midline incision from the suprasternal notch to the pubic bone is made even if thoracic organs are not going to be retrieved [22,23]. Figure 21.4 shows the initial exposure. Diathermy should be used for all of the following steps. Proper hemostasis is particularly important as brain death is linked to a systemic peripheral vasodilatation and compromised hemostasis [24].

After performing a median sternotomy with a sternal saw and achieving hemostasis with bone wax in addition to diathermy, a self-retaining thoracic retractor is inserted. The large Belfour abdominal retractor with teeth can be inserted following the separation of the ligamentum teres hepatis and the falciform ligament. The diaphragm to the left and right of the pericardium should be incised to reduce tension. The pericardium should be divided in coordination with the cardiothoracic team from the apex to the innominate vein. Subsequently, the pericardium will be fixated suprasternally in every corner.

All abdominal and thoracic organs are then examined thoroughly for apparent pathologies. If no evidence for potential contraindications to organ procurement is found and ABO and tissue compatibility have been confirmed, the recipient's center should be informed that the implant procedure may be initiated [25].

Abdominal organs

General considerations

There are two distinct techniques for the procurement of abdominal organs. First, the "rapid perfusion technique with dissection in the cold," which reduces operating time and is recommended for hemodynamically unstable donors and when procuring organs from circulatory death donors (DCD) [26]. In this approach, the abdominal aorta is isolated and cannulated, with no further dissection until core cooling of the organs is achieved in situ. The aim is to minimize organ ischemia by rapid aortic cannulation, exsanguination, and cold perfusion. Following administration of 30 000 IU of heparin, expeditious access to the abdominal cavity is obtained by sharp dissection using a knife and making a midline incision from the xiphoid to the pubis. The Belfour retractor is placed and the retroperitoneal aorta just above the bifurcation is exposed. The aorta or common iliac artery and inferior vena cava (IVC) are cannulated and the flush is initiated. For rapid exsanguination, the suprahepatic vena cava is divided inferior to the right atrium. The pericardium is then opened and the descending thoracic aorta is cross-clamped. Ice and slush are packed around the liver and kidney, which may require further mobilization of the colon so that the kidneys are adequately cooled. Subsequent dissection is carried out after completion of cold perfusion [26]. This procedure is diagramed and discussed in detail in the Chapter 22.

Second, in the standard "warm dissection technique" an anatomical dissection is performed prior to cannulation and perfusion. Some evidence suggests that this technique is associated with more parenchymal and vascular damage due to vasospasm, potentially leading to inferior initial graft function [27]. A recovery period of 30–60 min after dissection might be able to reverse some of these adverse effects. Dissection in the cold on the other hand might be complicated by a more challenging anatomy.

There are many different approaches to the donor operation. Some surgeons prefer to perform all their procurements using the rapid technique. Others use varying degrees of "cold" and "warm" dissection. Irrespective of the technique, multiorgan procurement should be performed meticulously such that all organs can be removed without being compromised. The basic principles of the donor operation include dissection of the great vessels of the chest and abdomen, isolation of the aorta in preparation for cross-clamping, perfusion and core cooling in situ, and rapid recovery to prevent ischemia.

Preparatory steps and cannulation

The peritoneal attachments of the distal ileum and cecum are divided and the retroperitoneal space is exposed with the mobilization of the right hemicolon. Next, the duodenum is mobilized through a Kocher's maneuver and the infrahepatic IVC and abdominal aorta are exposed down to the aortic bifurcation and

the common iliac arteries. At this point, some surgeons prefer to isolate the superior mesenteric artery (SMA) at the root of the small bowel mesentery, and place a vessel loop around it. This is used to control the amount of aortic flush coursing through the pancreas to 1–2 L. The inferior mesenteric artery (IMA) may be ligated and divided at this stage, taking care not to injure any accessory renal arteries [28]. In 1–3% of individuals, lower pole renal arteries can arise from the common iliac artery [29]. The common iliac artery has to be cannulated distally to insure the perfusion of accessory distal renal arteries in these cases. To insure a successful vascular reconstruction, especially in pediatric or split liver transplantations, the IMA should be preserved. In case of severe arteriosclerosis or aneurysms of the distal aorta, the thoracic aorta can be cannulated for perfusion.

The liver is mobilized by dividing the round ligament between heavy silk or Vicryl sutures. Subsequently, the attachments of the falciform, left triangular, and gastrohepatic ligaments are released. Prior to proceeding with the dissection of the gastrohepatic ligament, the presence of an accessory/replaced left hepatic artery originating from the left gastric artery should be explored. This anatomical variation is present in 15–23% of donors and should be carefully preserved [30].

The common bile duct is visualized just above the duodenum and the presence of a replaced right hepatic artery can be palpated posterior to the liver hilum. This anatomical variation is found in 10% of donors. The hepatoduodenal ligament is dissected next, and the common bile duct is exposed, proceeding from lateral to medial by staying as distal and close to the duodenum as possible. The distal bile duct is ligated with 2-0 silk or Vicryl and divided.

The gallbladder is either removed from its bed after dividing the cystic duct between 3-0 Vicryl or Silk sutures, or incised and flushed with saline until the affluent from the divided common bile duct is clear. This step prevents autolysis of the bile duct epithelium during cold preservation [31].

The supraceliac aorta is then dissected in preparation for cross-clamping. This is done by incising the preaortic fascia, retracting the esophagus to the left, and separating the diaphragmatic fibers of the right crus. The supraceliac aorta is encircled and an umbilical tape is placed around it.

Heparin (30 000 IU or 300 IU/kg) is administered at least 3 min prior to cannulation. While waiting for the thoracic team, abdominal organs should be protected with wet abdominal gauzes.

The abdominal aorta is cannulated by tying the distal umbilical tape at the level of the bifurcation of the common iliac arteries. The second tape is then lifted up and the abdominal aorta is incised while occluding the proximal aorta. The perfusion cannula is inserted and the assistant secures the umbilical tape around the cannula. The tip of the cannula should be positioned below the origin of the renal arteries. A second cannula for the portal flush can now be placed [32].

The superior mesenteric vein (SMV) may be used for the portal flush as long as the venotomy is made well away from the margin of the pancreas. Alternatively, the inferior mesenteric vein (IMV) is dissected and isolated at the mesenteric root of the transverse colon and cannulated for the portal flush. Care should be taken not to push the cannula too far, thus preventing injury to the splenic vein.

The surgeon should inform the entire team that preliminary dissection and cannulation has been completed, indicating that it should prepare for cold perfusion and organ removal.

Perfusion

The supraceliac aorta is cross-clamped with a long, straight aortic clamp at the same time as the surgeon transects the vena cava or the right atrium and exsanguinates the abdominal organs into the abdomen or right thoracic cavity. The aortic and portal cannulas are opened to begin perfusion and the organs are packed with slush and ice. Topical cooling of all abdominal organs using ice and cold saline should be applied immediately following perfusion. Pockets are created in the retroperitoneum and the lesser sac to allow for adequate cooling of all organs.

Removal of liver and pancreas

Once the effluent emerging from the transected IVC is clear, the surgeon begins the process of removing the abdominal organs. The pericardium and diaphragm are incised bilaterally: on the left, extending to the esophagus, and on the right, extending posterior the right lobe of the liver, adrenal gland, and IVC. This allows the liver to fall back into the chest. Always keep the liver covered with slush and ice. Figure 21.5a,b details the anatomy relevant to the procurement of the liver and pancreas.

The gastroduodenal artery is identified at the superior border of the duodenum and followed proximally to the common hepatic artery. Once the origin of the common hepatic artery from the celiac artery is identified, the gastroduodenal artery can be ligated. Dissecting proximally on the common hepatic artery will lead to the splenic artery, which is isolated and divided close to its origin from the celiac artery. The splenic artery should not be dissected into pancreatic tissue as this may injure the dorsal pancreatic branch.

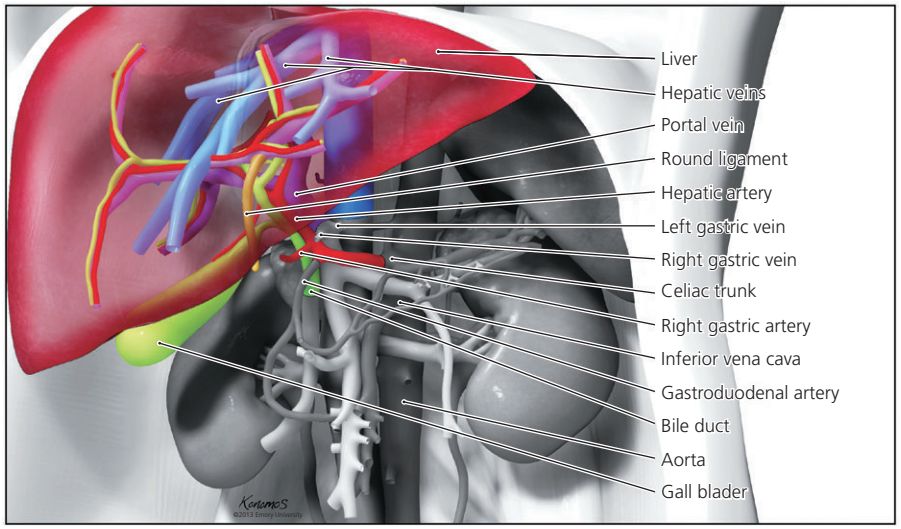
Using sharp dissection along the greater curvature of the stomach, the short gastric vessels are divided up to the diaphragmatic hiatus. The proximal duodenum is stapled just distal to the pylorus and staple lines are thoroughly disinfected. Stomach and duodenum are flushed with betadine and an antifungal solution via the nasogastric tube prior to cross-clamping.

If an accessory hepatic artery has been ruled out, the left gastric artery is divided and transected. All splenic attachments are then divided and the spleen is mobilized completely. The proximal jejunum distal to the ligament of Treitz is stapled, divided, and disinfected; next, the splenocolic ligament is divided while lifting up the tail of the pancreas, with the spleen used as a handle. The dorsal side of the pancreas is dissected using the same technique.

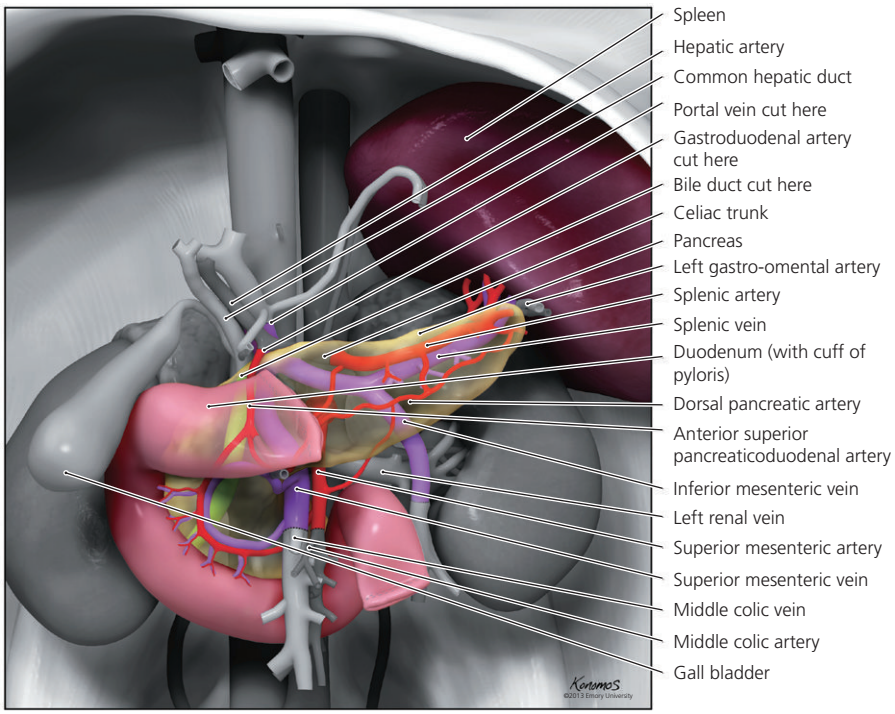
The root of the small bowel mesentery is exposed. The SMA and SMV can then be individually ligated, or stapled and divided, taking care to keep the mesenteric staple line well away from the head of the pancreas and the uncinate process to avoid injury to the inferior pancreaticoduodenal arcade.

The portal vein is divided at the level of the coronary vein, ensuring sufficient venous length for both, liver and pancreas. If the pancreas is not being procured the portal vein should be divided at the splenic confluence.

The SMA is then excised with an aortic patch under direct vision, ensuring the identification of the orifices of the renal arteries, which are usually located just below the origin of the SMA. The infrahepatic IVC is now divided just above the renal veins. To release liver and pancreas en bloc, the right and left paravertebral muscle layers (including the right and left adrenal glands) are divided. The SMA is then excised with the proximal aorta and the celiac trunk. Stiff neural tissue around the celiac trunk might complicate this step. If a replaced right hepatic artery is present, the SMA should be divided distal to the origin of the replaced artery. In rare



(A)



(B)

Figure 21.5. Anatomical relationships of importance for (A) liver procurement and (B) pancreas procurement. The procured anatomy is shown in color.

circumstances the accessory hepatic artery will traverse the substance of the pancreas, in which case the organ may not be utilized [33].

Nephrectomy

The kidneys may be removed en bloc or individually. After removal of the liver and pancreas, the colon and duodenum are mobilized and retracted up into the upper abdomen. The ureters are identified, dissected with sufficient periureteral tissue to insure blood supply and divided as distally as possible. The IVC is divided just above the bifurcation of the common iliac veins, and the aorta is transected at the level of the aortic cannulation. The kidneys are then removed en bloc by incising the prevertebral fascia and mobilizing the kidneys from lateral to medial with Gerota's fascia, using sharp dissection [25]. Kidneys from donors younger than 5 years should always be procured en bloc with a complete abdominal aorta and inferior caval vein.

The pertinent anatomical relationships to kidney procurement are shown in Figure 21.6.

Back table preparation of the kidney

The left renal vein is divided with a cuff of the IVC and the right kidney is preserved with a complete vena cava. The aorta is incised between the paired lumbar arteries, and after visualizing of the

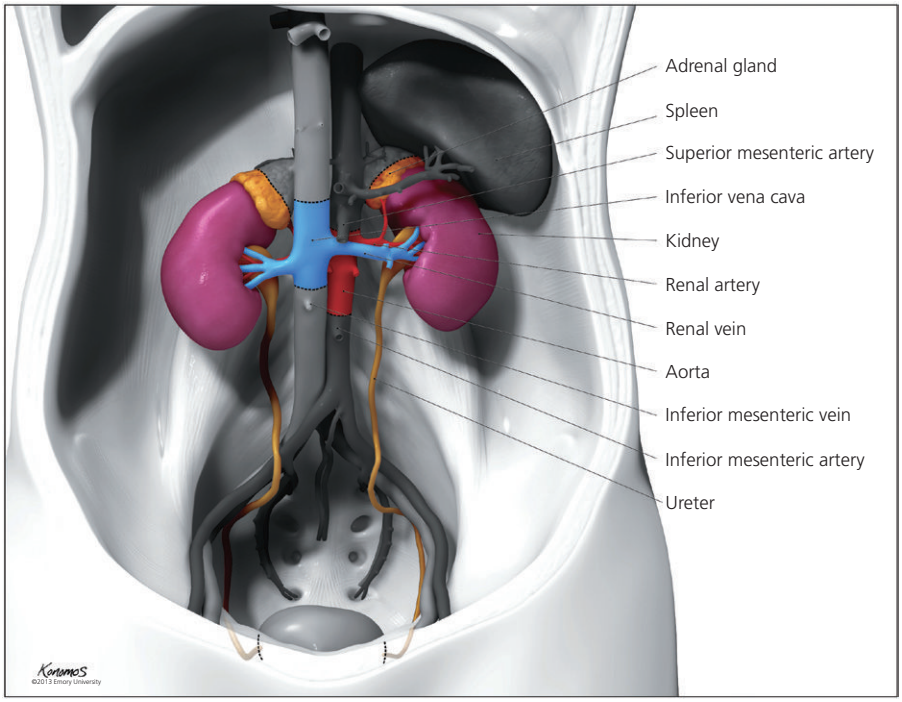


Figure 21.6. Anatomical details of importance in procurement and preparation of the right and left kidneys. The procured anatomy is shown in color.

orifices of the renal arteries from within, the anterior portion of the aorta is divided and the kidneys can be separated. Further preparation of the ureter should not be performed as this might compromise the ureter's blood supply. Placing a hemostat on the tip of the ureter to facilitate its identification throughout the dissection is commonly practiced; however, the ureter should remain cooled and not draped outside the preservation solution for prolonged periods of time.

Removal of iliac arteries and veins

Common, external, and interior iliac arteries and veins should always be procured and need to be sent with the pancreas and/or liver. The iliac bifurcation should be dissected gently to avoid traction injuries. Procurement of the internal iliac artery should include second-degree branches. In case of severe arteriosclerosis, the brachiocephalic trunks should also be procured.

Small intestine

Following the midline sternolaparotomy, the small intestine is thoroughly inspected for apparent pathologies, such as congestion and hematoma, edema, adhesions, petechial hemorrhages, angiodysplasias, or signs of contusion and peritonitis. The motility of the small bowel should be evaluated. The small bowel is wrapped in a moist abdominal pad. Selective gut decontamination with antibiotics (amphotericin B/gentamicin, and polymyxin E) via the nasogastric tube is instituted at the time of procurement.

The abdominal aorta and IVC are exposed from the bifurcation cephalad to the SMA following the mobilization of the right hemicolon and a Kocher's maneuver (see Chapter 62 for illustrations of the procurement technique and details of intestinal procurement). The distal duodenum and the ligament of Treitz are then mobilized. The IMA and IMV are divided and the SMA is exposed with a vessel loop. The distal abdominal aorta and distal IVC are encircled with two 0-Vicryl or Silk sutures. The greater omentum is now divided and the left colon is mobilized and the colic vessels are identified. Antegrade decompression of the intestine is controversial and has become widely discouraged [22].

A complete colectomy is performed after stapler transection of the terminal ileum close to the ileocecal valve. The cecum and right colon are mobilized, preserving the ileal branches of the ileocolic artery. The colon is mobilized further by dividing the middle colic, left colic, and inferior mesenteric arteries. The sigmoid colon is stapled and transected to allow a complete exposure of the mesenteric root. After a left-sided medial visceral rotation (the so-called Mattox maneuver), the tail of the pancreas and the spleen are mobilized as described in detail earlier. The mesenteric root of the proximal jejunum is now divided and the jejunum is divided with a stapler 1–2 inches distal to the ligament of Treitz. The SMA and SMV are exposed by transverse dissection of the mesenteric root distal to the level of the middle colic artery and vein [34]. Critical is the preservation of the SMA–portal vein axis.

An early branching of the SMA requires close attention if the pancreas and small intestine are procured simultaneously. En-bloc removal with back table preparation should be preferred in these cases. The inferior pancreaticoduodenal artery (IPA), which originates just proximal to the origin of the middle colic artery, should be preserved with the pancreas. Injury to the IPA will devascularize the head and parts of the uncinate process of the pancreas as the superior pancreatciduodenal artery (SPA) is sacrificed while removing the liver.

The anterior side of the mesenteric root is marked with a suture prior to stapler transection of the duodenum directly distal to the pylorus. The left gastric artery can then be ligated and the short gastric vessels to the spleen are transected. The abdominal aorta is tied off distal to the diaphragm, and heparin is administered as detailed earlier. The abdominal aorta is cross-clamped and the

perfusion is initiated. The small intestine is the first abdominal organ to be procured after perfusion. The subpancreatic portions of the SMA and SMV are ligated afterwards to prevent backflow of blood into the pancreas graft.

Multivisceral procurement

Following the medial visceral rotation and mobilization of pancreas and spleen, the left liver lobe is mobilized up to the left hepatic vein. The lesser omentum is divided while paying attention to potential left hepatic arteries. The gastrolineal ligament is divided and the distal esophagus is exposed. The cardia can then be transected with a stapler. Next, the abdominal aorta is exposed distal to the diaphragm and clamped or ligated following the application of heparin. Cannulation and perfusion as detailed above follow. Abdominal organs (liver, pancreas, small intestine, stomach, and colon) can then be removed en bloc [35]. Additional detail regarding multivisceral procurement and transplantation can be found in Chapter 74.

Thoracic organs

General preparation

Following a midline thoracolaparotomy as detailed above, the pericardium is opened and fixed to the suprasternal skin. The ascending aorta (up to the aortic arch) and the pulmonary artery are exposed subsequently.

The superior vena cava (SVC) and IVC are exposed and two heavy silk ligatures are positioned around the SVC cephalad to the azygos vein. Sharp dissection of the Sondergaard (Waterston) interatrial groove is then performed to facilitate the differentiation of cardiac and pulmonary left atrial cuffs. After separating the aorta and pulmonary artery, a pearl string suture is placed on the anterior mid-portion of the ascending aorta for the insertion of the perfusion cannula. The perfusion cannula can then be inserted into the ascending aorta after heparin (30 000 IU) has been administered for a minimum of 3 min. A cannulation suture is placed in the distal main pulmonary artery using a similar procedure.

If deemed necessary by the lung team, prostaglandins can be administered intravenously at this point. Prostaglandin E_1 is a strong vasodilator and has been shown in some studies to reduce ischemia/reperfusion injury of the lungs. A shift to anti-inflammatory cytokines might partially be responsible for this effect [36].

The relevant anatomy is displayed in Figure 21.7a,b.

The SVC is then ligated twice after all central venous lines have been removed. In coordination with the abdominal team, the ascending aorta is then cross-clamped as distally as possible to increase the amount of graft tissue. The clamped IVC is incised. If only the heart is to be removed, either the left or right upper pulmonary vein is incised; if both the heart and lung are to be

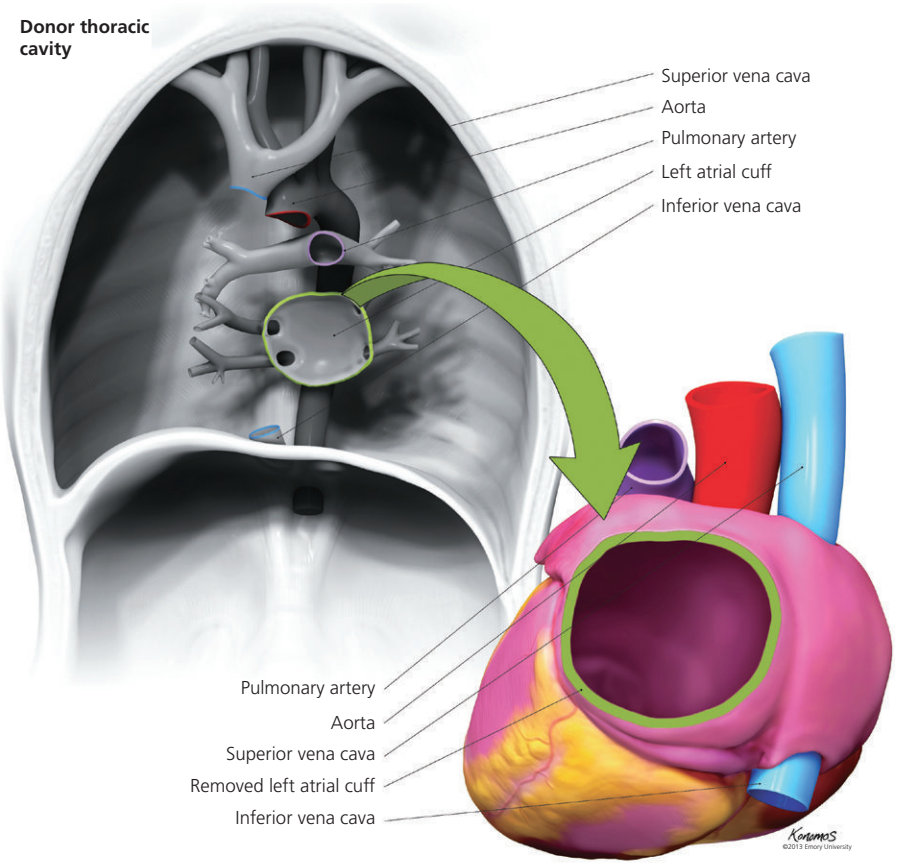


Figure 21.7. The anatomical relationships of importance in (A) heart (before lungs are also procured) and (B) lung procurement (after heart has already been procured). The procured anatomy is shown in color.

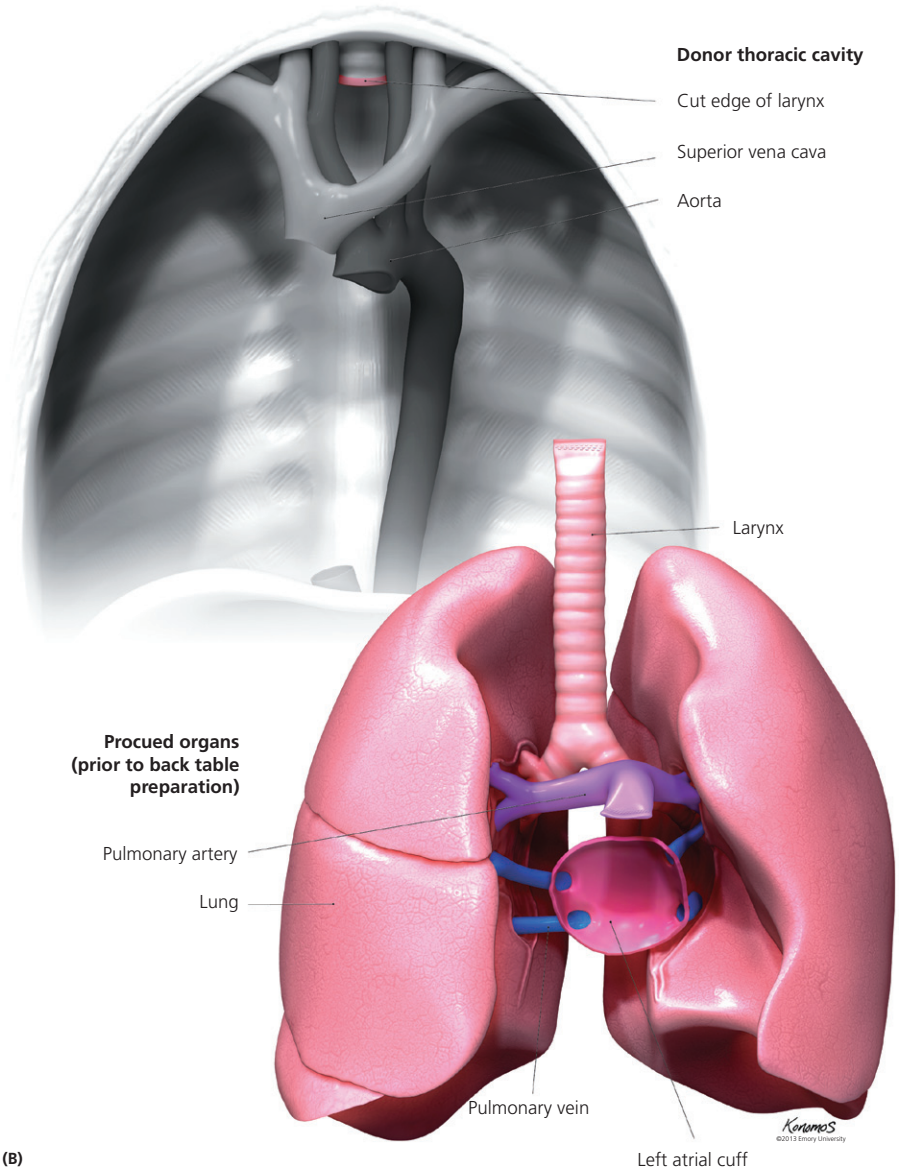


Figure 21.7. (*Continued*)

procured, the left atrial appendage is incised. The main trunk of the pulmonary artery is incised if the lung is to be perfused in a retrograde fashion.

A single flush preservation is started after letting the heart beat empty. The abdominal perfusion can be started in parallel. The pulmonary perfusate is administered through the pulmonary arteries at gravity pressure while the cardioplegic solution is infused at a pressure of 80 mmHg. Local cooling with ice-cold water is started immediately.

An improvement of external cooling of the lungs can be achieved with ice saline slush placed into both pleural spaces through a small reduction of the ventilatory tidal volume. In turn, cardiac cooling is facilitated by the administration of cold saline solution through the pericardium. Pulmoplegia requires approximately 5 L of cold Perfadex® lung preservation solution, while cardioplegia requires a minimum of 1 L of cold crystalloid cardioplegic solution. If both cardioplegia and pulmoplegia are to be administered, cardioplegic solution should be continuously infused for the duration of pulmoplegia. Aortic root pressure and absence of ventricular distension must be continually monitored during infusion of cardioplegic solution. Rapid termination of contractile and electrical activities are hallmarks of adequate cardioplegia. A continuing electrical activity can be related to an unknown coronary artery disease or left ventricular hypertrophy. More and longer cardioplegia should be used in this case. During pulmoplegia, the perfusate must be uniformly distributed throughout both lungs and free flow of gradually clearing solution from the left atrial vent site should be carefully monitored.

Following cardioplegia, the perfusion cannula is removed and the pearl string suture is closed. The incision of the posterior pericardium between the SVC and ascending aorta allows dissection of the trachea above the carina. The anterior, lateral, and medial surfaces of the trachea can be cleared to enable faster tracheal access during the lung excision.

Heart removal

The IVC and SVC are divided between ligatures. If the heart alone is to be procured, the pulmonary veins are also divided between ligatures. If both the heart and lung are to be removed, a transverse incision is made into the left atrium, leaving a sufficiently large cuff of the left atrium around the pulmonary veins. The incision is started at the midpoint between the left inferior pulmonary vein and the coronary sinus, and extended towards the base of the left atrial appendage and towards the inferior edge of the IVC on the right. The left atrium can then be released from the posterior mediastinum by separating it from the posterior pericardium.

The SVC and azygos vein are transected immediately below the supra-azygos and SVC ligature. The innominate and left carotid arteries are separated. The aorta and pulmonary artery should be divided as distally as possible. The heart can then be removed and a final inspection should follow on the back table. Additional detail and illustrations can be found in Chapter 58.

Lung removal

Prior to the beginning of the procurement procedure, a bronchoscopy should be performed to examine the lung for inflammation, edema, lacerations, and amount and consistence of the sputum. A sputum sample should be obtained and a bronchial lavage can be performed. In addition, the arterial blood gas data and chest X-ray should be reviewed.

The ventilator should be disconnected temporarily prior to sternotomy to avoid damage to the lungs during inflation.

Following a midline sternotomy, access to both pleural spaces is gained without cauterization to prevent injury to the lungs. The lungs are then palpated and examined thoroughly, which requires both lungs to be elevated. Parameters and pathologies of interest include size and appearance, adhesions, atelectasis, consolidation, air leakage, bullae or edematous swelling, and palpation for any lesions. Anomalies that can compromise the transplant procedure or organ function following transplant should be immediately reported to the recipient's implant team along with the anticipated cross-clamp time. Additional detail and illustrations can be found in Chapter 70.

The lungs should continue to be ventilated even after the heart has been explanted.

The main pulmonary artery is dissected away from the ascending aorta and the SVC is separated from the right pulmonary artery. After cannulation, all central venous lines are removed and ventilation continues. The SVC is ligated and the IVC is incised right above the diaphragm.

The left atrial appendage is opened to decompress the left ventricle. If the heart is not going to be procured, the left atrium is incised. The ascending aorta is then cross-clamped and perfused with a cardioplegic solution. After a left ventricular distension has been ruled out, the pulmonary perfusion can be initiated. Topical cooling should start immediately and strong suction is used to remove any effluent from the perfusion solution.

After cardio- and pulmo-plegia, both cannulas are removed, the ventilator is disconnected, and the pleura opened widely on both sides. The anterior part of the pericardium is removed along the phrenic nerve anterior to the pulmonary hilum. The posterior part of the pericardium is then excised transversely. The inferior pulmonary ligament on the left side is transected and the left lung is exposed and mobilized to the right. The descending aorta can now be transected below the origin of the left subclavian artery after dissecting upwards close to the esophagus. This step is repeated for the right lung where the dissection is extended up to the level of the azygos vein.

All neck vessels away from the trachea are then divided. Both lungs are gently massaged and manually inflated up to a continuous 30 cmH_2O pressure until all atelectatic tissue is fully expanded. Inflation is then held at a mid-ventilatory level and the trachea is stapled as far cephalad as possible after removal of the endotracheal tube. Two more stapler lines are placed prior to dividing the trachea between the two proximal lines. All remaining mediastinal connections are divided and the double lung block is removed from the thoracic cavity [37,38].

Back table preparation of the lungs

The explanted lungs are re-inspected for all the aforementioned apparent pathologies. Retrograde flushing of the pulmonary arteries by inserting urinary catheters into the pulmonary veins should be performed in order to remove any thromboembolic material and to insure uniform distribution of the preservation solution to both lungs.

Further dissection of the left and right bronchi should be avoided to insure proper vascularization. Only if the left and right lungs are to be transplanted separately, both bronchi should be divided with an additional stapler line at the level of the carina.

Heart and lung en-bloc removal

Pleura and pericardium are excised up to the pulmonary hilum. The SVC, IVC, and aorta are then divided, leaving as much vessel length for the heart graft as possible.

The trachea is then divided between three stapler lines as described above. To remove the heart and lung en bloc, the connective tissue above the esophagus, aorta, azygos vein, and the inferior pulmonary ligament are divided [39].

Organ packing

Following removal from the field, organs are placed into a sterile bag with perfusion solution at 4 °C. This bag is placed into a bag filled with sterile, ice-cold Ringer's lactate or saline and a third empty bag is placed around these two bags, which is then carefully labeled and placed into a transport box with crushed ice. Kidneys, single lungs, and split livers are stored separately and unambiguous labels are attached to the third bag. Vessels are placed into a separate bag with perfusion solution. Blood specimens and segments of the spleen (or, if absent, lymph nodes) are placed in the box with cold Ringer's lactate or saline. Any relevant information about the explanted organ or donor should be updated and included or attached to the transport box. The implant team should then be informed that the organ procurement procedure has been completed.

Care of the donor after procurement

Upon completion of the organ procurement procedure, all foreign materials and fluids are removed and all wounds are closed carefully with watertight sutures before wound dressing is applied. Care should be taken that no derivations are visible before the corpus is transferred in a respectful manner.

Summary

Organ procurement is a technically demanding procedure performed under conditions of temporal pressure and at times

marked hemodynamic instability. The procedure is typically conducted in an operating room with which the surgeon is unfamiliar, at times of night that are taxing, and with other surgical colleagues with whom the surgeon has not operated before. The procedure thus requires exceptional knowledge regarding the entirety of thoracic and abdominal anatomy and specific understanding of the anticipatable consequences of brain death. Operative planning should be intentionally facilitated prior to incision and potential conflicts resolved prior to conduct of the case. Understanding of the donor procurement is the first step to a successful transplant for numerous recipients, and also an opportunity to highlight the transplant field to guest hospitals. It is without doubt an exceptionally important part of the transplant field, and indeed serves as the foundation for success in every deceased donor transplant.

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CHAPTER 22

Techniques for Organ Procurement after Circulatory Death

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Introduction

Prior to the adoption of brain death criteria in 1968, most cases of organ donation were primarily of the non-heart beating donor category [1]. The concept of brain death as an endpoint definition of death was advanced largely through the efforts of an ad-hoc committee on the topic from Harvard University, and the general acceptance of this principle has precipitated the current state in which most organ donation in western countries is either from brain dead donors or living donors. In both of these circumstances, organ procurement proceeds while tissue perfusion continues. In the early 1990s, with the persistent shortage of organs and continuing wait-list deaths, there was a renewed interest in donation after circulatory death (DCD), that is the removal of organs from individuals whose death is commensurate with cardiopulmonary arrest, and as such, is accompanied by progressive warm ischemia. This chapter will review the techniques for procurement of organs from DCD donors. Specific treatment of organ procurement from brain dead donors or living kidney or liver donors, can be found in Chapters 21, 23, and 24, respectively.

Definitions

DCD donors are patients who have suffered catastrophic and irreversible neurological injury, but do not meet criteria for brain death; or whose circumstances of death preclude formal brain death declaration. Death is determined by loss of cardiopulmonary function and classified according to the Maastricht criteria [2]:

Category 1 – dead on arrival to the hospital
Category 2 – death following resuscitation
Category 3 – death following planned withdrawal of life support
Category 4 – cardiac arrest while brain dead

DCD can be divided in to two categories: controlled and uncontrolled. In an uncontrolled setting, patients usually have sustained cardiopulmonary arrest prior to arrival at the hospital, and arrive without cardiorespiratory function either with or without intervening attempts at cardiopulmonary resuscitation. In selected centers, and under the guidance of established protocols, some patients who have been declared dead in the field by cardiorespiratory criteria are placed on cardiopulmonary bypass to maintain some degree of perfusion to the organs, typically excluding cerebrovascular reperfusion [3]. In the controlled setting, patients undergo withdrawal of care in an intensive care unit (ICU) or operating room (OR) setting. Death is declared by virtue of witnessed cardiopulmonary arrest, and organ procurement proceeds thereafter. Our experience is drawn largely from the controlled setting. Nevertheless, the technical details of procurement are similar in both settings, and driven by the need for expedient organ procurement, perfusion with preservation solution, and cooling.

Outcomes

The critical issue in DCD donation has been that of warm ischemia affecting the organs as a result of circulatory dysfunction, arrest, and rapid perfusion and cooling at the time of recovery. This impacts organ viability and graft function. However, there is mounting evidence that brain death might have its own deleterious effect on the quality of organs (see Chapter 21). Organs recovered from a DCD donor might not be subjected to this same inflammatory cascade [4]. A more complete discussion regarding the outcomes of DCD donor organs relative to other categories of donor organs is found in Chapter 65.

The results are more encouraging for DCD kidneys than livers. Studies by Weber et al. have shown that, despite the high rate of early graft dysfunction in DCD kidneys, the long-term graft and patient survivals are equivalent to those for brain dead donor-derived kidneys [5]. Graft survival for DCD donors was noted to be 78% at 10 years; compared to 76% for brain dead donor-derived kidneys.

Another study by Rudich et al. compared 708 DCD organ recipients with 97 990 brain dead recipients in the US between 1993 and 2000 [6]. DCD kidney recipients had a higher delayed graft function rate (42.4%) compared with recipients who received organs from a brain dead donor (23.3%). However, the rate of allograft survival was similar at the end of 6 years (73.2% for DCD, 72.5% for brain dead).

The results for liver transplantation from DCD donors have been inferior to those obtained for kidneys. Nonetheless, DCD donors serve an important role in narrowing the gap between demand and supply of livers for transplant, particularly when the individualized risk–benefit assessment is specifically considered and found to be favorable. Abt et al. reviewed the results obtained from the UNOS database [7]. They compared livers procured from 144 DCD donors with those from 26 856 brain dead donors. The 1-year graft survival rate was lower in the DCD group (80% vs. 85%). The DCD

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recipients also had a higher rate of retransplantation compared to the brain dead group (14% vs. 8%).

Foley et al. also reported a higher rate of vascular and biliary complications in livers obtained from DCD donors [8]. The rate of biliary strictures was higher in the DCD group at the end of 1 year (33% vs. 10%). However, the incidence of hepatic artery thrombosis and primary non-function was the same in both the groups. This study also found that the graft and patient survivals were worse in the DCD group in comparison with the brain dead group. A subset analysis of the DCD group revealed better results with DCD donors under 40 years of age compared to donors over 40 years. Three-year graft survival for livers from DCD donors under 40 years was better than that from the over 40-year-old DCD donor group (65% vs. 45%), although this was not statistically significant. The same was true for patient survival rates at 3 years.

Results from pancreas transplantation using DCD donors have been acceptable, although likely subject to significant selection bias. Qureshi et al. compared patients who received pancreata from DCD donors with those receiving them from brain dead donors between 2008 and 2011 in the UK [9]. They noted no difference in graft thrombosis rates or any difference in hemoglobin $A1_c$ levels at 1 year between the groups. The pancreas graft survival rates were also not significantly different.

A larger study done by Bellingham et al. at the University of Wisconsin had similar results [10]. They compared patients from 1993 to 2008. The pancreas graft and patient survival rates at 1, 3, and 10 years were similar between the DCD and brain dead groups. In addition, there were no differences in perioperative complications like thrombosis, enzyme leak, and abscess formation between the groups.

Preoperative phase

Potential donors are identified after referral to the organ procurement organization (OPO). These patients have had catastrophic brain injury but have not met brain death criteria. Such have patients have been determined to have no significant opportunity for meaningful recovery. It has to be made clear that the transplant team has no involvement in these medical decisions. Also, the patient who is a potential candidate for a DCD is not legally dead or a donor until he/she has been declared dead after cardiac arrest.

Utility of the University of Wisconsin DCD Tool

Crucial to a successful DCD recovery is the ability to predict if clinical death will occur within 2 h of the withdrawal of support. The University of Wisconsin has developed a tool to assess a potential DCD donor and predict if the patient will expire within a set time, thus making him/her a DCD donor (Table 22.1)[11]. A score is calculated based on the following: patient's age, body mass index (BMI), O_2 saturation, method of intubation, level of spontaneous respiration, and pressor requirements. If the score is high, there is a higher likelihood that the patient will expire in the appropriate time period after withdrawal of support. Other models exist, but no prospective comparison between the models has validated one or another as clearly superior.

Table 22.1. The University of Wisconsin criteria for predicting asystole following withdrawal of life support (evaluation tool for donation after death)

Criteria	Assigned points	Patient score
Spontaneous respirations after 10 min		
Rate > 12	1	
Rate < 12	3	
TV > 200 mL	1	
TV < 200 mL	3	
NIF > 20	1	
NIF < 20	3	
No spontaneous respirations	9	
Body mass index		
25	1	
25–29	2	
> 30	3	
Vasopressors		
No vasopressors	1	
Single vasopressor	2	
Multiple vasopressors	3	
Patient age		
0-30	1	
31–50	2	
51 +	3	
Intubation		
Endotracheal tube	3	
Tracheostomy	1	
Oxygenation after 10 min		
O_2 saturation > 90%	1	
O_2 saturation 80–89%	2	
O_2 saturation < 79%	3	
Final score		
Date of extubation time of extubation		
Date of expiration time of expiration		
Total time		
TV = tidal volume; NIF = negative inspiratory force.		
Scoring		
8–12 – High risk for continuing to breathe after extubation;		
13–18 – Moderate risk for continuing to breathe after extubation;		
19–24 – Low risk for continuing to breathe after extubation.		

Reproduced from Lewis et al. [11], with permission from NATCO.

Consents

Organ donation is only discussed after the family, in consultation with the primary treatment team, has made a decision to withdraw support. Once the patient is deemed to be a donor, detailed consents are obtained from the next of kin or their legal surrogate. Consent may be obtained for procedures to be performed prior to death, such as the placement of femoral artery and vein catheters, and the administration of agents such as heparin, phentolamine, amphotericin B, mucomyst, vitamin E, and prednisone (Anderson M, personal communication)

The family is also assured that organ donation would not occur until the patient has expired and been declared dead by a physician who is caring for the patient and who is independent of the transplant team. The family is also told of the possibility of the patient not expiring within a set amount of time, usually 2 h, and that this would preclude the patient from being an organ donor. If this were to happen, the patient would be taken back to the ICU where he/she would expire without organ donation. There is variation in practice regarding the duration of this waiting period, depending on the specific OPO protocol, the hemodynamic status of the donor, and whether the donor is a potential liver donor, among other factors. Importantly, these regional variations are critically important to maintaining the integrity of the DCD process. As such, members of a procurement team must be cognizant and respectful of the specific guidelines governing procurement in their current setting.

Premortem administration of pharmacological agents

As discussed above, the OPO may obtain specific consents from a patient's family for administration of medications prior to

withdrawal of support. These medications may minimize the ischemia/reperfusion injury and have the potential to improve organ function after implantation. There is some evidence to indicate that premortem treatment may have a protective effect on the vascular endothelium and thus have a beneficial effect on the transplanted organ [12].

Heparin is administered prior to withdrawal of support to reduce the risk of thrombi in the recovered organ. There is a theoretical risk that heparin might hasten death, but evidence for this is lacking. Phentolamine may be infused prior to withdrawal to prevent vasospasm and facilitate an adequate organ flush. This agent may cause a transient drop in blood pressure; however, this is not believed to promote the progression to circulatory death. Regardless, the overriding principle is that the transplant team cannot interfere with the progression to death and doubt in this regard should be resolved so as to eliminate any concern from the family or participating members of the donor's care team.

Surgical technique

The goal of a DCD recovery is the rapid procurement of organs to minimize warm ischemia, using a technique that insures that the organs are not injured and are usable for transplantation. Clear communication between the surgical recovery team and the donor hospital OR team is crucial.

Different methods of rapid procurement have been described previously in the literature. We will describe the recovery method used at the University of Wisconsin [13].

A DCD recovery in the OR is preferred. This minimizes time lost in transporting the patient to the OR and prepping the patient for recovery after he/she has been declared dead. The patient's family members are given the opportunity to be with the donor prior to and after withdrawal of support.

All maneuvers actuating the withdrawal of life support from the patient are performed entirely at the discretion of the physician responsible for the care of the patient. The surgical recovery team is in no manner involved in these decisions. As mentioned before, prior to withdrawal of support, and in the setting of consent from the family and care team, we administer 30 000 IU of heparin, 10–20 mg of phentolamine, and 12.5–25 g of mannitol. Consent for medication administration, femoral artery/vein cutdown, and possible cannulation prior to withdrawal will have been obtained from the family. Sedatives, local anesthetics, or narcotics are used at the discretion of the primary care team, but are not to be administered by the transplant team or given in such a way as to promote progression to cardiorespiratory arrest.

Once the patient has been brought to the OR, he/she is prepped and draped from chin to the proximal thigh. We expose the femoral artery and vein and prepare it for cannulation prior to withdrawal of support (Figure 22.1). This cutdown is performed using local anesthesia. During this time, it is important to recognize the potential DCD donor is a patient who is still alive. The 18–20-Fr cannulas are kept ready for cannulation, which is usually done after the patient has been declared dead and the mandatory 5-min waiting period has elapsed. The chilled preservation solution is also kept ready, with the tubing primed for rapid infusion.

Once the premortem interventions as described above are performed, members of the surgical recovery leave the OR. A member of the OPO is usually present in the operating room during the withdrawal process. He/she records hemodynamic measurements every minute after withdrawal of support, along with times of declaration of death, the 5-min waiting period, when the recovery was begun, and the times when the organs were flushed. These data are crucial for the transplant team to decide if the organs can be used for transplant.

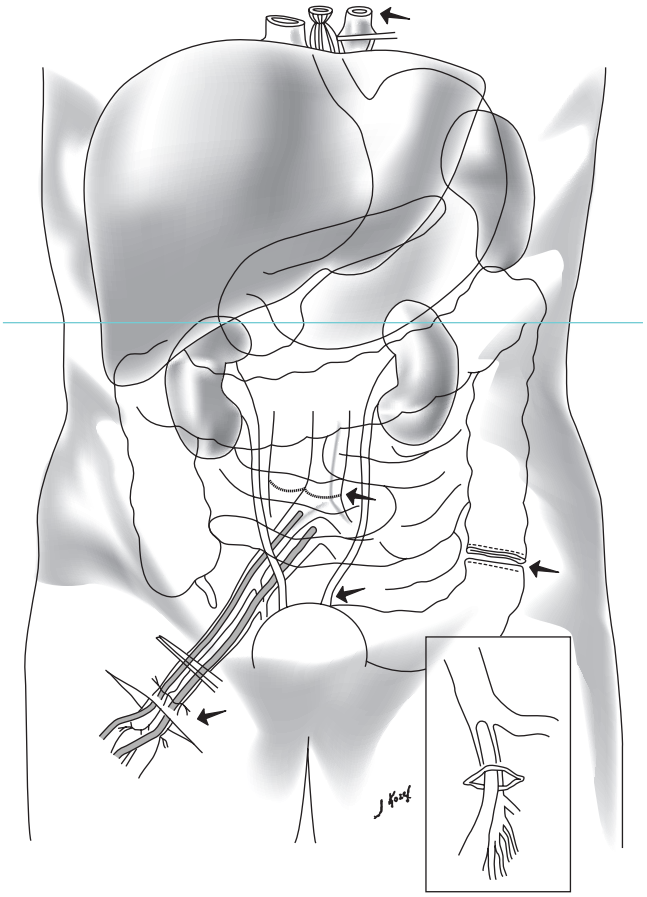

Figure 22.1. Technique of rapid en-bloc removal of all intra-abdominal organs. Arrows indicate major steps and insert depicts ex-vivo superior mesenteric/portal vein flush out. (Redrawn from D'Alessandro et al. [13], with permission from Wolters Kluwer Health)

The patient's primary treatment team withdraws support. This team monitors the patient and notes the time of cessation of cardiorespiratory function. Once the donor is declared dead by the physician, we wait for an additional 5 min to elapse prior to beginning the procurement and infusing the preservation solution. The 5-min waiting period is per the Institute of Medicine guidelines published in 1997 [14]. The issue of the waiting period to assure irreversible death has been under debate. The waiting period is to assure that there is no autoresuscitation after cardiorespiratory arrest has occurred. DeVita et al. reviewed published case reports on 108 patients from 1912 to 1970 who had expired while their vital signs were monitored [15]. They did not find any evidence of autoresuscitation 65 s after cardiopulmonary arrest was noted. The Society of Critical Care Medicine has thus endorsed a 2-min waiting period [16]. Thus, the debate in this area continues. As there is no evidence that an additional 3 min alters outcome, erring on the longer time is prudent.

After the waiting period has elapsed, the surgical recovery team returns to the OR. The femoral artery is cannulated with a preselected cannula. The cannula is inserted to approximately the

aortoiliac junction. A rapid flush with cold preservation solution is begun. Concurrently, a median sternotomy is performed and the abdomen is opened sharply from the xiphoid to the pubic symphysis. The pericardium is opened and the right atrium is incised. This serves as a vent to the flush solution. The femoral vein can also be incised to serve as a vent while the chest and abdomen are being opened. The thoracic aorta is then identified and clamped. This insures that the abdominal organs get the majority of the cold flush. Two to 3 L of preservation solution are infused. The abdomen is filled with ice.

Once the flush is complete and the effluent is clear, retrieval of the abdominal organs en bloc proceeds. The esophagus is divided using a GIA stapler in the chest. A large clamp is placed on the thoracic aorta; this serves as a useful retraction tool. Dissection starts at the level of the right atrium and all retroperitoneal attachments are sharply divided along with the diaphragm. Care is taken to remain anterior to the vertebral bodies and posterior to the aorta and the vena cava. This dissection plane is carried down to the aortic bifurcation.

The organs then are placed in their anatomical position. The lateral attachments of the left and right colon are taken down. The ureters are identified and divided close to the bladder. Dissection is carried to a level just above the aortic bifurcation. Hemostats are placed on the ureters to help with their easy identification. The distal aorta and cava are divided just cephalad to the bifurcation. The sigmoid colon is then identified and divided using a GIA stapler. Any remaining retroperitoneal attachments are divided. The abdominal viscera is then removed en bloc and placed in a large basin with ice. The usual operative time for this portion of the procedure is less than 15–20 min.

Once the organs have been removed en bloc, either the inferior mesenteric vein or a branch of the superior mesenteric vein is identified and cannulated. One liter of preservation solution is flushed through the portal system. The common bile duct is also identified and divided close to the duodenum. The biliary system is flushed with 50 mL of preservation solution. The gall bladder is also opened and its contents emptied. This is also flushed with cold normal saline.

The posterior wall of the aorta is opened longitudinally. The celiac, superior mesenteric artery (SMA), and renal artery orifices are identified. The celiac and SMA arteries are each flushed with 500 mL of preservation solution. The right and left renal arteries are also flushed with 500 mL of preservation solution. The organs en bloc are then stored in preservation solution at 4 °C and transported to the transplant center for further back table dissection. As an alternative, the organs may be divided in the donor operating room for transport to multiple transplant centers.

Prior to completion of the donor operation, the iliac veins and arteries are procured for possible vascular reconstructions. The choice of preservation solutions remains a matter of debate, as does the choice of static versus pulsatile perfusion of the organs, particularly the kidneys, after procurement. Further discussion of these points can be found in Chapters 25 and 26.

Casavilla et al. from the Pittsburgh group have described a "super-rapid" technique (Figures 22.2 and 22.3) [17]. In this technique, the abdomen is opened from the xiphoid to the pubic symphysis. The distal aorta is identified and is cannulated. Perfusion of the organs with cold preservation solution is started. The chest is then opened via a median sternotomy. The thoracic aorta is then cross-clamped and the vena cava is opened to vent. The inferior mesenteric vein is then cannulated to perfuse the

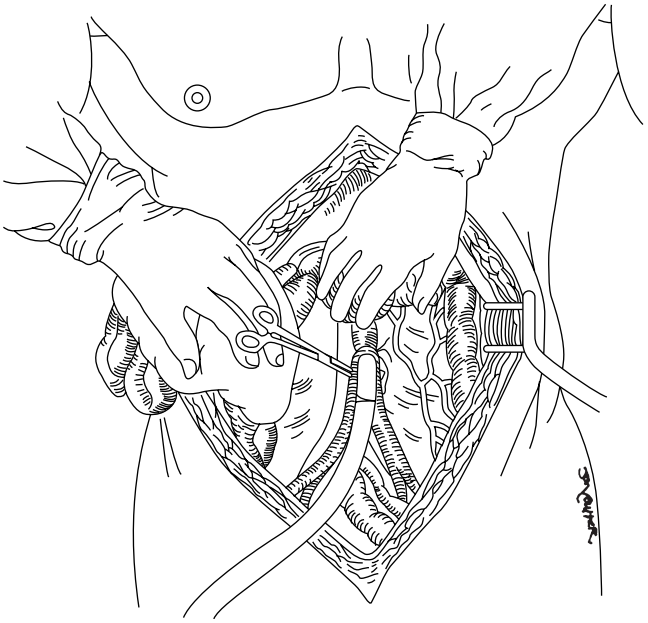

Figure 22.2. The super-rapid technique. Midline abdominal incision and aortic cannulation for immediate prefusion of cold preservation solution. (Redrawn from Casavilla et al. [17], with permission from Wolters Kluwer Health)

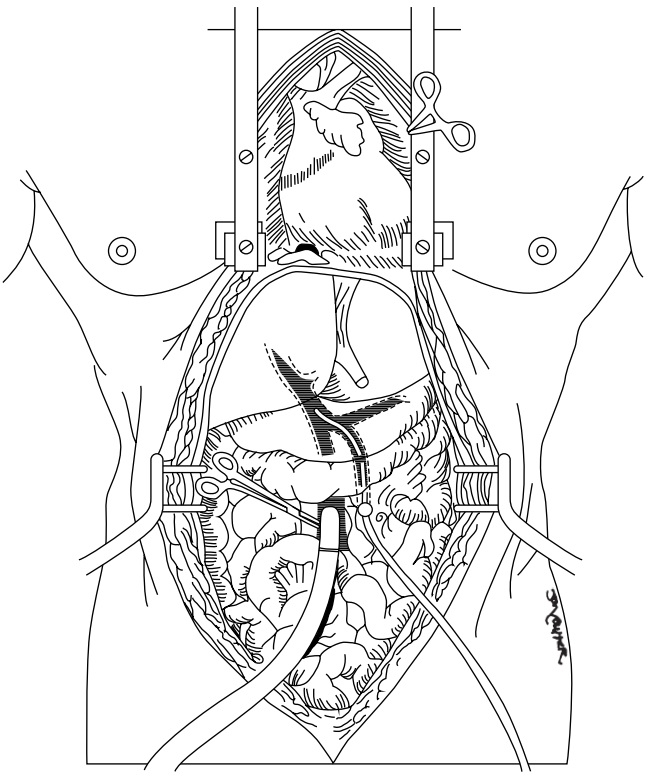

Figure 22.3. Cannulation of the inferior mesenteric vein for cooling of the portal system. (Redrawn from Casavilla et al. [17], with permission from Wolters Kluwer Health)

portal system. Once the organs have been flushed and cooled adequately, a hepatectomy is performed. This is followed by en-bloc nephrectomies.

Reich has described a similar recovery procedure to the "super-rapid" technique mentioned above [18]. He describes in detail how a hepatectomy is performed in situ in the cold and cautions against spending time procuring the pancreas after weighing the risk of injuring a possible replaced or accessory right hepatic artery. In such cases, the liver is procured along with the head of the pancreas. An alternative, the author suggests, is procuring the liver and pancreas en bloc. Once the liver is removed, bilateral nephrectomies are then performed.

Summary

The gap between the number of patients on the waiting list and the number of organs available for transplant is not decreasing. In fact, one may argue that the demand is increasing with supply still lagging. DCD donors present an acceptable source of organs and add to the number of organs available for transplant. Results have shown that the kidneys and pancreata from DCD donors do well in the long term in properly selected circumstances. The results for livers from DCD donors show a higher rate of complications, but nonetheless are acceptable in terms of patient and graft survival when the individual risk–benefit balance for the recipient is properly assessed.

The operative strategy for DCD procurement is somewhat different from that for a brain dead donor. Emphasis is on a safe, expeditious recovery once the patient has been declared dead, without inflicting injury to the organs being procured. The techniques described above should serve as a guide to achieving this.

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CHAPTER 23

Techniques for Living Donor Kidney Procurement

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Introduction

Living donor kidney transplantation is now widely recognized as the most effective treatment for patients with renal failure. Compared to dialysis, it provides excellent function and improved quality of life. Compared to deceased donor transplantation, it provides a reduced risk of delayed graft function, improved long-term function, lower rates of rejection, and increased flexibility in preparing recipients for transplantation. However, as living donor transplantation places a healthy person at risk, the proper use of this modality has remained a matter of some debate since its introduction in 1954. The pressures impelling greater use of living donor kidneys are many and include growing waitlists for deceased donor kidneys, increasing degrees of sensitization amongst potential recipients, and worsening co-morbid conditions of recipients. This is juxtaposed against a generally aging population of potential donors with increasing co-morbid conditions of their own, making the risk of the procedure, particularly when considering long-term kidney function, more concerning. Thus, proper patient selection and operative technique is paramount in the conduct of a living donor transplant to provide a quality organ for an appropriate recipient, while providing safe passage of the donor through a major operative procedure from which he/she has no potential for physical gain. This chapter will cover the evolution of living donor kidney transplantation, examining patient selection, the technical aspects of the donor nephrectomy, and issues likely to influence its outcome. Additional discussion of the ethical aspects of living donation can be found in Chapter 138.

History and current status of living kidney donation

On December 23, 1954, a team lead by Dr. Joseph Murray transplanted a kidney from the then 23-year-old Ronald Herrick into his identical twin brother, Richard. The success of this procedure was a watershed event in the development of both kidney transplantation and living organ donation. Ronald Herrick's selfless gift provided his brother with 8 years of quality life; an event beyond reasonable expectation for patients with end-stage renal disease before the advent of chronic dialysis. Ronald Herrick went on to live a rich and complete life prior to his death at the age of 79 [1].

From this remarkable beginning, kidney transplantation and living organ donation have grown steadily in terms of both the number of procedures performed and their success. Since 1998 there has been a total of 112 012 living kidney donors in the US [2]. During this time period the number of living kidney donor transplants expanded steadily from approximately 2000 to on average >6000 per year. While the factors fueling this growth remain uncertain, it seems likely growing public awareness of the benefits of living donor renal transplantation, new surgical techniques, and changing donor demographics were each important. The demonstration that kidney transplantation confers a significant survival benefit for patients with end-stage renal disease (ESRD) relative to dialysis, together with the knowledge that the short- and long-term survival of kidneys from living donors was vastly superior to that of kidneys from deceased donors, certainly contributed to the growth of living kidney transplantation (also see Chapter 103 for an in-depth discussion of long-term kidney transplant outcomes). Steadily increasing waiting periods for renal transplantation (from 1276 days for unsensitized patients listed in 1999–2000 to 1382 for patients listed in 2003–2004) have encouraged patients with ESRD and their relatives and friends to increasingly consider living donation as an alternative to the transplantation with organs from deceased donors. During this time it was also reported that renal transplantation prior to the institution of dialysis (pre-emptive renal transplantation) was associated with improved graft survival [3]. For the majority of patients with developing kidney failure, living donor renal transplantation represents the only opportunity to undergo a pre-emptive transplant. The introduction of minimally invasive procedures to perform nephrectomies in living kidney donors, while not significantly changing the risks of the surgical procedure, have been shown to reduce postoperative pain and expedite the recovery of the donor, and thus almost certainly have contributed to increased numbers of living kidney donors. This may be particularly germane for the increasing number of donors who are not closely related to their recipient. In this vein it should be noted that much of the growth of living kidney donation has been associated with changes in donor demographics. The most striking trend in this regard is the increase in unrelated and distantly related directed donors, as well as spouse/partner donors observed between 1998 and 2008 [2,4]. This increase has been partially offset by a modest decline in related living kidney

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donors. There is also a trend toward an increase in the age of living kidney donors.

Despite these positive trends in living kidney donation, it is concerning that after peaking at 6647 in 2004, the number of living donors declined each year to 5968 in 2008. After increasing again in 2009 and 2010 (6387 and 6278, respectively), the number of living donors fell again to 5770 in 2011. As the factors driving the increase in the number of living donors in the 1990s and early 2000s are poorly understood, so too are the factors responsible for the decline in the number of living kidney donors in the mid to late 2000s a matter of speculation. Some have speculated that this may be related to the decline in the US economy during this time and the consequent lesser ability of potential donors to cover the costs associated with living kidney donation. Others have reasoned that the increasing age of the population with ESRD listed for kidney transplantation may be associated with more limited numbers of potential living kidney donors. A less speculative factor that has been associated with significantly decreased rates of living donor transplants in children is the Share 35 rule. This initiative prioritizes kidneys from deceased organ donors younger than 35 years of age to patients listed for transplantation prior to their 18th birthday in all cases aside from zero HLA mismatches, allocation of the kidney with life-saving, non-renal organs, or highly sensitized patients. While the Share 35 rule had the intended effect of decreasing the waiting time of children for kidneys from deceased organ donors, it had the unintended consequence of reducing the number of living donor kidney transplants performed in children. Finally, geographic and program-specific differences in the practice of living kidney transplantation may influence the availability of living kidney donation. Generally speaking, rates of living donor kidney transplantation are highest in New England and the north central US, and lowest in the south-east [4]. Finally, it has been reported that the use of medically complex living kidney donors varies widely by program, with the largest programs having a significantly higher proportion of living donors categorized as medically complex [defined as donors with a reduced glomerular filtration rate (GFR), obesity, or hypertension] [5]. It seems highly likely that there are similar differences in the willingness of programs to accept technically or surgically complex donors. The use of medically and surgically complex donors is a controversial area that merits careful study. However, greater dissemination of data and broader consensus about the acceptable medical and surgical criteria for living kidney donation have the potential to increase the access of patients with ESRD to living donor kidney transplantation.

Several other initiatives to increase access to living donor kidney transplantation are being implemented or considered. Approximately one-third of all potential living donor pairs are ABO incompatible. This combined with the high frequency of sensitized recipients waiting for a kidney transplant results in many recipients with medically suitable donors being unable to undergo living donor renal transplantation using standard approaches. Two strategies specifically aimed at increasing the opportunity of highly sensitized patients to undergo living donor kidney transplantation are desensitization and paired donor exchange programs [6]. Critical advances facilitating each of these approaches include the introduction of solid phase assays to detect anti-HLA antibodies (also see Chapter 36) and the potential to more effectively treat antibody-mediated rejection using agents such as the proteasome inhibitor bortezomib or eculizumab, an antibody targeting the complement protein C5 (covered in more depth in Chapter 99) [7]. Desensitization is most easily applied in the setting of living donation and directly addresses the pre-existing antibodies specific for blood group antigens or HLA molecules. The two most common desensitization protocols make use of high-dose intravenous immunoglobulin G (IVIG) alone or low-dose IVIG in combination with plasmapheresis. Rituximab, an anti-CD20 monoclonal antibody, has been added to each of these regimens in an attempt to deplete the B cells responsible for producing the donor-directed antibodies, thereby increasing the effectiveness of each therapy. There are now numerous studies demonstrating that desensitization using either method results in acceptable short-term patient and graft survival. However, the observed rate of acute cellular and antibody-mediated rejection is higher than that observed in recipients without evidence of donor-directed humoral immunity. Furthermore, emerging data suggest that long-term outcomes following desensitization may be inferior to those observed in transplants between individuals without pre-existing donor-specific immunity.

As detailed in Chapter 27, an alternative approach, kidney paired donation (KPD), avoids the deleterious effects associated with immunologic memory by exchanging donor kidneys between the originally intended, immunologically incompatible pairs such that each recipient receives a kidney from an immunologically compatible donor who originally intended to donate to a different recipient [8,9]. The exchange between the incompatible donor and recipient pairs is organized such as to ensure that each recipient with an incompatible donor receives an immunologically more suitable kidney, ideally resulting in a negative cross-match. While making the medical management of the recipient substantially easier and almost certainly improving graft function and long-term survival, the implementation of KPD is mathematically and organizationally complex. Although individual programs have reported remarkable successes [10], the large number of incompatible pairs necessary to optimize KPD has lead to the creation of regional and national KPD programs such as the National Kidney Registry, the Alliance for Paired Donation, and a pilot KPD program operated by the Organ Procurement and Transplantation Network (OPTN) and administered by the United Network for Organ Sharing (UNOS). The introduction of altruistic, non-directed living donors into KPD programs has allowed the creation of transplant chains that have exceeded 30 donor–recipient pairs. Despite the appeal of KPD, mathematical modeling based on large KPD programs suggests that highly sensitized recipients and O recipients with A donors will be relatively disadvantaged. As experience with KPD has increased, it has become increasingly apparent that no single approach is optimal for all groups of patients. As the risk of rejection has been shown to be associated with the strength of the donor-specific antibodies (DSA), desensitization offers the potential for timely transplantation with good outcomes of recipients who have relatively weak DSA. For recipients who are not broadly sensitized but have strong DSA, KPD can provide the realistic option of receiving a living donor kidney without an increased risk of immune-mediated injury. For those patients who are both broadly sensitized and have numerous high-strength anti-HLA antibodies, a hybrid approach using KPD to identify the best potential donor in terms of known DSA together with desensitization to minimize the risk imposed by low-strength DSA may offer the best approach.

An additional initiative under consideration is some form of donor compensation aimed at offsetting at least partially the financial barriers and health risks associated with living kidney

donation. The results of surveys indicating donors' concerns about the potential negative impact of kidney donation on their health and financial status suggest that these concerns are significant barriers to living kidney donation and lend support to careful consideration of limited compensation of living organ donors. However, the National Organ Transplant Act of 1984 (NOTA, see Chapter 19) has been viewed as precluding any form of compensation to living donors. The NOTA makes it illegal to buy or sell organs and further stipulates that it is a crime "for any person to knowingly acquire, receive, or otherwise transfer any human organ for valuable consideration for use in human transplantation." The realization that living kidney donors incur significant personal expenses in terms of travel, lost wages, and potentially future medical risk and expenses has lead some to suggest providing limited compensation to living donors in terms of health and life insurance, as well as a small amount of cash to offset the indirect costs related to kidney donation [11,12]. It is of note that although the Amsterdam Consensus Conference explicitly states opposition to compensating living donors, it does mandate lifetime health insurance, a form of compensation, be provided to living donors [13]. Of all the major industrialized nations, only the US has failed to meet this expectation. Thus, while legal, ethical, and emotional concerns persist, a growing group expresses support for consideration of a limited and regulated system of compensation to offset the financial costs and risks assumed by living kidney donors.

Evaluation, selection, and post-donation follow-up of living kidney donors

Evaluation of the potential living donor begins with a conversation aimed at obtaining basic medical information to determine the potential donor's general health status and to begin the process of educating him/her about the potential risks, both operative and long-term, associated with living kidney donation. It is important to emphasize that the process of obtaining "informed consent" is not an isolated event, but rather a means of initiating an ongoing dialogue that continues and evolves throughout the evaluation and donation process. Consistent with this approach, obtaining informed consent is not the responsibility of any single member of the living donor team, but instead is a process requiring the participation and input of multiple team members, including but not limited to the nephrologist, surgeon, living donor advocate, social worker, and a mental health expert. An important component of the informed consent dialogue is being certain that the potential donor understands that the evaluation/donation process is entirely voluntary and that he/she may terminate the process at any point independent of a specific medical reason. In this event, the transplant team generally assumes responsibility for this decision by indicating that the potential donor was found not to be a suitable candidate for kidney donation, rather than informing the intended recipient that the donor had changed his/her mind about proceeding. In addition to providing potential kidney donors with information related to the possible surgical and long-term medical risks resulting from kidney , it is necessary to inform the individuals being evaluated for living kidney donation about the risks of the evaluation process itself. To those not intimately involved in the process of living kidney donation, the risks of the evaluation process may not be intuitively obvious. However, in addition to the potential risks of medical procedures that constitute the evaluation, such as allergic reactions to the contrast agents used for abdominal imaging, the information obtained in the course of the evaluation itself may be harmful or unwanted. Examples include the discovery of infections or malignancies unknown to the potential donor that may impact his/her established relationships, employment, and future access to healthcare insurance. In some cases these conditions may require that health agencies be informed of the finding. It is also possible that these findings could have significant financial implications for the potential donor. Alternatively, the early diagnosis of these conditions at a time when they may be more amenable to treatment may be seen as a benefit to the potential donor. Another potentially harmful consequence of the evaluation process is related to the results of HLA typing that may demonstrate that believed family relationships are incorrect [14]. For these reasons, it is common practice in living donor kidney transplantation to obtain separate consents for the evaluation process and the surgical procedure. In order for the donor and recipient to determine the balance of the potential risks and benefits of living kidney donation and transplantation it is important that they be provided with both national outcome data and the data of the individual transplant center. As far as possible, this should include a discussion of unique donor or recipient factors that could be expected to modify the outcomes predicted by these data.

The specific goals of the evaluation process are to examine all aspects of the potential donor's medical and psychosocial health in order to determine any conditions that may increase the risk of kidney donation. A complete psychosocial evaluation by a psychiatrist, psychologist, or social worker with experience in transplantation is mandated by the bylaws of the UNOS and the OPTN. The psychosocial assessment of living kidney donors focuses on determining whether the individual has adequate social support systems and financial resources to safely proceed with living kidney donation, as well as assessing the donor for psychiatric diseases and behavioral disorders. Information impacting this assessment includes determining the patient's employment and insurance status, as well as the status of interpersonal relationships with their spouse, family, and close friends. The psychosocial evaluation also assesses the patient's ability to understand the potential short- and long-term consequences of living kidney donation and his/her ability to make an independent, informed decision. The final component of the psychosocial evaluation is an assessment of current or past drug or substance abuse.

Central components of the medical examination include the history and physical examination, with a special focus on a family history of kidney diseases, laboratory testing to include determination of the immunological compatibility with the intended recipient and the presence of transmissible infectious diseases, abdominal imaging to define the renal anatomy, and the results of age-appropriate health screening [15]. The medical evaluation is usually structured to facilitate the early detection of medical conditions that would preclude living kidney donation, thereby minimizing the cost of the evaluation as well as the time spent evaluating unsuitable donors, which in turn allows new donors to be identified and more rapidly evaluated. The components of a standard living kidney donor evaluation are listed in Table 23.1.

The medical evaluation usually begins with a focused medical history aimed at determining if the donor has a history of kidney disease (including proteinuria, renal calculi, frequent urinary tract infections, or chronic use of nephrotoxic drugs such as non-steroidal anti-inflammatory drugs), cardiovascular or cerebrovascular disease, hypertension (including eclampsia or pre-eclampsia),

Table 23.1. Components of a standard living kidney donor evaluation

Studies that may be performed prior to visiting the transplant center
1 Initial interview
2 Completion of a detailed health questionnaire by the donor
3 Informed consent provided by donor to be evaluated as a potential living kidney donor
4 Donor ABO typing
5 Cross-match performed
6 HLA typing of the donor may be done at this time
7 24-h urine collection to quantify renal function and assess for proteinuria
8 At least three random blood pressures provided
9 Other testing as indicated by the history:
  (a) 24-h blood pressure monitoring study
  (b) 2-h oral glucose tolerance test ± a HbA1c
  (c) Metabolic work-up for donors with a history of renal stones
Studies performed during a visit to the transplant center
10 Detailed history and physical examinations by transplant nephrologists and surgeons to include at least three sets of complete vital signs
11 Detailed interviews by the independent living donor advocate and a mental health expert
12 Laboratory studies to include:
  (a) Complete blood count with platelet count and differential
  (b) Electrolytes
  (c) Comprehensive metabolic panel to include fasting serum glucose and measurement of transaminases
  (d) Fasting lipid profile
  (e) Coagulation studies to include the prothrombin time (PT), international normalized ratio (INR), and partial thromboplastin time (PTT)
  (f) Urinalysis and culture
  (g) Pregnancy test if indicated
  (h) Prostate-specific antigen (recommendations based on donor age and family history)
  (i) Serologies for HIV, hepatitis B and C
  (j) rapid plasma reagin (RPR)
  (k) Testing for tuberculosis – TB skin testing or Quantiferon – TB Gold
  (l) Testing for strongyloides, *Trypanosoma cruizi*, and West Nile virus for donors from endemic areas
  (m) Electrocardiogram (ECG)
  (n) Chest X-ray
  (o) Abdominal imaging using CTA or MRA
  (p) Echocardiography and cardiac stress testing as indicated
  (q) Pulmonary function studies and CT scanning of the chest as indicated
Additional studies to be reviewed by the living donor evaluation committee
13 Results of age appropriate cancer screening
  (a) Gynecologic examination with PAP smear
  (b) Colonoscopy
  (c) Mammogram

diabetes mellitus (including gestational diabetes), autoimmune disease, chronic infections, or bleeding/thrombotic/embolic disorders. Individuals without apparent contraindications to organ donation then undergo testing to assess their immunologic compatibility with the intended recipient. This includes determination of the donor's ABO group (and comparison with that of the recipient), a cross-match to determine the presence and, if present, to quantify the amount of donor-directed anti-HLA antibodies, and in some settings HLA typing. HLA typing early in the evaluation process is most common in the setting of multiple, potential donors; it may be deferred until near the end of the process with single donors to avoid the expense in the fraction of individuals who are determined not to be eligible kidney donors. At this stage incompatible donor–recipient pairs wishing to proceed may be offered the option of paired donor exchange programs, desensitization, or a combination of the two approaches as an alternative to identifying other donors or waiting for a kidney from a deceased organ donor. Compatible donors may then proceed with a 24-h urine collection to assess both renal function (creatinine clearance) and proteinuria. The medical history may dictate additional studies, such as an oral glucose tolerance or hemoglobin A1c (HbA1c) in individuals at increased risk of diabetes, a 24-h blood pressure study in those at increased risk of hypertension, and a urine collection to assess the risk of stone formation in individuals with a history of renal calculi. Routine laboratory studies, including a complete blood count, a comprehensive metabolic profile, a lipid profile, a urinalysis, coagulation studies, hepatitis and HIV serologies, a rapid plasma reagin (RPR) test for syphilis, and testing for tuberculosis (TB skin testing or an interferon-γ release assay (e.g. Quaniferon®–TB Gold), and a urine culture, may also be attained at this time. Additional testing for strongyloides, *Trypanosoma cruzi*, and West Nile virus is indicated for potential donors from endemic areas (see Chapter 92 for additional consideration of donor-derived infections). The potential donor should also provide a series of blood pressure values at random times.

Individuals whose test results are compatible with living kidney donation may then present to the transplant center to undergo further diagnostic studies and imaging, which at a minimum would include an electrocardiography (ECG), chest X-ray, abdominal imaging, and completion of any of the laboratory testing described above that were previously deferred. Individuals over the age of 50 years or those over 40 years with risk factors for coronary artery disease, such as a history of tobacco use, hypertension controlled by a single agent, an abnormal ECG, or a strong family history of early coronary disease, should undergo cardiac stress testing. Potential donors found to have a murmur should undergo echocardiography. Donors with a history of syncope, dizziness, or palpitations should undergo both an echocardiogram and Holter monitoring. Potential donors with a sustained history of cigarette smoking or an abnormal chest X-ray may require pulmonary function testing or a chest computed tomography (CT) for further evaluation. Based on their gender, age, and family history, potential donors should also undergo screening for cervical, breast, prostate, colon, and skin cancer in accordance with the American Cancer Society recommendations.

Abdominal imaging is a critical component of the evaluation of potential living kidney donors. It is usually preformed later in the course of the evaluation to minimize cost and avoid exposing potential donors who are deemed not to be candidates for other reasons to radiation and contrast agents. The one exception to this approach may be the early use of ultrasound to screen potential donors with a family history of polycystic kidney disease to exclude those with obvious renal cysts from further evaluation. The choice of the imaging modality used has evolved with the introduction of new technologies and the improvement of existing approaches to abdominal imaging. Angiography, once the mainstay of imaging for living donors, is now rarely if ever used due to its inherent risks and its limited potential to detect incidental findings involving organs other than the kidneys that may influence the decision about an individual's candidacy for living kidney donation. Computed tomography angiography (CTA) and magnetic resonance angiography (MRA) are each widely used for imaging the abdominal organs of potential living donors. Both define the vascular anatomy of the kidneys with a high degree of accuracy and are similarly effective for assessing other abdominal organs and tissues for anatomic or pathologic entities that may influence an individual's suitability to be a living kidney donor. CTA has the disadvantage of exposing potential donors to ionizing radiation and iodinated contrast agents that may provoke an ana-

phylactic reaction in susceptible individuals. These concerns are mitigated to some extent by improvement in scanning protocols that reduce radiation exposure as well as the introduction of safer contrast agents. One advantage of CTA compared to MRA is its ability to detect small-to-moderate sized renal and ureteral calculi not visualized by MRA. Aside from avoiding exposure to radiation and iodinated contrast agents, evolving MRA protocols may allow for the quantification of renal perfusion and function. Given the current capabilities of CTA and MRA, the choice between the two modalities for imaging potential living kidney donors likely depends on institutional considerations, including the available equipment and the necessary interpretive expertise. Radioisotope renography using agents such as ^{99m}Tc-DTPA or ^{99m}Tc-labeled MAG3 also allow for assessment of renal perfusion and function. As the capabilities of cross-sectional imaging modalities have improved, nuclear medicine renograms are used less frequently, but still may contribute useful information in the setting of significant size discrepancies that may suggest differences in the functional contributions of the right and left kidneys.

Review of the results of the living kidney donor evaluation by a multidisciplinary evaluation committee is a critical component of the evaluation process. The composition of this evaluation panel varies by institution, but should generally include a nephrologist, surgeon, living donor coordinator, an independent living donor advocate, social worker and/or mental health expert with knowledge about kidney transplantation and donation. The members of this group should be independent of the team evaluating potential renal transplant recipients in order to objectively assess the risks to the donor without being biased by considering the potential benefits to the recipient. Although criteria vary somewhat by program, commonly accepted exclusion criteria for living donation are shown in Table 23.2. The areas of special focus during the committee's deliberations are described below [16].

Table 23.2. Potential contraindications to living kidney donation

- Age <18 years or a mental or psychiatric condition that impairs the prospective donor's ability to assess the potential risk or make an autonomous decision
- Established diabetes mellitus or impaired glucose tolerance
- Hypertension
  - Requiring multiple agents or high doses of single agents for control
  - With evidence of end-organ injury
  - In populations at increased risk for kidney damage
- Cancer current or treated but at significant risk for recurrence, excluding non-pigmented skin cancers that have been adequately treated
- Communicable infectious diseases such as HIV, hepatitis B, hepatitis C, West Nile virus, and Chagas disease
- Evidence of renal disease, including a reduced creatinine clearance, proteinuria, or hematuria
- Documented risk for inherited renal diseases
- Multiple or recurrent renal calculi of a metabolic condition that predisposes to the recurrence of renal calculi
- Renal abnormalities, including significant discrepancy in the kidney sizes or anomalies of the renal vasculature that predispose the recipient or donor to increased technical complications
- Coronary or peripheral vascular disease
- Significant valvular heart disease
- Autoimmune diseases such as rheumatoid arthritis, Crohn's disease, or ulcerative colitis
- Chronic lung disease
- Morbid obesity or obesity with co-morbid conditions
- History of major thrombotic or hemorrhagic events
- History of substance abuse without completion of an appropriate rehabilitation program

Donor age

Results of a survey reported in 2007 showed a trend towards the acceptance of older individuals as living kidney donors [15]. Of the 132 centers surveyed in the US, approximately 21% excluded living donor candidates over 65 years of age, while 60% of the programs had no set upper age limit. Simultaneously, transplant programs are more closely scrutinizing younger donors. An age of younger than 18 years is considered a contraindication to living kidney donation by virtually all programs and donors aged 18 years–21 are more closely evaluated, particularly with respect to their maturity and motivation to donate. This more cautious approach is motivated by their relatively longer expected survival, concerns about the rate of decline in renal function with increasing age, and the obligation to be certain that their decision to donate is made autonomously. The living donor advocate's role is central to this process.

Hypertension

While hypertension was previously considered an absolute contraindication to living kidney donation, programs are increasingly considering and evaluating potential living donors who may have risk factors for the development of hypertension or may even have been diagnosed with mild, well-treated hypertension. Studies have demonstrated that a significant percentage of individuals classified as hypertensive based on measurements of blood pressure performed in the clinic are found to be normotensive based on 24-h ambulatory blood pressure monitoring studies [17]. For the purpose of classifying living donor candidates an average blood pressure of >135/85 mmHg is considered diagnostic of hypertension. Regarding those accurately diagnosed as having hypertension, an increasing number of programs now consider individuals achieving good blood pressure control with a relatively low dose of a single antihypertensive agent (or more rarely two agents) as appropriate candidates for living kidney donation, assuming the absence of end-organ injury such as left ventricular hypertrophy, retinopathy, or proteinuria. Hypertensive individuals with risk factors for cardiovascular disease, such as hypercholesterolemia, obesity, smoking, and a strong family history of coronary artery disease, would also be excluded from living donation. The trend toward accepting selected individuals with well-controlled hypertension as living kidney donors is based on reports that with short-to-intermediate term follow-up, living donors with mild-to-moderate essential hypertension and normal renal function did not experience worse outcomes with respect to blood pressure, proteinuria, or renal function when compared to non-hypertensive donors [18,19]. Frequent exclusion criteria for potential donors with mild, well-controlled hypertension include relatively younger donors (age <45–50 years), African–American donors, and those donating to more distantly related/non-related recipients under the assumption that they will derive a lesser psychological/emotional benefit. Those donors with pre-existing hypertension who meet the selection criteria for living kidney donation should be informed of the relatively incomplete safety data related to the short-term follow-up of hypertensive donors to date, and be encouraged to make the appropriate lifestyle modifications, including adhering to an appropriate diet, engaging in moderate exercise, and committing to routine, scheduled medical care.

Diabetes

Overt diabetes, defined by the American Diabetes Association as a fasting plasma glucose of >126 mg/dL, a plasma glucose level of

>200 mg/dL 2 h after a 75-g oral glucose challenge, or a HbA1c of >6.5, is a contraindication to living kidney donation. Similarly, impaired glucose tolerance also precludes donation at most centers due to its association with an increased risk of developing diabetes in the future. Impaired glucose tolerance is defined as a fasting plasma glucose between 100 and 126 mg/dL, a glucose between 140 and 200 mg/dL in a 2-h oral glucose tolerance test, or an HbA1c ranging from 5.7 and 6.5. While a normal fasting glucose value is sufficient to exclude diabetes and impaired glucose tolerance in most donors, individuals with a past medical history of impaired glucose tolerance, gestational diabetes, delivery of a child with a birth weight of >9 lb, first-degree relatives with diabetes, African–Americans, and individuals with a body mass index (BMI) of >27 kg/m^2 should all undergo a 2-h oral glucose tolerance test to more accurately assess their future risk of diabetes. In addition to a glucose tolerance test, measurement of their HbA1c may provide useful information in assessing potential donors with risk factors for diabetes mellitus who do not meet the diagnostic criteria for diabetes or impaired glucose tolerance. In addition, individuals with multiple risk factors for the metabolic syndrome, including high blood pressure, triglyceride levels of >150 mg/dL, high-density lipoprotein levels of <40 mg/dL for a man and <50 mg/dL for a woman, should undergo screening for glucose intolerance. Individuals at risk of developing diabetes mellitus but not meeting the criteria for impaired glucose tolerance may proceed with donation given an appropriate understanding of the future risks and counseling related to the need for lifestyle modifications. Weight loss may significantly improve glucose homeostasis and metabolic parameters, although concerns about the donor's ability to maintain the lifestyle associated with these changes must be carefully assessed.

Obesity

Some evidence suggests that obese and morbidly obese living donors are at increased risk for renal dysfunction following kidney donation [20]. They are definitely more likely to have proteinuria, hypertension, and hypercholesterolemia; each of which increases their life-time risk of cardiovascular disease. In fact, irrespective of kidney donation, obese individuals have decreased survival relative to those with normal weights. Although a point of contention, in consideration of these risks, many programs impose an upper limit on the BMI of living donors. The value most widely used among centers imposing size limits is a BMI of 35 kg/m^2, although a small number of centers restrict living donation candidacy to individuals with a BMI of <30 kg/m^2. One intermediate approach is to allow individuals with a BMI of up to 35 kg/m^2 to proceed with donation in the absence of other conditions related to obesity, while those with borderline hypertension, borderline glucose intolerance, hypercholesterolemia, and ongoing use of cigarettes are required to attain a BMI of ≤30 kg/m^2.

Assessment of donors for underlying renal diseases

Renal function is perhaps the best single predictor of kidney health and renal diseases. Determination of an individual's creatinine clearance (CrCl) is the most widely used measure of renal function. The use of formulas to estimate CrCl may provide some insights that help in deciding which potential living kidney donors to evaluate, but are not sufficiently accurate to serve as the sole means of assessing renal function in prospective living kidney donors. When equations are used, the CKD-epi equation is preferred as it provides a more accurate estimate of CrCls in the normal range as compared to the MDRD of Cockrault–Gault equations. A 24-h urine collection is the most widely used method to measure the CrCl of prospective kidney donors. However, this relatively simple test is subject to numerous errors, including over- and under-collection, that make its results variable and <100% reliable. Another approach is to use radioisotopes or iodinated tracers to directly measure the CrCl. Although conceptually more accurate in that the test is directly supervised, many centers do not use this methodology due to its logistic complexities. Regardless of the method used to determine a potential donor's level of renal function, a threshold value for CrCl must be selected. Historically, many programs selected a value of 80 mL/min as the threshold. With the definition of the stages of chronic kidney disease (CKD), many programs adopted the threshold of 90 mL/min, which defines stage II CKD. Regardless of the value chosen, the measure of renal function should be normalized for donor size by expressing it as the value per 1.73 m^2. An increasingly common approach is to define acceptable thresholds as being within 2 standard deviations of the normal value for the potential donor's age and sex, thereby taking into account the effect of these variables on renal function.

Significant proteinuria and hematuria each are strong indicators of serious underlying glomerular pathology and renal disease. Proteinuria can be quantified by determining the amount of protein present in a 24-h urine collection or by measuring the albumin-to-creatinine ratio on a spot urine sample. Values of >250–300 mg/24 h or >30 mg/g, respectively, are generally considered indicative of possible significant underlying pathology and would exclude donation in the absence of known benign causes such as postural proteinuria. Reasons for falsely elevated measurement of proteinuria based on a 24h urine collection include intensive exercise immediately prior to the collection period and over-collections, which can be estimated based on the amount of excreted creatinine (15–20 mg/kg for women and 20–25 mg/kg for men). Like proteinuria, hematuria can be indicative of serious underlying renal diseases. The definition of microscopic hematuria varies somewhat by transplant program, but typically is defined as five or more red blood cells (RBCs) per high power field. The first step in evaluating hematuria is to obtain an accurate history. In females who are menstruating or men or women who have been exercising intensively, collection of a repeat urine sample in the absence of the potentially confounding event is indicated. Urinary tract infections and renal stones can be excluded as the cause of microscopic hematuria by obtaining a urine culture and CT scan of the abdomen and pelvis. If these diagnostic studies fail to reveal the cause of the hematuria and it persists, urine cytology and cystoscopy are indicated. If these studies are not informative, individuals who remain interested in kidney donation should undergo kidney biopsy to exclude intrinsic kidney disorders as the cause of the hematuria. In the absence of a documented benign cause such as thin basement membrane disease of mild severity, potential donors with persistent hematuria should be counseled against living kidney donation.

Screening for hereditary renal diseases

For potential living donors with a significant family history of renal disease, genetic testing and a kidney biopsy in addition to abdominal imaging studies may be necessary to assess their future risk of developing kidney disease. Examples of renal diseases with signifi-

cant genetic components include adult polycystic kidney disease (ADPKD), systemic lupus erythematosus (SLE), thin basement membrane disease (TBM), Alport's syndrome, and IgA nephropathy. ADPKD is the fourth leading cause of renal failure in the US and is the most commonly inherited renal disease. Loci related to ADPKD include mutations on chromosome 16 (*PKD1* locus, 85% of affected patients) and chromosome 4 (*PKD2* locus, 15% of affected patients). Patients with the *PKD2* mutation may present later in life. Individuals 30 years of age or older who do not have renal cysts as determined by high-resolution, cross-sectional abdominal imaging can be considered free from the risk of developing ADPKD. Potential donors younger than 30 years old require genetic testing to determine whether they are at risk for ADPKD in the future. SLE may occur in approximately 12% or more of individuals with an affected first-degree relative. The evaluation of living donors with a family member affected by SLE should include studies to detect the presence of antinuclear antibodies, antiphospholipid antibodies, and measurement of complement levels. Persons thought to be at risk for developing SLE based on these studies should be excluded from donation. Familial kidney diseases that present with hematuria include TBM disease, Alport's syndrome, and IgA nephropathy. TBM generally has an autosomal dominant pattern of inheritance. First-degree relatives of affected individuals should be screened for hematuria as well as hypercalciuria and hyperuricosuria. Individuals who do not have severe thinning of their basement membranes on renal biopsy, do not have proteinuria or hypertension, and do not have concurrent IgA nephropathy or Alport's syndrome may proceed with kidney donation, although the potential donor should be counseled that the long-term risk of renal disease in this setting is not known with absolute certainty. Alport's syndrome has different modes of inheritance with most being X-linked. Components of Alport's syndrome include sensorineural hearing loss, ocular findings such as lenticonus, cataracts and retinal lesions, hematuria, and hypertension. Males aged 20 years or older found not to have any of these conditions can proceed to donation. Female siblings of affected individuals with normal urinary results may also proceed to donation.

Renal calculi

Results of a recent survey indicate that 77% of the responding programs allowed individuals with a history of kidney stones to be considered for living kidney donation [21]. Patients with a history of documented kidney stones should undergo a metabolic evaluation to determine whether there is a metabolic condition, such as hypercalciuria, hypocitraturia, hyperuricemia, hyperoxaluria, or metabolic acidosis, that places them at increased risk for stone formation in the future. Most programs exclude potential donors with a metabolic cause for kidney stone formation that cannot be corrected and those who have formed multiple stones or developed their first renal stone at a young age, as these individuals are at increased risk for recurrence.

The final component of the living kidney donation process is an attempt to ensure that living donors are instructed about the need for maintaining a healthy lifestyle. This discussion should address a healthy diet, moderate exercise, and routine age-appropriate medical care. At a minimum, this should include routine measurements of blood pressure and laboratory studies, including a serum creatinine, urinalysis, fasting blood glucose, and fasting lipid profile.

Operative technique

Living organ donation is one of the few procedures that surgeons perform where the intent is not to improve the condition of the patient undergoing the procedure; instead, it actually places his/her health at risk for the benefit of another individual. While there are tangible and intangible benefits as a result of the donor evaluation process, the majority of the risk associated with donation is related to the operative procedure. Given the dictum of medicine is "primum non nocere" or "first, do no harm," it particularly behooves the surgeon to understand and ultimately optimize the operative techniques employed.

As mentioned above, the evaluation process for living donation can be quite complex. While the medical and psychosocial metrics are extremely important and may contribute to predicting long-term success, it is the donor's fitness for surgery and anatomic considerations that are paramount in the surgical preoperative assessment.

While the preparatory medical evaluation has previously been discussed, including specific populations warranting additional testing, there are also anatomic considerations that must be taken into account regardless of the specific surgical technique that is employed. The use of preoperative abdominal imaging in this day and age is considered mandatory. The choice of technology, either CT or magnetic resonance imaging, is primarily determined by the expertise present at one's institution. We prefer CT imaging with three-dimensional reconstructions, which impart superb depictions of both arterial and venous anatomy (Figure 23.1). While most early experiences with minimally invasive donor nephrectomy considered vascular anomalies such as multiple renal arteries or circumaortic renal veins to be a contraindication to donation, this is not the case in the current era. Currently, most centers will proceed with nephrectomy with two or even three renal arteries or when there are complex venous aberrations [22]. Preoperative imaging, both CT and MRI, has been shown to accurately predict eventual operative findings and provide critical information to the donor surgeon [23]. In addition to providing clarity to both arterial and venous anatomy, preoperative imaging can provide excellent definition of the renal parenchyma as well as relay information about the collecting system and ureter with delayed sequences during excretion of filtered contrast. Lastly, but of paramount importance, abdominal imaging can alert the surgeon to any occult intra-abdominal pathology that might prevent donation.

Once it has been determined that there are no medical, psychological, or anatomic contraindications to donation, the surgeon can proceed with finalizing an individualized operative plan. Current techniques of living donor nephrectomy vary among centers and have significantly evolved over time as newer strategies have been introduced. We will discuss the traditional "open" operative technique as well as the more recently described minimally invasive approaches to safely remove the donor kidney without jeopardizing patient safety or donor organ quality.

Open donor nephrectomy

When Ronald Herrick stepped forward to donate his kidney to his twin brother over 50 years ago, Joseph Murray and his surgical team procured the kidney via an "open" donor nephrectomy. Similar to other traditional operative procedures of the day, there was no thought of minimizing the invasiveness of the procedure. Open donor nephrectomy is a well-established procedure to safely procure a kidney from a transplant donor. The patient is positioned with the right or left flank elevated and exposed (depending on

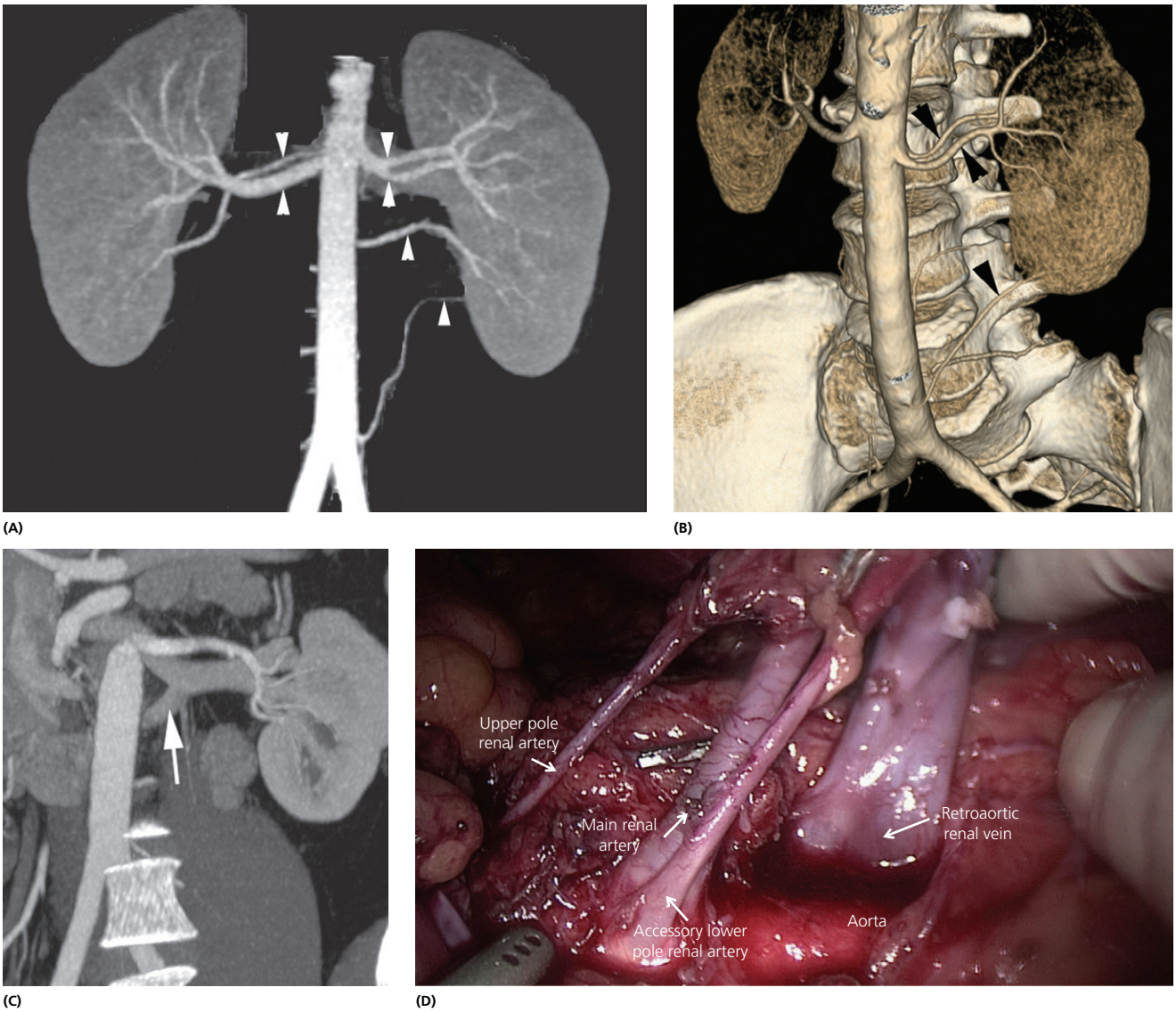

Figure 23.1. Reconstruction of abdominal computed tomography (CT) scans demonstrating aberrant anatomy. (A) The renal arterial anatomy for a potential donor with two right and four left renal arteries (each arrowhead marks a separate artery). Of note, the smaller right renal artery originates cephalad to the main renal artery but supplies the low pole of the kidney. On the left there is a small lower pole renal artery arising at the level of the aortic bifurcation. This arterial anatomy may increase the risks of technical complications with use of either the right or left kidney. (B) A donor with three left renal arteries, including a small artery supplying the lower pole that originates near the aortic bifurcation (each arrowhead marks a separate artery). Of note, there is a single right renal artery making the right kidney potentially more suitable for donation and transplantation. (C) CT reconstruction from a donor with a circumaortic left renal vein (the arrow marks the retroaortic component of the circumaortic vein). (D) An intraoperative picture of three renal arteries and a retroaortic renal vein. While it is important for the surgeon to be aware of this aberrant anatomy prior to surgery, this finding does not significantly increase the risks of technical complications or impact the choice of which kidney to use.

which kidney is to be removed). The incision begins at the tip of the 12th rib and curves anteriorly in the direction of the umbilicus until reaching the lateral border of the rectus sheath. The surgeon then continues to divide the muscular layers of the abdominal wall—external and internal oblique muscles as well as the transversalis. If needed, the latissimus dorsi muscle is divided and retracted posteriorly. Occasionally, a portion of the 12th rib may need to be removed for exposure. Deep to the muscular layers, the peritoneum is identified and pushed medially to expose the retroperitoneal space. The ureter is identified emanating from the lower pole of the kidney and coursing along the anterior surface of the psoas muscle. It is carefully mobilized down to the pelvis with a generous packet of periureteral tissue to ensure adequate blood supply. A vessel loop or umbilical tape may be passed around the mobilized ureter to aid with gentle retraction. Gerota's fascia is then incised and the kidney is separated from the surrounding perinephric fat. The renal vein is then identified and mobilized medially towards the inferior vena cava (IVC). When harvesting a left kidney, the vein is freed to the point where it crosses the aorta. The adrenal vein must be divided on the superior aspect, while the gonadal and one or more lumbar

veins must be identified and divided on the inferior aspect. The shorter right renal vein does not usually have any connecting branches and needs to be mobilized to the point where it enters the IVC. The renal artery is skeletonized near its origin from the aorta after lifting the kidney and reflecting it anteriorly. If the surgeon prefers, a dose of systemic heparin may be given at this time. The ureter is then ligated distally and transected. The renal artery and then vein are clamped and divided, leaving a sufficient cuff of donor vessel to allow for adequate ligation. The donor kidney is removed from the field and handed to the waiting recipient surgeon. The donor renal artery is carefully cannulated and a cold perfusate is used to flush the organ in preparation for its implantation. The wound is then closed in layers with an absorbable suture and the patient is awoken from anesthesia and returned to the recovery room.

The early postoperative care of donors is very similar to that of anyone undergoing a major intra-abdominal procedure. Volume status, electrolytes, as well as urine output are closely monitored. Pain and other postoperative symptoms such as nausea should be adequately addressed. Donors should be closely observed for any potential surgical complications, including postoperative bleeding, bowel injury, or wound infections. They should be encouraged to ambulate early to minimize the risk of pulmonary complications or deep venous thrombosis. The usual hospital stay is 3–5 days, with resumption of employment within 4 weeks if strenuous physical activity is not required.

Much like for other traditional operative techniques, open donor nephrectomy is associated with a longer hospital stay, prolonged postoperative pain, poorer cosmesis, and slower convalescence when compared to newer less invasive techniques [24]. As in the field of general surgery as a whole, in the past 20 years there has been a persistent drive to develop minimally invasive techniques in transplantation as well. Even more so than for open cholecystectomy or traditional appendectomy, in the current era open donor nephrectomy is a procedure that has mostly been relegated to the textbooks and reserved only for unique circumstances.

Laparoscopic donor nephrectomy

While laparoscopy had been employed for simple gynecologic procedures for many years, it was not until the development of the computer chip color camera that the indications of laparoscopy expanded to include more complex procedures. The early 1990s ushered in the era of laparoscopy for general surgery with the introduction of laparoscopic cholecystectomy and appendectomy. With the eventual success and acceptance of these procedures, the indications for laparoscopic surgery expanded to include solid organs. The first laparoscopic nephrectomy was performed in 1991 [25]. Reports thereafter demonstrated that the new, minimally invasive technique for nephrectomy resulted in less postoperative pain, shorter hospital stays, and diminished recovery periods when compared with open donor nephrectomy. Within a short period of time, the technique for laparoscopic donor nephrectomy was developed. First described in a large animal model, the initial experience in humans was detailed closely thereafter in 1995 by Ratner et al. [26]. Since that time, several variations in the technique have arisen. These include hand-assisted, full laparoscopic, lap retroperitoneal, hand-assisted lap retroperitoneal, robotic-assisted, and laparoendoscopic single site donor nephrectomy. While there are several minimally invasive approaches to donor nephrectomy, the full laparoscopic and the hand-assisted laparoscopic approaches are currently the most widely employed. Ultimately, the technique chosen depends on the individual patient and the surgeon's expertise and preference. We will describe in detail the hand-assisted laparoscopic approach for a left-sided kidney since that is what we favor at our own institution.

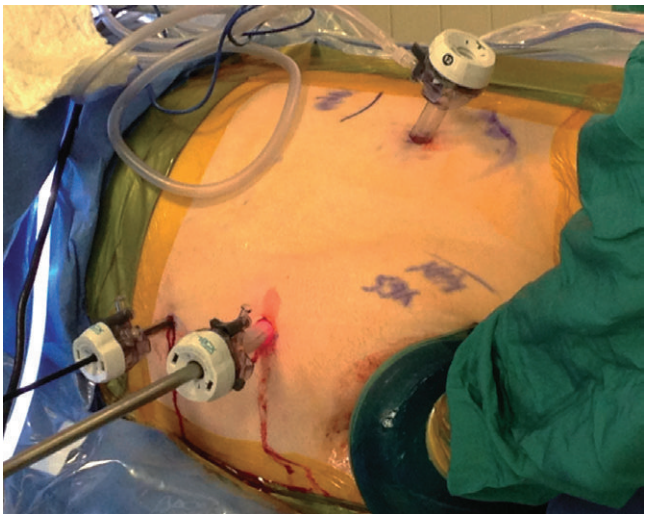

Figure 23.2. Typical positioning and port site placement for left-sided, hand-assisted laparoscopic donor nephrectomy. The patient is placed in a modified lateral decubitus position, ensuring appropriate precautions are taken to avoid iatrogenic injuries associated with nerve traction or skin pressure.

Hand-assisted laparoscopic donor nephrectomy

The patient is placed in a modified lateral decubitus position, ensuring appropriate precautions are taken to avoid iatrogenic injuries associated with nerve traction or skin pressure. (Figure 23.2) The side of interest is placed in the upright position; this is usually the left as most donors undergo left-sided donor nephrectomy. The table is flexed to expand the area between the costal margin and the iliac crest. If so equipped, a kidney bar may be raised to help with this. The patient is secured in place with a beanbag and tape or other methods as needed. The table can then be rotated safely as the procedure requires. A 7-cm midline incision is made below the umbilicus. A gelport or similar handport device is inserted and a pneumoperitoneum is established. Additional ports are then inserted with the hand inside the abdomen. For a left nephrectomy, we ordinarily place three additional ports under direct vision: a 12-mm port (dissecting port) in the anterior axillary line midway between the iliac crest and costal margin; a 5-mm port (assistant port for retraction) just lateral to the midline and two to three finger breadths below the costal margin; and an additional 12-mm port (camera port) superior and just lateral to the umbilicus. A similar set of trocars is required for right-sided procedures and commonly an additional 5-mm port may be placed for a liver retractor.

For left-sided procedures, the descending and sigmoid colon are taken down from their lateral attachments using an ultrasonic device such as the harmonic scalpel. If necessary, the attachments of the splenic flexure may be divided and occasionally the diaphragmatic attachments of the spleen may need to be mobilized to allow the spleen to fall forward and medial away from the kidney. The colon is the reflected medially in order to expose the

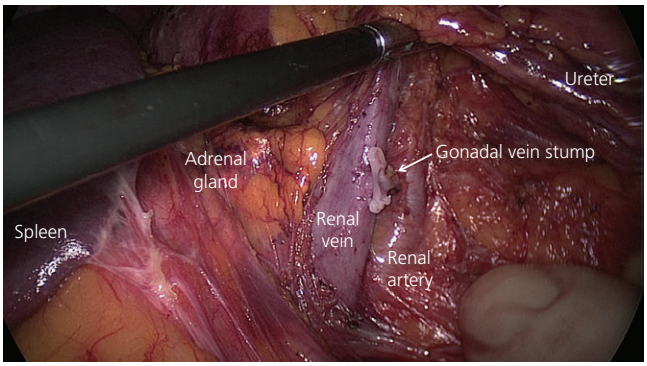

Figure 23.3. Operative picture illustrating the relationship between the gonadal vein (now divided), renal vein, ureter, and renal artery.

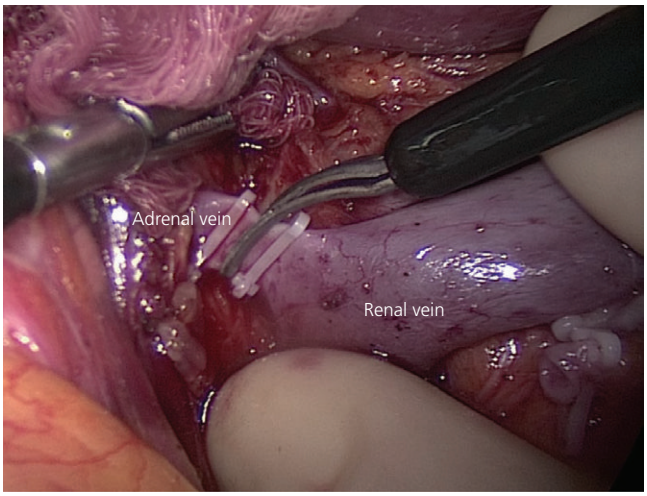

Figure 23.4. Division of the adrenal vein between clips on the superior aspect of the renal vein.

kidney enclosed in Gerota's fascia. Care is then taken to develop the natural plane between the mesocolon and retroperitoneum. Much of the dissection can be accomplished bluntly by sweeping the tissue medially, paying particular attention to avoid creating a buttonhole within the mesocolon. Once the colon and mesocolon are sufficiently medialized, the gonadal vein and ureter are identified. After confirming that the structure is indeed the gonadal vein, tissue from the anterior surface of the vein is dissected free from the pelvis to the level where the gonadal vein enters the left renal vein on its inferior edge. This dissection continues in a cephalad direction to expose the anterior surface of the renal vein. The ureter is identified during this dissection and the gonadal vein along with the ureter are swept laterally as a bundle to ensure an adequate packet of periureteral tissue is harvested. Superiorly, the gonadal vein may be used for retraction as the renal vein is mobilized and dissected circumferentially, but care must be taken not to avulse it from its origin on the renal vein. Eventually, it is often easier to divide the gonadal vein, near the confluence with the renal vein, allowing for greater visualization of posterior lumbar branches that must be clipped and divided (Figure 23.3). As the renal vein is exposed, the adrenal vein will appear on the superior aspect and will need to be ligated with clips and divided (Figure 23.4). The renal vein is then mobilized medially to ensure adequate

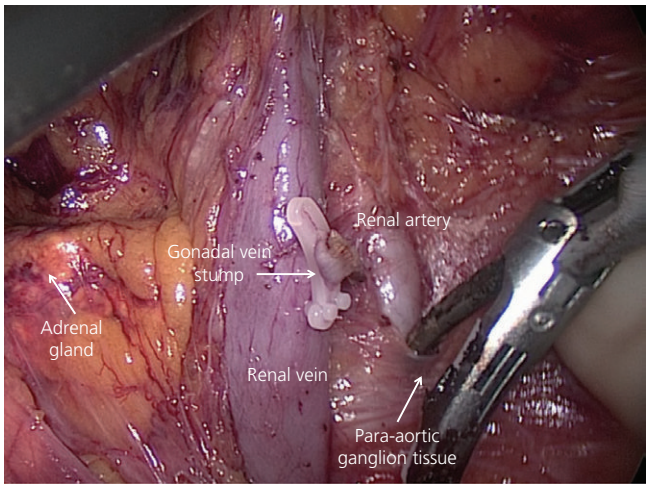

Figure 23.5. Anatomic relationship of the renal artery and renal vein. The renal artery lies posterior to the renal vein. To fully expose the renal artery, the surrounding ganglion tissue must be divided to the level where the artery meets the aorta.

length, usually to the point where it passes anterior to the aorta is sufficient. The renal artery lies posterior to the renal vein and can be exposed by lifting the lower pole of the kidney with a blunt grasper or by rotating the kidney medially and approaching the vessels from the posterior. To fully expose the renal artery, the surrounding ganglion tissue must be divided carefully down to the level where the artery meets the aorta (Figure 23.5) At this point, with the vascular anatomy defined, it is helpful to mobilize the kidney from its attachments.

Now that the ureter/gonadal vein complex has been mobilized medially, it is necessary to free it laterally. The ureter is traced distally near the pelvic inlet where it crosses the left iliac artery. The ureter can then be gently grasped and retracted medially as a packet of tissue with the gonadal vein and investing fat and connective tissue. The lateral attachments of the mesoureter are then divided with the harmonic scalpel in a caudad to cephalad direction, moving along the top of the psoas muscle towards the lower pole of the kidney. The plane of dissection is continued along the lateral surface of the kidney, dividing Gerota's fascia to expose the kidney. The posterior attachments are then divided, freeing the kidney from its shell and driving towards the upper pole. In order to divide the upper pole attachments many times, it is necessary to grasp the kidney and pull it inferiorly and rotate it from side to side. The last plane of dissection proceeds along the superior edge of the renal vein between the adrenal vein and the upper pole of the kidney. Vascularized tissue in this region is divided between clips, and using the harmonic scalpel the final attachments to the upper pole of the kidney are liberated.

The kidney is then completely mobilized from the upper to lower poles and along its lateral aspect. It should be noted that care should be taken not to disturb the triangle of tissue between the ureter and lower pole of the kidney as dissection can jeopardize blood flow to the ureter. Similarly, it is important to preserve, if present, any accessory renal artery supplying the lower pole of the kidney, as it is also responsible for supplying the ureter (Figure 23.6). With the kidney completely mobilized and all of the vascular structures defined and freed, it is now time for removal. After administering a dose of systemic heparin, we first divide the ureter distally where

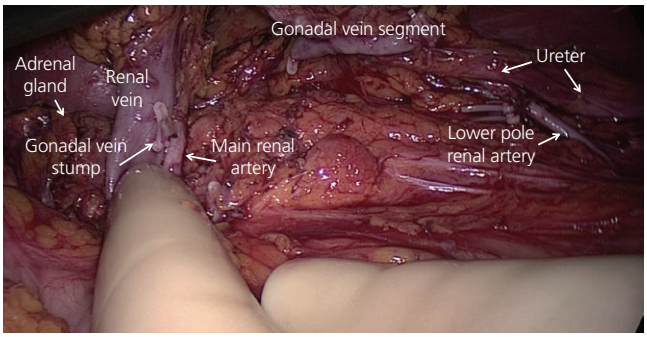

Figure 23.6. Donor kidney prepared using the hand-assisted laparoscopic technique. The gonadal vein has been divided at its origin on the renal vein and distally near the pelvic inlet. The isolated segment is removed with the ureter and periureteral tissue. Care should be taken during the dissection to preserve any accessory vessels such as the lower pole artery, as illustrated here.

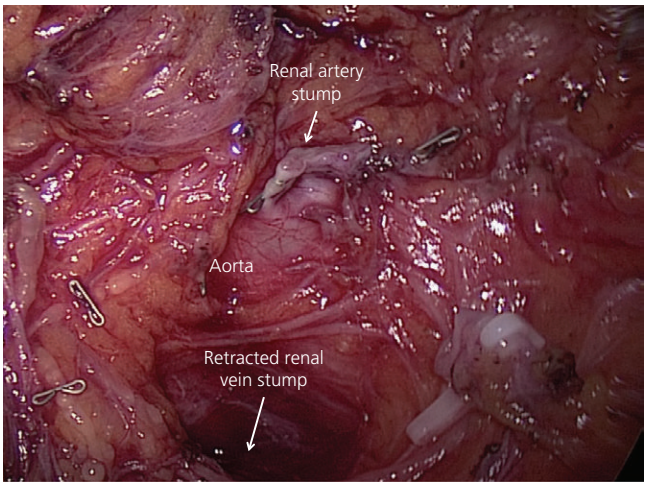

Figure 23.8. Inspection of staple lines on the renal artery stump and retracted left renal vein.

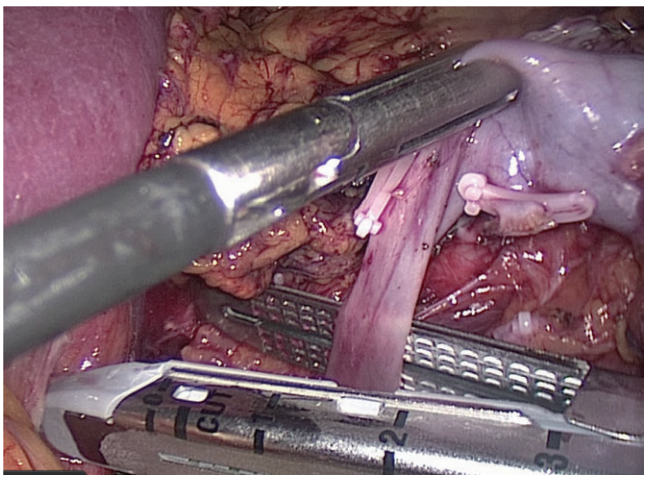

Figure 23.7. Division of the renal vein using an endovascular stapler. The vein can be retracted laterally and the stapler is then used to divide the vein as it drapes over the aorta, ensuring adequate length for the reconstruction in the recipient.

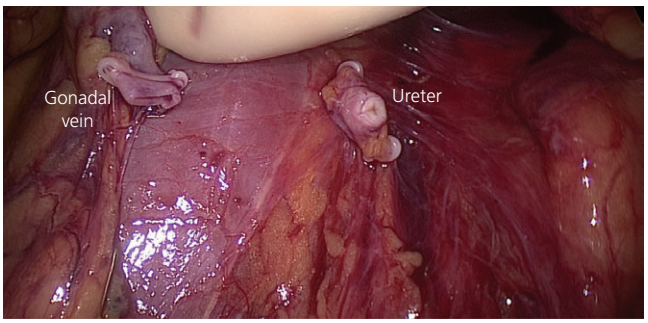

Figure 23.9. Cut ends of the ureter and gonadal vein are examined for any evidence of bleeding.

it crosses the iliac artery; a clip is used to control the distal side which will stay with the patient. The gonadal vein is ligated and divided distally if this has not been done earlier. The kidney is then either grasped and lifted up by the surgeon or retracted laterally by the assistant in order to stretch out the renal artery and vein. Retracting the vein cephalad provides excellent exposure to the renal artery. Alternatively, the kidney may be rotated medially to expose the origin of the renal artery from the posterior. The artery is then divided using a laparoscopic vascular stapler. While the stapler is removed and reloaded, the kidney is rotated laterally if needed and the vein is grasped and stretched by the assistant. The vein can then be divided with the stapler as it crosses over the aorta, ensuring adequate length (Figure 23.7). The kidney is then removed through the handport and transferred to the waiting recipient surgeon for flushing. A careful check is then made for hemostasis, specifically at the staple line on the renal artery stump as well as the location for the staple line where the renal vein was divided (Figure 23.8). Finally, the divided ureter and gonadal vein stumps are inspected (Figure 23.9). The fascial defects at all of the larger ports sites are closed with absorbable suture, as is the lower midline incision.

Intraoperative/postoperative management

Similar to any surgical procedure, the anesthetic management of a living donor is of critical importance and requires effective communication between the anesthesiologist and the surgeon. Like any laparoscopic procedure, the patient must be kept completely relaxed to provide adequate working space. This is particularly important during the time when the renal vessels are about to be transected and the kidney extracted. We find it helpful to communicate directly with the anesthesia team prior to this event to ensure that removal of the kidney remains uneventful. In the past, we routinely treated the donor with a dose of furosemide prior to explant to ensure "adequate diuresis" in the recipient. This was followed by a repeat dose of furosemide and additional administration of mannitol to the recipient near the time of reperfusion. We have since terminated this practice and have chosen to keep the donor euvolemic, titrating fluid to an adequate urine output instead of aiming for a brisk diuresis.

Most donors undergoing laparoscopic nephrectomy remain in the hospital for 2–3 days after surgery. During the first 2 weeks following donation, they gradually return to their normal daily

activities. At 2 weeks, most donors are able to tolerate mild exercise. We advise that they wait 4–6 weeks before reinitiating any strenuous activity or heavy lifting. We favor utilizing intravenous ketorolac to minimize postoperative pain and narcotic requirements, thereby decreasing ileus and nausea.

Impact of laparoscopic techniques

Much like other laparoscopic techniques in general surgery, minimally invasive techniques to remove a donor kidney have quickly gained favor at most transplant centers. Today, laparoscopic donor nephrectomy is considered the standard of care almost universally. Several studies have shown that laparoscopic donors experience less pain, as indicated by a decreased use of narcotics after surgery, and enjoy shorter hospital stays and a quicker return to full activity and work [24,27–31]. Short-term follow-up of laparoscopic donors has failed to reveal any increased risk of complications when compared with open donors. Long-term outcomes, however, remain unknown and await further study.

The advantages of the hand-assisted technique when compared to conventional laparoscopy include tactile feedback, a perceived margin of safety with the ability to use digital pressure to control bleeding, potentially better exposure and dissection of structures, as well as rapid removal of the kidney, resulting in shorter warm ischemia times [32–35]. These features are particularly appealing in centers where training programs exist. Most studies describing hand-assisted laparoscopic donor nephrectomy conclude that the hand-assisted technique is superior to the full laparoscopic technique with regards to operative time and blood loss [34,36–41]. In addition, one meta-analysis suggested that the risk of open conversion is less with the hand-assisted technique [42].

The disadvantages of the hand-assisted techniques include a slightly larger incision than would be required to deliver the kidney and restricted areas where the incision can be placed to facilitate reaching the kidney. The pure laparoscopic technique offers the advantage of placing the incision low, where it is better concealed and usually a smaller incision size since the kidney is delivered using a bag. There is some theoretical suggestion that a lower, smaller incision may lead to less postoperative, pain but there are no studies to support this.

Other techniques

There are various other techniques that have been described and are employed at a number of centers throughout the US and Europe. One of the more commonly described is a retroperitoneal approach that can be performed with and without hand assistance. The retroperitoneoscopic approach has an advantage over the standard transperitoneal laparoscopic technique in that the peritoneum remains intact with a lower theoretical risk of visceral injuries and perhaps a lower rate of ileus. In a few small studies, the hand-assisted retroperitoneoscopic approach had significantly shorter operative time compared to standard laparoscopic donor nephrectomy [43–45]. Overall, other clinical outcomes were similar and this may reflect patient selection or surgeon familiarity with the procedure. Currently, the retroperitoneal approaches have not gained widespread favor.

More recently, various surgical subspecialties have employed robotic assistance in their procedures. There are several reports of early use of robotic-assisted donor nephrectomy and we anticipate that this will be an increasingly common technique [46–48]. The theoretical advantages of the robotic-assisted technique includes increased degree of freedom for movement facilitated by the 360° articulating wrist of the operating arms, resulting in greater flexibility and maneuverability. An additional advantage is the benefit of a tremor-free operation due to the built-in stabilization system. Decreased fatigue and operative technique-related injuries to the surgeon are also potential advantages to the robotic-assisted approach. One significant disadvantage to the robotic technique is the required financial investment as well as the learning curve associated with the device.

Lastly, recent advances in minimally invasive surgery have focused on single incision/site laparoscopic surgery (SILS) and natural orifice translumenal endoscopic surgery (NOTES). There are several studies examining the utility of single-site laparoendoscopic donor nephrectomy [49–51]. As expected, the total incision length was less and there was a suggestion of a quicker recovery time in recipients undergoing the single-site procedure. These studies also pointed to longer operative times and possibly a higher conversion rate to the standard technique [52,53]. There was limited sample size in these studies, impacting the power and our ability to draw any concrete conclusions. There is currently a randomized, prospective clinical trial sponsored by Cornell University evaluating the use of conventional laparoscopic donor nephrectomy versus laparoendoscopic single-site donor nephrectomy. Endpoints in the trial include operative times, pain scores, analgesic requirements, length of stay, surgical scar satisfaction, as well as function and survival of the transplanted kidney. It will be interesting to see if there are any appreciable, significant differences between these techniques. Future techniques will push the envelope of technique and technology using unique approaches such as natural orifice surgery or NOTES. The first report of natural orifice surgery in a donor nephrectomy described a right-sided nephrectomy procured laparoscopically, but instead of extracting the kidney through a standard Pfannenstiel incision, the surgeon elected to remove it through the vagina in a woman who had previously undergone a hysterectomy [54]. Interestingly, another group recently described combining the robotic technique with transvaginal extraction of the donor kidney with the uterus in place [55]. In both reports, the donor did not receive any postoperative narcotic intravenous analgesia and was discharged home after 24 h.

The future of living donor kidney surgery will likely include some iteration of these techniques. We must, however, ensure that whichever technique we employ, it must remain as safe as humanly possible for those who choose to undergo a procedure not for their own benefit, but for the betterment of someone else.

Donor outcomes following living kidney donation

Unlike most types of surgical procedures that have acceptable rates of major complications and mortality, the fact that living kidney donors derive no physical benefit from the surgical procedure has resulted in a zero tolerance policy for major morbidity and mortality related to either the surgical procedure itself or the act of kidney donation. While not achievable in practice, this mindset likely reflects appropriate concerns within the transplant community that potential living donors be safeguarded from excessive risk. Transplant programs are mandated to report donor deaths and acute complications related to the donor procedure (defined as those that occur within 6 weeks of donation or before the donor is discharged from surgical care) to the OPTN. The risk of procedure-related donor death is estimated to be approximately 1

in 3000. This is consistent with reports that of the 51 153 living kidney donor surgeries performed between October 1999 and December 2008 there were 14 deaths identified by center reporting and examination of the Social Security Master Death File [56]. During this time period, an additional 39 donors died within 1 year of donation with the identified causes reflecting conditions common in the general population, such as cancer, accidents, cerebrovascular and cardiovascular disease, homicide, and suicide. There is some evidence to indicate that the operative and early postoperative risks of living kidney donation are decreasing. OPTN data indicate that there were only three deaths occurring within 30 days of the procedure for living donor nephrectomies performed between 2005 and 2009 [2]. During this time period, the number of living donor deaths occurring for any reason within 1 year of donation ranged from 0.03% to 0.08%. With respect to acute complications of living kidney donation, the most common occurrence is hemorrhage requiring transfusion, which occurs in approximately 2% of all donors. The need for reoperation is approximately 1 in 100 living donor nephrectomies. Aside from hemorrhage, the most common complications requiring reoperation include incisional hernias and bowel obstruction, each reported to occur in approximately 1% of living kidney donors. As the vast majority of donor nephrectomies are performed laparoscopically, conversion to an open procedure is considered a complication and was reported to have occurred in $<1\%$ of all laparoscopic donor nephrectomies performed in 2009. In the current era, readmission rates are also low, with approximately 1% of living donors requiring readmission primarily related to intra-abdominal concerns such as abdominal pain, constipation, ileus, nausea, or vomiting. Other less commonly reported causes for readmission include deep venous thrombosis, pulmonary embolism, chylous ascites, pancreatitis, or intra-abdominal infection. Of note, there is no evidence to suggest that the choice of the operative approach affects the incidence of complications in the donor or the function of the transplanted kidney.

In contrast to the procedure-related risks that are generally captured due to uniform reporting requirements mandated by policy, the long-term consequences of living kidney donation have been much less thoroughly studied. This is in part due to the perception that living donors are healthy prior to donation and return to a healthy lifestyle following recovery from the surgical procedure. Practical issues also confound efforts to track the health of kidney donors over long periods of time. Many donors live relatively long distances from the transplant center that performed their surgery, making it difficult to return to the transplant center for routine health assessments following kidney donation. Second, a significant fraction of living donors do not have health insurance. As funds are not set aside to cover the long-term medical expenses (including routine healthcare maintenance) of former living donors, there are significant financial impediments for both the donors and the transplant centers with respect to conducting comprehensive, long-term follow-up of living kidney donors. Lastly, living kidney donors frequently view themselves as being healthy, as do their physicians, and do not see the value in the time spent in routine health assessments following kidney donation. Consistent with this gestalt since 1996, only 280 prior living kidney donors have been listed for a kidney transplant. In view of their former living donor status, these rare individuals receive priority for transplantation. At the time of listing they are awarded four additional points, placing them ahead of the vast majority of recipients, excluding zero HLA-mismatched recipients and recipients of extrarenal organs. While the barriers to monitoring the long-term health of living kidney donors are formidable, there is a rapidly growing consensus that society and the transplant community are responsible for ensuring that information about the long-term consequences of living kidney donation are collected and disseminated. The need for studying the long-term consequences of living kidney donation is made even more pressing by the changing demographics and increasing medical complexity of living kidney donors in the 2000s. As discussed, the trend is for living kidney donors to be older, more racially and ethnically diverse, and less closely related to their recipients than in times past. An increasing number of living donors also have, or are at risk for, health conditions such as hypertension, obesity, and abnormalities of lipid and glucose homeostasis, each of which increases their risk of cardiovascular and renal disease [56].

Despite concerns about the inadequacy of existing data to inform potential living kidney donors about the associated risks, numerous studies do provide useful information. A study of 62 veterans who underwent unilateral nephrectomy for trauma during the Second World War failed to reveal an increase in mortality or hypertension relative to servicemen of the same age who had not undergone nephrectomy [57]. Kidney disease among deceased subjects or dysfunction among living individuals was attributed by the authors to causes other than nephrectomy, suggesting that uninephrectomy in young males had few major adverse sequella over the 45-year follow-up period. One of the earliest efforts to define the effects of kidney donation on the long-term health of donors examined 57 donors followed for a minimum of 20 years after donation [58]. In comparison to 65 siblings, there was no difference in serum creatinine, blood urea and nitrogen, creatinine clearance, mean blood pressure, or the frequency of hypertension or proteinuria treatment. A subsequent study of a significantly larger number of former kidney donors from the same institution confirmed the relative safety of living kidney donation [59]. In this second study, the authors determined the vital status and risk of ESRD in 3698 individuals who donated kidneys between 1963 and 2007. They also performed specific measurements of renal function, renal injury, hypertension, general health status, and quality of life in a subset of 255 patients who donated between 2003 and 2007. Their results indicate that the survival of living donors was comparable to that of control individuals matched for age, sex, race, and ethnicity. Similarly, there was no significant difference in the risk of ESRD for donors as compared to the general population. The quality of life and general health status of living kidney donors was not distinguishable from that of individuals obtained from the National Health and Nutrition Examination Survey (NHANES) who were matched for important variables. Based upon these findings, the authors concluded that the development of significant health issues among living kidney donors was similar to that observed in the general population. While reassuring, it is uncertain to what degree these findings from the study of primarily Caucasian donors, many of whom donated in the 1970s and 1980s, can be extrapolated to the more racially and ethnically diverse donors of today who may have different health risks given the increasing prevalence of obesity and its related health problems. In order to address the impact of changing donor demographics and the general health status of individuals in the US, in recent times a second group compared national registry data for 80 347 living donors who underwent nephrectomy between 1994 and 2009 to 9364 participants in NHANES III, in which those having contraindications to kidney donation were excluded [60]. While the duration of follow-up was obviously

shorter (just over 6 years), the mortality rate of living donors was no different from that of age-, sex-, and race-matched participants in NHANES III. A third recent report directly compared the effect of race on health following living donation [61]. This group examined the medical records of 4650 individuals who were kidney donors between 1987 and 2007 and were insured by a single company at some point between 2000 and 2007. The health status of these living kidney donors was compared to participants in NHANES. This analysis demonstrated that the risk of developing hypertension, diabetes mellitus, or CKD was increased in black and Hispanic living kidney donors relative to white kidney donors. However, these risks were not increased relative to demographically-matched control individuals from NHANES, indicating that race and ethnicity affect the risk of hypertension, diabetes, and CKD but that living kidney donation does not further impact these differential risks. The finding that ESRD, although rare following living kidney donation, is more common in African–Americans warrants further study. One possible contributing factor is the recent description of two mutations in the gene encoding APOL1. These mutations termed *G1* and *G2* are quite common, occurring in nearly one-third of all African–Americans without known kidney disease and two-thirds of those with kidney disease. While the presence of a single at-risk allele, either *G1* or *G2*, conferred protection against the *Trypanosoma* parasite responsible for "sleeping sickness," two copies of the at-risk alleles more than doubled the risk of ESRD. This has caused some to implement screening of African–American potential kidney donors for the presence of the at-risk alleles of APOL1 [62]. While this is relatively inexpensive, in the absence of data about the impact of these mutations on living kidney donors, it may serve to further disadvantage African–Americans with respect to access to living donor kidney transplantation.

When taken together, these and other studies provide some reassurance that living kidney donation is not prohibitively risky from either the standpoint of operative risk or long-term consequences of having a single kidney. However, nearly all of the studies performed to date suffer from the lack of a control population that truly reflects the excellent health status of living kidney donors. In contrast to the exhaustive testing performed on living kidney donor candidates, most studies out of necessity have used control groups comprised of patients who identify themselves as healthy based on medical conditions currently known to them or at best limited testing of small numbers of patients. Attempts to address this issue by using approved living kidney donors who for one reason or another did not undergo living donor nephrectomy have proven difficult in that many of these medically suitable donors who ultimately did not complete donation are not interested in participating in long-term studies of their current and future health for a variety of reasons. Studies defining the life-time risks of living kidney donation are further complicated by the infrequent occurrence of serious, donation-related adverse events and their potential/probable occurrence at time points remote from donation. The impact of these observations on study design requires that in order to be informative, a study would need to follow a large number of living donors for a prolonged period of time. This has obvious financial implications in that there are currently limited funding options to offset the costs of this additional effort. In an attempt to address these limitations and concerns, the National Institutes of Health has sponsored two studies of living donor outcomes. ALTOLD (Assessing Long-term Outcomes of Living Kidney Donation) is a multicenter, 3-year study of 200 living kidney donors and 200 matched controls for the development of renal, cardiovascular, bone, and mineral disease. This study is limited in that it is not designed to detect rare, but serious, adverse events or those that occur more than 3 years after donation. RELIVE (Renal and Lung Living Donors Evaluation) is a study that aims to examine national databases to determine the vital status and occurrence of ESRD in 8951 prior living donors. Subsets of this large population will be selected to undergo additional testing to determine the incidence of renal dysfunction, cardiovascular disease, and changes in quality of life. Several additional multicenter, federally funded studies are currently ongoing, including the Kidney Donor Outcomes Cohort (KDOC) study (ClinicalTrials.gov identifier NCT01427452), the Long-term Effects of Becoming a Living Kidney Donor Study (ClinicalTrials.gov identifier NCT00936078), and the Live Kidney Donor Study – Cross-Sectional and Historical Cohort Study (ClinicalTrials.gov identifier NCT00951977). Despite these initiatives and the widespread opinion of transplant professionals, patients, representatives of payer organizations, and the federal government that these types of studies are critical to inform potential donors about the risks of living donation [63], it is unlikely that optimally designed studies will be undertaken until appropriate financial support is available to offset the disincentives of participating in a long-term study for which there is no apparent medical benefit.

Summary

The past two decades have seen tremendous changes in living kidney donation and transplantation. Technical innovations such as laparoscopic donor nephrectomy, robotic donor nephrectomy, and laparoendoscopic single-site nephrectomy offer prospective donors the realistic expectation of less postoperative pain and a more rapid recovery. Protocols to effectively desensitize recipients together with paired donor exchange programs are extending the benefits of living donor kidney transplantation to an increasing number of sensitized recipients. Changes in donor demographics combined with the willingness of transplant centers to consider more medically and surgically complex individuals for living kidney donation have also contributed to a nearly three-fold growth of living kidney donation in the last 20 years. While the preponderance of existing data suggest that living kidney donation is associated with acceptable short- and long-term risks, the changes in the practice of living kidney donation described above challenge the field to design and implement strategies to accurately monitor the long-term outcomes of living kidney donors so as to adequately inform future donors of the associated risks.

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CHAPTER 24

Techniques for Living Donor Liver Procurement

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Introduction

Faced with an ever-increasing donor shortage, surgeons caring for patients with end-stage liver disease have been forced to expand utilization beyond whole organ liver grafts from standard criteria brain dead donors. One important option is the use of partial grafts from living donors. The goal of living donor liver transplantation (LDLT) is to create a partial functional liver graft for recipients, leaving enough remnant liver mass to maintain the donor's health with normal liver function. However, the practical application of LDLT is mitigated to some extent by the ethical principle of "primum non nocere"; first do no harm. Living organ donation is one of the only fields in medicine in which a healthy person undergoes a major surgical procedure without a pathological condition. In full cognizance of this concern, the initial era of LDLT involved only adults donating a small portion of their liver to small pediatric recipients.

In 1988, the first attempt was made for a small child with her mother as a living donor. The patient died 6 days after the transplant due to severe hemolysis and renal failure [1]. The first successful LDLT was performed for a pediatric patient in Australia in 1989 with a left lateral segment graft [2]. Left lateral segmentectomy removes only 20–25% of the donor liver and is well tolerated by adult donors. Adult-to-pediatric LDLT gained wide acceptance as both relatively safe for the donor and highly efficacious for the child.

With this success, the indications for LDLT were slowly broadened to adult recipients. Makuuchi performed the first successful adult-to-adult LDLT in 1993. A left lobe graft was utilized [3]. Unfortunately, the early experiences of adult-to-adult LDLT did not yield results equivalent to those demonstrated in pediatric recipients. The results were hamstrung by the theoretical and actual amount of liver mass needed by the recipient. To overcome this limitation, surgeons began to use the larger right lobe. In 1996, the first adult-to-adult LDLT using a right lobe graft was performed in Hong Kong [4] and this technique then rapidly became the most common procedure in adult-to-adult LDLT [5].

The use of the right lobe graft lead to the rapid increase of LDLT in both eastern and western countries. While this lead to significant improvement in recipient outcomes, it was also fraught with an increasing incidence of morbidity and mortality for the donors [6–9]. There is now sound consensus that donation of the right lobe is associated with an increased risk of donor morbidity and mortality compared to the left-sided graft [10]. In 2002, the combination of a well-publicized donor death and the change to the Model for End-stage Liver Disease (MELD) era of deceased donor allocation, the demand and popularity of adult-to-adult LDLT diminished dramatically in the US and Europe. In contrast, the demand and need for LDLT in eastern countries with poorly developed deceased donation systems has continued to rise. In 2010, while LDLT accounted only for 4.5% of liver transplantations performed in the US [11], it accounted for >90% of liver transplantations in Asia.

Thus today, while LDLT remains safe and effective in children, the aggressive application of adult-to-adult LDLT is still controversial, especially in parts of the world where there are well-developed organ procurement systems and the need for its application in adults is relative. Although several centers, mostly in Asia, utilize LDLT for critically ill patients with high MELD scores, in the west it is generally reserved for patients with low-to-moderate MELD scores [12,13]. In the US, right lobe adult-to-adult LDLT has demonstrated a significant survival advantage when compared in an intent-to-treat analysis to those who must wait for a deceased donor [14,15]. This advantage is sustained even in those with a MELD score of <15, a group that generally does not benefit from deceased donor liver transplantation [16]. Therefore, as new technologies that maximize donor safety evolve, the practice of LDLT has a potential to be revitalized.

In this chapter, we will start with a review of the donor evaluation and graft selection in LDLT. Surgical aspects of living donor hepatectomy will be reviewed, including conventional open techniques as well as laparoscopic donor hepatectomy. Finally, we will outline perioperative donor management and donor complications. Specifics of the recipient procedure for LDLT are covered in the companion Chapter 57. Technical aspects of deceased donor liver procurement from donation after brain death or donation after circulatory death can be found in Chapters 21 and 22, respectively.

Donor evaluation

Donor safety is paramount; there can be no exception to this rule. To prevent unnecessary morbidity, vigilant evaluation of potential donors is crucial. Ideally, the evaluation of the donor should be conducted by a designated donor advocacy team, which independently assesses donor candidacy to avoid a conflict of interest [17]. The aim of the donor evaluation is to assess whether the donor is suitable for living donation medically, surgically, emotionally,

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psychologically, and financially. Equally important is the need to identify anatomical variants that could increase donor risks and jeopardize the recovery of either the donor or recipient. A variety of criteria may make potential donors unsuitable for living donation (Table 24.1).

The donor must be fully informed. A thorough assessment must be made to ensure that there is no coercion. The donor must make the decision voluntarily, without any direct or indirect financial gain deriving from the donation. During the evaluation, the donor is educated regarding the risks of the procedure. This includes discussion of the morbidity and mortality rates reported in the medical literature, as well as outcomes of the center that will perform the operation [18]. Particular effort is needed in the psychosocial evaluation of potential donors. In this process, a psychiatrist and social worker conduct a thorough evaluation on an individual basis. Significant psychiatric and emotional disorders are a contraindication for donation. Although ambivalence is common in living donors, the majority of them can go through the evaluation process and donor operation with a careful team support [19]. The donor has the right to withdraw his/her willingness to donate up to the time of surgery. The reader is referred to Chapter 138 for a more extensive discussion of the ethics of living donation.

Table 24.1. Possible exclusion criteria for living liver donation

- Age <18 years or >55 years (>60 years in case a recipient needs a left lateral segment)
- Unable to make an informed consent due to mental or psychiatric disorders
- Morbid obesity
- Poorly controlled medical conditions (e.g. hypertension, diabetes)
- History of coagulation disorder (e.g. Factor V Leiden)
- History of bleeding disorder
- Significant cardiac history (coronary artery disease, valvular disease)
- Peripheral vascular disease
- Chronic pulmonary disease
- Recent history of malignancy with long time to recurrence (e.g. breast cancer)
- History of melanoma
- Active infection
- History of recurrent infection
- History of liver disease including hepatitis
- Fatty liver
- Asymptomatic ZZ alpha-1 antitrypsin genotype
- Significant donor/recipient size mismatch (donor < recipient)
- Donor future liver remnant <30% (generally, 35% is considered as a safety margin)
- Vascular or biliary anomaly unsuitable for living donation

The first step of the evaluation begins with a thorough medical history and physical examination. Generally, potential donors should be completely healthy and between 18 and 55 years of age. When the recipient needs a left lateral segment, donors up to the age of 60 years can be considered. The donor and recipient should be blood group identical or compatible. ABO-incompatible blood groups can be accepted in extreme situations where deceased donation is extremely scarce. It is preferable for the donor to have a clear and established relationship with the recipient; however, volunteers who are physically and mentally healthy can, on occasion, be Good Samaritan donors. The donor must not have significant co-morbidities. However, medical conditions such as mild hypertension can be considered to be acceptable if they are medically well controlled. In female donor candidates, oral contraceptives must be discontinued. The past medical history of a hypercoagulable state is a contraindication for living donation due to the risk of fatal thromboembolic events. Active smokers and drinkers should not be considered for donation. If a potential donor's body mass index (BMI) is $>30\,kg/m^2$, a liver biopsy should be performed to rule out the presence of fatty liver, which can negatively affect donor safety as well as recipient outcome.

The next step of the evaluation includes extensive lab profiling and serologic testing. The donor should have normal liver function. Systematic screening for coagulation disorders is important to identify hidden thrombophilic states [20]. A lipid profile is also important; there is a close relationship between hyperlipidemia and fatty liver disease [21].

If the donor is deemed to be a good candidate from a medical and psychosocial perspective, then the next step is to assess the anatomical and surgical aspects. In the assessment of the anatomy, invasive studies should be avoided. In the early eras of LDLT, donors were routinely evaluated with angiography and endoscopic retrograde cholangiography. However, these invasive procedures carried a certain risk that could not be justified as part of a routine evaluation of a healthy donor. Recent advances in the three-dimensional reconstruction of the liver using multiphase computed tomography (CT) scans have contributed to a precise, non-invasive mapping of the important vascular structures, allowing for a preoperative simulation of the graft procurement (Figure 24.1a). This enables surgeons not only to visualize detailed liver anatomy but

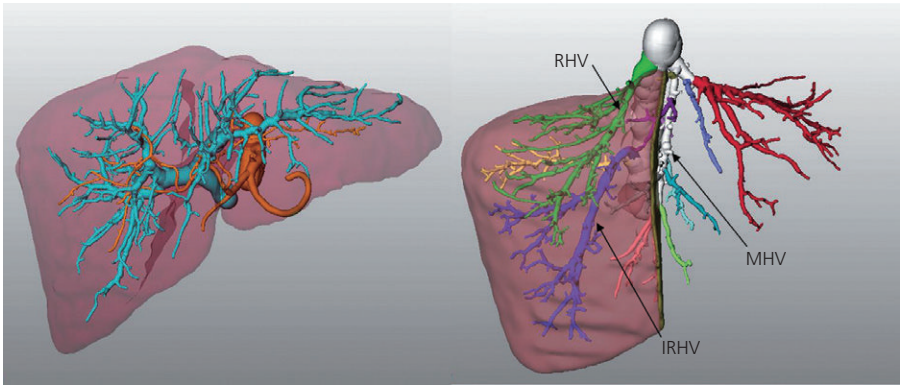

Figure 24.1. Three-dimensional reconstruction of the (A) hepatic artery and portal vein and (B) estimated right lobe graft without middle hepatic vein using multiphase CT scan. RHV, right hepatic vein; MHV, middle hepatic vein; IRHV, inferior right hepatic vein.

also to estimate graft and remnant liver volumes (Figure 24.1b). Biliary imaging can be performed with magnetic resonance (MR) cholangiography or CT cholangiography to exclude significant anatomical variants that jeopardize candidacy (see Anatomical variants below). At the end of the evaluation process, only 30–40% of aspiring donors will be considered good candidates for living donation.

Graft selection

In order to understand the type of grafts used in LDLT, it is important to define two concepts: the future liver remnant (FLR) and the graft-to-recipient body weight ratio (GRWR). The FLR is the proportion of the donor liver that is estimated to remain after the donation. An FLR of 30–35% is considered a safe and acceptable lower limit under which donation takes on a higher risk of the development of postoperative liver failure [22]. The GRWR is the ratio between the donor graft weight and the recipient body weight. The lower limit of graft acceptability in adult-to-adult LDLT is considered to be approximately 0.6–0.8%, which is equivalent to 30–40% of recipient standard liver volume [22]. However, many centers prefer a GRWR of at least 1% to give a safety margin to the recipient in case of certain specific technical complexities. A GRWR below 0.6–0.8% increases the chance of developing graft dysfunction, known as small-for-size syndrome (SFSS). The ideal graft is one that leaves a donor with a FLR of >35% and at the same time provides an adequate liver mass with respect to the recipient.

There are essentially three types of grafts that are commonly used in LDLT (Figure 24.2); the left lateral segment (segment 2–3); the left lobe with middle hepatic vein (segment 1–4); and the right lobe without middle hepatic vein (segment 5–8). The smallest conventional graft is represented by the left lateral segment, which usually provides 20–25% of the total liver volume. This graft is usually reserved for pediatric recipients. The left lobe, which represents 30–40% of the total liver volume, is usually offered to teenagers or small adults. Finally, the right lobe, which represents 60–70% of the total liver volume, is reserved for the remainder of the adult population. This is the largest graft and offers the most consistent results in adult recipients. However, it is also the one that is associated with the highest morbidity and mortality in the donor [10]. The left lobe usually has a single venous outflow (common channel of left and middle hepatic veins), single left branch of the portal vein, and single left hepatic duct. However, the left lobe graft often requires two small anastomoses of hepatic arteries [23]. In contrast, the right lobe graft usually has a single right hepatic artery, but frequently requires multiple and complex reconstructions of the venous outflow and the bile ducts [24].

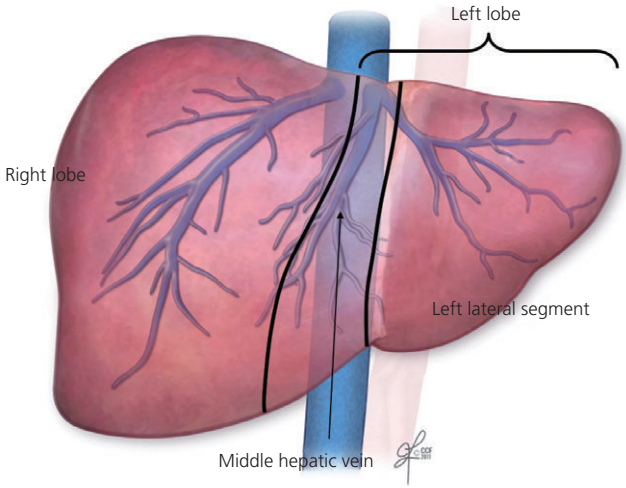

Figure 24.2. Graft types commonly used in living donor liver transplantation.

Graft selection is generally guided by three important factors: liver anatomy; donor–recipient size matching; and severity of recipient liver disease. In determining whether a donor liver can provide sufficient liver function to the recipient, it is important to estimate the functional capacity of the graft. This estimate is known as the "functional graft size," which is a composite function of actual graft size modified by the severity of the recipient's condition, the degree of portal hypertension, and the degree to which a graft outflow might be impaired [5]. The larger graft (right lobe) is more likely to provide enough liver mass to meet the recipient's metabolic demands and withstand the hyperdynamic splanchnic flow commonly seen in adult cirrhotics. However, the right lobe often needs complex venous reconstruction to ensure optimal graft outflow. In using the left lobe graft, actual graft size may not always exceed the 0.8% of GRWR or the 40% of standard liver volume threshold. Even in such cases, the left lobe graft has excellent venous outflow and can provide adequate functional liver mass if the recipient's portal hypertension is limited. If small left lobe grafts are used for patients with high MELD scores or severe portal hypertension, the risk of graft failure is very high.

Several centers routinely use the extended right lobe grafts including the middle hepatic vein [25]. This graft provides a larger liver mass and better venous drainage for the recipient, but donor safety is of increased concern compared to the other graft variants. Right posterior segment (segment 6–7) grafts have been used in some rare instances. This graft requires complex surgical techniques and is considered only when the donor has a disproportionately small left lobe with a rare anatomical variant of the portal vein [26,27]. Mono-segment grafts are used for small infants (body weight of <10 kg) to prevent a large-for-size graft failure [28,29]. In general, when the left lateral segment is too large for such small infants, lateral and caudal portions are removed to down-size the graft. These three grafts are technically more challenging, but can provide excellent outcomes in experienced centers.

Anatomical variants

Hepatic artery

Hepatic artery reconstruction remains one of the most important and challenging steps in LDLT. Preoperative evaluation of hepatic arterial anatomy is mandatory both in the donor and recipient. Arterial variants are commonly encountered in living liver donors (about 50%). Identification of the origin of the middle hepatic artery (A4) is important. When the A4 arises from the right hepatic artery, the left lobe graft has two separate hepatic arteries that need to be reconstructed. If there are intrahepatic arterial collaterals, a smaller branch can be sacrificed [30]. The risk of biliary complications can be reduced, however, by reconstructing both branches [23]. The presence of separate branches of segment 2 and 3 requires anastomosis of each branch with a smaller caliber (1–2 mm) and further increases the risk of thrombosis in the left-sided graft.

Portal vein

Anatomical variants of the portal vein that would require multiple anastomoses or be a contraindication for donation occur less frequently than those of the hepatic artery or hepatic vein [31]. Portal vein variants occur in about 20% of the general population, with trifurcation the most common variant [32]. The right anterior branch can arise from the left portal vein. However, this branch can be identified in the hilar area and is not an absolute contraindication despite its increased complexity; it requires the reconstruction of two separate branches in right lobe LDLT. When a branch of the right lobe arises from the umbilical portion of the left portal vein, the origin of this portal branch can be difficult to dissect and reconstruct.

Hepatic veins

Ensuring perfect venous outflow is a key in partial grafting because venous drainage is a very important determinant of graft function and, accordingly, of functional graft size [5]. Foreknowledge of hepatic venous anatomy enables preoperative determination of surgical plans for optimal venous reconstruction. Since the left hepatic vein is almost always (92%) dominant for the left lobe, a standard left lobe graft retaining both the left and middle hepatic veins usually promises optimal outflow [33]. In contrast, various anatomical variants of right and middle hepatic vein anatomy can create the need for demanding technical reconstructions when using right lobe grafts [34]. Modern three-dimensional CT technology can provide fine estimates of the volume of potentially congested segments. As a general rule, the right anterior segment (segments 5 and 8) is predominantly drained by the middle hepatic vein. In donor right lobectomy without the middle hepatic vein, ligation of segment 5 and 8 veins (V5 and V8) can cause severe venous congestion. This causes two problems: (1) the congested area does not fully function, reducing the functional graft volume; and (2) the right anterior portal vein becomes an outflow tract that can cause portal hyperperfusion of the right posterior segment. This combination can cause serious graft dysfunction and SFSS. When a significant volume of graft congestion is predicted on imaging, major branches of the middle hepatic vein should be reconstructed.

A significant inferior right hepatic vein (>5 mm) exists in approximately 20–40% of donors [35,36]. In these cases, the vein should be preserved for subsequent reconstruction in recipients. An inferior right hepatic vein of <4 mm might also be significant and independently responsible for venous drainage of segment 6. Therefore, it is worthwhile to temporarily clamp the vein to assess whether the corresponding segment becomes congested before it is sacrificed [22]. When an estimated congestion area is minimal, there is minimal portal hypertension, or the GRWR is large, a standard right lobe graft without complex venous reconstruction suffices. In contrast, when a significant volume of graft congestion is anticipated, reconstruction of the inferior right hepatic vein is strongly recommended.

Bile duct

Biliary complications are the most common surgical complications, accounting for complication rates of up to 25% in donors [8,37,38] and 40% in recipients [5,12,39]. Abnormal or non-standard biliary anatomy poses significant technical challenges in LDLT. Surgical challenges are greater when multiple ducts are present and bile duct caliber is very small. MR or CT cholangiography is useful for preoperative evaluation of bile duct anatomy [40], but intraoperative cholangiography is still important to rule out anatomical variants not demonstrated on preoperative imaging.

The biliary anatomy can be classified into two major categories with respect to the presence of the main right hepatic duct [41,42]. The presence of the main right hepatic duct makes the donor suitable for either left or right lobectomy, leaving a single hepatic duct to be reconstructed. In the absence of the main right hepatic duct, the anterior and posterior branches take off separately from the left hepatic duct and/or common hepatic duct. Trifurcation of the left, right anterior, and right posterior branches (12%) is included in this variant. In the absence of the main right hepatic duct, two separate hepatic ducts are identified in right lobe grafts, necessitating complex biliary reconstruction. The left lobe graft almost always presents with a single duct [24]. The biliary transection plane for the left lateral segment graft is in the umbilical fissure and may be distal to the left duct's bifurcation into segmental ducts.

Special attention should be paid to rule out critical variations of the bile duct anatomy. For example, it is best to be aware prior to cholecystectomy and division of the cystic duct of an aberrant right hepatic duct that arises from the cystic duct 2–3% of the time [43]. Accessory left (1–2%) or right hepatic ducts (2%) may also preclude donor hepatectomy.

Surgical techniques: conventional open procedures

Left lateral segment graft (Figure 24.3a)

The laparotomy is performed through an upper midline incision with or without a short right lateral extension. The falciform ligament is divided up to the suprahepatic vena cava. After a brief evaluation of the abdominal organs, the left triangular and coronary ligaments are divided. The gastrohepatic ligament is divided to open the lesser sac, but if a left hepatic artery arises from the left gastric artery, it must be preserved. The base of the left hepatic vein is dissected, but it is unnecessary to encircle it at this point.

Surgical sponges are placed behind the right lobe of the liver to mobilize the liver medially. There is no need to surgically mobilize the right lobe. At the hepatic hilum, the left hepatic artery is dissected free. The left portal vein is identified posterior to the left hepatic artery. This is also dissected free. The caudate branch of the portal vein is tied and divided to free the entire left portal vein. The left hepatic duct is encircled and divided sharply in the plane of the umbilical fissure. Bleeding from small periductal arteries is controlled with fine sutures.

The transection line is marked with electrocautery at 0.5 cm to the right of the falciform ligament. The transection of the liver parenchyma can be performed by a variety of methods [e.g. cavitron ultrasonic surgical aspirator (CUSA), clamp-crushing technique, water-jet, bipolar coagulator); the choice depends on operator experience and confidence, but should be with the aim of minimizing blood loss and maximizing anatomical precision. The parenchyma is divided until the left hepatic vein is reached. By dividing the cranial end of the arantius ligament, access to the left hepatic vein becomes easier.

After administrating 3000 U of heparin intravenously, the left hepatic artery is ligated and transected. The left portal vein is also ligated and transected. The left hepatic vein–middle hepatic vein junction is controlled with a vascular clamp and the left hepatic vein is transected. The graft is immediately removed to the back table and flushed with cold preservation solution. While the

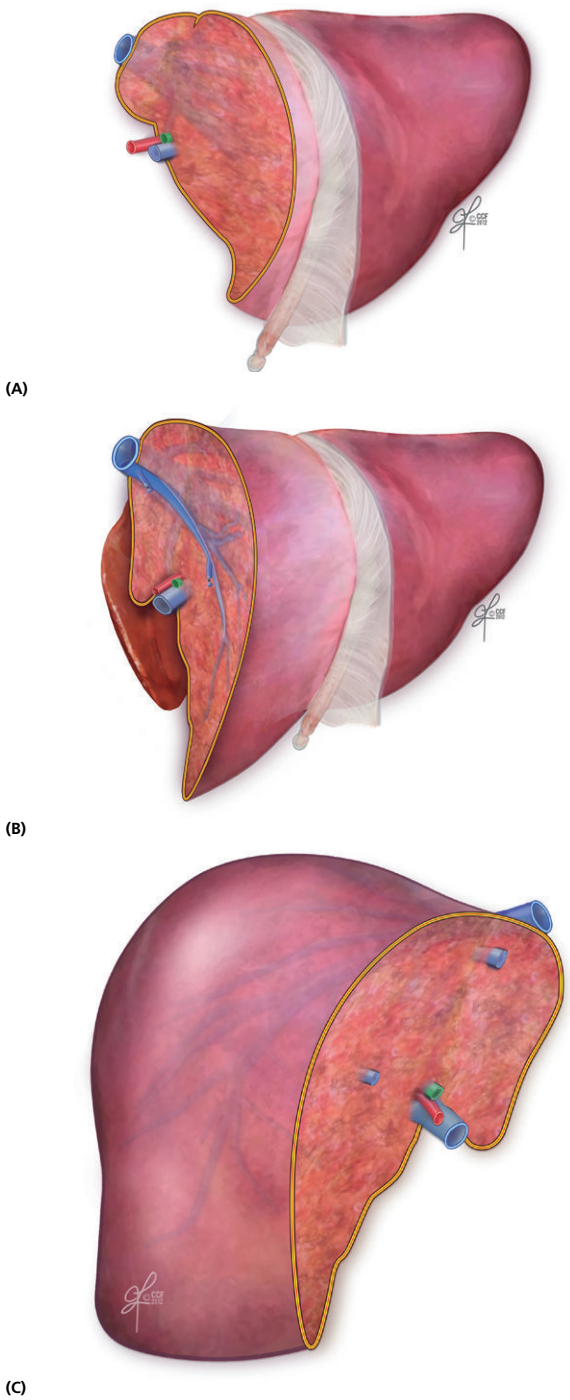

Figure 24.3. (A) Left lateral segment graft, (B) left lobe graft, and (C) right lobe graft.

back-table team perfuses the graft, the stump of the hepatic vein is oversewn with monofilament suture.

Left lobe graft (Figure 24.3b)

An upper midline incision with a right lateral extension is made for optimal exposure. A left lateral extension is usually unnecessary. The left lobe is mobilized as described in the left lateral segmentectomy. The falciform ligament tissue should be preserved on the liver side so that it can be used to fix the graft to the recipient's abdominal wall to prevent graft malposition into the right subphrenic space. The caudate lobe is retracted up to expose the retrohepatic vena cava by dividing the retroperitoneum.

A cholecystectomy is performed and the cystic duct is cannulated to perform a cholangiogram. Special attention must be paid to rule out anatomical variants that were not identified in preoperative imaging studies.

During the hilar dissection, unnecessary dissection must be avoided in the right-sided structures. The left hepatic artery is identified at the level of the bifurcation and dissected free. If the segment 4 hepatic artery arises from the right hepatic artery, arterial dissection is extended toward the right side of the bifurcation. The left portal vein is then identified posterior to the left hepatic artery and dissected free. An effort is made to preserve a small caudate branch to ensure its blood supply.

The left hepatic duct is identified within the hepatic hilar plate. A marker clamp is placed approximately 0.5 cm to the left of the bifurcation of the left and right ducts and a repeat cholangiogram is obtained. It is imperative that if a right posterior duct originates from the left duct, the eventual transection point is to the left of the take-off of that duct. The duct and entire hilar plate are then transected.

The short hepatic veins between the caudate lobe and the vena cava are either tied and divided or transected with a sealing devise such as the Ligasure or harmonic scalpel. There is usually a dominant caudate vein >5 mm and it can be preserved with a small vena caval patch so that it can be reconstructed in the recipient surgery to prevent venous congestion of the caudate lobe [44].

The confluence of the left and middle hepatic vein is dissected to its base at the entrance to the vena cava. This requires the ligation and transection of the left phrenic vein and careful mobilization of the confluence from the diaphragmatic attachments. A tape is then passed from the groove between the middle and right hepatic veins, and passed behind the hilar structures, to isolate the liver parenchyma from the vena cava and the hilar structures. This allows for the liver hanging maneuver, which can facilitate exposure, minimize blood loss, and guide the direction of parenchymal transection [45].

An intraoperative ultrasound is performed to delineate the anatomy of the middle hepatic vein. The location of the middle hepatic vein tributaries is also confirmed. Because the transection line is determined based on the anatomy of the middle hepatic vein and the gallbladder fossa, it is unnecessary to confirm the demarcation line by a temporary hemihepatic inflow occlusion. The line is marked with electrocautery on the right side of the middle hepatic vein coming caudad to the gallbladder fossa and down the undersurface of the liver on the left of the gallbladder fossa to the point of biliary transection. Parenchymal transection then proceeds with the surgeon's technique and tool of choice. Once the middle hepatic vein is identified, the parenchymal transection line is best at 1–2 mm to the right of the middle hepatic vein with a thin layer of liver parenchyma between the dissection plane and the vein proper. This can prevent bothersome bleeding from small branches of the middle hepatic vein. During the final parts of the parenchymal transection, both ends of the umbilical tape are pulled gently to give upward traction. The transection is then completed and hemostasis is completely obtained.

After administrating heparin, the left hepatic artery and left portal vein are both tied and transected. Subsequently, a staple

device is applied to the base of the left and middle hepatic vein, and the vein is transected on the graft side. The liver graft is immediately removed and flushed on the back table with cold preservation solution.

After the stump of the left hepatic duct is oversewn, a final cholangiogram is performed through the cystic duct to ensure the integrity of the remaining bile duct.

On the back table, the left lobe graft usually needs a venoplasty to optimize venous outflow. A septum between the left and middle hepatic veins is cut vertically and removed. The defect of intima is closed with a monofilament suture. This technique also helps enlarge the diameter of a venous orifice [46].

Right lobe graft (Figure 24.3c)

An upper midline incision with a right lateral extension is made. The round ligament is divided and then the falciform ligament is divided all the way up to the suprahepatic vena cava. After an evaluation of the abdominal viscera, the right triangular and coronary ligaments are divided. The left lobe should not be mobilized because unnecessary mobilization increases the risk of malposition of the remnant liver and can cause postoperative liver insufficiency in donors. The right lobe is rotated medially to mobilize it until the retrohepatic vena cava appears.

A cholecystectomy is performed and followed by a cholangiogram through the cystic duct. Hilar dissection is started at the right lateral aspect of the hilum. This allows dissection of the bifurcation of the hepatic artery without touching it to ensure an unperturbed blood supply to the common hepatic duct [47]. The right hepatic artery is dissected free. The right portal vein is then dissected. The caudate branch is tied and divided to ensure the necessary length for placing a vascular clamp when the liver graft is removed. The right hepatic duct is encircled with the hepatic hilar plate. After localization cholangiogram, the duct and hilar plate are transected sharply 0.5 cm from the bifurcation.

Attention is then returned to the caval dissection. The caval ligament is carefully isolated and divided. The short hepatic veins are divided on the right side of the vena cava. The groove between the middle and right hepatic veins is dissected and tunneled. The right hepatic vein is isolated and an umbilical tape is brought through this groove for the liver hanging maneuver (Figure 24.4a) [48]. The caudal end of the umbilical tape is passed behind the hilar structures to isolate the liver parenchyma from the vena cava and the hilar structures (Figure 24.4b).

The transection line is determined as for the left lobe graft. The parenchymal transection then proceeds in an identical fashion to that of the left lobectomy (see above). The only difference is that the significant V5 and V8 draining into the middle hepatic vein from the anterior segment needs to be preserved (see Anatomical variants above). These veins are tied on the remnant side and clipped on the right lobe side.

After the transection is complete and full hemostasis achieved, intravenous heparin is administered. The right hepatic artery is then tied and transected, as is the right portal vein. The right hepatic vein is then stapled vertically along the edge of the vena cava and sharply divided on the graft side. The liver graft is immediately removed and flushed with cold preservation solution.

The stump(s) of the right hepatic duct(s) are oversewn with monofilament suture. A final cholangiogram is performed through the cystic duct to confirm the integrity of the remnant bile duct.

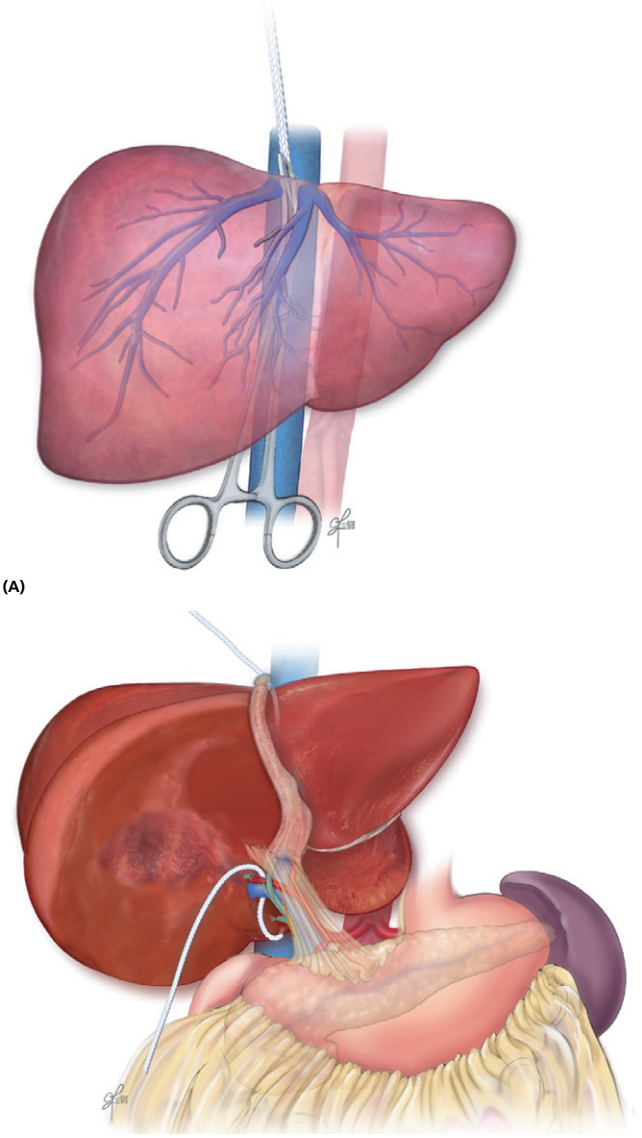

Figure 24.4. Liver hanging maneuver to facilitate parenchymal transection.

On the back table, the V5 and V8 are reconstructed if necessary. This technique provides a functional liver mass comparable to an extended right lobe graft with middle hepatic vein. A vascular graft is necessary for reconstructing the V5 and V8. Unused fresh iliac artery and vein grafts in deceased donor liver transplantation are a good source of vascular grafts. Autologous grafts such as the intrahepatic portal vein of a recipient native liver, inferior mesenteric vein, gonadal vein, and recannalized umbilical vein can be used with good patency [49]. If these are not available, cryopreserved vascular grafts from cadaveric donors are another alternative [50]. Excellent outcomes have been reported using expanded polytetrafluoroethylene (ePTFE) grafts for the V5 and V8 reconstruction [51]. In that series, the patency of ePTFE grafts was 80% at 1 month and 40% at 4 months. Recipients with early obstruction of the ePTFE graft had significant congestion in the anterior

segment, but those with late obstruction were asymptomatic. Patient and graft survival rates were as good as those of the extended right lobe graft (with middle hepatic vein). As long as early patency is achieved, late venous graft obstruction seems to have no impact on congestion in the anterior segment or survival. Because there is no limit to the availability of artificial vascular grafts, they may be a useful alternative for anterior segment drainage.

Laparoscopic donor hepatectomy

Minimally invasive techniques have been successfully applied for living donor renal procurements, and their deployment has made the procedure more appealing to many potential donors. In general, minimally invasive surgery promises better pain control, shorter length of hospital stay, quicker functional recovery, improved cosmesis, and lower risk of surgical complications. In fact, since the introduction of laparoscopic donor nephrectomy [52], the benefits of the laparoscopic procedure (described in Chapter 23) have been widely conceived and this technique has rapidly become the standard for living donor kidney transplantation worldwide [53,54]. It has been proven to be relatively safe, although there have been reports of sporadic donor deaths from faulty clip application to the renal artery [55]. In attempts to follow in the footsteps of minimally invasive renal procurement, some centers began performing laparoscopic-assisted living donor hepatectomies. These techniques include laparoscopic and hand-assisted laparoscopic surgery.

With respect to laparoscopic liver surgery, many reports have suggested its safety and feasibility for resection of benign and malignant liver tumors. With the development of laparoscopic liver surgery, its application for living donor hepatectomy has been raised and the first attempt was made for a left lateral segmentectomy in France [56]. This report was followed by a successful series of 16 donors [57]. More recently, a Korean group reported 11 donors who underwent a laparoscopic left lateral segmentectomy; they achieved shorter hospital stay and earlier oral diet without surgical complications when compared to those with conventional open donor hepatectomy [58].

The first laparoscopic-assisted donor right hepatectomy was reported from Northwestern University in 2006 [59]. Four donors successfully underwent a laparoscopic-assisted procedure without complications. Another Korean group reported nine laparoscopic right donor hepatectomies with the only major surgical complication being an intra-abdominal abscess in one patient [60]. Further, a Japanese group reported 13 successful laparoscopic hemiliver procurements, including three right hepatectomies. They used a gasless abdominal wall-lifting technique rather than pneumoperitoneum to reduce the risk of air embolism [61].

With regard to the surgical technique, pure laparoscopic donor hepatectomy is rarely performed. Instead, the "hybrid" technique is commonly used. This technique consists of a hand-assisted laparoscopic mobilization of the liver followed by an open technique to perform hilar dissection, parenchymal transection, and graft retrieval. In addition to small port incisions, this technique requires a 10–12-cm long epigastric midline incision for a hand port and graft extraction. By avoiding a subcostal incision, the hybrid technique may reduce donor pain and morbidity and shorten the recovery time.

Despite the recent reports of these techniques, surgical stress on living donors seems to be dependent on the amount of liver volume removed. In addition, there remains concern over the ability to rapidly control inadvertent hemorrhage through a small incision if it should occur. Therefore, it appears that routine application of this approach is still controversial. The combination of extensive experience both in laparoscopic surgery and conventional donor hepatectomy is essential to the successful application of this approach. Further careful assessment and discussion will be needed to reach a general consensus [62,63].

Perioperative donor management

On the day prior to planned surgery, the transplant team meets to go through a final checklist to assure the evaluation is complete and nothing has been overlooked. A "huddle" should be performed routinely just prior to the induction of anesthesia to make sure the entire team is fully ready and all equipment is available. If deficiencies are found at either meeting, further evaluations should be performed and the procedure postponed or aborted.

Living liver donors should receive intensive care immediately and for about 24 h after the surgery. Close monitoring of vital signs is essential and laboratory data should be periodically checked. After thorough assessment by the transplant team, the donor is allowed to be transferred to the regular transplant nursing floor where the medical staff is familiar with the care of liver resection and liver transplant patients. These teams should be familiar with common complications associated with donor surgery such as intra-abdominal bleeding, wound infection, acute renal failure, gastric distension, electrolyte imbalance and deep venous thrombosis. Both the intensive care unit and regular nursing floor should have appropriate monitoring in place to detect these complications before they cause significant problems. Donors with unexpected changes in vital signs and laboratory data require immediate evaluation.

A multidisciplinary team lead by an attending transplant surgeon examines and assesses the donor on a daily basis until the day of discharge. Pain management is a major issue that needs to be addressed. Epidural anesthesia can provide good pain management, but there is a risk of complications such as acute spinal compression secondary to a hematoma [64–66] and a case of paraplegia has been reported. Instead of epidural analgesia, intravenous patient-controlled analgesia is a choice in the early postoperative days. Some centers, including our own, prefer to use a very short course of narcotics (24 h) followed by ketorolac (Toradol). Since prolonged use of narcotics increases the risk of complications, early switching to non-narcotics is strongly recommended.

The transplant surgeon should perform outpatient follow-up to check wound healing, signs of infection, and recovery of liver function. Any abnormalities in the physical examination and laboratory data should be further evaluated. Liver function tests should be monitored at least until they normalize. The donor should have some sort of imaging study to confirm adequate liver regeneration and to rule out any surgical complications, including bile leak, biliary stricture, thrombosis, and fluid collection. Without clinical indications, imaging studies do not need to be repeated.

Comprehensive and accurate follow-up data on living donors are critical to ensuring their safety. Currently, the United Network for Organ Sharing (UNOS) requires transplant centers to submit information about the status of each living donor for a minimum of 2 years after donation [67]. Follow-up protocols may vary among

programs, but most centers continue to follow donors annually unless they have any complications.

Donor complications

Despite donor safety being of paramount importance, finite morbidity and mortality rates have been reported worldwide. While the systematic utilization of right lobe grafts allowed for the rapid expansion of adult-to-adult LDLT with good outcomes in recipients, it soon became clear that there was far more morbidity and mortality in right lobe donors compared to left lobe donors. The reported rates of donor complications after left lateral segment, left lobe, and right lobe donation are approximately 15%, 25%, and 35–40% [8,9,37,68,69]. The estimated donor mortality rate is 0.1% for left lobe and 0.5% for right lobe donation [10]. The difference in morbidity and mortality between left lobe and right lobe donation can be attributed to two factors: (1) leaving a larger functional liver remnant after left lobectomy and (2) the commonly encountered multiple bile ducts after right lobectomy. The advantage of a single bile duct should not be overlooked because morbidity in right lobe recipients is often caused by complications associated with multiple bile ducts.

It is important to report these complications according to the Clavien system, which scores them according to five categories of severity (Table 24.2) [70]. Despite this, reported complications vary significantly among different programs, possibly due to different experience or reporting methodology.

Biliary complications represent the most common morbidity occurring in living liver donors. Wound infection is the second most common complication. Vascular complications are less common, but are accompanied by significant morbidity and mortality. Gastric stasis is rare (3%), but severely compromises quality of life in the left lobe donor [9]. Complications associated with anesthesia and postoperative recovery (deep vein thrombosis, pulmonary embolism, etc.) have been reported. These risks must be discussed in detail with donors and their families when considering LDLT and during the consent process. Some donors require social and psychological supports either in the early or late stage after donation [71,72]. Social workers and psychiatrists play an important role in supporting and treating them. Medical treatment may need to be considered in donors with significant problems. Close communication between the transplant team and the donor's primary care physicians is also important in taking best care of these issues.

Table 24.2. Classification of complications according to the Clavien system

Grade 1	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions. Allowed therapeutic regimens are drugs such as antiemetics, antipyretics, analgesics, diuretics, electrolytes, and physiotherapy. This grade also includes wound infections opened at the bedside
Grade 2	Complications requiring pharmacological treatment with drugs other than those allowed for grade 1 complications. Blood transfusions and total parenteral nutrition are also included
Grade 3	Complications requiring surgical, endoscopic, or radiological intervention
Grade 3a	Intervention not under general anesthesia
Grade 3b	Intervention under general anesthesia
Grade 4	Life-threatening complications (including central nervous system complications) requiring intensive care unit stay
Grade 4a	Single organ dysfunction (including dialysis)
Grade 4b	Multiorgan dysfunction
Grade 5	Death of the patient

Reproduced from Dindo et al. [70], with permission from Wolters Kluwer Health.

Summary

Living donor liver transplantation has evolved to become a viable, albeit ethically and technically complex, option for patients with end-stage liver disease. The primary factors driving its successful implementation are proper donor selection and exquisite attention to the anatomical details of the procedure. Careful consideration of the impact of the hepatectomy on the donor and its adequacy for the recipient must be made well in advance of the procedure, with continued assessment intraoperatively. The ideal graft provides the donor with a future liver remnant exceeding 35% and the recipient with a graft-to-recipient weight ratio of >0.8%, simultaneously. In general, living donation remains an open procedure, although laparoscopic approaches are being explored. Perioperative management by a multidisciplinary team is important, and the practice of LDLT should be limited to dedicated, properly trained and resourced teams. Its implementation in the near term will be governed by the availability of such teams and the shortage of deceased donor organs.

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CHAPTER 25

Abdominal Organ Preservation and Resuscitation

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Introduction

Modern organ transplantation, particularly deceased donor transplantation, is dependent on methods to maintain the viability of explanted organs until their ultimate revascularization in the recipient. Indeed, the quality of preservation is a major factor dictating subsequent graft function, and almost all of the innovations in organ allocation are made possible by the ability to preserve organs for significant periods of time. This chapter will introduce the prevailing concepts that have emerged as important in organ preservation, and highlight clinical data supporting the preferred methods of abdominal organ preservation and transport. It complements Chapter 26, which covers similar concepts adapted to the unique requirements of thoracic organs. Both of these chapters introduce the emerging concepts involved in organ resuscitation: the maneuvers used to improve the quality and function of an organ while it is ex vivo. Indeed, this later concept represents the future, in that the expectations that organs can merely be preserved in their previous state are giving way to a realization that organs can in fact be improved over time and made more suitable for transplantation through ex-vivo manipulation.

Factors shaping the development of modern organ preservation

In the early days of clinical transplantation, donor and recipient were within the same hospital and in the majority of cases in adjacent operating theatres. This allowed the direct transfer of the organ to be transplanted and hence no method of preservation was necessary beyond the flush-out of blood. As programs have developed into national and even international schemes, the preservation period has become longer to facilitate transportation of the organ from the donor to the recipient as well as HLA tissue matching to reduce the chance of graft failure due to rejection. Adequate preservation during this period is essential to ensure that a functional organ with life-sustaining potential is implanted at time of transplantation. A simple method was therefore adopted to bridge the organ from donor explant to recipient transplant: that of rapidly cooling and storing the organ on ice. However, this hypothermic and hypoxic preservation is not without its harmful side-effects, and preservation methods have been designed to counteract these mechanisms of tissue damage. In addition, in the past decade the type and condition of organ donors have significantly changed, with for example more older, hemodynamically unstable and even non-heart beating donors.

Maintaining organ viability during preservation is an important prerequisite for successful outcome after transplantation. Currently, most centers use static cold storage to preserve organs. This preservation method, however, was developed in an era characterized by the availability of younger and higher quality donor organs [1]. The increasing gap between those who need organ transplantation due to end-stage organ failure and the number of high quality organs available from deceased donation after brain death (DBD) forced the transplant community to accept organs from alternative sources. For kidney transplantation, the living donor program has partially bridged this gap in many countries. For other organs, this type of donation is either impossible or with carries a substantial risk for the donor.

Given the lower availability of high quality donors and the persistent need for transplantable organs, acceptance criteria have been continuously extended. These extended criteria donor (ECD; covered in depth in Chapter 53) organs are DBD transplants and the average recipient survival, graft survival, and organ function are inferior when compared to transplant of standard criteria DBD organs. A DBD kidney is considered to be an ECD organ if the estimated adjusted relative risk of graft failure is $\geq$1.70. The donor characteristics of an ECD kidney include age >60 years, or age 50–59 years plus two of the following features: cerebrovascular accident as the cause of death, pre-existing hypertension, or terminal serum creatinine >1.5 mg/dL. The characteristics of an ECD liver are age >60 years, cold storage time >12 h, extent of steatosis as measured histologically, and use of vasopressors.

The increased use of these types of donors in kidney transplantation resulted in an increased incidence of delayed graft failure (DGF) from 15% in the late 1980s to >25% in the latest reports from the Organ Procurement and Transplantation Network (OPTN). DGF has been shown to negatively affect graft survival with a relative risk of 1.7, leading to 15% reduction in survival compared to standard criteria donors (SCDs) after 1 year.

In an attempt to further increase the donor pool, especially in recent years, the use of donation after circulatory death (DCD;

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

discussed in Chapter 22) has increased. DCD donors are categorized according to the Maastricht criteria. Whilst in most countries only Maastricht category 3 donors or controlled DCD are used, some countries introduced the concept of uncontrolled DCD (categories 1 and 2). The controlled DCD donors are patients with extensive irreversible brain injury where further medical treatment is futile, however, they do not meet the criteria for brain death. As a consequence of withdrawal of ventilation support, the patient will die due to cessation of circulation with cardiac arrest. After a period of "no touch," organs can be retrieved and preserved.

Uncontrolled donors do not suffer from brain injury but die after unsuccessful resuscitation after their primary cardiac arrest. In both controlled and uncontrolled situations, there are substantial warm ischemic periods that lead to injury of the potential graft.

Kidneys from DCD donors have a higher risk of primary nonfunction (PNF) and suffer from DGF. Interestingly, despite the higher incidence of poor function after transplantation, long-term graft survival is equivalent or only slightly lower than that for kidneys derived from DBD donors, which could be due to selection.

The use of DCD donors is less accepted in renal transplantation than in renal transplantation due to inferior outcomes and frequent biliary complications leading to retransplantation. Some centers, however, have equivalent outcomes for DBD and DCD liver transplantation, indicating that with proper donor management and adequate preservation techniques these donors can be a good source to reduce mortality on the waitlists.

Principles of cold preservation

After circulatory arrest, tissue metabolism continues for some time; however, the absence of oxygen and nutrients rapidly leads to major metabolic problems within renal cells. The suppression of metabolism is therefore essential to maintain organ viability during the preservation period.

The reduction in metabolic rate in biological systems as well as chemical reactions is dependent on the reduction in temperature. The Q10 temperature co-efficient is a measure of the rate of change of a biological or chemical system as a consequence of decreasing the temperature by 10 °C [2]. Applying this to organ preservation, this means that reduction of the core temperature of an organ to 4 °C will reduce the metabolism to approximately 10% in the majority of cells and will diminish enzyme activity (Figure 25.1). Calne et al. showed that simply cooling of kidneys in ice water preserved renal function for 12 h [3]. The development of preservation solutions that targeted harmful pathways during cold preservation enabled longer storage times and preservation quality improved [4]. Despite the beneficial concept of hypothermia, unwanted side-effects in the

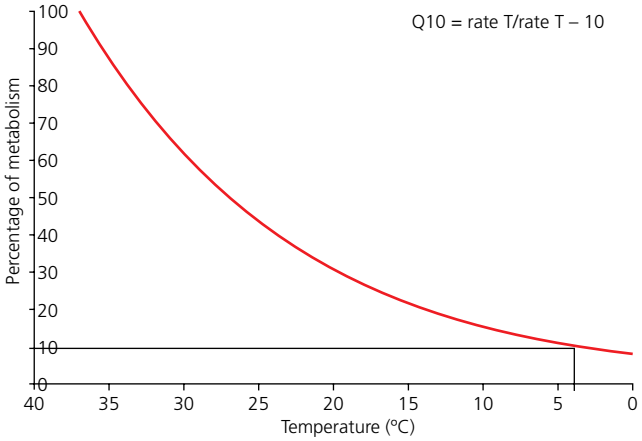


Figure 25.1. Relationship between temperature and metabolic activity.

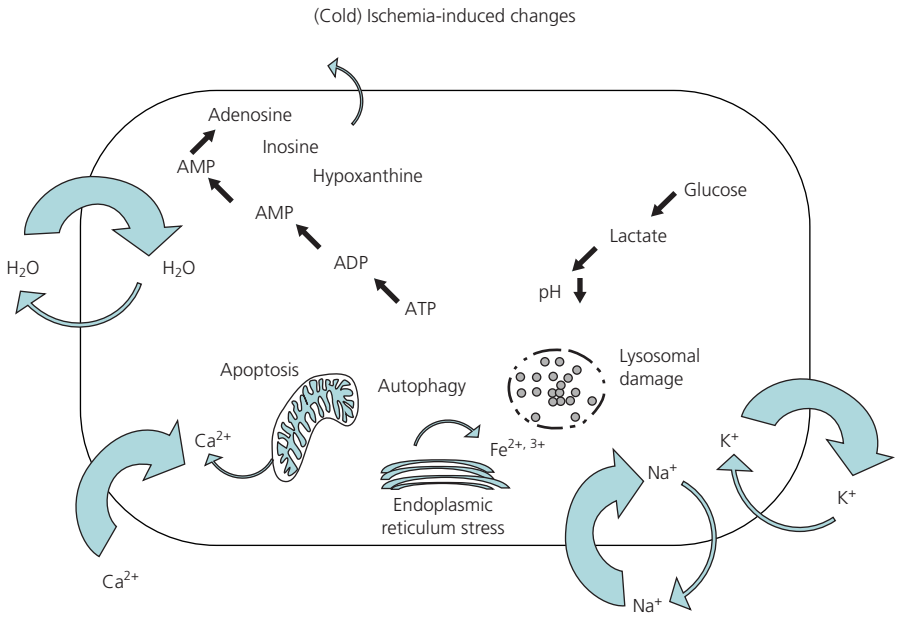


Figure 25.2. Negative effects of cold ischemia: breakdown of ATP, acidosis, release of lysosomal enzymes, endoplasmic reticulum stress, increased Na^+ and Ca^{2+} influx into the cell, and subsequent cell swelling.

preserved organ still occur (Figure 25.2). These effects can only be partly be counteracted by preservation solutions.

Energy and acidosis

Tissue hypoxia during organ preservation results in a rapid fall in intracellular adenosine triphosphate (ATP) levels. Even at temperatures as low as 0–4 °C, cellular ATP content is rapidly depleted and cells switch to anaerobic metabolism to support cellular processes. This leads to a much less efficient production of ATP, but also to the production of lactic acid and acidosis [5,6]. The contribution of acidosis to ischemic injury is pH dependent. Severe acidosis activates phospholipases and proteases, causing lysosomal damage and eventually cell death [7–9]. Mild acidosis (pH 6.9–7.0), however, has been suggested to have a protective effect by inhibiting phosphofructokinase, the rate-limiting step in glycolysis [7,8]. Adequate control of pH is therefore an important function of preservation solutions.

Cell swelling

The changes in cellular structures observed during preservation are cell swelling and formation of protruding pockets [9]. The mechanism underlying these changes is the impaired activity of the Na^+/K^+-ATP-dependent pump. As a result, sodium excretion is reduced and sodium passively enters the cell, attracted by the negative charge of cytoplasmic proteins. This creates a hyperosmolar intracellular environment and subsequently an influx of water. To re-establish the disturbed Donnan equilibrium and to prevent cell swelling, impermeants and colloids are added to preservation solutions.

Effective impermeants are saccharides and non-saccharide anions. Molecular size determines the success of extracellular impermeants in preservation solutions, with larger molecules being the most successful [10–12]. Colloids such as starches are large molecules that are retained in the vascular compartment.

Reactive oxygen species

Reactive oxygen species (ROS) are generated by several processes in ischemic and post-ischemic reperfused organs [13]. An extensively studied generator of ROS is xanthine oxidase, which simultaneously produces hydrogen peroxide (H_2O_2) and the superoxide anion (O^{2-}) [14]. The subsequent reduction of H_2O_2, catalyzed by iron, leads to hydroxyl radical formation (OH^-). ROS react rapidly with other molecules, which results in severe damage to lipids, nucleic acids, and proteins during reperfusion, the ischemia–reperfusion injury (IRI) [15,16]. In addition to xanthine oxidase, which in human renal transplantation may be of minor importance since it is not abundant in human cells, in contrast to rodents [17], several other sources of ROS are important. Infiltration of the graft by leukocytes after reperfusion results in the production of mainly superoxides (known as the respiratory burst).

Mitochondrial malfunctioning resulting from partial reduction of the respiratory chain is an important contributor to ROS formation after reperfusion. The formation of ROS has long been considered to contribute to cellular injury during the reperfusion phase but not during cold preservation) [15,16]. However, some reports suggest that oxygen radicals are formed during reperfusion as well as during cold preservation [18].

Calcium

In normal circumstances a large difference in free Ca^{2+} concentration exists between the intracellular and extracellular space fluids. This difference is maintained by active transport of Ca^{2+} by several ATP-dependent processes, including Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger [19]. During cold preservation, cellular ATP concentrations fall, leading to increased intracellular Ca^{2+}. Accumulation of Ca^{2+} in the cold phase will lead to activation of calcium-dependent processes, such as calpain activation and protein kinase C signaling. Calpain activation leads to loss of cell structure by breakdown of the cytoskeletal spectrin [20]. Calpain activity has been shown to be increased in cold stored hepatocytes, and further increased during rewarming [21].

Enzymes

Intracellular proteases are involved in the breakdown of proteins during preservation, most likely due to the absence of oxygen. Also, matrix metalloproteinases (MMPs) may be activated during cold preservation, leading to detachment of endothelial cells from the underlying matrix. This phenomenon has been predominantly studied in the liver, but also occurs in renal preservation [22,23]. To reduce this detrimental effect by blocking MMPs (especially MMP 2 and 9), the addition of the often disputed colloid, hydroxyethyl starch (HES), into the University of Wisconsin (UW) solution has been shown to play an important role [23].

Another relevant family of enzymes activated during cold preservation are apoptosis-related caspases [24–26]. During prolonged preservation, free iron is released from cytochrome P450 [27]. The release of free iron will, in combination with hydrogen peroxide, lead to severe production of ROS [28,29]. Recently, changes in autophagy fluxes have been postulated as a possible mechanism of increasing injury with increasing cold ischemia times. Although most studied in the liver [30,31], preclinical work indicates that autophagy may also be important in kidneys [32]. In relation to this, Minor et al. [33,34] have also postulated that endoplasmic reticulum stress might be involved in cold ischemia-induced injury. Peralta and Brenner subsequently confirmed this [35].

As described above, during cold preservation, cellular homeostasis is no longer sustained. Major derangements are shifts in electrolytes, changes in pH, intracellular enzymes and proteins, and increase in intracellular water. Preservation solutions are therefore composed to counteract these processes.

Hypothermic storage

With the establishment of kidney transplant programs, the need for preservation became evident. Belzer and his team thought that continuous cooling of the kidney throughout the preservation period would be the most natural way to do this and showed good results. Due to the hydrostatic pressure and the absence of cellular mechanisms counteracting fluid entrance into the interstitial space and cells, tissue edema was a serious problem. This edema was counteracted by using fluids including oncotic agents. For this purpose, human albumin or cryoprecipitated plasma was used. Belzer and Southard later developed a synthetic machine perfusion (MP) fluid (Belzer MPS) that would eventually also provide the basis for the UW cold storage solution. The complexity of the procedure initiated the search for alternative and easy methods. Many different solutions were tried, varying in complexity and efficacy. With the development of better preservation solutions, most centers now use cold storage as their main preservation methods, although recently hypothermic machine perfusion (HMP) has gained renewed interest as a result of the use of ECD and DCD donors.

Table 25.1. Composition of preservation solutions

		EC	UW	HTK	HOC	Celsior	IGL-1
Buffers (mM)	K_2HPO_4	15	–	–	–	–	–
	KH_2PO_4	43	25	–	–	–	25
	$NaHCO_3$	10	–	–	10	–	–
	Histidine	–	–	198	–	30	–
Impermeants (mM)	Glucose	195	–	–	–	–	–
	Lactobionate	–	100	–	–	80	100
	Citrate	–	–	–	80	–	–
	Mannitol	–	–	38	185	60	–
	Raffinose	–	30	–	–	–	30
Electrolytes (mM)	Chloride	15	20	32	–	42	20
	Calcium	–	–	0.0015	–	0.25	–
	Magnesium	–	5	4	40	13	5
	Potassium	115	120	9	84	15	25
	Sodium	10	30	15	84	100	120
ROS scavengers (mM)	Allopurinol	–	1	–	–	–	1
	Glutathione	–	3	–	–	3	3
	Tryptophan	–	–	2	–	–	–
Nutrients (mM)	Adenosine	–	5	–	–	–	5
	Glutamate	–	–	–	–	20	–
	Ketoglutarate	–	–	1	–	–	–
Colloids (mM)	HES	–	0.25	–	–	–	–
	PEG	–	–	–	–	–	0.03
Osmolality (mOsm)		406	320	310	400	255	320

EC, Eurocollins solution; HOC, hyperosmolar citrate solution; HTK, histidine–tryptophan–ketoglutarate solution; IGL-1, Institut Georges Lopez-1 solution; PEG, polyethylene glycol.

Composition of clinically used cold storage solutions

Numerous preservation solutions have been evaluated for the static cold storage of kidneys for transplantation [36]. They differ considerably in terms of their chemical composition (Table 25.1). A few examples of the most important solutions in international clinical practice are described below.

Eurocollins solution

The first static cold storage solution was developed by Collins in the late 1960s [37,38]. A minor modification by the Eurotransplant Foundation in 1976, eliminating magnesium, resulted in the Eurocollins (EC) solution that was used mainly in the era before the UW solution had been developed.

EC solution is a simple intracellular-like preservation solution. Phosphate is used for pH buffering and glucose serves as the osmotic agent. As glucose is able to cross the cell membrane, EC solution provides a source for ATP and lactate in an anaerobic environment, reducing its effectiveness against impermeants after a number of hours [39,40]. Most centers have now switched to using the UW solution.

University of Wisconsin solution

Continuous and systematic research by Belzer and Southard in the 1980s led to the development of the UW solution and its clinical introduction in 1987. Metabolically inert substrates such as lactobionate and raffinose serve as osmotic agents (Table 25.1). HES is used as a colloid. The colloids were originally added to hypothermic machine preservation solutions to prevent tissue edema due to hydrostatic pressure. Belzer and Southard used diafiltrated HES in UW solution as they originally aimed at developing one solution suitable for both cold storage and HMP. The feasibility of HES as a colloid in UW solution has been extensively debated. HES prevents interstitial edema and has a beneficial effect on MMPs, but increases viscosity [41,42]. Analyzing the effect of HES on red blood cells (RBC), several authors have shown an increased RBC aggregation when large molecular weight HES is present [41,42]. This effect could partially explain the slower washout of blood and initially patchy reperfusion of organs when UW solution is used [43]. In UW, the compounds allopurinol and glutathione (GSH) are included to prevent formation of ROS. Allopurinol inhibits xanthine oxidase, which improves kidney preservation, while liver or pancreas preservation are almost unaffected [44,45].

GSH is a tripeptide that has free radical trapping properties. This important antioxidant is oxidized to glutathione disulfide together with converting peroxides. Experimental studies have shown the importance of GSH in models of both renal tubular injury and isolated perfused liver [44,46]. In the absence of GSH, less ATP was generated and more lactate dehydrogenase (LDH) was released [44,46]. Subsequent studies have shown the benefit of GSH after kidney transplantation and that GSH is especially important in long-term liver preservation [46].

To date, UW solution is considered the gold standard preservation solution for the kidney, liver, pancreas, and small bowel [39,47–53].

Histidine–tryptophan–ketoglutarate solution

Histidine–tryptophan–ketoglutarate solution (HTK) was initially introduced as a cardioplegic solution in open heart surgery by Bretschneider in the 1970s [54,55]. The basic design is that of a very potent buffer: histidine combined with two amino acids (Table 25.1). Tryptophan serves as a membrane stabilizer and antioxidant, through its oxidative metabolites in the kyunerine pathway, such as 5-hydroxy-tryptophan [56,57], while ketoglutarate acts as a substrate for anaerobic metabolism during preservation [58]. Tryptophan protects the organs against ROS-mediated damage. Mannitol

is a slightly larger monosaccharide than glucose, but unlike glucose it cannot be metabolized and does not pass through the cell membrane easily. Furthermore, it is added for its beneficial effect as a free-radical scavenger. . In a cultured rat hepatocyte experiment, the amount of thiobarbituric acid reactive substances (TBARS) as a marker for ROS-mediated injury was measured. After 24-h preservation TBARS were significantly higher in HTK solution compared to UW solution preserved hepatocytes, suggesting superior antioxidant capacity of UW solution due to the combination of GSH and allopurinol [59].

HTK solution has a low viscosity and to achieve complete tissue equilibration according to Bretschneider, high volumes (~15 L) have to be rinsed through the organs at low flow rates.

Hyperosmolar citrate solution

Hyperosmolar citrate (HOC, also known as Marshall's solution) is used for renal transplantation primarily in the UK and Australia [60,61]. HOC solution is a citrate/$NaHCO_3$-buffered solution with mannitol as the impermeant. Due to its composition as a hypertonic solution, HOC solution prevents the entry of fluid into cells [62] and has been shown to be effective in experimental kidney preservation [38].

Celsior solution

Celsior solution was developed initially for cardiac preservation in the early 1990s. It is similar to UW solution in composition and it has since proven to be of use in the preservation of kidneys, liver, and pancreas [40,48,63–73]. Unlike UW, it has a high sodium content (100 mM) and low potassium content (15 mM), and is relatively low in viscosity, as it does not contain HES. It combines the inert osmotic agent philosophy of UW solution together with the strong buffering capacity of HTK solution. Furthermore, Celsior solution contains lactobionate and mannitol as impermeants, along with reduced glutathione as an antioxidant.

Institut-Georges-Lopez-1 (IGL-1) solution

The HES controversy initiated a search for other colloids, e.g. dextran and polyethylene glycol (PEG) [71,72,74–77]. The high viscosity associated with HES, as well as its tendency to cause red cell aggregation in animal experimental models [42,64] and in vitro [41,65,72,78], justified the search for alternatives such as PEG. PEG is a neutral, water-soluble, non-toxic polymer. Although chemically not a true colloid, it acts like a colloid by the binding water. In addition, PEG can bind to cell membranes and create layers of "structured water." This induces a type of immune camouflage by interfering with the identification of the graft as foreign by cells of the immune system [79,80]. PEG prevents the activation of reperfusion-induced inflammation, which can have a long-term effect on graft outcome, particularly through interference with danger signal production. Several studies, both experimental and clinical, have now confirmed the efficacy of PEG for liver [55,81,82], kidney [83,84], pancreas [85], small bowel [86], and heart preservation [87].

Clinical use of hypothermic preservation

It is difficult to answer the question as to which solution provides the best preservation method using cold storage. The use of a preservation solution is a matter of availability, cost, and "couleur locale." Even in kidney transplantation, only a limited number of high-quality randomized controlled trials (RCTs) have been performed [36].

This following section describes preservation methods for the different organs.

Renal preservation

Nowadays, static cold preservation is the most prevalent storage methods irrespective of the donor type. With the exception of the US, where approximately 20% of kidneys from deceased donors are pumped, most countries primarily use cold storage. While the preservation solution is almost exclusively Belzer MPS for CS, more variation is seen for pump perfusion, with HTK and UW solutions being most commonly used. Since the early 1990s, UW solution has been the gold standard in kidney preservation, replacing EC solution in Europe as the preferred solution. This was the result of a RCT comparing EC solution with UW solution and showing that DGF was significantly lower in the UW group (23% vs. 33%). Also, 1-year graft survival was found to be significantly higher in the UW group [63,88]. Shortly following this trial, another RCT in the same Eurotransplant region showed that HTK solution was superior to EC solution in reducing DGF, but without an effect on graft survival. In the same study, no difference were found between HTK and UW solutions. Registry analysis, however, indicates that UW solution is associated with a lower incidence of PNF and risk of graft loss, particularly when storage times are >18 h..

A multicenter randomized prospective trial comparing UW versus HTK solution in kidney preservation showed equal results in terms of the incidence of DGF (33% vs. 33%) [49,74]. For prolonged cold storage times with HTK solution (>24 h), however, little data are available. One single-center study reported a significantly higher incidence of DGF with HTK solution than UW solution (50% vs. 24%) when cold ischemia time was >24 h [86,89]. The opposite was reported in a more recent study, with much a higher DGF rate associated with HTK solution than with UW solution (56% vs. 16%) [87,90]. Direct comparison of these conflicting findings is impossible due to the different definition of DGF in each of the studies.

Other solutions that were tested against UW solution are Celsior solution and IGL-1 solution, which both can be considered "look-alikes" of UW. Celsior solution was shown in relatively small studies to be comparable to UW solution in terms of DGF and graft survival [40,64,91]. In a small cohort study comparing IGL-1 and UW solutions, no differences were found in DGF or graft survival. Postoperative serum creatinine levels, however, were lower in IGL-1 solution stored kidneys, possibly related to the presence of PEG in that solution [84].

A recent systematic analysis in the UK of preservation solutions used in clinical practice revealed no major differences in transplant outcome between HOC and UW solution or HOC and Celsior solutions [92], although recent work by Kay et al. in experimental transplantation showed more edema in kidneys flushed with HOC solution compared to UW solution [93].

With the increasing use of more marginal kidneys from ECD and DCD donors, a re-emergence of interest in hypothermic machine perfusion (HMP) has been seen. Retrospective data from UNOS showed it had a beneficial effect in reducing DGF, especially in high-risk groups [73]. A large meta-analysis from Wight et al. concluded that HMP results in a 20% decrease in DGF. Retrospective analysis from the US registry also showed it improved DGF rates

and graft survival [75]. Although indicative only, recent Level A evidence became from the Machine Perfusion (MP) trial, was executed in the Eurotransplant region, showed that HMP is beneficial for SCDs, ECDs, and DCDs; the rate of DGF was lower and 1-year and 3-year survival were better [76,77]. Interestingly, the impact of DGF on graft survival seemed to be lower in MP stored kidneys. In an extension of the MP trial, Treckman et al. reported reduced risk of DGF and improved 1-year graft survival and function in ECD kidneys [78].

Jochmans et al. concentrated on DCD donors (all Maastricht category 3) and showed that HMP was associated with a reduced risk of DGF and better early graft function up to 1 month after transplantation. DGF rates decreased from 70% to 54% and graft survivals were identical [80]. In contrast to this study Watson et al. in a study in the UK were unable to show any effect of MP on outcome of DCD kidneys [94]. A likely explanation for the differences observed in the UK and the Eurotransplant region is that in the UK study kidneys were cold stored prior to pumping. In the UK study, similar DGF rates were found in both arms (56% vs. 58%), rates that are comparable to the those found in the Eurotransplant trial. In a study by Hosgood et al., renal resistance of kidneys that were pumped immediately following their retrieval was lower than the resistance of kidneys that were put on the machine after hours of cold storage [95]. The relationship between renal resistance (RR) and DGF has been noted by many centers pumping kidneys and is frequently given as a reason to discard kidneys. In the MP trial where researchers were blinded for RR, the relationship between RR and DGF was confirmed with a high odds ratio; however, predictive value was poor and the authors therefore concluded that RR as a stand-alone quality assessment tool cannot be used to predict outcome with sufficient precision [96,97].

Another potential interesting application of MP is in the use of perfusate biomarkers for selection or predictive value of outcome. The most used and measured biomarker is glutathione-S-transferase (GST), an enzyme localized in the renal tubules. It is involved in deconjugation of waste products and excreted into the urine [97]. Total GST has been shown to reliably reflect renal tubular injury and correlates with warm ischemia time [98,99] and with DGF [97]. In the latter study, NAG and H-FABP were independent predictors of delayed graft function, but not of primary non-function and graft survival. Other potential biomarkers, like lactate dehydrogenase, aspartate aminotransferase, and alanine-aminopeptidase, have no independent prognostic potential for any of the endpoints. Perfusate biomarker concentrations had no relevant correlation with cold ischemic time or renal vascular resistance on the pump [97].

Liver preservation

Similar to renal preservation, cold storage became the preferred preservation method for the liver. The solutions used are also similar to those used for kidney preservation, and for a long time UW solution has been used by most centers, although HTK and Celsior solutions are increasingly used [100]. It was shown that with the preservation capacity of UW solution was equal to that of HTK solution or Marshall's solution, even with longer preservation periods [101–103]. Graft survival and immediate function were improved and UW solution quickly became the preservation solution of choice. In liver transplantation, no high quality studies are available comparing preservation solutions; many of those that have been published have <100 cases in one arm and thus are likely to be underpowered. Most studies have found similar outcomes when comparing HTK and UW solutions, although some studies suggest that HTK solution is more effective for biliary tract flush and prevention of biliary complications [53,104–108]. Retrospective analysis from the UNOS database and a number of recent single-center reports, however, indicate that HTK solution is associated with a higher risk of graft loss, in particular with cold ischemia times longer than 8 h and in DCD donors [109].

The indication that UW solution might be less efficient in preserving the biliary tree can be attributed to the higher potassium content in UW, which results in vasospasm. Alternatively, the type of colloid may also be of relevance. HES has been shown to form aggregates with red blood cells of up to 5 μm^2 in size, which is large enough to block small capillaries [41]. In IGl-1 solution, potassium levels are low and HES is substituted for PEG. In a controlled trial in France, however, comparable results to the standard of care were found, although no report on bile flow or bile composition was given [81].

Comparisons between Celsior and UW solutions showed no differences in PNF rate or graft survival at 1 and 3 years [48,64,70,91,110]. Recently, Garcia-Gil et al. reported a reduced incidence of post-reperfusion syndrome, defined as a decrease in the mean arterial pressure of $>30\%$ of the baseline value for more than 1 min during the first 5 min after graft reperfusion, from 21.6% in the UW solution preserved group to 5.9% in the Celsior solution preserved group [40]. In the same study, no effect on 5-year graft survival was seen. In the same study, HTK and Celsior solutions were compared and showed similar outcomes [40].

Following the developments in MP with kidneys, there also has been renewed interest in the MP of livers. As elegantly summarized by Monbaliu et al., liver perfusion is more complex than kidney perfusion [111]. Factors like hepatic and portal flow competition, exclusive arterial supply to the biliary tree, susceptibility of hepatic sinusoidal endothelial cells, and high metabolic rate of the liver are complicating issues in MP of the liver [33]. Several groups, building on the legacy of Starzl, Belzer, and Bretschneider in the 1960s, are developing new concepts applicable to liver perfusion [112–115], and novel systems that show efficacy in MP in preclinical studies are on the edge of entering the clinical arena. The first and only recently published clinical study using hypothermic liver preservation was performed by Guarera et al. with a home-built MP set-up in 20 patients; the results are encouraging. Peak serum injury markers were lower in the HMP preserved livers and early allograft dysfunction was reduced from 25% in cold stored livers to 5% in HMP stored livers ($P = 0.08$) [116].

Larger studies, however, will be needed to show the benefit of HMP in liver preservation, especially in ECD and DCD donors. Remaining questions about flow rates and perfusion pressures, rate of oxygenation, single or dual vessel, composition of the perfusion fluid, and, probably most debated, perfusion temperature are still under investigation.

Pancreas preservation

Preservation of the pancreas can be divided into two parts, i.e. preservation of the whole pancreas and preservation of islet cells. In clinical practice a pancreas considered unsuitable for whole pancreas transplantation is used for the isolation of islets (details of islet production and preservation are covered in Chapter 61). For both whole pancreas transplantation and isolated cell transplantation (ICT), static cold storage is the predominant method of preservation. Whole organ preservation of the pancreas has almost

exclusively been performed with UW solution since it was developed by Southard and Belzer and proven to be a successful preservative even before it was applied in other organs [117]. Recently, reports have suggested that other preservation solutions may be effective alternatives to UW.

Comparisons between HTK and UW solutions are mostly retrospective and/or single center observations. The only somewhat larger RCT performed by Schneeberger et al. reported equivalent outcome in graft survival and graft function [118]. In a registry data analysis of 4392 pancreas transplantations, it was found that use of HTK solution was an independent risk factor for graft loss (hazard ratio 1.30; $P = 0.014$), especially with cold ischemia times of >12 h (hazard ratio 1.42; $P = 0.017$). Some authors also report higher rates of graft pancreatitis, possibly due to high volume use of HTK solution leading to edema [119,120].

The first prospective RCT comparing UW solution (n = 50) with Celsior solution (n = 50) for clinical pancreas transplants demonstrated that Celsior and UW solutions had similar safety profiles for pancreas preservation. A smaller RCT from Brazil of only 30 transplants found no differences [65]. A retrospective analysis from Italy indicated that EC solution is comparable to UW solution in respect to surgical complications [121]. Another study comparing Celsior solution (n = 28) with UW solution (n = 44) for pancreas preservation demonstrated that 2-year recipient survival rates, 2-year graft survival rates, pancreas leakage rates, and clinical graft pancreatitis rates were similar in both groups [67].

Preservation of pancreatic tissue with the aim to isolate islets is mostly performed using UW solution, although other methods and solutions are available. A meta-analysis by Kaddis et al. from 2010 revealed that storage in UW solution improves outcome [122]. A recent report from the University of Illinois at Chicago indicated that HTK and UW solutions produce a similar islet yield [123]. Likewise, a report from Geneva retrospectively analyzing 376 islet isolations from pancreases flushed and transported with IGL-1 solution (n = 95), UW solution (n = 204), or cold storage (n = 77) showed no differences in islet graft function [124].

Report of HMP of the pancreas is limited to that by Leeser et al. Four human pancreata were procured from deceased donors and HMP was compared with cold storage using UW. This pilot study indicated that low-flow pulsatile MP of the pancreas with prolonged cold ischemia time could result in excellent yield, viability, and function. Experimental studies in pigs also indicate improved preservation and yield with MP [125].

The two-layer method (TLM) is almost exclusively used in pancreas preservation. In the TLM, the pancreas is directly oxygenated by perflurocarbon during reservation and this maintains a high level of adenosine triphosphate (ATP) in tissues, which is thought to be due to the maintenance of cellular integrity and retention of parenchymal and non-parenchymal viability [20,59,126,127]. Most studies report a beneficial effect of TLM, although two recent large-scale studies showed no beneficial effect of TLM or storage on human islet isolation and transplantation [126,128].

Small bowel preservation

Preservation of intestinal grafts is adequate but most likely not optimal [129]. Preservation of the intestine is difficult since the mucosa is extremely sensitive to ischemia [130]. Disruption of the intestinal barrier leads to post-transplant bacterial translocation [131]. To prevent post-transplant complications, cold ischemia times must be kept to a minimum.

As it is with other abdominal organs, UW solution is most commonly used in small bowel preservation [132]. The only clinical report comparing in retrospect UW and HTK solutions found no difference in function or acute rejection [133]. Experimental studies with human intestine by de Roover et al. indicated that UW and Celsior solutions are equal in preservation potential [134].

Experimental models comparing different solutions demonstrate potential improvements with the addition of nutrients [68,135] and luminal preservation [136–138]. Also, the addition of oxygen is suggested to be beneficial [139–141].

Resuscitation protocols

In-situ perfusion of DCD donors

Given the availability of potential DCD Maastricht category 1 and 2 donors, use of organs from this source would shorten the waitlist [142,143]. In 2006, the US Institute of Medicine recommended the transplant community to undertake active efforts to increase donation from Maastricht category 2 DCD donors in particular, estimating that this group could expand the donor pool by 22 000 per year. However, due to the unknown extent of ischemic injury, assessment of organ quality is difficult and risk of poor function is considerable. In Spain especially, where organ donation per million inhabitants is the highest in the world, organs from DCD donors are used extensively. Using strict selection criteria, one center in Madrid, Spain, has successfully transplanted kidneys from Maastricht category 1, 2, and 4 donors [144–146]. Following irreversible cardiac arrest in suitable donors, a femoral arterial and venous catheter is inserted, allowing the maintenance of grafts by extracorporeal membrane oxygenation (ECMO). ECMO systems are widely used to support patients with cardiorespiratory failure on intensive care units. In the setting of organ donation, a double balloon is inflated immediately above the diaphragm and used to prevent recirculation through the brain or heart. This allows continued circulation of warm, oxygenated blood through the abdominal organs after cardiac arrest.

This method has demonstrated good results, with 1- and 5-year graft survivals comparable to those for DBD kidneys at the same institution during the same time period [146]. Category 2 kidneys undergo a variable amount of warm ischemia of up to 2 h, and the discard rate for retrieved kidneys is therefore high at approximately 33%. Even after acceptance, the DGF (68–80%) and PNF rates (6%) are substantial [144,145]. In a small series of category 2 and 4 donors in Barcelona, Spain, normothermic ECMO was compared with hypothermic ECMO and with in-situ cold perfusion by EC solution, where the incidence of DGF and PNF was significantly lower in kidneys perfused with normothermic ECMO [147]. A series of kidneys from Maastricht category 1 and 2 DCD donors in Paris, France, with a longer warm ischemic time of up to 150 min, demonstrated higher discard (43%) and DGF rates (92%), but a lower PNF rate (2%) [148]. Graft survival was 89% at 6 months. The normothermic ECMO group had lower rates of DGF compared to the other groups and less PNF than the in-situ group.

Whilst ECMO is used for uncontrolled DCD, it has not been widely tested in controlled DCD. ECMO provides a possible method by which the acidosis and low venous oxygen levels present after the withdrawal of life-supporting treatment could be reversed in controlled DCD. Following a decision to withdraw treatment on the ICU, family consent for donation is sought, and if agreed to, a femoral catheter is inserted and supportive treatment withdrawn. Five minutes after cardiac arrest, the aortic occlusion balloon is

inflated and ECMO is initiated. In a case series from Michigan, USA, this method has provided low rates of DGF (8%) and PNF (0%) for Maastricht category 3 DCD donors [149]. Another group from North Carolina, USA, transplanted a series of kidneys following sub-normothermic (22 °C) ECMO after controlled DCD [150]. DGF rates were higher in this study (57%) and 1-year graft survival was 87%. A large case series from Taipei, Taiwan, demonstrated that ECMO for kidneys from Maastricht category 1, 2, and 4 donors could result in excellent 5-year graft survival rates of approximately 88% [151]. This survival rate is comparable to that for live donor kidneys (89%) and DBD kidneys (83%) transplanted at the same institution during the same time period.

For livers, the ECMO technique is used only for uncontrolled donors. Fondevila et al. and the Barcelona group reported the first case–control study of ECMO for uncontrolled DCD liver preservation [152]. The group required strict criteria to accept and transplant organs, resulting in only 10 transplants from 40 donors. Livers were rejected if macroscopically steatotic on inspection, or on the grounds of elevated transaminases or poor perfusion. Donors with prolonged warm ischemia time were also excluded. Patient and graft survivals were comparable to matched DBD livers transplanted at the same unit, with an average of 23 months of follow-up.

Another case–control series from Spain examined the results of uncontrolled DCD liver allografts [113,153]. Jimenez-Galanes et al. and the Madrid group have strict criteria for accepting these organs: donors must be younger than 50 years old, have an arrest time of $<$15 min, and have a warm ischemia time of $<$50 min. Maximum acceptable ECMO time is 270 min. Twenty liver allografts were transplanted from 43 potential donors. The 1-year graft survival was 80% and was comparable to that for matched DBD livers transplanted at the same unit. PNF rates were also comparable in this small series (10% vs. 5%). Retransplantation rate, however, was higher for the uncontrolled DCD group (15% vs. 0%) as were the peak aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels.

Oxygenated machine perfusion at different temperatures

Future developments in kidney preservation will aim to repair damaged organs by providing mechanisms to support metabolism and remove waste products, counteracting the effects of ischemia–reperfusion injury (IRI). New preservation fluids may allow static storage at normothermic or sub-normothermic temperatures, as has been demonstrated in animal studies. AQIX, a non–phosphate-buffered solution, has been used for short-term, sub-normothermic, static preservation in a porcine experimental model [154]. Improved outcomes following static cold preservation may also be possible with the development of new solutions. Polysol, a low-viscosity solution, has been tested in porcine autotransplant models, showing improved renal blood flow and tissue oxygenation compared to UW solution [155] and improved creatinine clearance compared to HTK solution [155,156].

Delivery of oxygen to the graft during the preservation period has been the focus of several studies and various methods, other than the delivery of oxygenated whole blood, have proven to be promising in animal and experimental studies. Persufflation of gaseous oxygen directly through the vasculature of the kidney has been tested as a rescue therapy for ischemically damaged kidneys. Filtered and humidified gaseous oxygen can be administered through the renal vein and allowed to escape from the surface of the kidney through fine pin-pricks made with a microvascular needle. In animal studies, autotransplanted kidneys displayed better creatinine clearance following oxygen persufflation as compared with cold static or perfusion preservation alone [157,158]. The Groningen and Poitiers groups in collaboration have tested an oxygenated perfusate to preserve kidneys during HMP, and this has been tested in animal models of DCD and in autotransplant models [159,160]. Membrane oxygenators within MP systems have been shown to promote ATP synthesis even following 30 min of warm ischemia [161]. Oxygenation of machine perfusates with 100% oxygen to achieve very high oxygen partial pressure has also been tested as an adjunct to MP, and shown to be able to recondition kidneys previously stored for 18 h by SCS [162]. On reperfusion, kidneys previously recirculated with oxygenated perfusate showed improved urine production and creatinine clearance. Oxygen could also be delivered to tissues via soluble oxygen carriers. The respiratory pigment Hemarina-M101, a large hemoglobin molecule derived from marine invertebrates, has been used to supplement standard preservation fluids in a static cold preservation animal study [163]. Hemarina-M101 improved metabolic activity of preserved cell lines, increased ATP levels, improved creatinine clearance, and reduced graft fibrosis following transplantation of preserved whole organs [163].

The next logical step would seem to be the combination of normothermic preservation with continuous or pulsatile perfusion to remove metabolites and maintain constant intravascular conditions. Synthetic fluids could possibly be combined with normothermic perfusion. Lifor, a solution incorporating a non-protein oxygen carrier, has been tested for short-term in-situ normothermic preservation [164] and in a porcine model of sub-normothermic renal perfusion [165]. Lifor demonstrated a better preservation of renal function [164] and reduced renal resistance compared to UW solution [165]. A hemoglobin-supplemented fluid allowed the resuscitation of kidneys despite 2 h of warm ischemia, providing life-sustaining function [166]. Another option would be to use blood itself and in an animal study, normothermic perfusion with heparinized whole blood been shown to preserve renal function during 6 h of perfusion, despite a prior 40 min of warm ischemia [167].

It is possible that a period of normothermia and oxygenation may be beneficial at the beginning or end of a cold preservation phase. Theoretically it would offer some degree of ischemic preconditioning at the beginning of the preservation period, or allow cytoprotective mechanisms to aid the recovery of the organ. Two hours of normothermic reperfusion with autologous blood after 16 h of SCS was shown to improve renal blood flow to the level of kidneys statically stored for only 2 h [168]. This technique was at least equivalent to HMP for 18 h [91,168]. Extracorporeal normothermic perfusion has recently been tested in human renal transplantation [169]. The first published case was that of an ECD declined by six UK centers before being accepted in Leicester. One kidney was perfused at normothermic temperatures with plasma-free, RBC-based solution for 35 min prior to transplantation, while the other kidney was stored by static cold preservation alone. Both kidneys had acute tubular necrosis (ATN) and rejection on protocol biopsy at 1 week; however, the recipient of the reperfused kidney had primary function, while the recipient of the static cold preserved kidney required dialysis for a further 4 weeks. The Leicester group has used this method of preimplantation normothermic recirculation in a case series of 16 transplants, with promising results so far [170].

One option in the prevention of IRI may be "immune camouflage," whereby selected polymers interact with allograft cell membranes to reduce immune cell invasion of the graft. Polyethylene glycol (PEG), a constituent of the IGL-1 solution, is one such polymer. Animal studies have suggested that plasma with PEG may have better static preservation effects than IGL-1 or UW solutions, with less immune cell infiltration and improved graft survival [171].

HMP of the liver as a resuscitation modality for previously untransplantable livers has now entered preclinical testing. Guarera et al. preserved 10 discarded human livers with pulsatile perfusion for up to 10 h to test the viability of the technique [114]. Alongside this study, the same group preserved porcine livers modeling DCD and completed three transplantations from livers preserved by HMP. All recipients showed initial graft function with reduction in serum enzymes [116]. Vekemans et al. also tested HMP for the rescue of discarded human livers in an experimental model of ischemia–reperfusion [172]. HMP was used for 4 h after a period of SCS, and was compared to SCS alone. During normothermic reperfusion with blood after the cold preservation, HMP was associated with reduced AST and lactate dehydrogenase (LDH) release, and reduced mitogen-activated protein kinase (MAPK) levels. Histological examination of liver biopsies from both preservation modalities was similar [172].

The potential for HMP to act as a resuscitation therapy for DCD livers may be further extended by the oxygenation of the perfusate during the perfusion period [hypothermic oxygenated perfusion (HOPE)]. This has been tested in animal models of DCD, with HOPE used at the end of a period of SCS to precondition the organs [115,173]. Preconditioning with oxygenated perfusate resulted in reduced cellular damage compared to SCS alone, with reduced necrosis and lipid peroxidation and increased cellular ATP recovery and bile flow. HOPE-treated livers also had improved graft survival, although experimental animals did not survive beyond 18 h whether HOPE was used or not [173]. The use of oxygenated machine perfusate has been further tested using a sanguineous fluid at both normothermic and hypothermic temperatures [115]. HOPE in this form also reversed the microscopic and macroscopic damage present in these livers, reducing AST release, increasing bile flow after reperfusion, reducing serum lactate, and improving recipient survival [115]. In this study, all experimental animals died within 24 h if the liver had suffered 120 min of warm ischemia [115].

Future developments in liver preservation will have to address the demands of the transplant waitlist by making previously untransplantable organs viable. It is speculated that the addition of oxygen to preservation modalities may make this possible through maintaining metabolism. Similarly to kidneys, various methods have been tested to deliver increased oxygen concentrations to livers ex vivo. Minor et al. have tested gaseous oxygen persufflation in a pig transplant model. Following 10 h of SCS, oxygen bubbles are passed into the vena cava for 2 h prior to implantation [174]. Livers receiving oxygen persufflation had greatly improved 1-week graft survival of 83% (in the control group it was 0%). Histological studies at autopsy showed preserved liver architecture in the study group. Serum liver enzymes and clotting parameters were also all within normal ranges. Post-conditioning with persufflated gaseous oxygen has also been tested in a small series of human livers that were otherwise rejected due to previous warm ischemic damage [175]. This resuscitation permitted the transplantation of five livers, and all patients were alive and well at 2 years' follow-up, without retransplantation. There was a significant increase in cellular ATP levels after oxygen persufflation. Persufflation has also shown positive results as a rescue therapy in experimental models following long periods of SCS (18 h), reducing serum liver enzymes, LDH, and tumor necrosis factor (TNF)-alpha, and increasing bile production [162]. The first RCT of resuscitative oxygen persufflation is currently recruiting [176]. When compared with oxygenated HMP, oxygen persufflation has shown slightly more promising results in terms of hepatic enzyme release and bile production in an animal model of DCD [177]. Further adaptations to HMP have included the use of a hyperbaric oxygen chamber to enclose the pump and increase the partial pressure of oxygen within the tissues. In a small experimental series, this technique has shown the benefit of increased cellular ATP and bile production and reduced ALT release after reperfusion [178]. The two-layer method, which was developed initially for pancreas preservation (see above) has also been tested in liver preservation. A continuously oxygenated perfluorocarbon layer lies underneath and in direct contact with the preservation fluid (typically HTK or UW solution). In liver experimental studies, this method improved cellular ATP levels but not cellular morphology [179].

Normothermic reperfusion

Normothermic machine perfusion (NMP) provides an intuitive and possibly better alternative to HMP, but has associated complexities and technological challenges. Provision of a physiological environment for continued metabolism allows assessment of function and recovery of the organ ex vivo. Schön and the Berlin group were the first to demonstrate, in an animal model, that 4 h of ex-vivo normothermic preservation of the liver was safe prior to transplantation [180]. Compared to SCS, this technique also seemed to have increased benefit when following a period of warm ischemia [180]. Friend and the Oxford group followed on from this work to show that porcine livers could be preserved for much longer periods of time (72 h) by NMP with oxygenation of the perfusate [181]. Further development of these experimental models allowed testing of liver synthetic function during a simulated reperfusion phase. This group then compared SCS in UW solution to NMP with oxygenation of the perfusate. NMP resulted in increased bile and glucose production, and galactose clearance, with reduced release of hepatic enzymes and histological damage [182]. The same group used a model of DCD liver preservation (60-min warm ischemia time) to show that NMP could recover function; in comparison, SCS showed no evidence of viability, with no bile production or substrate utilization [183]. In animal transplantation experiments mimicking both DBD and DCD, NMP has shown the potential to reduce hepatic enzyme release, increase patient survival, and improve hepatic histology. This benefit was consistent only if the preservation period was long (20 h) [184]. Currently, the Oxford group in close collaboration with King's College and Birmingham have finalised a first-in-man pilot study applying NMP and using a novel device in 20 liver transplants including both DBD, DCD and ECD. First results appear to be very promising (personal communication). The Barcelona group, in collaboration with the Groningen group, has performed experiments with NMP in animal models of uncontrolled DCD, with long warm ischemia time (90 min). In this study, NMP after a period of ECMO showed the potential to further improve upon the results obtained with ECMO followed by SCS [185]. Improvements were seen in function, and macroscopic and histological appearances of the livers [185]. Tolboom et al. used animal transplantation studies with long warm ischemia time (60 min) to show

that ischemically damaged livers preserved with NMP could be transplanted with excellent (92%) 4-week survival [186]. This is comparable to the survival in controls receiving healthy livers preserved by SCS or NMP [186]. Animals receiving ischemically damaged livers preserved by SCS alone all died within 12 h of transplantation. The same group moved on to examine sub-NMP, again following 60 min of warm ischemia time [187]. Machine perfusion at both 20 and 30 °C recovered ischemically damaged livers, and all animals survived beyond 1 month [187]. Steatotic livers are particularly susceptible to IRI, and given that they make up an increasing proportion of the donor pool, their preservation and resuscitation is of increasing importance. NMP has the potential to reduce the level of evident hepatic injury in these livers, which SCS does not [188].

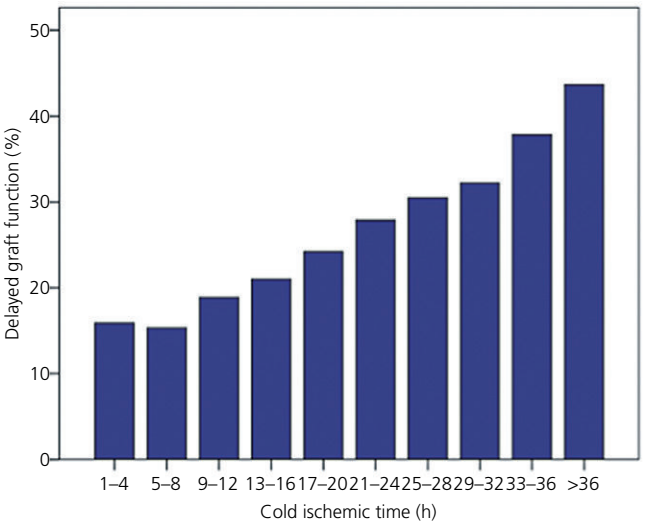
Hibernation strategies

During preservation, donor organs are cooled to 4 °C after procurement to prevent injury. However, deceased donor organs cannot be maintained at 4 °C indefinitely. In kidneys, the risk of DGF increases progressively as cold ischemia time increases [189]. For every 6-h increment of cold ischemia, the risk of DGF increases by 23% and prolonged cold ischemia time is negatively associated with graft survival (Figure 25.3).

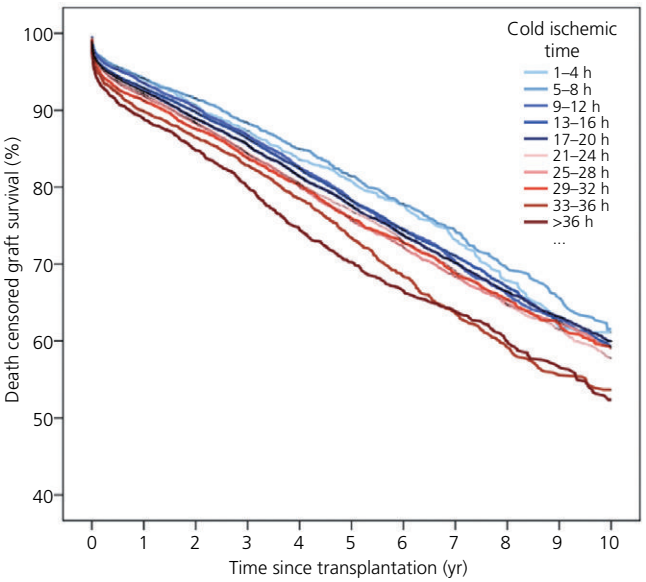
Tubular cell death during human kidney transplantation is apoptotic during cold preservation [32] . During warm ischemia–reperfusion at implantation, both apoptotic and necrotic cell death occur [18,190]. A number of investigators have suggested that hibernation represents a unique natural model of organ preservation and transplantation [191–193], where torpor can be viewed as a natural equivalent of donor kidney storage at 4 °C, whereas arousal is a natural equivalent of the warm reperfusion that occurs during implantation of a graft. Mammalian hibernators undergo extreme reductions in core body temperature (CBT) every winter. During torpor CBT can fall for several days from 38 °C to as low as −3 °C in arctic hibernators or 2–10 °C in temperate-zone hibernators [194] and hibernators undergo physiological changes, reducing their heart rate from a summertime level of 200–300 bpm to 3–5 bpm and their respiratory rate from 100–200 breaths/min to 4–6 breaths/min [194]. Interestingly, during hibernation animals are less susceptible to IRI, possibly related to a reduced activation of the immune system [195,196]. The recent discovery that hydrogen sulfide induces a torpor-like state, referred to as suspended animation in mice by Blackstone et al. [197], lead to the idea that hydrogen sulfide might be protective in IRI [198,199]. Application of non-toxic dosages of hydrogen sulfide gas results in physiological changes similar to those observed during natural hibernation [197,200]. In experimental models it was shown that hydrogen sulfide application results in a reduction of IRI in kidneys and livers [201]. Translation of hibernation strategies to the clinical situation is however complicated since experiments in pigs did not show clear protection [202,203].

Summary

After an initial innovative and active period in the 1960s when organ preservation methods became established, further developments in this important field for transplantation fell almost silent until the late 1980s. Centers had a clear clinical preference for cold storage of organs submerged in a preservation solution. Simple static cold storage appeared to be sufficient to allow recovery after preservation and adequate function using standard criteria donors.



(A)



(B)

Figure 25.3. Impact of cold ischemia time on (A) delayed graft failure and (B) graft survival. (Data from UNOS/OPTN.)

The current challenges due to the changes in donor type, including the use of older and high-risk donors, has now propelled perfusion and preservation into a new period of development and investigation of exciting novel strategies. The use of continuous HMP, normothermic reperfusion in situ, and ex-vivo resuscitation using miniaturized technology and incorporating biochemical strategies is proof of the fact that we are entering a new phase of development. The prior goal to bridge from donor to recipient with only minimal injury and loss of function has given way to a concept of organ improvement during storage and transport. This may be achievable as more sophisticated technologies to reduce injury and initiate repair become available. To unravel some of the clinically relevant questions in preservation recently the Consortium on Organ Preservation in Europe (COPE) was established and supported by the European Commission to focus on three clinical trials in liver and kidney preservation using novel technology and oxygenated

hypothermic or continuous normothermic perfusion. Preservation technology will continue to be crucial in reducing the organ shortage and minimizing organ discard, whilst allowing previously untransplantable organs to be transplanted.

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CHAPTER 26

Thoracic Organ Preservation and Resuscitation

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Introduction

Significant advances in lung preservation have been made since the first successful clinical lung transplant was reported in 1983. Lung preservation has evolved from needing adjacent operating rooms for both donor and recipient operations in that era, to cold static immersion for transport, to today's clinical standard: hypothermic flushed inflated preservation (hypothermic, aerobic, static preservation). However, the ideal preservation strategy for donor lungs during the obligate, yet unnatural, phase spent outside of a body remains to be identified. Historically, the central tenet to organ preservation was to "slow the death" of the organ until reimplantation into the recipient could occur. Today, the concept of "maintaining life" of the organ during preservation is emerging and novel technologies and strategies are being developed towards that end. This opens the door to move beyond just preservation of the organ into the era of "personalized medicine for the organ," where an opportunity for diagnosis and targeted treatment of donor lungs can occur. In this chapter, we will review current lung preservation strategies and discuss potential future approaches to lung preservation and resuscitation.

Though lung preservation is often considered the time spent outside of the donor body, preservation strategies in fact must be employed from the time a donor is identified until well into the post-transplant period. As such, this chapter naturally bridges content in Chapters 21, 22, 58, and 59, which deal specifically with organ procurement from deceased donors and donors after circulatory death, and the thoracic transplant procedures themselves, respectively. Prior to organ retrieval and the onset of ischemia, active donor management has helped increase organ recovery and has been shown to improve lung oxygenation from the time of initial brain death to the time of organ retrieval, resulting in improved lung and cardiac recovery rates [1–3].

Lung preservation

In the preretrieval phase

During the preretrieval phase, particular attention should be paid towards maintaining a euvolemic state. This helps avoid aggravating neurogenic pulmonary edema and the development of hypervolemic edema, particularly in the context of brain death physiology [4]. Therefore, potential donors should have central venous pressure (CVP) monitoring to maintain the CVP between 4 and 10 mmHg [5] and a pulmonary artery catheter should also be considered for wedge pressure measurements when left heart dysfunction is suspected. If needed, dopamine (<10 μg/kg/min) and vasopressin (<2.4 U/h) have been shown to be the vasopressors of first and second choice, respectively [6], as norepinephrine and epinephrine have been associated with lung dysfunction [7,8]. In addition, vasopressin infusion has the added benefit of improving hypotension not only due to its vasopressor action, but also due to its antidiuretic hormone action when diabetes insipidus is present [9–11]. It should be titrated to a systemic vascular resistance (SVR) of 800–1200 dyn·s/cm^5 when a pulmonary artery catheter is present [12].

Another principle of donor lung protection is to avoid barotrauma. Generally, protective lung ventilation strategies similar to the ARDSnet strategy [tidal volume, 6–8 mL/kg; positive end-expiratory pressure (PEEP), 5 cm H_2O; fractional inspired oxygen, <0.5] have been employed for ventilation of the donor [13]. However, a recent randomized controlled trial by Mascia et al. has demonstrated an increase in the number of lungs utilized from donors who were ventilated at a tidal volume of 6–8 mL/kg of predicted body weight and PEEP of 8–10 cmH_2O [14]. Lungs are also particularly susceptible to atelectasis and pneumonia. Frequent turning and suctioning for pulmonary toilet is important. Regular recruitment maneuvers should be performed to avoid atelectasis and bronchoscopy for the removal of mucous plugs and bronchoalveolar lavage (BAL) specimens should also be routine. Serial chest radiographs are obtained to monitor for the development of edema or any possible infiltrates [1,6,12].

A methylprednisolone bolus at 15 mg/kg has been shown to improve lung function and post-transplant outcomes even if given hours after brain death [3,15]. However, it is currently unclear whether this is a result of its anti-inflammatory effect or of steroid replacement in the setting of adrenocorticotropic hormone (ACTH) deficiency following brain death. The effect of other hormone replacement therapies, such as triiodothyronine and L-thyroxine have been explored in the literature, but no definite benefit has yet been shown in lung transplantation [3]. The use of beta$_2$-adrenergic agents has been shown to speed up alveolar fluid clearance in ex-vivo perfused human and rat lungs [16]. This finding formed the basis of the Beta-agonists for Oxygenation in Lung Donors (BOLD) study, a randomized, placebo-controlled clinical trial of nebulized albuterol versus placebo in 500 organ donors [17]. Interestingly,

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treatment with high dose inhaled albuterol did not lead to better donor oxygenation or increased lung utilization, and the routine use of beta$_2$-adrenergic agents cannot be recommended at this time [18].

Angel et al. have used retrospective data to show the impact of a standardized donor management protocol on the resuscitation of poor quality donors [2]. In the 4-year period following initiation of their protocol, of 254 donors initially classified as "poor," 135 were able to be reclassified as "extended" or "ideal" at the end of donor management. Ultimately, 21% of donors originally classified as "poor" were actually used for lung transplantation. In comparison, prior to the initiation of the standardized donor protocol, only 10% of lungs were used from donors originally classified as "poor." Gabbay et al. have also had success with improving the Pao_2/Fio_2 (P/F) ratio with a donor management strategy [1]. Of 140 consecutive transplants, 20 donors who originally would have been rejected were used successfully without impact on 30-day and 3-year survival. Others have also shown increased yield with aggressive donor management [3,19,20].

At retrieval

In the current paradigm of lung transplantation, the decision to utilize an organ for transplantation is made at the time of donor surgery. Once the decision to utilize a donor lung is made, the lungs are procured from the donor and the obligate ex-vivo phase begins. Most often, the recipient operation will begin in parallel with the decision to utilize the lung; thus, minimizing injury to the organ during the ex-vivo phase is important.

At the time of procurement, many strategies are employed in an attempt to better preserve the donor lung [21]. As in the preprocurement phase, a lung protective strategy similar to ARDSnet is utilized for ventilation to avoid injury from barotrauma. Full anticoagulation of the donor (300 U of heparin/kg) is given to minimize the risk of intravascular clot formation during the retrieval. Once the assessment of the donor is complete, a dose of 500 μg of prostaglandin E_1 (PGE_1) is given into the pulmonary artery to lower the pulmonary vascular resistance (PVR) by dilating the pulmonary vasculature. This facilitates the subsequent homogenous flushing of the pulmonary vasculature. PGE_1 has also been found to also downregulate proinflammatory cytokine expression, which may further help to reduce primary graft dysfunction (PGD) [22]. Response to PGE_1 can be recognized by a subsequent fall in the systemic blood pressure due to pulmonary vasodilation. With this fall, aortic crossclamp of the donor is commenced. The heart is cardiopleged and organ recovery can begin. The vasculature of the lung is flushed to cool the lung tissue and to remove blood from the pulmonary vasculature, further minimizing the potential for clot formation and allowing for the removal of inflammatory and immune cells. Early in the experience of lung transplantation, the use of an extracellular-type (i.e. low potassium) solution was found to be beneficial to lung preservation as opposed to the intracellular-type solutions used in other organs [23]. The intracellular type solutions led to significant arterial vasoconstriction, limiting the effectiveness of the flush. Dextran 40 was also found to be a key ingredient in the lung flush solution and serves two purposes [24]. First, it acts as an oncotic agent to help keep fluid within the intravascular space. Second, it has the ability to reduce the aggregation of erythrocytes, neutrophils, and thrombocytes. This can help preserve flow through the microvasculature after reperfusion, particularly in the bronchial microcirculation, and may play a role in reducing bronchial anastomotic complications. Another key ingredient in the flush solution is glucose. Because the lungs are stored inflated with oxygen, a unique situation arises during storage where the lungs are ischemic but not hypoxic. Glucose helps support aerobic metabolism in the lung during preservation. Multiple retrospective studies have demonstrated the superiority of low potassium dextran glucose (LPDG) solution to intracellular-type solutions [25–29].

The flush solution is first administered anterograde into the pulmonary artery, and then retrograde into each of the main pulmonary veins. This additional retrograde flush can help remove embolic clot from the pulmonary arteries and also helps to perfuse bronchial vessels via the bronchopulmonary shunt [30]. Furthermore, retrograde flush will possibly better flush areas of the lung affected by hypoxic pulmonary artery vasoconstriction [31]. Approximately 50–60 mL/kg of perfusate is utilized for the anterograde flush and 1 L for the retrograde flush. The desired flush pressure is a balance between too high a pressure leading to injury of the pulmonary vasculature and too low a pressure leading to inhomogeneous flushing. In practice, the flush solution is hung 30 cm above the patient and driven by gravity. Following the flush, the lungs are removed, inflated at an airway pressure of 20 cmH_2O with 50% oxygen, and stored on ice. Inflation of the lungs serves two purposes. First, it provides oxygen to the lung parenchyma for aerobic metabolism and second, it preserves the alveolar structure and surfactant during storage. Accordingly, van Raemdonck et al. have shown that inflation, even with nitrogen, is superior to atelectatic storage [32]. An airway pressure of 20 cmH_2O has been found to be ideal. In the case of donor lung transport by air, extra care should be taken to not overinflate the lungs as the low atmospheric pressure in flight, despite pressurized cabins, will result in gas expansion and may cause barotrauma to the lung during transport.

Once the lungs have been removed from the body, reduction of the metabolic rate by cooling of the lungs remains the cornerstone strategy for lung preservation today. Kayano et al. have shown in a rat model that the optimal temperature for lung preservation is approximately 10 °C [33,34]. However, to simplify transport logistics, 4 °C, the temperature of ice, is most commonly used. Once removed from the body, transplantation into the recipient is generally performed as soon as possible. PGD and 30-day mortality have been reported to increase with cold ischemic times longer than 8 h [35]. While lungs with 10–12 h cold ischemic times have been transplanted with success, these selected lungs have typically had fewer donor and recipient risk factors.

At Transplantation

Throughout the entire procurement process, warm ischemia is carefully avoided. However, during implantation, this is largely unavoidable. Date et al. showed that a significant increase in temperature occurs during implantation and that use of a cooling jacket to surround the lung during implantation can greatly reduce this warming [36]. Though Stammberger et al. showed that 2 h of warm ischemia made no difference to post-transplant outcomes in a pig model of lung transplantation, these lungs were not injured at baseline and the follow-up time was short [37]. Use of a cooling jacket during implantation is largely unobstructive and has potential benefit; therefore, we routinely utilize a cooling jacket during implantation.

Reperfusion is a crucial part of lung transplantation where additional injury could occur and thus should be performed with particular care. Gradual reinstitution of pulmonary arterial blood flow over a period of 10 min by the slow release of the pulmonary artery

clamp after implantation has been shown to improve post-transplant outcomes [38]. Some have advocated the use of a special perfusion solution to perfuse the lung just prior to release of the pulmonary artery clamp; however, this is not standard practice in most centers [39]. Once reperfusion is commenced, ventilation of the new lung is performed with a low fraction of inspired oxygen (usually FiO_2 21%) to avoid oxygen-related lung injury. For patients transplanted on cardiopulmonary bypass, it is important to leave some pulmonary artery ejection/pulsatility (PAP 10–15 mmHg) to reperfuse the already transplanted lung during implantation of the second lung. An extension of this strategy of gradual, protected reperfusion has been described by the Vienna group: Aigner et al described the use of extracorporeal membrane oxygenation (ECMO) support rather than cardiopulmonary bypass during lung transplantation for all patients experiencing hemodynamic or respiratory instability during transplantation [40]. Of 130 patients who underwent intraoperative ECMO support, 51 patients had their ECMO extended into the perioperative period, allowing for a very gradual increase of pulmonary artery flow over hours or days rather than minutes.

Post-transplantation

Following reperfusion, we routinely utilize heparin (100 U/h) and Rheomacrodex (10% dextran 40, 500 mL/24 h) as an intravenous infusion for 7 days to improve the pulmonary and bronchial microcirculation [41,42].

In donation after circulatory death

The use of controlled donation after circulatory death (DCD) lungs (Maastricht category III or IV) is now well described in the literature, with equivalent or near-equivalent outcomes to conventional donor lungs in international case series and demonstrating promise to increase lung transplant activity [43–48]. However, the use of DCD lungs creates new challenges in lung preservation due to the additional warm ischemic time accrued between cardiac arrest and cold flush. Fortunately, lungs have been shown to tolerate a relatively long period of warm ischemia and this is likely due to the low metabolic demand of the lung combined with the presence of oxygen in the lungs [49,50]. Indeed, Miyoshi et al. recently demonstrated the importance of oxygen in the alveoli in a porcine model [51]. In their study, hypoventilation rather than circulatory arrest contributed most to poor post-transplant outcomes. Clinically, a warm ischemic time of less than 1 h has been considered to be acceptable for transplantation [49,52–55]. In general, this 1-h timeframe is adequate for the retrieval of donor organs in controlled DCD. However, the Lund group has reintroduced the concept of topical in-situ cooling in an attempt to better preserve DCD organs [56]. Two chest tubes are placed and then cold Perfadex® is used to cool the lungs to 18 °C. In animal models and case reports, topical cooling has been shown to safely prolong DCD lung preservation in situ for up to an additional 6 h after 1 h of warm ischemia time. However, the practical need for such a strategy is probably best suited to uncontrolled DCD lungs (Maastricht category I or II).

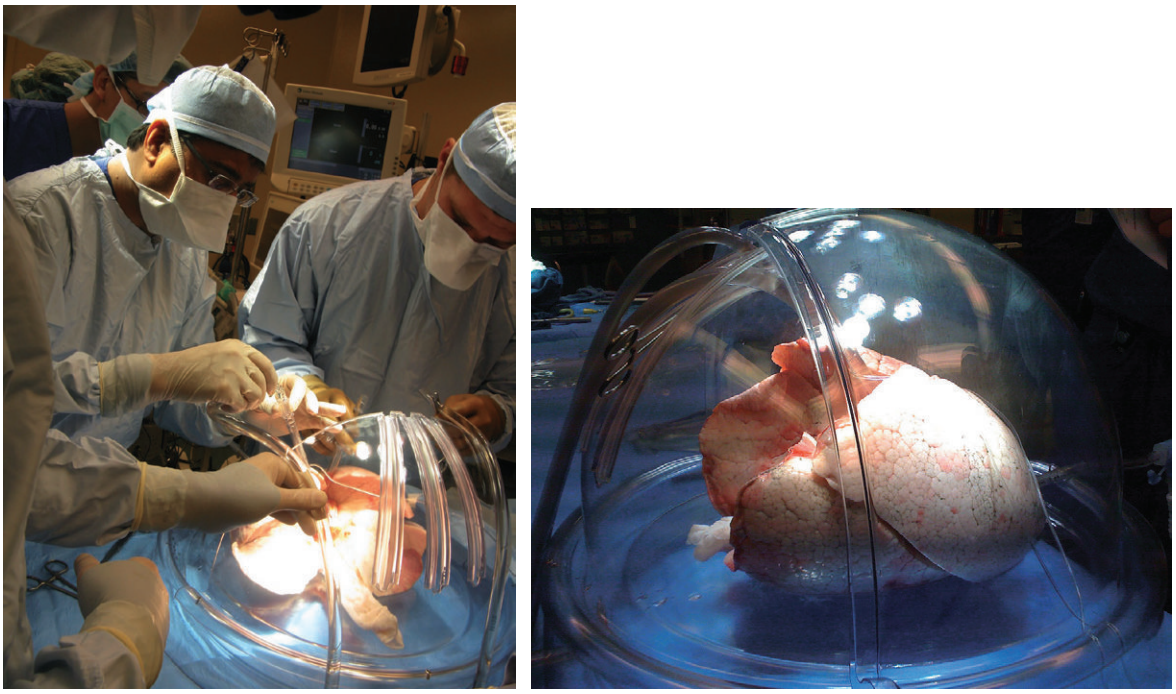
The use of full heparinization prior to circulatory arrest and cold flush in the brain dead donor is well established. However, the use of heparin in DCD may not always be possible due to potential ethical issues, as it is an intervention aimed solely at organ preservation and is given prior to donor death. To circumvent this, post-arrest heparinization given by injection into the pulmonary artery and followed by a few beats of cardiac massage to help distribution is included in most DCD retrieval protocols. Okazaki et al. have shown in a canine lung transplant model that post-mortem heparinization can be beneficial, supporting this approach [57]. Clinical data are also now emerging demonstrating that no difference in outcomes are seen in donors who received preretrieval heparinization versus those who did not [58]. Retrograde flush, however, remains a particularly important strategy to remove embolized or in-situ clot in the pulmonary artery in DCD lungs.

Normothermic lung preservation

While the current cornerstone of clinical lung preservation is to limit the metabolic rate by hypothermia, this strategy best serves lungs meeting ideal acceptance criteria. With the current donor organ shortage, most programs now utilize increasing numbers of extended criteria organs where lung function is not as assured as in ideal lungs. Ideally, further evaluation and even resuscitation of the lungs would be possible during the ex-vivo phase of the organ before transplantation into the recipient. As limitation of the metabolic rate by hypothermic preservation precludes the possibility of meaningful lung evaluation and recovery, preservation of donor organs would need to occur at normothermic or near-normothermic conditions to achieve these goals. One such strategy has been that of ex-vivo lung perfusion (EVLP; Figure 26.1). This strategy attempts to simulate the in-vivo situation by ventilation and perfusion of the donor lung graft. Originally proposed as early as 1938 by Carrel for organs in general and then in 1970 by Jirsch et al. for the evaluation and preservation of lungs in cases of distant procurement, attempts in those eras failed due to an inability to maintain the air–fluid barrier within the lung, leading to the development of edema and increased PVR in the donor lung during EVLP [59,60].

Driven by the objective of better evaluation of DCD lungs, Steen et al. in Lund, Sweden developed a modern ex-vivo perfusion system with the intent of short-term evaluation of lung function of this population of lungs ex vivo [61]. In doing so, they developed a buffered, extracellular solution with an optimal colloid osmotic pressure to act as the lung perfusate (Steen Solution™, Vitrolife). This solution helps hold fluid within the intravascular space during perfusion and provides nutrients needed to maintain lung viability. The composition of Steen solution is quite similar to the current clinically utilized preservation solution of LPDG (Perfadex®, Vitrolife), with human albumin as the major additional constituent. This protein is meant to maintain a higher oncotic pressure to reduce the development of pulmonary edema during perfusion. Steen et al. utilized this solution mixed with red blood cells in combination with their circuit and were able to successfully perfuse and evaluate lungs in a large animal model for 1 h without the development of pulmonary edema; subsequent transplantation was successful [61]. Following their work in large animals, Steen's group was the first in 2007 to publish a case report of successful transplantation of a non-acceptable lung following a brief period of EVLP [62]. Subsequently, Steen's group has published a case series of six cases using short-term perfusion to evaluate rejected donor lungs [63].

The ultimate goal of Steen's studies was to utilize EVLP as a method for lung evaluation and thus the perfusion times have been short. For the application of EVLP for preservation, improved evaluation, and the goals of lung recovery and repair, much more time is required. Erasmus et al. first attempted to extend the EVLP duration to 6 h; however, circuit-induced injury again became problematic with increased PVR and airway pressures in the lung near the end of 6 h [64]. We first described successful long-term (12 h)



(A) (B)

Figure 26.1. Ex-vivo lung perfusion (EVLP). (A) A lung preparation being placed on an EVLP device. The capacity to actively manipulate the organ, including to perform bronchoscopic lavage, is a significant advantage of EVLP over cold storage. (B) Human lungs undergoing EVLP resuscitation. Ventilation and perfusion can both be maintained during storage.

EVLP using a lung protective strategy for acellular normothermic perfusion and ventilation [65].

To attain stable 12-h perfusion, several key lung protective strategies were employed [65]. First, an acellular perfusate was utilized. We hypothesized that oxygen delivery to the lung could occur via the ventilator rather than via the vasculature. This concept is also supported by Egan's group, where mere ventilation of a donor lung with room air at normothermia was demonstrated to preserve cell viability for 24 h [66,67]. In addition, acellular perfusion is logistically simpler for clinical use and also avoids the problem of limited lifespan of red blood cells within the harsh environment of the perfusion circuit. Second, rather than subject the lungs to perfusion at 100% of cardiac output, maximal flow was limited to 40%. This lower flow aids in the reduction of hydrostatic edema caused by perfusion and, despite lower flows to non-dependent areas of the lung, histology and post-transplant function in EVLP lungs were shown to be normal. Third, we found that maintenance of a positive left atrial pressure of 3–5 mmHg to be important for the success of long-term perfusion. This small, but positive left atrial pressure tents open the capillaries and post-capillary venules and prevents collapse of the microvessels from occurring during increases in airway pressures and decreases of flow at inspiration [68]. Absence of positive left atrial pressures can lead to unstable alveolar geometry and results in decreased lung compliance [69]. Finally, we noted the importance of using a centrifugal pump. With ventilation, distension of the alveoli will place pressure upon the perialveolar vessels, leading to cyclical increases in PVR with every breath. As a consequence of how a centrifugal pump functions, increased afterload to the pump will result in decreased rotation and flow. Thus, the pump will back off during times of increased resistance rather than forcing fluid through, potentially causing injury or edema as a roller pump might do. During perfusion, oxygen is removed and carbon dioxide is supplied via a membrane oxygenator as a simulation of cellular metabolism. Removal of oxygen allows for the measure of lung function by taking the difference between post-lung and pre-lung perfusate Po_2, and addition of carbon dioxide helps maintain the stability of the pH of the perfusate. Using this strategy, reproducible, safe 12-h normothermic ex-vivo perfusion has been demonstrated in porcine and human lungs, and this strategy of EVLP has been shown to interrupt ischemic damage caused by prolonged cold ischemia [65,70,71].

Ex-vivo lung evaluation

Current lung evaluation is a clinical process greatly dependent on the judgment of the surgeon. While some evaluation does occur prior to retrieval, i.e. chest X-rays and intensive care unit (ICU) bronchoscopy, the majority of the evaluation leading to the decision about utilization occurs at a single time point: the time of organ retrieval. Lungs that may be injured but have not yet had time to express that injury in the form of edema and lower P/F ratios may still be utilized, inadvertently. Furthermore, donor physiology during retrieval may not be entirely conducive to accurate lung evaluation as blood pressure is often labile and under-recruitment of the lung parenchyma may give falsely low Pao_2. Injury, as represented by the development of edema during EVLP, is reflected in changes in compliance and airway pressure, which precede the effect on perfusate Po_2 [72]. Thus, during EVLP, these parameters should be monitored carefully and EVLP allowed to proceed over a period of at least a few hours to allow for trends in compliance and airway pressure to be detected. To reduce the effect of atelectasis that might occur during donor lung transport, the baseline time

point should be 1 h after warming the perfusate and after careful recruitment of the lung. All subsequent physiological measurements (compliance, airway pressures, PVR, and perfusate Po_2) can be compared to this time point. A cut-off, or normal value, is often sought for lung evaluation, but compliance and resultant airway pressure are based in part on lung volume and thus can fall within a large range of values. With this strategy, the trend of values becomes more important than the absolute values themselves, e.g. a lung that has a compliance of 80 mL/cmH_2O at the start but 40 mL/cm H_2O after 4 h is more concerning than a lung that starts and ends with a compliance of 40 mL/cm H_2O. When an acellular perfusate is utilized, its effect on Po_2 interpretation also needs to be understood. Because the Po_2–oxygen content curve is linear in the situation of ex-vivo acellular perfusion, mixtures of perfusates of different Po_2, such as in a shunt situation, results in higher measured total Po_2 [72].

Using our EVLP strategy described by Cypel et al. and the evaluation strategy described above, a clinical trial was performed by the Toronto Lung Transplant program using EVLP for the assessment of high-risk lungs that otherwise would not be used [73]. Lungs that originally did not meet acceptance criteria but during EVLP evaluation demonstrated stable or improved PVR, dynamic compliance, and/or peak inspiratory pressure and Po_2/fraction of inspired oxygen >350 mmHg were transplanted and resulted in post-transplant outcomes equivalent to those of contemporary controls.

Commercially available platforms for EVLP are now in various stages of development. Among them are the XPS system by XVIVO Perfusion, the LS1 by Vivoline Medical, and the OCS Lung by Transmedics. Of the three, the XPS system is the most faithful replication of the Toronto strategy and differs only in the addition of various in-line monitors to streamline organ assessment. The LS1 system resembles most closely Steen et al's system for lung evaluation and differs from the Toronto strategy in the use of a roller pump, an open atrium, and a blood-based cellular perfusate. A clinical trial is currently underway in the UK using this device [74]. The OCS Lung device is marketed as a mobile lung preservation system. It provides an additional strategy for lung preservation that Transmedics has termed the "preservation mode," where the perfusate is swept with an oxygenated gas (12% O_2, 5.5% CO_2, 82.5% N_2) and a captive breath is recirculated. This minimizes the amount of gas volume needed and therefore simplifies transport of the system, but the lung is not actively functioning for assessment. The remaining "continuous monitoring" and "sequential monitoring" modes are the more conventional perfusion strategies, where the lung is ventilated with room air and a venous perfusate provided to the pulmonary artery to allow for assessment. Whereas the continuous monitoring mode operates similar to the Toronto and Lund strategies, where the lung oxygenates a continuously deoxygenated perfusate, the "sequential monitoring" mode measures the time needed to oxygenate a preset pool of perfusate at a venous saturation of 73% and arterial saturation of 93% in an automated manner without further deoxygenation. This system also differs from the Toronto strategy in the use of an open atrium, a roller pump, bellows instead of a ventilator, and a blood-based cellular perfusate. Results following OCS lung preservation have been limited to case reports, but this is now the subject of the international INSPIRE trial [75,76]. The predication that cold ischemia is detrimental to the donor lung underlies the development of mobile normothermic perfusion solutions such as the OCS Lung system. However, it remains to be proven whether limited cold ischemia is truly detrimental, particularly when 30 years of clinical experience with short cold ischemia as the sole strategy of lung preservation has shown good outcomes. Even if normothermic preservation is utilized from retrieval to implantation, the need for some cold ischemia is inevitable; cold flush and topical cooling at the time of donor cross-clamp and preimplantation cooling are utilized to protect the lung and minimize warm ischemia during the actual surgical implantation. Compelling experimental and clinical data demonstrating that continuous mobile normothermic perfusion is superior to a combination of short intervals of cold ischemic preservation and normothermic evaluation and treatment will be needed to justify the conversion to this strategy, considering the logistical challenges and the added economic expense needed for mobile normothermic perfusion of lungs, whether for standard or "extended" criteria organs.

Rather than aiming solely at minimizing cold ischemia, normothermic preservation instead demonstrates great promise for resuscitating injured donor lungs. Given that the majority of potential donor lungs are injured by a variety of mechanisms, including brain death, contusion, aspiration, infection, edema, and atelectasis, one could imagine that targeted therapies for each of these injuries could be delivered ex vivo for repair and greatly increase the donor lung pool. The requirements for perfusion for repair compared to perfusion solely for evaluation differ by time. While the majority of lungs can be evaluated within 2–4 h of perfusion, repair will require longer non-injurious stable perfusion while potential treatments are administered. The HELP study by the Toronto Lung Transplant Program perfused 23 initially rejected donor lungs and was able to recover 20 of them for transplantation with equivalent short- and medium-term outcomes. This experience has now been extended to more than 50 clinical lung transplants after EVLP with improved outcomes; the experience continues to grow [77].

Other early studies in the use of EVLP for lung repair have been reported, many still only in abstract form. Each of these studies was targeted at a different form of donor lung injury and it is this breadth of exploration that will ultimately result in an arsenal of ex-vivo lung therapy techniques applicable to each uniquely injured donor lung. Pulmonary edema is a common injury in donor lungs due to brain death physiology and/or ICU fluid management prior to retrieval. Unlike the in-vivo situation, use of terbutiline was found to accelerate the clearance of alveolar fluid during perfusion [78]. Another common mechanism of injury is aspiration. Inci et al. have attempted to improve porcine lungs injured by acid aspiration [79]. By lavaging the donor lung with surfactant during EVLP, they were able to achieve improved graft function when compared with controls. Due to the significant number of lungs rejected for suspicion of infection or pneumonia, delivery of high doses of antibiotics during EVLP is attractive. Both the Newcastle and Toronto groups have early data showing potential reduction in the burden of infection following EVLP antimicrobial therapy [80,81].

EVLP-based gene and cellular therapy have also been explored. We have shown that ex-vivo gene therapy with an adenoviral vector is effective and additionally attractive because of reduced vector-associated inflammation. Furthermore, this strategy can easily fit into the logistical flow of clinical lung transplantation, simplifying adoption [82]. We have demonstrated that EVLP-based interleukin (IL)-10 gene therapy of rejected human donor lungs resulted in improved function and reduced biomarkers of inflammation, suggesting that IL-10 gene therapy could possibly increase the resilience of all donor lungs to reperfusion [71]. Lee et al. have shown that the delivery of mesenchymal stem cells to EVLP lungs can

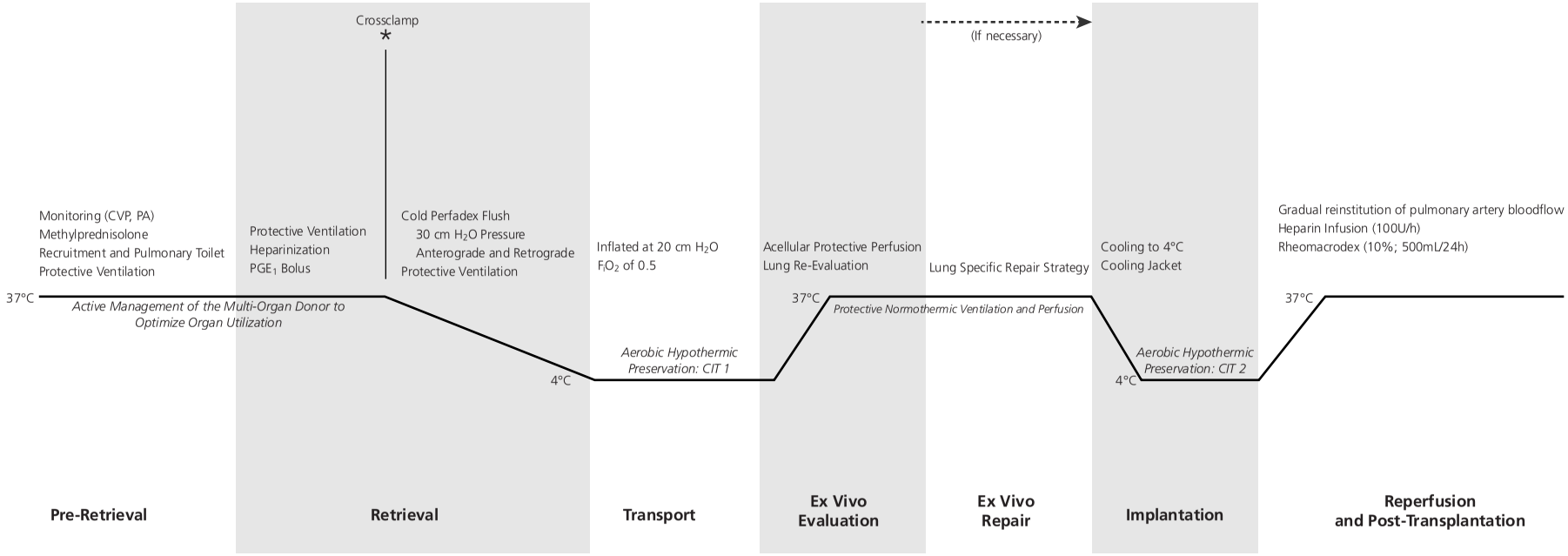


Figure 26.2. Trajectory of contemporary lung preservation techniques. Lung preservation begins in vivo in the multiorgan donor and continues through the ex-vivo phase of aerobic hypothermia and possible aerobic normothermia, finishing with reperfusion in the recipient. At each step there is potential for lung injury and opportunity to prevent or repair the injury. Temperature variation is used as a tool to provide hypothermic static preservation and normothermia for active therapy as needed. CVP, central venous pressure; PA, pulmonary artery.

restore endothelial barrier permeability and alveolar fluid balance after endotoxin-induced lung injury [83].

Heart preservation solutions

A major factor limiting the size of the donor pool is the duration of tolerable ischemic time. The most common method of heart preservation currently is antegrade perfusion with potassium-containing crystalloid solution at 4°C. The organ is then transported on ice and the graft reperfused within 4 h. Prolonging the ischemic time has been a focus of intense investigation. New methods to circumvent ischemic injury include variations in preservation solution and use of warm, heart beating strategies is being tested currently as described next.

Initial experience with cardiac allograft preservation involved use of solutions originally developed for use in abdominal organ transplantation, such UW solution and Euro-Collins solution. The electrolyte composition in these solutions is similar to intracellular fluid with low sodium and high potassium concentrations. Additionally, these solutions contain a number of impermeable molecules that minimize cellular edema. It became evident to heart surgeons that the preservation solution used for cardiac allografts must provide not only myocardial but also endothelial protection. A high potassium concentration in the preservation solution delivered to a warm graft can damage endothelium and predispose to chronic allograft vasculopathy [84–87]. To address this issue, newer solutions such as Histidine-Tryptophan-Ketoglutarate (HTK; Custodiol® or Bretschneider) and Celsior were developed. These solutions were based more on the extracellular milieu with lower potassium concentrations and additional substrates for energy production. Alternatively, some groups advocate the use of blood-based cardioplegic solutions. This requires a significant volume of blood to be withdrawn from the donor, which is not always tolerated. Nevertheless, published results suggest that this strategy provides good endothelial preservation and does not prevent the successful procurement of other organs [88]. Several comparisons have evaluated the merits of the various solutions and the weight of evidence demonstrates relatively equivalent and effective myocardial protection. Many novel preservation solution additives have been investigated, including antifreeze proteins to allow subzero preservation [89], endothelin antagonists to enhance endothelial preservation [90], antioxidants to blunt the deleterious effects of ischemia and reperfusion [91], and agents to maximize vascular smooth muscle relaxation [92], post-ischemic myocardial contractility [93], and preservation of mitochondrial energy stores [93]. While the majority of these modifications are not clinically available, it is likely that enhanced preservation solutions will one day improve graft function or prolong tolerable ischemic time.

Normothermic heart perfusion

Use of continuous oxygenated perfusion may improve graft preservation and ventricular function [93]. It allows for ongoing aerobic metabolism and washout of products of ischemia such as lactate and adenosine [94], thereby producing less substrate for free radical generation [95,96]. Also, since the myocardium tolerates higher temperatures better than lower, normothermic preservation should prevent thermal damage [97]. A preservation technique incorporating these two strategies has demonstrated that myocardial recovery and function after warm, machine perfusion are superior to cold static preservation at 4 h [98]. A practical way to transport a donor heart warm and beating using machine perfusion is being evaluated in the US in the Proceed II trial [99].

Summary

Ever since the development of clinical heart and lung transplantation, transplant clinicians and scientists have sought to reduce injury and maximize safe preservation time during the storage and transport of donor organs. Key advancements in lung preservation in the form of hypothermia, inflated storage, and Perfadex® flush have culminated in the maturation of lung transplantation into a standard of care for end-stage lung disease around the world. The state of cardiac transplantation remains considerably more limited by time. Today, the emphasis in lung preservation is shifting from slowing down organ death to that of facilitating organ recovery and regeneration prior to implantation. This has led to the emergence of normothermic EVLP as a strategy for lung preservation. Though EVLP is effective for lung preservation alone, its true potential lies in facilitating lung recovery and repair. Therefore, the thoughtful manipulation of preservation temperature in the context of the lung transplant process is important; i.e. protective hypothermia for retrieval and implantation and normothermia for recovery and repair (Figure 26.2). The development of ex-vivo lung repair strategies for the broad spectrum of donor lung injury is a burgeoning and important area for research. Development of an ex-vivo treatment arsenal ranging in complexity from pharmacological to gene and cellular therapies will hopefully one day allow for a "personalized medicine approach to the donor organ" – the "personalized" or targeted repair of donor lung injuries specific to each individual lung, finally allowing clinicians to utilize the full potential of the donor organ pool.

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CHAPTER 27

Kidney Paired Donation Networks

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Introduction

The waitlist for kidney transplantation continues to grow at a rapid rate across the world. In the (US, the wailist has doubled in the past 10 years with no significant increase in deceased donor organ availability [1]. Approximately 4500 Americans die on the kidney transplant waitlist each year [1]. Thus, the advantage of living donor kidney transplantation has increased over the years, offering patients the chance of receiving a kidney without waiting years on the list. Indeed, a living donor may be the only route to transplant for some older patients who can do well once transplanted but who cannot wait years for a deceased donor organ. Other advantages to living donor transplant include the chance for better immunologic compatibility, and improved early and long-term renal allograft function compared to deceased donor transplantation [2,3]. Furthermore, the transplant operation becomes elective surgery, allowing for optimal patient preparation and the opportunity to perform pre-emptive transplantation prior to the initiation of dialysis.

A significant barrier for many patients to receiving a living donor kidney transplant is that they must be immunologically compatible with their donors. Compatibility testing consists of blood type (ABO) as well as human leukocyte antigen (HLA) testing. It is estimated that there are at least 6000 patients on the kidney transplant waitlist with willing healthy donors who are not compatible [4]. The concept of kidney paired donation (KPD), in which incompatible pairs exchange donors to create compatible combinations, was first proposed in 1986 by Rapaport as a means to achieve compatible living donor transplantation for otherwise incompatible pairs [5]. The concept exchanges two incompatible donor pairs to create two compatible combinations (Figure 27.1a) A three-way exchange (Figure 27.1b) is more complex but allows more possible permutations of matching [6]. More recently, non-directed donors have been utilized to begin "chain transplants" (Figure 27.1c), resulting in large numbers of transplants from a single non-directed donor who is willing to donate a kidney to any recipient in need. While the concept of KPD to expand living donor transplant options had been well received, significant ethical, legal, immunologic, and logistical barriers needed to be overcome for this concept to become a reality. This chapter will cover these issues and describe how they are being successfully dealt with in modern paired exchange programs. The chapter should be considered along with Chapters 28 and 37 that cover the general considerations in accepting a patient for kidney transplantation and the management of patients awaiting kidney transplantation in general, respectively.

Which recipients are most likely to benefit from kidney paired donation?

Segev et al. published computer simulations of a cohort of incompatible donor–recipient pairs in a KPD program to predict matching rates for different subgroups of patients. These data have been an important predictive foundation for which pairs may be most likely to benefit from KPD and which factors predict such a benefit. They found that for recipient candidates with a panel reactive antibody (PRA) of <80%, the likelihood of a match is approximately 50% for most blood types and that there is only a small increase in benefit with a larger cohort size of incompatible pairs. As expected, moderately sensitized candidates with non-O blood types or with blood type O donors are expected to have short waiting times for a match. Not surprisingly, very few pairs with blood type AB donors are ever likely to be matched. In the group with a PRA of >80%, the likelihood of a match is significantly impacted by the cohort size of the incompatible database for almost all blood types. As expected, highly sensitized candidates who are blood group AB or with blood type O donors are more likely to be matched.

The simulations demonstrate that there are two major barriers to more matches. First, a significant imbalance of blood groups develops in KPD programs; the recipient pool becomes enriched with O blood type and the donor pool becomes enriched with non-O blood types. Of course, blood type O donors will usually be attached to highly sensitized recipients if they do not match quickly in a large cohort of incompatible pairs. Second, highly sensitized recipients in the cohort are in competition to find rare donor HLA types who may provide a match. For example, donors who are homozygous at multiple HLA loci will be more likely to match with sensitized patients, but such HLA types are uncommon.

Kidney paired donation matching algorithms

The matching software system should be optimally designed to predict matches with at least one-, two-, and three-way matching algorithms. Statistical modeling has shown that three-way exchanges have the opportunity to make the best use of blood type O donors and help more highly sensitized recipients compared with two–way exchanges [6]. Four-way or greater closed loop exchanges add significant complexity to the logistics of exchanges and add less benefit, and thus are not routinely performed. Utilization of non-directed donors to initiate a non-simultaneous, extended, altruistic

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

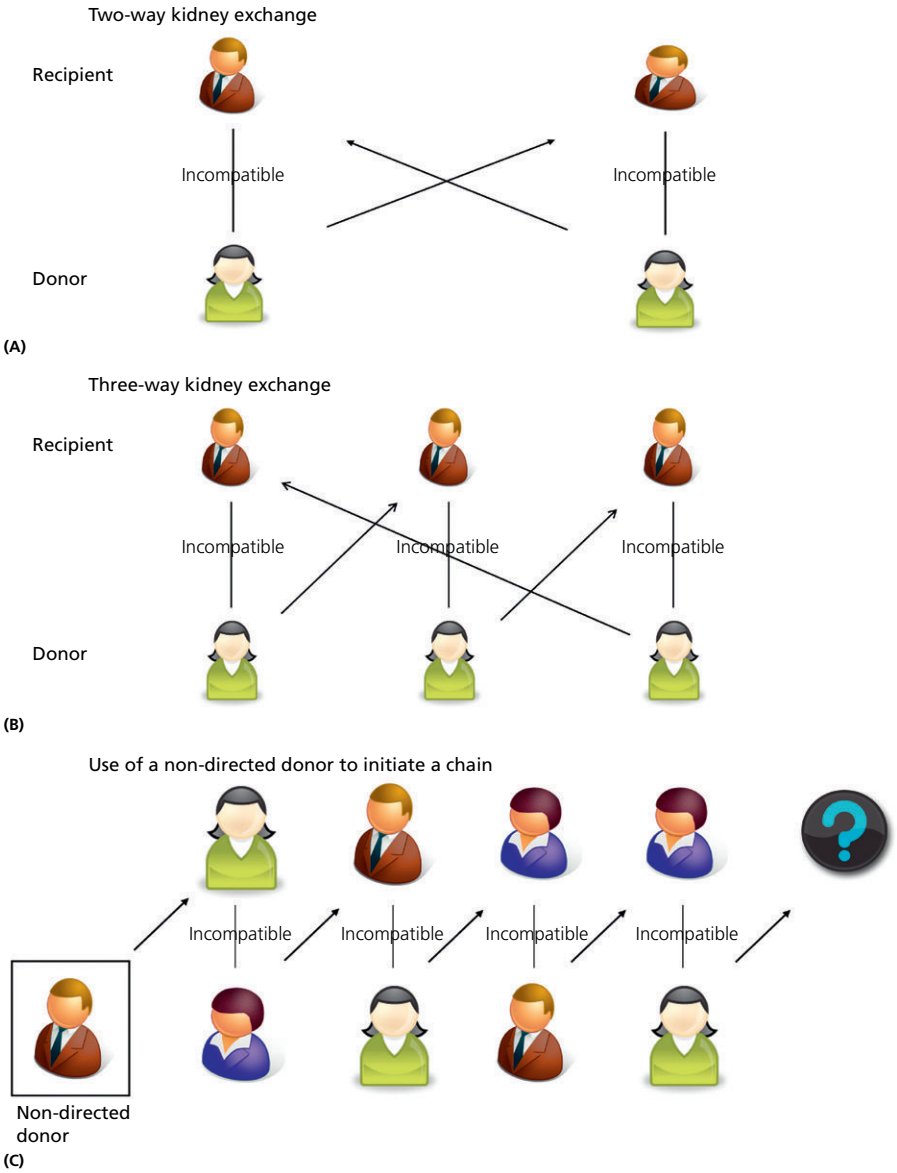

Figure 27.1. Common types of kidney paired donation transplants. (A) A closed loop two-way exchange between incompatible pairs. (B) A three-way closed loop exchange between incompatible pairs. (C) A one-way "chain" exchange started with a non-directed donor and which can terminate in either donation to the deceased donor waitlist or kept as a "bridge donor" to initiate another chain.

donor (NEAD) chain has benefitted large numbers of patients. Rees et al. reported the first NEAD chain in March 2009 in which a single non-directed donor facilitated 10 transplants involving six transplant centers in five different states over a period of 8 months [7]. Subsequent larger chains involving other regional and single-center programs have been reported, including a chain with a total of 46 individuals (23 donors and 23 recipients) at a single center [8,9]. There are two possible ways to end a chain: (1) the last living donor donates directly to the deceased donor waitlist thus ending the chain; and (2) a "bridge donor" is held and thus another chain is started at a later date. It has not been definitively established which method generates the greatest number of transplants. Computer models generated by Gentry et al. show that donating directly to the list generates more transplants over time if the chain length is held to three pairs within a concurrent segment [10]. However, simulations generated by Ashlagi et al. utilizing unrestricted chain lengths demonstrated that using "bridge donors" generates more transplants despite the risk of a donor reneging [11,12]. Clearly, more data and experience with both of these modalities is necessary to determine which chain termination mechanism generates the greatest number of transplants over time.

The kidney paired donation database, software matching program, and use of solid phase antibody testing

A successful KPD program depends upon a large number of incompatible pairs and a sophisticated software program to predict matching. All recipient and donor candidates should be consented for inclusion into a KPD database to maximize the pool size. The critical pool size to generate substantial numbers of matches is not known, but has been estimated to be at least 100 pairs [13]. Early

education about immune compatibility and the option to enroll both donor and recipient candidates in a KPD program are important aspects of KPD programs to maximize enrollment. Inclusion of multiple donors for a single recipient may be encouraged in an effort to enroll more blood type O donors or donors with more favorable HLA types into the program. Many programs, especially regional and national programs, mandate complete evaluation of all donors prior to enrollment into the KPD program, which limits the number of donors who may otherwise participate if not for the cost and time involved in each evaluation process.

The essential elements that are necessary to enter into any KPD system include donor and recipient demographic information, such as age and gender, as well as blood type and histocompatibility information. Both donor and recipient HLA types should be entered to allow for the identification of mismatching between pairs; these data are utilized by many (but not all) KPD programs. Indeed, the US program gives considerable points in the national algorithm to zero mismatch pairs. The majority of candidates in large KPD databases are sensitized and thus difficult to match with a compatible donor. Therefore, the most critical information entered into the KPD database is the assigned unacceptable antigens based upon very sensitive HLA antibody identification assays. These assays are the basis for predicting matched pairs for sensitized candidates and as such have had a profound impact on the development of KPD. Thus, to understand KPD, one must have an understanding of HLA antibody detection techniques (comprehensively reviewed in Chapters 36 and 89).

Role of HLA antibody identification

Advances in immunogenetics such as DNA-based HLA testing, solid phase antibody characterization, and flow cytometry cross-matching have refined the ability to identify acceptable donor–recipient profiles [14,15]. The most widely used technique to determine anti-HLA antibodies in the US uses a solid phase assay in which fluorescent microbeads are coated with individual HLA molecules. Fluorescence detection is then used as a marker for antibody detection. Identification of anti-HLA antibodies in recipient candidates is used in a "virtual cross-match" as the basis to predict actual cross-match results with a given donor [16,17]. In KPD, all sensitized candidates must have these antibodies to "unacceptable antigens" assigned in the database; matching software can then preclude a match with any donor with the "unacceptable antigens." Careful assignment of unacceptable antigens allows for accurate prediction of cross-match results. By contrast, over-assignment of unacceptable antigens, especially in highly sensitized candidates, will limit their transplant options and may prevent a successful transplant combination [18]. Ideally, assignment of unacceptable antigens in a KPD database should be sufficiently stringent to prevent a positive cross-match that would preclude transplantation (and thus interfere with a planned KPD transplant), but not so stringent as to prevent a transplant combination that may be acceptable [19,20]. Indeed, the goal of anti-HLA antibody identification is to lead to *more* successful transplants, and not to prevent transplantation of candidates with acceptable levels of donor-specific antibodies or antibodies that may not be clinically relevant. This is an area in which significant work is left to be done; this work will have a profound impact on the transplantation of highly sensitized patients by KPD. This concept of "unacceptable antigens" is used in the US; however, the European Transplant Program utilizes the reverse system and assigns "acceptable antigens" [21]. While the approach differs in Europe, the concept of utilizing a virtual cross-match to predict actual cross-match results is the same.

Global kidney paired donation experience

KPD has had a global impact on living donor transplantation. Many transplant centers worldwide have adopted this strategy to increase access to transplantation in single-center, regional, or national programs.

The United States experience

In the US, though firm foundation had been laid for lawful living donor transplantation for many decades, a clause in the National Organ Transplant Act (NOTA) of 1984 caused concern for the legality of paired donation. It stated, in part, "it shall be unlawful for any person to knowingly acquire, receive or otherwise transfer any human organ for valuable consideration for use in human transplantation" [22] (see Chapter 138 for a more detailed discussion of the ethics of living donation). There was concern that the kidney exchange process, whereby an incompatible donor gives a kidney to a different recipient in exchange for the kidney from the other incompatible donor, was tantamount to valuable consideration and thus unlawful. In response to lobbying efforts by the transplant community and transplant organizations, the Charlie W. Norwood Living Organ Donation Act was passed in December 2007, which amended the NOTA by adding that the clause regarding valuable consideration "does not apply with respect to human organ paired donation" [23]. Other countries continue to face legal barriers to KPD that have limited its international growth.

In addition to legal obstacles to KPD, ethical issued also needed to be addressed. In 1997, Ross et al. from the University of Chicago published a discussion of the ethics of a paired kidney exchange program [24]. Other subsequent publications have also laid an ethical foundation for paired donation [25,26]. The four overriding biomedical ethical principles that guide the justification of living donor transplantation are beneficence, non-malfeasance, justice, and autonomy [27]. It is felt that "indirect donation," by means of paired donor exchange, does not weaken or change these guiding principles. However, concerns such as the potential for coercion and the maintenance of anonymity have special consideration in paired donation. For example, a reluctant donor may be relieved by an incompatible result, but faced with the option for paired donation, no longer has the incompatible result as a reason for not proceeding with donation. Thus, extra measures and care should be taken with donors enrolled in paired donation to be certain that the decision to donate is purely voluntary. Of course, all participants have a right to withdraw consent at any time, and thus paired donor transplants must proceed in a sequential fashion such that a potential recipient's donor will not donate until the recipient has been transplanted. Thus, it is felt that consents and policies should be in place at all US centers performing KPD to ensure that such special considerations are managed appropriately.

In addition, since two or more pairs are involved in the paired donation process, it is felt in the US that protocols should be established to maintain privacy and confidentiality between pairs. These protocols should encompass clinic visits, all phases of the operation including preoperative, operative, and postoperative care units, and all family waiting rooms. With the legal framework in place and strict adherence to the guiding principles of biomedical ethics and attention to these special considerations, the public has largely come to accept KPD as acceptable in the US.

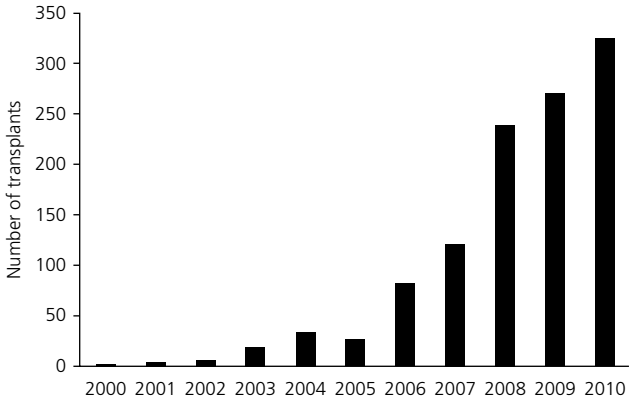

Figure 27.2. Number of reported kidney paired donation transplants performed in the US from 2000 to 2010.

Table 27.1. Scoring system proposed in the United States National KPD program

Match characteristic	Points
Match between a candidate and a potential living donor	200
Zero antigen mismatches between candidate and potential donor	200
Candidate who is a prior living organ donor	150
Highly sensitized candidate with PRA ≥80%	125
Candidates ≤18 years of age	100
Matches between candidates and potential donors at the same center	75
Matches between candidates and potential donors in the same donation service area	50
Matches between candidates and potential donors in the same region	25
Candidates who have participated in previous match runs but were not transplanted	2
Candidates who have one or more antibody assignments against the potential donor	−5

PRA, panel reactive antibody.

KPD in the US has been utilized by single centers, multiregional programs, and more recently by a national program that was initiated in 2010 as a pilot project with four coordinating centers [12,28,29]. Since KPD was initiated in the US, >1000 KPD transplants have been performed (Figure 27.2.) [1,30,31]. Johns Hopkins initiated the first large single-center KPD program in 2000 and reported their 100th KPD transplant in March 2010. The Methodist San Antonio program began in 2008 and has done 250 KPD transplants through December 2013. Each of these single-center experiences has been aided by computerized matching software developed by Inessa Kaplan [32]. Several multiregional programs have also been quite successful, including the Alliance for Paired Donation, New England Program for Kidney Exchange, Paired Donation Network, and National Kidney Registry [7,29,33,34]. Each of these multiregional programs has distinct rules for participation, unique matching algorithms, and differing levels of success and participation. Importantly, the outcomes of KPD transplant recipients have been shown to be comparable to those for non-KPD living donor transplants [35]. In an effort to standardize and hopefully expand KPD in the US, the United Network for Organ Sharing (UNOS) initiated a pilot program in 2010, which has subsequently been approved for utilization by all approved US transplant centers.

The operational guidelines for the US National KPD program were updated in 2011 and set out the rules for participation [36]. All pairs must be consented and fully evaluated and approved for transplant and donation prior to entrance into the KPD program. Unacceptable antigens are identified by the local center according to guidelines that include the use of solid phase, single antigen bead testing. The matching algorithm uses a scoring system to prioritize matches based on a weighted point system (Table 27.1). All cross-match testing is done by the local transplant candidate's participating center. If a center identifies repeated positive cross-matches that preclude an exchange from occurring, the center may be required to submit samples to a referral laboratory for testing in order to participate further in the program. Donor operations should occur simultaneously except in the case of chain operations in which separate rules are employed for the order of operations. Anonymity amongst pairs must be preserved prior to the exchange operations. It is anticipated that most donor kidneys will be shipped to avoid the logistics of travel for donors and to avoid separation of candidates and their donors.

As of December 2011, 86 US transplant centers were participating in the national program and 10 transplants had been performed as part of the national program. Since then, broader participation by more centers, along with the recent addition of chain transplants, have significantly expanded the program.

The Korean experience

The first paired donor transplant in the world was a two-way exchange in Korea in 1991 between recipient pairs who had HLA incompatibility [37,38]. In Korea, deceased donor transplantation is limited, which has enhanced efforts to expand living donor transplantation options. The Korean criteria for acceptable living unrelated (LURD) transplantation require either a two- of four-antigen match at HLA A/B or at least a one-antigen match at HLA DR [39]. The reasons for exchange transplantation in Korea include blood-type incompatibility, unacceptable HLA matching according to policy, or positive cross-match. Anonymity between pairs has not been required. At a single transplant center in Korea, 1090 living donor kidney transplants were reported between 1995 and 2006, including 692 living related transplants (LRD) and 398 LURD transplants [39]. The LURD transplants included 129 exchange recipients and 269 non-exchange recipients. Of the 129 exchange recipients, 84 (65%) were done for blood-type incompatibility, 39 (30%) to conform to HLA matching criteria, and six (5%) for positive cross-match. Sensitization levels of exchange and non-exchange donors have not been reported by the Koreans, but interestingly, the proportion of retransplant patients was significantly lower in the exchange group compared with the non-exchange group. Graft survival and patient survival data were comparable between the exchange and non-exchange groups of LURD recipients. The KPD program increased the overall percentage of living donor transplants by 11.8%. The success of the Korean experience has been driven by large single centers and not a national program, and is expected to continue to increase in the future due to the more recent utilization of more non-directed donors [40,41].

The Romanian experience

Romania also has very limited deceased donor transplantation and thus a strong willingness to pursue alternative strategies for living donor transplantation. There are two active kidney transplant programs, one of which initiated a KPD program in 2001 [42]. Inclusion criteria for the KPD program include an incompatibility due

to ABO or positive cross-match, informed consent, and an emotionally-related living donor. From January 2001 to December 2005, a total of 26 paired donor transplants were performed, including 23 two-way exchanges, two three-way exchanges, and one four-way exchange. Operations were simultaneous for two- and three-way exchanges and on subsequent days for the four-way exchange. All pairs were acquainted preoperatively, as anonymity was felt to be unnecessary and unrealistic. Neither the proportion of ABO or cross-match incompatible pairs transplanted, nor sensitization levels of recipients, were reported. Graft survival rates between the KPD recipients (98.2%) and the direct living donor cohort (97.9%) were very similar and the difference in acute rejection rates between the groups was not statistically significant. This Romanian experience was entirely driven by a single transplant center and thus also highlights the capacity of single-center paired donation.

The Dutch experience

It is clear that the success of KPD is related to the number of pairs in the incompatible pool. To this end, the Netherlands, a country with a population of approximately 16 million, formed a highly successful national program in 2004 in which all seven kidney transplant programs participate [43–49]. An independent organization, The Dutch Transplantation Foundation, was formed and manages the KPD allocation system according to a set of rules agreed upon by all centers. First, all donors are evaluated by a common protocol and pairs are entered into the system four times a year if medically suitable. Second, a computerized matching algorithm is used to select best matches according to present conditions, including: (1) a maximum number of matched pairs; (2) blood type identical before blood type compatible matches; (3) most difficult-to-match (highly sensitized) recipients first; (4) short chains preferred; (5) pairs distributed over multiple centers; and (6) time on dialysis. The program considers that such a relatively simple algorithm that does not account for degree of HLA matching, donor age, gender or size, or other variables is important to the success of the program. Third, all compatibility testing between matched pairs is done at a national HLA reference laboratory, which allows for standardization of cross-matching. Finally, paired donor surgical procedures are done simultaneously and all donors must travel to recipient centers. The matching algorithm originally accommodated only two-way exchanges but was adapted to provide three-way exchanges in 2005 and to include maximum possible chain length in 2007. Between January 2004 and December 2008, 312 pairs were registered, including 54% from ABO incompatible pairs and 46% from cross-match incompatible pairs. Exchanges were identified for 169 pairs and 131 exchange transplants were performed, making the Netherlands' experience the most successful national program to date.

The United Kingdom experience

The legal framework to establish a KPD program in the UK developed in 2004 and is regulated by the Human Tissue Act, which set up the Human Tissue Authority (HTA) to regulate all living donor transplantations. Independent assessors evaluate all living donors and make recommendations to the HTA with regard to suitability. In the case of KPD, donor approval is required from a panel of three HTA members. With this structure to approve KPD donors in place, a matching scheme was developed by UK Transplant and is carried out amongst the 23 kidney transplant centers on a nationwide basis [50]. When ABO and cross-match incompatible pairs are identified, they are fully evaluated and, after approval, are entered into the program. An algorithm was developed to identify all potential two-way and three-way exchanges and then to score the matches based upon four criteria: (1) geographical proximity of donor to recipient; (2) calculated antibody reaction frequency; (3) HLA A, B, and DR mismatch; and (4) donor–donor age difference. Match runs are generated every 3 months and cross-match testing is organized and carried out by the participating centers. Strict anonymity between pairs is a requirement of the program. It is strongly recommended that donor kidneys are shipped and it is required that donor operations are performed simultaneously. Recently, UK Transplant adopted a scheme to allow for altruistic non-directed donation and initiation of chain transplants. Between April 2007 when the initial KPD transplant was performed by UK Transplant and April 2010, a total of 64 KPD transplants have been performed. This initial productivity was less than originally anticipated, but it is hoped the recent initiation of chain paired donation utilizing non-directed donors will further enhance the productivity in the future.

The Australian experience

The Western Australian (WA) KPD program was established in October 2007 as an initiative of a single transplant center in Perth which performs approximately 80 living donor kidney transplants annually [49,51]. Thirteen months after initiation of the program, nine KPD transplants had been done, including one two-way exchange, one three-way exchange, one two recipient chain initiated by a non-directed donor, and one three recipient chain initiated by a non-directed donor. Five of the recipients were sensitized, of whom three had a PRA of >70%. This KPD experience increased living donor transplantation by approximately 10% in Western Australia. The WA KPD experience was the basis for developing a national program, including all 14 Australian transplant centers, to increase access to living donor transplantation in Australia. To ensure equitable matching and allocation for all transplant centers, the National Organ Matching System (NOMS) performs the match runs generated by a computer program that was developed based on the guidelines of the Dutch algorithm. It is anticipated that the national experience will mimic the WA experience in expanding access to living donor transplant by 7–10%, but such national data has not yet been published.

Future expansion

Utilizing compatible pairs

In an effort to expand KPD, it has been suggested that non-HLA identical compatible pairs should be considered for inclusion into KPD programs. The utilization of compatible pairs to facilitate KPD transplants has been termed altruistic unbalanced paired kidney exchange (AUPKE) [52]. Ratner et al. have studied attitudes toward AUPKE and found that many pairs may favor such an arrangement, especially if there is a perceived benefit for the recipient [53,54]. One example of such a perceived benefit would be if the compatible exchange recipient were to receive a kidney from a younger donor. Several groups have utilized compatible pairs in KPD simulations and shown significant benefit [55,56]. Addition of compatible pairs increases the population of blood type O donors with unsensitized recipients, thus addressing the blood type imbalance that exists in the KPD pool. For example, each unsensitized blood type A candidate with a blood type O donor could facilitate a transplant between an incompatible blood type O recipient with a younger blood type

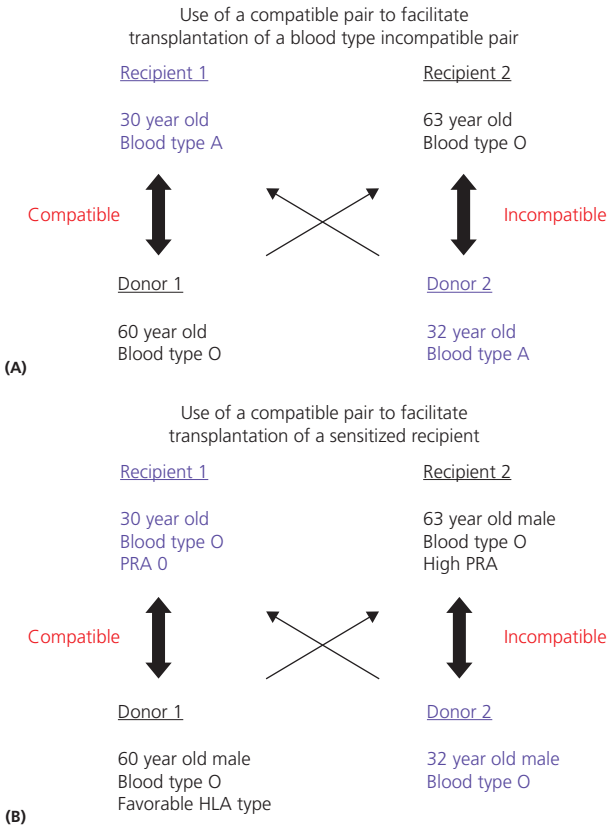

Figure 27.3. Utilization of a compatible pair to facilitate kidney paired donation transplants. (A) Use of a compatible pair to facilitate transplantation of a recipient with a blood-type incompatible donor. (B) Use of a compatible pair to facilitate transplantation of a sensitized recipient with a cross-match incompatible donor.
PRA, panel reactive antibody.

A donor (Figure 27.3a). Additionally, donors with rare, favorable HLA types can be used to match otherwise very difficult-to-match highly sensitized recipients (Figure 27.3b). Inclusion of compatible pairs into large KPD programs may be expected to double the match rate for incompatible pairs and thus may have a dramatic effect on further expansion of KPD. Ethical concerns, however, regarding the use of compatible pairs in KPD are an important consideration and may limit further expansion of such programs; further discussion and research are needed.

Combination of kidney paired donation with desensitization

KPD and desensitization have often been viewed as competing strategies to transplant incompatible pairs, but more recently they have been viewed as complementary [28,57,58]. The subset of candidates who are very highly sensitized (PRA $\leq$90%) or moderately sensitized with non-blood type O donors are very difficult to match with a compatible pair in KPD programs. These candidates may be ideally suited to a combination of KPD with desensitization. For example, a candidate with high antibody reactivity against an original donor may be able to find an exchange donor to whom they have less antibody reactivity that would be amenable to successful desensitization. Such a strategy has been widely employed by the transplant groups at Johns Hopkins, UCLA, and Methodist San Antonio, and is thought to be an important option for the most challenging to match candidates. Results utilizing this strategy have not been published and thus this concept will need to be studied further prior to wide implementation.

Internationalization

The international growth of KPD programs over the past 10 years has resulted in thousands of patients realizing the benefit of living donor kidney transplantation and who would otherwise have waited years to receive a deceased donor kidney transplant. Importantly, each KPD transplant that is performed results in one less candidate on a waitlist, allowing for more rapid transplantation for others who do not have donors. Now that many ethical, legal, and logistical barriers have been overcome, successful programs have now been established in Europe, Asia, Australia, and North America. Several reports have shown that shipment of living donor kidneys over very large distances has not been associated with significant delayed function or poor outcomes [59,60]. It is envisioned that the future may bring international co-operation and thus even larger exchange registries to benefit further highly sensitized patients around the world [61].

Summary

KPD has long been recognized as a potential solution to the shortage of suitable deceased donor kidneys and the factors maintaining patients on deceased donor waitlists for prolonged periods of time. However, it is only recently that the substantial logistical barriers preventing broad implementation of ethically sound KPD programs have been sufficiently addressed to allow for meaningful implementation of broad KPD sharing programs. As these programs emerge in the coming decade, it is likely that they will become increasingly utilized to provide the best option for sensitized patients awaiting transplantation.

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SECTION 4

Pre-operative Management of Transplant Patients

CHAPTER 28

Patient Selection and Indications for Kidney Transplantation

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Introduction

Though solid organ transplantation originated with kidney isografts and allografts, modern renal transplantation remains distinct due to several key attributes. First, as death was the consequence of irreversible renal failure in the 1950s, and the kidney is a paired organ, living donors enabled the first successful transplants, and remain a major source of transplantable kidneys a half-century later. Second, because there is now an alternative treatment for patients with irreversible kidney failure (maintenance dialysis is successful and widely available throughout the developed world), much of the urgency associated with transplantation of other organs does not apply to kidneys. Accordingly, though as with all solid organs demand far exceeds supply, acceptance of chronic dialysis as a therapeutic modality makes a decision by patient or provider not to pursue transplantation a reasonable one, as death is not the only alternative. Thus, defining indications and contraindications for transplantation and selecting appropriate transplant candidates occur in the context of renal replacement therapy (RRT), a very different scenario from patient selection for other solid organ transplants. This chapter will summarize the common indications that might bring a patient to consider transplantation as their option for renal replacement. The chapter complements information found in Chapter 37, which details waiting list management for patients awaiting transplantation.

Kidney transplantation as renal replacement therapy

End-stage renal disease (ESRD) is the most advanced stage of chronic kidney disease (CKD) (Table 28.1) [1]. CKD risk increases dramatically with age, with the vast majority of patients never progressing beyond Stage 3 due to mortality from associated co-morbidities. Ultimately, there are approximately 120 000 incident cases of ESRD in the US each year [2]. An almost uniformly fatal outcome was the norm until the 1960s, when dialysis and transplantation first became available. Originally, dialysis was thought to be only a temporizing solution, providing support until recovery for a patient with acute kidney failure or a bridge to transplantation for those with irreversible disease. However, due to dismal transplant outcomes and a limited supply of transplantable kidneys, chronic dialysis had by the 1970s become the more common therapy [3]. This paradigm became institutionalized in the US by the Social Security Amendments of 1972, federal legislation that created the Medicare ESRD program, to this day the only organ-specific entitlement in this country [4]. With benefits thought largely related to quality of life, indications for transplantation were limited (the young and healthy being considered the best candidates) and strict patient selection criteria were utilized to distinguish those most likely to benefit and withstand the hazards of chronic immunosuppression. There existed in most centers multidisciplinary selection committees entrusted with decision-making regarding access to dialysis, transplantation, or neither [5]. Selection criteria often included not just health status, but age, rehabilitation potential, and "value" to society [6]. Not surprisingly, the conundrums inherent in such a process led to the abandonment of these committees during the 1980s, though multidisciplinary assessment of "transplantability" persists in most transplant centers [7].

In the 1980s and 1990s, several major advances changed the dynamic of patient selection for transplantation. The availability of cyclosporine in 1983 ushered in the modern era of effective immunosuppression: rejection could be prevented, corticosteroid doses reduced dramatically, complications managed, and overall outcomes improved, particularly for recipients of deceased donor kidneys [8]. Less dependence on HLA matching between donor and recipient meant that transplantation could be offered to a much broader group of patients [9]. Advances in antimicrobial therapy, particularly antifungals and antivirals, allowed effective treatment of the infections that had caused the demise of many hardy and not-so-hardy recipients in earlier days [10]. Co-incidentally, a novel statistical model applied to a maturing Medicare database enabled comparison of outcomes with each modality of RRT [11]. To the surprise of more than a few, regardless of age, ethnicity, or original disease, not to mention the futility of trying to define an individual's value, kidney transplantation offered a substantial survival benefit over any other alternative: projected longevity compared to dialysis was essentially doubled by transplantation. In a more recent analysis designed to better inform allocation of deceased donor kidneys,

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 28.1. Chronic kidney disease (CKD) in the US

(a) Clinical stages of CKD according to estimated glomerular filtration rate (eGFR) [1,96]

Stage	Description	eGFR (mL/min/1.73m^2)	Prevalence (% US adults ≥20 years old)
1	Evidence of kidney damage with normal GFR (e.g. proteinuria, hematuria)	>90	5.7
2	Mild	60–89	5.4
3	Moderate	30–59	5.4
4	Severe	15–29	0.4 (Stage 4 or greater)
5	Kidney failure (end-stage renal disease)	<15	

(b) Common causes of end-stage renal disease (ESRD) with associated percentages of incident cases in 2009 [2]

Cause of ESRD	Percentage of incident cases
Diabetes mellitus (types 1 and 2)	44
Hypertension	28
Glomerulonephritis	7
Cystic kidney disease	2
Other or unknown	19

the concept of life years from transplant (LYFT) was developed. LYFT was derived utilizing patient characteristics in the USRDS database: projecting longevity on dialysis for any given patient, projecting longevity for that same patient with a kidney transplant, and subtracting the former from the latter [12]. Regardless of age, gender, ethnicity, original kidney disease, diabetic status, region, or insurance status, receiving a kidney from a deceased donor (with presumption of even greater benefit from a live donor kidney) substantially extends projected lifespan of most ESRD patients. It has also been estimated that a successful kidney transplant may save payers as much as $330 000 [adjusted to 2011 US dollars, including the value of quality-adjusted life years (QALYs)] over the life of the transplant [13]. Because of the life-sustaining and cost-effective nature of kidney transplantation, Medicare mandates assessment of transplant status for each covered ESRD patient. Recent data indicate extreme variability in how this policy is implemented from center to center [14].

In the current era, indications for transplantation in the ESRD population are now quite broad, and the selection process is focused on identifying the presence of a shrinking number of known contraindications. This "transplant evaluation" occurs after a CKD patient is referred to a transplant center by his/her nephrologist or other provider. Ideally, such referrals should occur at the same time as patients are referred for creation of dialysis access: during Stage 4 CKD [estimated glomerular filtration rate (eGFR), 20–29 mL/min), which allows completion of testing and identification of potential living donors early enough for the transplant to occur as primary therapy [15].

The guiding principle of transplant evaluation is determining the best option for each individual patient. Some would inject a competing ethic into the process, that of making the best use of the relatively limited number of kidneys available for transplantation (almost 100 000 candidates are listed, but only approximately 10 000 kidneys are recovered each year), attempting to ascertain the validity of one person's claim over that of another [16]. However, even granting that a provider might be capable of such a judgment, recent evidence suggests that while as many as 20% of those on the current waiting list may receive relatively little benefit from transplantation, the number of patients likely to live substantially longer with a transplant but lingering on dialysis without evaluation and listing may be ten-fold greater [17]. The solution would appear to be not limiting access but finding more transplantable kidneys.

Selecting a donor

As implied above, kidney transplantation, and to some extent all solid organ transplantation, owes its existence to the living donor. The first successful transplants were between twins, who are without important histocompatibility differences [18]. Subsequently, after development of effective immunosuppression, most of the first successful allografts originated in living donors [19]. In the current era, there remains substantial benefit of receiving a kidney from a living donor, including better kidney function and short- and long-term graft survival (61% versus 45% at 10 years) [16]. In addition, living donor transplantation offers elective surgical scheduling and excellent immediate function that may mitigate other risks (e.g. perioperative cardiac events, delayed graft function, acute rejection). With average time to transplant in excess of 3 years nationally (and longer in many locales) for a deceased donor kidney, the biggest advantage of living donor transplantation may be avoidance of prolonged time (and attendant dialysis co-morbidities) awaiting transplantation. For most potential recipients, living donor transplantation offers timely intervention and the best opportunity for a successful outcome, making it the donor option of choice in most situations.

Optimal timing of kidney transplantation

Although not totally without controversy, it is generally accepted that increasing duration of dialysis is associated with increased risk of kidney allograft failure [20,21]. Meier-Kriesche et al. reported in 2002 that time on dialysis was an important variable influencing risk for recipients of both living and deceased donor kidneys, with as little as 6 months of dialysis therapy significantly reducing long-term graft survival [22]. Subsequent work indicates any decrement in outcome is primarily reflective of reduced patient survival, not immunologic graft failure, and varies according to age and ethnicity [23]. A recent study by Schold et al. documented that the impact of time on dialysis could be broken down into time spent before and after wait listing [24]. When examined in this regard, the differential impact on outcome was entirely due to the time between onset of dialysis and wait listing, with the time between wait listing and transplantation having no impact. The authors interpreted the data as indicating pre-evaluation characteristics, including co-morbidities and socioeconomic status, as responsible for delayed referral and major factors contributing to increased mortality risk after transplantation.

Nuances aside, it is generally accepted that optimal timing for transplantation is before maintenance dialysis is initiated (pre-emptive), allowing patients to avoid associated co-morbidities, maintain functional status and employment, and promote optimal outcomes [15]. Given the number of candidates on the waiting list, pre-emptive transplantation typically requires the availability of a living donor, and only 2% of ESRD patients begin RRT with a transplant [16]. It is common among third-party payers and transplant centers to require recipients be at late stage 4 CKD (eGFR ≤20 mL/min) before proceeding with living donor transplantation;

Table 28.2. Medical assessment of the kidney transplant candidate

1 Comprehensive history and physical examination
2 Diagnostic evaluation:
  (a) Electrocardiography
  (b) Chest radiography
  (c) Colonoscopy (over 50 years of age)
  (d) Ultrasound of urinary tract and native kidneys
  (e) Females:
    Pap smear
    Mammography (age over 40 years or family history of breast cancer)
  (f) Cardiac evaluation (see text)
  (g) Urologic evaluation (history of bladder/voiding dysfunction or recurrent urinary tract infections)
  (h) Vascular evaluation: non-contrast pelvic CT (age >50 years or >5 years on dialysis)
3 Laboratory evaluation:
  (a) Routine: CBC with differential and platelet counts, comprehensive metabolic panel, prothrombin time, partial thromboplastin time
  (b) Urinalysis, urine culture
  (c) Serologic tests: HIV, HBV (core and surface antibody, surface antigen), HCV, CMV, EBV, varicella, rapid plasma reagin (RPR)
  (d) Prostate-specific antigen in men over age 50 years
  (e) Immunologic testing:
    ABO blood group
    HLA typing and determination of preformed anti-HLA antibodies
    Cross-matching with potential donors

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Table 28.3. Psychosocial and financial assessment of the kidney transplant candidate

1 Personal assessment:
  (a) Physical functioning
  (b) Intellectual capacity
  (c) Sexual activity
  (d) Coping mechanisms
  (e) Substance abuse or dependence
  (f) Medical adherence
2 Illness assessment
  (a) Impact of ESRD on functional status and psychosocial adjustment
  (b) Candidate's understanding of treatment options and the transplant process
3 Education: highest level attained
4 Occupation: employment length and stability
5 Financial: sources of income and adequacy of other resources (e.g. insurance) during pre- and post-transplant periods
6 Support system: family and social
7 Environmental: housing, transportation, relocation/travel needs

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current United Network for Organ Sharing (UNOS) guidelines require a eGFR of $\leq$20 mL/min to accrue allocation points on the waiting list [25]. There is no indication that pretransplant eGFR exerts any influence on either post-transplant allograft function or graft survival; the benefit of pre-emptive transplantation appears to be related entirely to avoiding dialysis and its attendant co-morbidities [26]. Thus, optimal timing for transplantation would be in a candidate with an eGFR of $\leq$20 mL/min, with little or no uremic symptomatology, and before initiation of chronic dialysis. Again, achieving this goal requires referral for transplantation early enough (eGFR 20–29 mL/min) to allow completion of the pretransplant process in a timely fashion [27].

The transplant evaluation

The transplant evaluation itself is designed to include:

- An educational component in which prospective candidates learn about risks, benefits, measures necessary to promote successful kidney transplantation, and strategies to identify potential living donors.
- Medical testing to identify co-morbidity that either requires therapy or may preclude transplantation (Table 28.2), as well as provide risk stratification for appropriate patient counseling.
- Psychosocial evaluation to assess ability of a potential candidate and his/her support network to understand and follow treatment plans (Table 28.3).
- Financial counseling to ensure access to resources (Medicare, Medicaid, private insurance, personal finances) sufficient to maintain a healthy allograft [28].

At most centers, referral results in triage based on patient history; obvious contraindications preclude further testing. The subsequent evaluation process typically involves a multidisciplinary team consisting of a coordinator, nephrologist, surgeon, mental health professional, social worker, and financial counselor. The medical screening is typically straightforward, with interpretation of results of testing geared toward addressing the issues noted below. A positive screening test (Table 28.2) will generally result in additional testing.

As is common in dealing with chronic diseases, successful transplantation is often accompanied by significant psychologic and socioeconomic concerns. In its early years, psychiatric problems were considered a major contraindication to kidney transplantation. Now, psychiatric illnesses, if appropriately treated, rarely preclude transplantation [29]. Rather, most transplant centers would require that psychiatric disturbances be adequately treated and have a reasonably benign prognosis before proceeding [30]. Initial assessment of a potential transplant candidate must address level of cognitive function [29]. Granting informed consent requires comprehension of risks and benefits of transplantation relative to dialysis. After transplantation, cognitive skills adequate to understand and follow complicated immunosuppressant and follow-up regimens are essential. At times, ESRD is associated with significant organic illness (anemia, vitamin deficiencies, etc.) that can impair cognition and these should be aggressively diagnosed and treated. In some ESRD patients, cognitive deficits may improve after transplantation with more definitive resolution of the uremic state. However, at times, even patients with severe, irreversible cognitive deficits may still benefit from transplantation with availability of a committed caregiver [31,32].

It is well established that non-medical variables (socioeconomic status, ethnicity, educational achievement, geographic distance to transplant center) have a substantial influence on access to transplantation as well as subsequent patient outcomes [33–35]. In the US, with its complex and often confusing system of healthcare financing, socioeconomic assessment is an important part of most pretransplant evaluations [36]. Usually, this task is performed by a social worker familiar with the requirements for post-transplant care, and able to provide counsel on an individual basis and assess the resources each candidate brings to the transplant process. The latter include insurance coverage, ability to travel to and from the transplant center, current employment, and prospects for future employment. For the ESRD patient, transplantation changes the dynamic of access to socioeconomic resources, removing them from the defined benefits associated with chronic dialysis. Terms of insurance coverage often change after transplantation and patients may lose disability benefits. Thorough socioeconomic assessment before transplantation simplifies coping with events afterwards.

Most ESRD patients identified as suitable transplant candidates remain under the care of a practicing nephrologist (not the

transplant center) while awaiting transplantation [27,37]. As time awaiting transplantation grows lengthier, new medical and psychosocial challenges may arise [38]. It is the treating physician's responsibility to ensure that newly emergent problems are addressed promptly and that the transplant center is notified of the change. Likewise, ongoing contact between the candidate and transplant center is increasingly the norm, with annual re-evaluation mandated by some payers and many transplant centers. Up to date knowledge of changes in medical status, cognitive function, insurance coverage or social support systems is crucial in determining whether to proceed with transplantation when an organ becomes available.

Contraindications to kidney transplantation

Any discussion of contraindications to kidney transplantation must be tempered by two considerations. The first is that contraindications evolve over time, due either to changes in standards of care that alter the potential impact of a given variable or specific approaches to a variable that mitigate its risk [39]. In the 1970s, contraindications included diabetes mellitus, age over 55 years and, among others, a history of gastrointestinal bleeding. The first two were jettisoned with changes in practice and the third with availability of effective medical therapy (H_2 blockers, etc.). Second, there is wide variation from center to center in how strictly contraindications are interpreted and implemented. It has been said that a limited projected lifespan may preclude transplantation; however, since for essentially all measurable parameters transplant offers extended longevity compared to dialysis, making such a determination, in the absence of a specific contraindication, is difficult [40]. Thus, medical, psychologic, and socioeconomic evaluation at most centers is designed to disclose contraindications; if none is found, evaluated patients are deemed acceptable candidates [28].

The list of absolute contraindications in 2014 is relatively short (Table 28.4), especially when compared to previous decades. These conditions typically pose significant barriers to one or both necessary and essential components of kidney allotransplantation: an open, subdiaphragmatic, vascular operation and long-term immunosuppression. The operation itself requires suitable vascular anatomy and a mechanism to provide adequate urinary outflow. While the latter is only rarely an issue (with surgical reconstruction of the urinary tract, if necessary, able to provide adequate outflow), the former often poses problems. Standard surgical approaches involve the iliac circulation. With worsening metastatic calcification often the consequence of increasing duration of CKD and dialytic care, it is not uncommon to find significant, circumferential vascular calcification that precludes anastomoses. Likewise, aortobifemoral bypass precludes common approaches to establish adequate circulation to the allograft. In some rare instances, however, creative surgeons may choose to proceed after endarterectomy or at alternate sites. In the case of aortoiliac stent grafting (which is increasing in popularity), detailed knowledge of the extent of distal landing sites of the stent graft is imperative. As waiting times (and resultant time on dialysis) increase, pelvic venous abnormalities may become more important. Candidates with a history of failed thigh arteriovenous grafts or long-term femoral catheters should have evaluation of their iliac veins and inferior vena cava to ensure patency for venous outflow.

Most transplant candidates undergo extensive cardiac testing as part of the evaluation process (see below). A contraindication based on advanced cardiac and/or pulmonary disease reflects significantly increased perioperative risk as well as potential impact on longevity even after a successful operation.

Table 28.4. Contraindications to kidney transplantation at the University of Alabama at Birmingham, 2014

1. Unsuitable vascular anatomy:
   - **(a)** Aortobifemoral bypass or aortoiliac stent grafting extending to both external iliac arteries
   - **(b)** Circumferential calcification of iliac vessels
   - **(c)** Thrombosis of the iliac veins and inferior vena cava
2. Advanced cardiopulmonary disease:
   - **(a)** Ischemic cardiomyopathy with left ventricular ejection fraction (LVEF) $\leq$20%
   - **(b)** Continuous home oxygen therapy
3. Invasive malignancy within 2 years of evaluation, excluding non-melanoma skin cancer and small (<2 cm) renal cell carcinomas
4. Obesity: body mass index (BMI) $\geq$40 kg/m^2
5. Active infection (e.g. tuberculosis, invasive fungal disease, osteomyelitis)
6. Active alcohol or substance abuse (excluding tobacco)
7. Care status: nursing home

Adherence

In the current era, daily ingestion of maintenance immunosuppressants is essential to successful transplantation. The inability to reliably take prescribed medications (for whatever reason) thus must be considered a contraindication. Because of the link between substance abuse and non-adherence to medical therapies, most consider active drug abuse or alcoholism an absolute contraindication to transplantation [28]. A history of non-adherence in the absence of substance abuse may be recognized as a relative contraindication, though interpretation varies widely from center to center [41]. Since the transplant center's exposure to potential candidates is limited, documentation and follow-up with the dialysis unit or nephrologist are essential in assessing and monitoring compliance history. This issue is particularly germane for teenagers being considered for transplantation. This particular topic is addressed in depth in Chapter 120.

Infection and malignancy

Long-term immunosuppression impairs the recipient's ability to deal effectively with infection and malignancy, its two principal complications. Again, while the long-standing tradition is eradication of infection before transplantation, the availability of effective antimicrobials has altered the standard somewhat towards control rather than eradication. Most centers defer transplantation of patients with invasive bacterial, fungal, or mycobacterial disease until an effective therapeutic course is completed. The approach to some viral infections [notably hepatitis C (HCV), hepatitis B (HBV), and human immunodeficiency virus (HIV)] has changed dramatically (see below). For HCV, recognition that patient survival with a transplant even in the presence of viremia exceeds survival on dialysis makes transplantation (in the absence of advanced liver disease or cirrhosis) the preferred therapy [42]. For HBV and HIV, availability of effective antiviral therapy allows control of viral replication despite ongoing maintenance immunosuppression [43,44].

Malignancy poses similar challenges. Non-melanoma skin cancers are common in the general population; even though immu-

Table 28.5. Impact of malignancy on waiting time for transplantation. Potential transplant candidates with a history of significant malignancy may require oncologic evaluation

No waiting time if thought cured at evaluation	
Incidental renal cell carcinoma	
In-situ carcinoma of bladder	
In-situ carcinoma of cervix	
Non-melanoma skin cancers	
Waiting time at least 2 years	
Most invasive tumors (including Wilms', invasive bladder and uterine)	
Waiting time beyond 2 years	
Melanoma*	5 years
Renal cell carcinoma	2 years if <5 cm 5 years if ≥5 cm
Breast carcinoma†	2–5 years
Lymphoma	2–5 years
Colorectal carcinoma	2–5 years
Invasive cervical carcinoma	2–5 years

*In-situ melanoma may require a shorter waiting period of 2 years.
†Early in-situ may only require 2-year wait. Individuals with advanced breast cancer (Stage III or IV) should be advised against transplantation.
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nosuppression may increase their frequency and severity, they are rarely life threatening and are not considered to preclude transplantation. However, with general acceptance that immunosuppression promotes tumor growth, other solid and hematogenous malignancies remain contraindications [45–48]. For a potential candidate with active cancer, transplantation should be avoided [28]. Traditionally, after appropriate therapy followed by a "waiting period" of variable length (determined by tumor characteristics and risk of recurrence) transplantation could be offered (Table 28.5) [29]. Again, there is some evolution towards shorter waiting periods for many tumors, driven by increased understanding of tumor biology as well as recognition of the survival benefit of transplantation over maintenance dialysis. Thus, the previously accepted waiting period of 5–10 years is now considered inappropriate for many candidates, especially given shorter projected longevity with maintenance dialysis. Where uncertainty exists, informed consent is essential, with oncologic evaluation and explicit acknowledgement that some tumors may recur regardless of waiting time.

Additional issues in patient selection

In the evaluation process, concerns arise that may not preclude transplantation, but require additional testing, treatment, and/or follow-up before transplantation can proceed. These "relative contraindications" are less universally accepted, potentially amenable to intervention, and more likely to lose significance as current therapies and understanding evolve over time. They can be either medical or psychosocial in nature, and though they may threaten the success of a transplant, do not themselves preclude either a surgical operation or long-term immunosuppression. For example, two decades ago (and more recently in some European countries) age (over 55, or 60, or 65, or 70 years) was considered a contraindication to kidney transplantation [49]. Currently, with data supporting a longevity benefit for recipients up to the age of 80 years, older chronologic age, if a consideration at all, is at most a relative contraindication, with greater emphasis on overall health status [17].

Table 28.6. Potential clinical approaches to kidney transplantation in patients with defined native kidney diseases

Native kidney disease	Recurrence rate	Management
Primary focal glomerulosclerosis	Up to 50%	Recurrence rate of up to 80% in those who lose an allograft to recurrent disease may influence retransplantation choices
Antiglomerular basement membrane disease	Low	Anti-GBM antibody in serum associated with increased risk
Wegener's granulomatosis	17%	ANCA presence not associated with risk of recurrence
IgA nephropathy	20–60%	Histologic recurrence common but graft failure rare
HUS/TTP	Up to 75%	Factor H mutation imparts poor prognosis

HUS, hemolytic-uremic syndrome; TTP, thrombocytopenic purpura, ANCA, antineutrophil cytoplasmic antibody.
Data from Golgert et al. [52].

Native kidney glomerulopathies

Recurrent glomerular disease is a common cause of allograft failure, but risk of recurrent disease is rarely a defining issue in the evaluation of transplant candidacy. In the past, diseases such as primary focal glomerulosclerosis were considered to recur with enough frequency that transplantation was discouraged [50–52]. Current data indicate recurrence rates perhaps less than previously thought, with disease recurrence often amenable to clinical management. The most likely native glomerulopathy to recur is primary focal glomerulosclerosis, with up to a 50% risk in young, Caucasian patients undergoing first transplants, and even higher in those undergoing retransplantation after an earlier graft was lost to recurrent disease (Table 28.6). However, the benefits of transplantation versus dialysis are so substantial that the risk of recurrence exerts only a minor impact on candidacy, and even donor selection, in most patients. While recurrent lupus nephritis is rare, most centers require quiescence of disease on minimal immunosuppression before proceeding with transplant; in addition, presence of anticardiolipin antibody (lupus anticoagulant) may require prophylactic anticoagulation.

Polycystic kidney disease

A consideration for patients with polycystic kidney disease (PKD) is the potential need for native nephrectomy either before or during the transplant procedure. While the majority of PKD patients will not require nephrectomy, some may benefit from having their native kidneys removed. Massive polycystic kidneys, extending into the pelvis, may not permit implantation of a transplant; physical examination and abdominal computed tomography (CT), which also allows assessment for malignant changes within native kidneys, enable appropriate assessment. Patients with significant symptoms or complications, including intractable pain, early satiety, recurrent urinary tract infections, and recurrent hematuria should also be considered for native nephrectomy. While this was traditionally performed prior to transplantation, there is increasing successful experience with synchronous bilateral native nephrectomy at the time of renal transplant [53].

Addressing perioperative complications

In general, most surgeons are reluctant to transplant nephrotic patients with ongoing protein loss and severe hypoalbuminemia

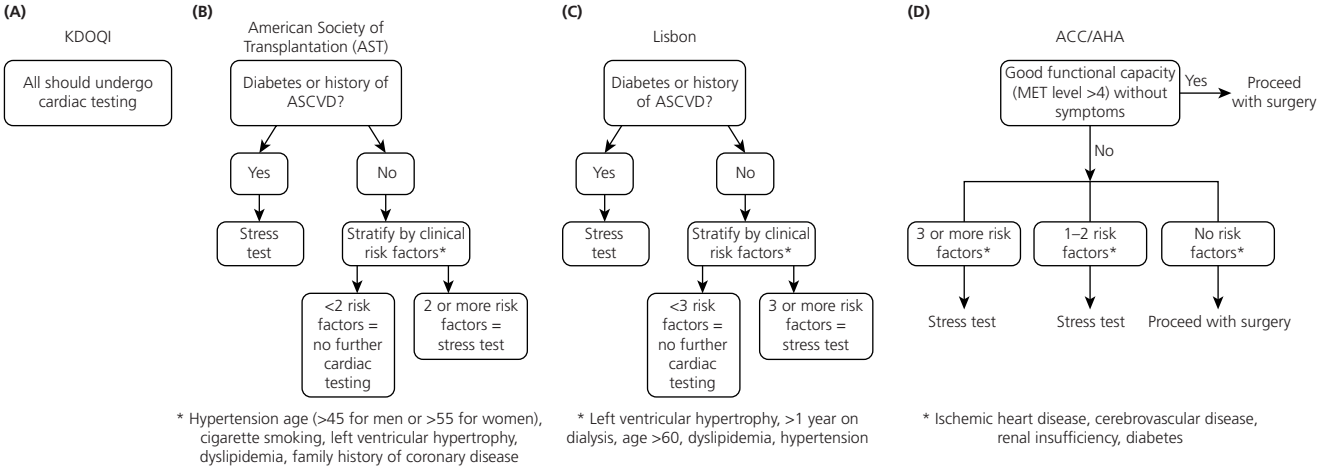

Figure 28.1. Overview of four pretransplant cardiac risk assessment guidelines: (A) Kidney Disease Outcomes Quality Initiative (K/DOQI); (B) American Society of Transplantation (AST); (C) Lisbon Conference on the Care of the Kidney Transplant Recipient (Lisbon); (D) American College of Cardiology/American Heart Association (ACC/AHA). ASCVD, atherosclerotic cardiovascular disease; MET, metabolic equivalent. (Source: Friedman et al. [62]. Reproduced with permission from the American Society of Nephrology.)

due to a perception of increased perioperative risk of complications. In a patient with advanced CKD, medical ablation of renal function with agents such as angiotensin-converting enzyme inhibitors or non-steroidal anti-inflammatory drugs usually controls urinary losses sufficiently to reverse the process, although native nephrectomy may be required in rare instances prior to transplantation. A history of venous or dialysis access thrombosis generally leads to consideration of studies of thrombotic tendencies, including assessment for Factor V Leiden mutations and/or deficiencies of protein C and protein S [54].

Cardiovascular disease

The cardiovascular burden associated with CKD is substantial, accounting for as many as half of patient deaths [2]. Even though transplantation is associated with a lower risk of cardiovascular death than maintenance dialysis, risk remains high compared to the general population [55,56]. It is widely recognized that traditional risk factors for coronary disease (as established in studies like Framingham) do not associate with risk after transplantation [57]. Hypertension, dyslipidemia, and cigarette smoking are relatively minor risk factors compared to pretransplant diabetes, previous coronary events, and post-transplant renal function [58]. In terms of patient selection, screening for cardiovascular disease, with implementation of corrective measures where possible, is an important part of the evaluation process in order to address two major concerns [59]. The first is defining individual risk associated with the transplant operation itself, with as many as 5% of recipients estimated to experience perioperative myocardial infarction. The second is assessing potential longevity of the recipient after a successful transplant; most would consider utilizing a kidney from either a deceased or living donor in a patient with irreversible cardiac dysfunction destined for early mortality as not being efficient use of a scarce resource. Unfortunately, there is no uniform standard applied to either purpose [60]. In fact, a recent provocative article suggested the potential adverse effects of routine screening of asymptomatic patients (needlessly delaying transplantation, risk of radiocontrast nephropathy, surgical bleeding from antiplatelet agents, psychologic burden, morbidity, and mortality associated with angiography and revascularization, and inefficient use of healthcare resources) as significant offsets to any potential benefits [61].

Defining the population to be screened (beyond the thorough history and physical examination, electrocardiography, and chest imaging that are part of every evaluation) is the first major challenge. Virtually all would agree that symptomatic patients (chest pain, dyspnea on exertion, limited exercise capacity, etc.) be studied. Given the extraordinary cardiovascular morbidity in those with advanced CKD even in the absence of symptoms, controversy resides in how asymptomatic patients should be addressed [61,62]. At the extremes are Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines indicating all candidates should undergo non-invasive testing and American College of Cardiology(ACA)/American Heart Association (AHA) recommendations that functional status tempered by risk factor profile should be the only major determinant of testing [63,64]. Intermediate levels of testing result from application of guidelines developed by more kidney-specific stakeholder groups (Figure 28.1) [29,65]. Recent surveys indicate most centers apply some version of the society-based recommendations to their patient population, with age, functional status, and risk factor profile determining level of testing for each individual. In a recent analysis of Medicare data, patients considered at high ischemic heart disease (IHD) risk (defined as diabetes, previous IHD, or >2 cardiac risk factors) had a 3-year post-transplant risk of acute myocardial infarction of 10%, versus 3% in patients not so characterized [58]. The lower-risk patients were substantially more likely to proceed to transplant without cardiac evaluation than the high-risk IHD group (20% versus 65%). Acute myocardial infarction risk was more influenced by IHD characterization than whether or not patients underwent screening, which indicates the influence of risk factor profiling not only on outcomes but also screening practices (Figure 28.2) [59,66]. The absence of a strong evidence base informing such practices supports those who advocate prospective controlled trials of screening protocols.

After deeming cardiovascular screening to be necessary in a transplant candidate, choosing the proper technique is the next challenge. Because non-invasive testing has significantly less pre-

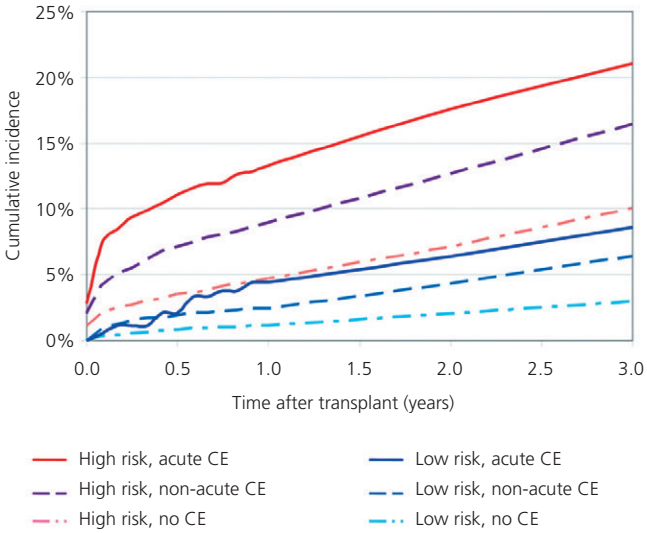

Figure 28.2. Observed incidence (Kaplan–Meier) of acute myocardial infarction after transplant according to clinical ischemic heart disease (IHD) risk group and pretransplant cardiac evaluation status. CE, cardiac evaluation. (Source: Lentine et al. [59]. Reproduced with permission from the American Society of Nephrology)

Table 28.7. Non-invasive tests utilized in assessing cardiac risk during pretransplant evaluation of potential kidney recipients. (Data from Friedman et al. [62])

- Stress echocardiography [using either exercise or pharmacologic stress, such as dobutamine stress echocardiography (DSE)]
- Myocardial perfusion scintigraphy (using either exercise or pharmacologic stress)
- Stress electrocardiography (EST)
- Electron beam computed tomography (EBCT)
- Resting electrocardiography (ECG)
- Conventional echocardiography
- Exercise ventriculography
- Digital subtraction fluorography (DSF)
- Carotid intimal medial thickness (CIMT)
- Cardiopulmonary exercise testing
- Computed tomography (CT) coronary angiography
- Magnetic resonance angiography
- Cardiac magnetic resonance imaging (MRI)

dictive value in the CKD population than the general population, early studies indicated angiography to be the preferred approach. While improvements in non-invasive testing may make these approaches preferable in triaging patients for coronary angiography and defining need for aggressive risk factor modification, there is still little consensus on the best way to risk-stratify transplant candidates. Studies of transplant candidates undergoing non-invasive testing followed by coronary angiography show considerable discordance, with angiographically demonstrable disease present in up to 40% of patients with negative stress tests [67,68]. At least two other observations may also be important. Work from the Mayo Clinic, not yet widely confirmed, indicates that a single measurement of serum troponin T at the time of evaluation (an indicator, perhaps, of subclinical ischemia) is a better predictor of subsequent mortality than other non-invasive testing [69]. Other work indicates that left ventricular dysfunction at evaluation, independent of results of other testing, is most predictive of waiting list mortality [70].

Applicable non-invasive tests are listed in Table 28.7. In general, no specific non-invasive study has been found to be ideal or superior. Given disparate expertise in cardiovascular testing among centers, the traditional recommendation is that whichever test can be performed most efficiently and reliably be utilized at a given center. A recent Cochrane meta-analysis supported such a recommendation, with dobutamine stress echocardiography (DSE) and myocardial perfusion studies (MPS) performing equally well in identifying those with ≥50% stenosis of a major coronary artery in published studies [71].

Positive results of non-invasive testing in most algorithms then leads to coronary angiography and, where indicated, revascularization. In this population, there is a strong preference for revascularization over medical management, based at least partially on studies performed decades ago on small numbers of transplant candidates [60,72]. Critics point to data from large studies in the general population applying modern medical interventions (aspirin, statins, beta-blockers, angiotensin blockade, etc.) with near -equivalent or superior outcomes to revascularization, except in triple vessel disease (or equivalent) [73,74]. Alternatively, recent studies in the CKD population have continued to document compromised therapeutic responses to medical management when compared to the general population [75].

Diabetic patients comprise a group at especially high risk for cardiac events after transplantation [56,76]. The relatively poor negative predictive value of non-invasive screening in this population has led some to advocate for cardiac catheterization as the only diagnostic test predictive of major coronary events in diabetics seeking transplantation.

Which findings at cardiovascular screening preclude transplantation? Again, this is a moving target. Currently, evidence of ongoing ischemia not amenable to revascularization would preclude transplantation at most centers. Likewise, significant left ventricular dysfunction (ejection fraction ≤20–25%) after optimal revascularization of severe coronary disease is associated with high mortality and may preclude transplantation. Alternatively, severe impairment of left ventricular systolic function in the absence of coronary disease is almost considered an indication for transplantation, with many reported cases in the adult and pediatric populations of remarkable improvement after restoration of renal function. Pulmonary hypertension is common in the dialysis population, seeming to worsen with duration of time on dialysis. Recent data have associated severe elevations [right ventricular systolic pressure, (RVSP) ≥50 mmHg] with increased risk of allograft failure and patient death [77]. Although these data have not been widely confirmed, some now see elevated RVSP as a relative contraindication to transplantation. Finally, severe valvular disease (critical aortic stenosis, symptomatic mitral stenosis) should be treated prior to transplantation [78].

Weight

Both severely underweight and overweight transplant candidates experience excess mortality rates on the waiting list and more complications after transplantation, though obesity garners significantly more attention in the evaluation process [79]. Even in the early 1970s, obesity was considered a contraindication to transplantation; at most centers, it remains one [80]. What has changed is the prevalence of obesity in the general and ESRD populations. Obesity increases the risk of CKD; recent National Health and Nutrition Examination Survey (NHANES) data indicate 35% of the US population is obese or morbidly obese [body mass index (BMI) ≥30 kg/m^2], and BMI at initiation of RRT is likewise increasing. Over 20% of kidney recipients have a BMI above 30 kg/m^2 [81].

Evidence indicates that increasing BMI is associated with increased risk of graft failure and mortality after transplantation. Risk is greatest for those with a BMI $\geq$36 kg/m^2, with increased risk for delayed graft function, acute rejection, wound infections, and major coronary events all contributing factors [82–84]. Nonetheless, for an ESRD patient with a BMI over 35 kg/m^2 or even over 40 kg/m^2, mortality and morbidity associated with kidney transplantation is lower than that associated with maintenance dialysis [81]. Thus, some choose to proceed with transplantation even in morbidly obese patients under certain circumstances. Most centers, however, ask patients to lose sufficient weight to result in a BMI of <36–38 kg/m^2, though a provocative study indicated little or no benefit on outcomes for obese patients who actually lose weight as prescribed pretransplant [79]. For the morbidly obese candidate, bariatric surgery may offer some benefit, although the risk and benefit of this operation in the ESRD population is not well established.

Age

Older age, if a concern at all, is at most a relative contraindication to transplantation. As noted above, there is substantial survival benefit of transplantation over dialysis at any age. Risk of complications increases with age and time spent on dialysis awaiting transplantation. In the US expanded criteria donor (ECD), and in some parts of Europe, Eurotransplant "old-for-old", allocation attempt to mitigate time on the list by offering kidneys from older donors preferentially to older candidates [85,86]. ECD kidneys in recipients over the age of 65 years have a 55% 5-year graft survival versus 64% for non-ECD kidneys from deceased donors, indicating relatively slight risk in this population. However, the attraction of this approach is limited by the number of potential candidates for ECD kidneys (35 000), and the number of ECD kidneys available each year (<1500) [16]. It also should be noted that recipients over 65 years demonstrate 77% graft survival at 5 years with kidneys from living donors. Given the length of the waiting list in many areas for both ECD and non-ECD kidneys, some offer transplantation to candidates over 70 years of age only if a suitable living donor can be identified.

HIV infection

Based on a presupposition that patients infected with HIV are already immunosuppressed and would not tolerate transplant protocols, HIV infection a decade ago was considered a contraindication to transplantation [87]. Now, HIV disease is considered a manageable chronic illness with extended survival due to modern antiviral therapy. Since HIV can cause kidney failure, the number of HIV-infected patients with ESRD is increasing. Though immunosuppressant management is challenging, with drug–drug interactions common, ESRD patients with HIV demonstrate better survival with transplantation than dialysis and are now offered transplantation at many centers [44].

Hepatitis B and C

Assessing kidney transplant candidacy in patients with exposure to viral hepatitis has become less demanding in recent years even as HBV infection has become less common and HCV almost epidemic. Ultimately, the goal in both scenarios is to avoid transplantation in patients with advanced hepatic fibrosis or cirrhosis unless the patient is considered a candidate for combined liver/kidney transplantation. Standard recommendations are that patients should undergo liver biopsy as part of the evaluation process if not already done [42]. Such an approach would allow identification of the patient with advanced fibrosis/cirrhosis, and implementation of appropriate therapies for chronic active hepatitis.

Routine liver biopsy is less uniformly practiced than before, for several reasons. First, hepatic fibrosis severe enough to preclude kidney transplantation is likely to be clinically evident based on imaging, physical findings, and laboratory abnormalities. If not, with liver allocation according to Model for End-Stage Liver Disease (MELD) criteria, such patients will be unlikely to receive enough priority to undergo transplantation. Second, therapy for HBV is now standardized, effective, and easily administered in the post-transplant setting, with little or no impact of HBV serostatus on outcomes [43]. Alternatively, therapy for HCV remains toxic and relatively ineffective when administered to patients with advanced CKD, and is contraindicated after transplantation; thus, with current therapies, treatment of HCV is not a major consideration for most transplant candidates. If treatment of HCV is desired, it should be administered before the transplant occurs [88]. Finally, as in other situations, survival of transplanted ESRD patients with either HBV or HCV exceeds their projected survival on dialysis, without evidence of worsening viral illness, again indicating transplantation to be the preferred option. Current practice at the University of Alabama at Birmingham in infected candidates is to monitor viral loads and hepatic enzymes, synthetic function, and serial imaging of the liver for changes indicative of worsening fibrosis or tumor development, and otherwise care for the patient as indicated.

Special considerations

Type 1 diabetes mellitus

Forty years ago, type 1 diabetes mellitus was considered an absolute contraindication to both transplantation and dialysis. Now, diabetes is the most common cause of ESRD, and young type 1 diabetics are a patient group that derives the greatest relative benefit from transplantation versus dialysis [12]. This finding is the consequence not of superior transplant outcomes among diabetic recipients, but of excess dialysis-related mortality in this population. Thus, in the current era, kidney transplantation is the preferred therapy for most type 1 diabetics with kidney failure, with pre-emptive transplantation of particular benefit [15].

A key aspect of evaluating the CKD patient with type 1 diabetes is choosing among approaches to transplantation (also see Chapters 32 and 41, which cover indications for the various types of pancreas transplants and the management of pancreas transplant candidates on the waiting list, respectively). In those undergoing kidney transplantation alone, there is substantial risk of recurrent diabetic nephropathy compromising long-term allograft survival, particularly beyond 10 years [89]. A kidney from a living donor offers at least two benefits over one from a deceased donor: greater average longevity of the allograft (51% vs. 36% 10-year allograft survival) and avoidance of lengthy waits on the list with correspondingly longer time on dialysis [16]. Outcomes with simultaneous pancreas–kidney (SPK) transplantation are excellent, with evidence that a successful pancreas transplant reduces long-term mortality and prevents progression of microvascular complications. However, waiting times of 2–3 years or longer are common for SPK transplantation, and long-term kidney graft survival is only marginally better than for living donor kidney transplantation alone (63% versus 51% at 10 years). For those who choose to undergo a living donor kidney transplant followed by pancreas-after-kidney (PAK)

transplantation, pancreas graft survival is inferior to that following SPK. However, there has been a steady improvement in pancreas graft survival over the past decade, with 55% 5-year survival (compared to 72% after SPK) [90].

How does one choose among these alternatives? In general, the greatest threat to the well-being of a type 1 diabetic with ESRD is time on dialysis. Thus, for the candidate with an identified living donor allowing pre-emptive or early transplantation, this may be the best choice, both for the candidate as well as all others awaiting a suitable organ. PAK transplantation can then be offered to the successful recipient who desires the advantages of a pancreas transplant in terms of glycemic control, often as soon as 2 months after the kidney. Risk factors for poorer outcome in PAK, including GFR <45 mL/min, interval from kidney to pancreas transplant >1 year, history of kidney rejection, and proteinuria, should be considered when planning for the subsequent pancreas transplant [91].

For the candidate with no living donor, SPK is probably the preferred choice: outcomes are better and time on the waiting list perhaps shorter than for a kidney transplant alone. It is not unreasonable, however, for the type 1 diabetic to demur on the living donor possibility in the hope of achieving the benefits of SPK transplantation, assuming he/she recognizes the trade-off of additional time on dialysis. Projected waiting time for SPK at the transplant center must be considered when choosing this option over a living donor kidney.

Patient with multiorgan failure

Kidney failure is accompanied by failure of a second organ in two principal circumstances. The first is that of renal insufficiency occurring against a backdrop of cardiac or hepatic failure in a patient considered a candidate for heart or liver transplantation. In 2009, 444 such transplants occurred (4% of all deceased donor kidneys transplanted), 82% of which were liver/kidney and 10% heart/kidney transplants [16]. The second is development of ESRD in a patient with a functional heart, lung, liver, or intestinal transplant. Such patients accounted for fewer than 1% of waitlisted candidates in 1995, but now comprise over 3% of the waiting list and account for 2% of recipients of deceased donor kidneys [92].

Assessing a patient with cardiorenal or hepatorenal syndromes for dual organ transplantation can be very difficult due to the high frequency of reversible renal dysfunction in severely ill patients. Even patients requiring dialysis may recover substantial function after receiving a healthy heart or liver. Conversely, outcomes of the primary transplant may be threatened by ongoing renal insufficiency. Typically, decision-making is influenced by the severity and longevity of renal dysfunction [93]. A recent consensus conference concluded that combined liver–kidney transplantation was indicated if:

1. ESRD with cirrhosis and symptomatic portal hypertension or hepatic vein wedge pressure gradient ≥ 10 mmHg;
2. liver failure and CKD with GFR ≤ 30 mL/min;
3. acute kidney injury or hepatorenal syndrome with creatinine ≥ 2.0 mg/dL and dialysis ≥ 8 weeks;
4. liver failure and CKD and biopsy demonstrating $>30\%$ glomerulosclerosis or 30% fibrosis [94].

For patients with end-stage cardiac failure, there is no consensus on how to address renal insufficiency. However, a recent analysis of UNOS data indicated that heart transplant candidates with an eGFR of ≤ 30 mL/min fared very poorly without the combined transplant [95].

In the patient with a successful solid organ transplant who subsequently develops ESRD, data indicate substantially poorer survival on maintenance dialysis than with kidney transplantation, perhaps reflecting the challenge of achieving optimal immunosuppression in such patients. Such patients are more likely than others to be listed pre-emptively, and principles of candidate evaluation mirror those in more standard situations.

Summary

Kidney transplantation remains the best option for most patients with ESRD. However, to achieve a result that substantially improves upon that achieved through dialysis, patient selection and preparation are critical. Optimal results are achieved when co-morbidities have been recognized and mollified, the patient has established realistic expectations for the procedure and the requirements for drug adherence, and contraindications have been sought and excluded. As a suitable donor organ remains a limiting resource, proper patient selection remains one of the most critical aspects of the transplant procedure, and a responsibility of all physicians caring for the patient with irreparable kidney failure.

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CHAPTER 29

Patient Selection and Indications for Liver Transplantation

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Introduction

The selection of patients for liver transplantation is guided by the overall medical philosophy of benefit to risk. At the very heart of every determination by the medical team is the weighing of overall benefit of transplantation to the patient while estimating risk to the individual. This often proves to be a challenging task for several reasons. The first is that we have limited data on individual outcomes for particular diseases. We have general data, which may apply to groups of patients with specific diseases but fail to be adequately specific for an individual patient. Second, the data that exist are insufficiently granular to understand the impact of multiple co-morbid conditions, such as is the case with an elderly patient with hepatitis C, renal insufficiency, diabetes, and a history of coronary disease. Although we may have some data with regards to each individual medical issue, their interactions are poorly understood. Lastly, we select patients with our best understanding of their individual benefit-to-risk ratio. However, the supply of organs for transplantation is limited. Therefore, our choice of any individual patient has implications for other patients waiting for or in need of transplantation.

To bring the later issue of population impact into focus, Organ Procurement and Transplantation Network (OPTN) data accessed on December 16, 2011 showed that of 16 148 unique candidates waiting for liver transplantation, only 4734 candidates had received liver transplantation as of that date [1]. In fact, the total number of liver transplants performed on a yearly basis over the last several years has been declining yearly from a peak of 6651 in 2006 to 4734 in December 2011 which was similar to the number performed in 1999. Therefore, when we select individuals who require liver transplantation, we do so with the understanding that the need of all patients in the US for liver transplantation continues to increase while supply appears to be decreasing. Indeed, these issues have become the domain of regulation that should be understood when selecting patients for the waitlist.

This chapter will cover the general indications and regulations at play when considering an adult patient for liver transplantation. Pediatric-specific issues will be covered in more depth in Chapter 114. The current chapter serves as a companion to Chapter 38, which deals with waitlist management prior to liver transplantation.

Indications for liver transplantation

General considerations

Currently, UNOS has seven liver diagnosis categories and 72 listed liver diagnoses as indications for liver transplantation [1]. In general, the beneficial impact of liver transplantation is primarily on the liver. Indeed, many other diseases that complicate the health of humans are made acutely worse by the process of transplantation and the treatment prescribed following the procedure. Exceptions exist, such as renal failure in hepatorenal syndrome; regardless, the procedure is formidable and takes its toll on the recipient. Therefore, we return to the basic assessment of benefit versus risk.

The major indications and contraindications for liver transplantation are listed in Table 29.1. In general, most patients with cirrhosis and acute liver failure (ALF) are deemed to be suitable candidates for evaluation when the risk of death with transplant is less than the risk of death without transplant. For these patients, liver failure is the issue and transplantation replaces liver function and prevents death from liver failure. In other categories, the indications may be more concerned with lifestyle or the prevention of complications from existing liver disease. For these patients, the outcome is based more upon improvement in health or reduction in future risk and less on overall survival probability.

Prior to the current allocation system in the US, the Model for End-stage Liver Disease (MELD) allocation system, livers were allocated based primarily upon the degree of liver dysfunction in combination with the length of time a patient had been waiting. To better understand and clarify listing for transplantation, a national conference was convened by the American Society of Transplant Physicians and the American Association for the Study of Liver Diseases in 1997 [2]. One-year survival in patients undergoing liver transplantation at that time approximated 90%. Therefore, the conference recommended listing a patient for liver transplant when the anticipated 1-year survival without transplant was 90% or less. Decompensated cirrhosis (defined as the presence of jaundice, encephalopathy, variceal bleeding, or ascites) is associated with 1-year survival of 70–75% [3]. With median survival in medically treated cirrhotics decreasing from 6.4 years in Child's A patients to 2 months in Child's C patients [4], the authors recommended listing for transplantation at a Child–Pugh score of $\geq$7. In patients with advanced liver disease and decompensation, the trigger for

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Table 29.1. Indications and contraindications for liver transplantation in adults

Indications
Acute liver Failure
• Virus
• Drug/medication
• Ischemia
• Wilson's disease
• Autoimmune
• Idiopathic
Cirrhosis with decompensation
• Hepatitis B or C
• Alcohol
• Non-alcoholic steatohepatitis
• Cholestatic disease (primary biliary cirrhosis, primary sclerosing cholangitis, secondary biliary cirrhosis,vanishing bile duct)
• Autoimmune
• Metabolic (alpha-1 antitrypsin deficiency, hemochromatosis, Wilson's disease)
• Malignancy (hepatocellular carcinoma, cholangiocarcinoma)
• Cryptogenic
Systemic complications of liver disease
• Hepatopulmonary syndrome
Benign liver conditions
• Polycystic liver disease
• Chronic Budd–Chiari
Metabolic conditions with systemic disease
• Primary oxaluria
• Familial amyloidosis
• Urea cycle deficiencies
• Glycogen storage disease
Retransplantation
• Primary non-function
• Recurrent disease with graft dysfunction
• Vascular complications
• Biliary complications
Contraindications
• Extrahepatic malignancy
• Hepatocellular cancer exceeding Milan or UCSF criteria
• Active substance or alcohol abuse
• Severe cardiopulmonary disease
• Inadequate social or financial support
• Poorly controlled psychiatric illness
• Anatomic abnormalities precluding adequate surgical reconstruction
• Poorly controlled HIV
• Non-compliance with medical recommendations

consideration of transplantation becomes risk of death without transplantation.

For patients without progressive liver failure, the need for transplantation may be based more upon risk of, for example, progressive disease (cancer), complications from the intrinsic liver abnormality (hereditary oxalosis, familial amyloid polyneuropathy), or improvement in health and well-being (Budd–Chiari, polycystic liver disease). Regardless of the indication, the evaluation process for each individual is designed to better inform the transplant team as to the potential for benefit as weighed against the overall risk of the procedure and recovery.

Viral hepatitis B

In the early years of liver transplantation, hepatitis B was a relative contraindication to selection for transplant. This was in large part due to the very high risk of recurrent disease, the risk of severe fibrosing cholestatic hepatitis, and increased mortality following transplant in these patients [5,6]. In 1993, a landmark paper from Europe showed that the absence of viral replication at the time of transplant and long-term immunoprophylaxis for hepatitis B virus (HBV) was associated with a reduced risk of recurrent HBV infection and reduced mortality [7].

A large study from Taiwan showed that lamivudine could significantly reduce disease progression in patients with hepatitis B over controls (7.8% vs. 17.7% over 32 months of treatment) [8]. In patients with evidence of hepatic decompensation and HBV replication, both lamivudine and adefovir have been shown to improve liver tests and reverse some clinical signs of liver failure [9–12]. A major concern with long-term lamivudine therapy has been the high rates of resistance (70% after 5 years of treatment) [13] and the potential for virologic breakthrough leading to alanine aminotransferase (ALT) flares and further decompensation [14]. More potent nucleos(t)ide analogs (entecavir and tenofovir) have been Food and Drug Administration (FDA) approved for the treatment of hepatitis B. Shim et al. [15] treated 70 patients with decompensated liver disease due to HBV with entecavir. The authors found that reduction in HBV replication and clearance of e-antigen were similar to those in treated patients with compensated disease and that entecavir improved hepatic reserve in patients with decompensated disease. No evidence of resistance or virologic breakthrough was observed during the study period. These studies and others have led to the recommendation from all three major liver societies to consider antiviral treatment in decompensated cirrhosis regardless of HBV DNA levels [16–18].

It would stand to reason that, given the availability of potent oral antiviral agents for HBV with proven efficacy and the recommendation to treat patients with cirrhosis, fewer patients with hepatitis B and decompensation would be presenting for transplantation. In fact, since 1990 the percentage of patients undergoing transplantation for hepatitis B has fallen from 5.7% to 1.5% [1]. A trend toward decreasing listing for end-stage liver disease (ESLD) and an increase in listing for hepatocellular cancer (HCC) in patients with hepatitis B has been shown [19]. Overall 5-year survival for patients with hepatitis B undergoing liver transplantation has increased from 53% between 1987 and 1991 to 85–94% currently [20,21].

In contrast to earlier years, hepatitis B in association with either liver decompensation or HCC is a clear indication for liver transplantation. Management of these patients both prior to and following transplantation are discussed in Chapters 38, 78, and 82.

Viral hepatitis C

The prevalence of hepatitis C virus (HCV) in the general population is highest in those born between 1945 and 1965 [22]. Due to the slow progressive nature of the disease, some authors project the burden of chronic complications due to HCV, including the need for liver transplantation, will continue to increase in the near future [23]. The earliest studies of the outcome of orthotopic liver transplantation (OLT) for HCV reported post-transplant patient and graft survival similar to those achieved following OLT for other chronic liver diseases. These were usually single-center reports limited by small numbers and relatively short follow-up. Several large registry analyses have recently reported reduced graft and patient survivals in recipients with HCV. Forman et al. [24] reported on the UNOS database which showed 3-year patient survival was 78% in 7459 HCV-positive recipients versus 82% in 20743 HCV-negative recipients ($P = 0.0001$). Likewise, 3- and 5-year graft survivals were significantly reduced in patients undergoing OLT with HCV as compared with non–HCV-infected patients.

Early studies showed approximately 10–15% of patients progressed to cirrhosis within 5 years of OLT. Later studies showed a significant increase in cirrhotic patients at 5 years, up to 40%,

leading many to suggest that HCV in recent years has become more aggressive in transplant recipients. It is hypothesized that HCV appears to be associated with worse results in recent years because of the more routine use of liver biopsies, stronger immunosuppressive agents, rapid steroid withdrawal, and increasing donor age [24]. Berenguer et al. have shown that patients with HCV have experienced a worsening of graft survival over time as compared to patients without HCV in whom graft survivals have consistently improved [25]. These data suggest that our change in practice may have negatively influenced HCV recurrence and/or progression.

Despite the negative impact of HCV on both morbidity and mortality following liver transplantation, it remains the most common indication for liver transplantation once patients have progressed to decompensation.

Alcoholic liver disease

In patients with advanced cirrhosis and decompensation due to alcohol, liver transplantation remains the best option for prolonged survival in appropriate candidates. Alcoholic liver disease is currently the second most common indication for liver transplantation in the US [1]. Survival (1–7 years) appears to be comparable to that in patients with other diagnoses at transplant in the setting of continued abstinence [26,27]. Overall indications for liver transplantation remain the same as in other forms of liver disease. However, the risk of recidivism causes additional concern with respect to patient evaluation.

Despite stringent selection criteria, rates of recidivism have varied widely in this patient group, likely in part due to the definition of relapse (any use vs. binge drinking for example) [28–30]. In one of the few prospective studies on recidivism, return to alcohol use at any level was reported in 22% of patients by 1 year post transplant [30]. Furthermore, at 5 years post transplant, 42% reported some use of alcohol, of whom 26% had a pattern consistent with harmful use.

Therefore, a major focus in the evaluation of patients with alcoholic liver disease remains the prediction of risk for recidivism. Several potential predictors have been studied, including family history, degree of social support, associated psychiatric diagnoses, prior relapse from treatment, and non-alcohol substance abuse. Results from studies have varied widely, making it difficult to use these predictors as absolute guides to selecting patients for transplantation. A meta-analysis of a large number of studies from 2008 showed a poor social support system, family history of alcohol abuse, and pretransplant abstinence of 6 months or less were significant predictors of recidivism [31]. Given the current limitations in the literature and the significant ramifications when patients are not selected, most centers have adopted a standard of abstinence (6 months or greater) as the major defining factor for selection in this patient group.

Metabolic syndrome

Metabolic syndrome has become increasingly prevalent in the US. Although the frequency of non-alcoholic fatty liver disease (NAFLD) and the incidence of cirrhosis in patients with NAFLD is not entirely clear, the frequency of NAFLD as an indication for liver transplantation continues to increase and is currently the third most common in the US [1]. The prevalence of obesity in the US is currently estimated to be 28.7% in men and 34.1% in women, with an increasing number of individuals moving into the most severe obesity categories (BMI 40–50 kg/m^2) [32,33]. These increases are likely to be mirrored by increases in NAFLD and non-alcoholic steatohepatitis (NASH); however, the rate at which these diseases progress to cirrhosis and liver decompensation is not clear. A review of the UNOS database showed that from 1988 to 1996, 16.8% of liver recipients had a BMI of $>30\,kg/m^2$, with 5.3% of these having a BMI of $>35\,kg/m^2$ and 2.1% a BMI of $>40\,kg/m^2$ [34]. Furthermore, from 2001 to 2004, 32.5% of liver recipients were obese with 8.4% of these patients were classified as severely obese and 3.2% as morbidly obese [35].

In a comprehensive analysis of the Scientific Registry of Transplant Recipients (SRTR) database, Charlton et al. [36] found that the frequency of NASH as a primary or secondary indication for liver transplantation is steadily increasing (1.2% in 2001 to 9.7% in 2009). Furthermore, several authors have suggested that the true frequency of NASH as an indication for liver transplantation may be underestimated as steatosis may be absent as cirrhosis develops. Phenotypically, patients with a pretransplant diagnosis of cryptogenic cirrhosis are quite similar to patients with cirrhosis due to NASH, making the classification of these patients suspect [37,38].

In selecting patients with metabolic syndrome or obesity for transplantation, one needs to take into consideration both the potential for recurrence of disease as well as the impact on patient and graft survival. NASH is well documented to recur following liver transplantation [39,40]. However, in the analysis of the SRTR database, Charlton et al. found that 3-year patient and graft survivals for recipients with a primary or secondary indication of NASH were similar to those for recipients with other indications [36]. Several authors have reported recurrent NASH-related cirrhosis and graft loss at 2.5–5%, with overall outcomes reported to be comparable to those for other indications [41,42].

In patients who are obese (BMI 30–35 kg/m^2) with NAFLD, short-term (30-day) mortality may be increased, with longer-term (1- and 3-year) mortality being similar to that in patients without NAFLD [43]. In patients with morbid obesity (BMI $>35\,kg/m^2$), 1- and 2-year mortality and primary non-function are worse as compared with other patients [44]. A review of the UNOS database from 1987 through 2007 found that patients who were very severely obese (BMI $>40\,kg/m^2$) had a higher number of infectious complications and cancer events ($P = 0.02$) leading to death as compared with control patients. In three different eras of liver transplantation, multivariable analysis showed that being severely underweight (BMI $<18.5\,kg/m^2$) or severely obese (BMI $>40\,kg/m^2$) were significant predictors of death [45]. In addition, many of these patients have associated diabetes mellitus, which has been shown to have a significant negative impact on survival in liver transplantation [46]. A case–control study that focused on the impact of diabetes in relation to transplantation found that patients with diabetes were 40% more likely to die within 5 years as compared with non-diabetics [47].

For patients with metabolic syndrome or obesity, both the impact of recurrent disease in the graft as well as the impact of associated co-morbidities on patient survival must be considered when selecting patients for transplantation.

Immune liver disease

Primary biliary cirrhosis

In a relatively recent study looking at trends in transplantation for primary biliary cirrhosis (PBC), the authors found that from 1995 to 2007, the absolute number of transplants that were performed for this diagnosis decreased by an average of 5.4 cases per year despite a steady rise in overall liver transplants [48]. The primary indication for transplantation in PBC remains the development of clinical

features of hepatic decompensation, which occurs in up to 26% of patients within 10 years of diagnosis [49]. The indication for intractable pruritus or debilitating fatigue is perhaps more specific to the cholestatic disorders [50]. Although pruritus is often treated with ursodeoxycholic acid, some studies have failed to show a significant effect in alleviating pruritus in these patients [51]. For those who fail to respond to pharmacologic therapy, transplantation may be the only option to improve their quality of life. Furthermore, chronic fatigue is reported in up to 80% of patients with PBC [52]. Although some patients without frank evidence of decompensation have been transplanted more for quality of life issues such as fatigue, one must be aware that fatigue has not been shown to consistently improve with liver transplantation in these patients [53].

Ideally, one would be able to identify patients as potential candidates on the basis that their 1-year survival with transplant would be higher than their 1-year survival with medical care alone. Traditionally, survival for patients with cholestatic liver disease has been the highest among all groups undergoing liver transplantation [1]. For most patients, the optimal benefit to risk of transplant occurs when they have reached a Child's B score or higher or a MELD score of $\geq$15. Serum bilirubin appears to be predictive of outcome in PBC and some authors have used an absolute value of 5.9 mg/dL or greater as an indication for consideration of liver transplantation [54]. Other authors have used a combination of factors, including bilirubin, albumin, prothrombin time, age, edema, and diuretic use (Mayo risk score), to identify patients with PBC in need of liver transplantation [55]. Centers may opt to use one or more of these tools in identifying those patients who may benefit from transplant.

Despite recurrence in up to a quarter of patients with PBC following liver transplantation, overall patient and graft survivals in this group are excellent [1]

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) typically presents in men under the age of 50 years. Although it may be a slowly progressive disease in many patients, liver transplantation should be a consideration in those patients who progress to decompensation or have complications related to biliary strictures such as recurrent cholangitis. Similar to the Mayo risk score for PBC, prognostic models have been developed to assist in determining timing of transplantation for PSC [56]. The Mayo risk score for PSC is based on age, serum bilirubin, serum albumin, and history of variceal bleeding [56]. MELD score has not been directly assessed against the Mayo risk score for either PBC or PSC, but does appear to remain valid regardless of disease etiology for patients [57].

Unique to PSC is the presence of biliary strictures, which can lead to additional complications, including accelerated jaundice, obstruction, and infection. In addition, patients are at significant increased risk of cholangiocarcinoma (see later) [58]. Patients may suffer from recurrent cholangitis requiring antibiotics and hospitalization. In some patients, this may occur with MELD scores of $<$15. For these patients, submission to a regional review board for additional points would be appropriate given the risk of sepsis and complications in patients with recurrent cholangitis. Although pruritus may be present in patients with disease, the indication for transplantation based upon pruritus alone would require careful consideration.

Although the disease may recur in up to 10–25% of recipients, outcomes for PSC, are excellent and similar to those for PBC [1,59,60].

Autoimmune hepatitis

The primary indication for liver transplantation in autoimmune hepatitis is evidence of hepatic decompensation. Many of these patients are likely to have been exposed to immunosuppression prior to transplantation. Therefore, a careful review of medications prior to transplantation is essential to assess potential risk. Metabolic complications from steroids may be increased in this population and should be reviewed as part of the overall evaluation prior to selection (see Evaluation).

Autoimmune hepatitis has been reported to recur in approximately 23% of patients following liver transplantation [61] and may lead to decompensation requiring retransplantation [62]. Similar to other immune diseases, although recurrence may occur following liver transplantation, outcomes for autoimmune hepatitis are excellent [1,61].

Inherited liver disease

Liver disease due to alpha-1 antitrypsin deficiency may present as cirrhosis in either childhood or adulthood [63]. Approximately 15% of patients with the PiZZ phenotype will develop liver disease and these patients are at increased risk of developing HCC [64]. There is no effective treatment for alpha-1 disease in the liver. In patients with liver involvement who develop evidence of decompensation, liver transplantation is the only therapeutic option. Because of the possible associated pulmonary involvement with the disease, patients considered for liver transplantation should undergo a thorough pulmonary evaluation. Overall survivals in patients who are transplanted for alpha-1 antitrypsin deficiency are excellent, with 1- and 3-year survivals of 88% and 84%, respectively [65].

Although the gene frequency for hemochromatosis is approximately 1 in 200, the need for transplantation in patients with this disorder is relatively uncommon [66]. Early detection and iron depletion can delay/prevent complications of advanced liver disease [67]. Patients with hemochromatosis and cirrhosis have an increased risk of HCC [68]. In patients with cirrhosis and decompensation, liver transplantation remains the only treatment. However, several authors have reported reduced survivals in patients undergoing liver transplantation for hemochromatosis [69,70]. Increased cardiovascular complications, infections, and recurrence of HCC in these patients likely reflect increased iron deposition in organs outside the liver [70]. In patients being considered for transplantation, thorough cardiovascular assessment and consideration of iron depletion should be made as part of the patient evaluation in this disease [71].

Patients with Wilson's disease may present with either cirrhosis and decompensation or ALF as an indication for transplantation. Oral or parenteral therapy to target copper depletion can result in long-term remission of the disease in some patients [72]. In patients with decompensated liver disease due to Wilson's disease or in patients presenting with ALF, liver transplantation is the best option. Following liver transplantation, most associated metabolic changes reverse, with the exception of severe neurologic dysfunction [73]. Despite this, overall survival in these patients appears to be very good [74].

Malignancy

Hepatocellular cancer

During the National Institutes for Health (NIH) consensus development conference in Washington DC in 1982, liver transplantation was accepted as a treatment modality for patients with ESLD

and malignant tumors of the liver. A quarter of a century later, OLT has become the standard of care for all forms of ESLD, including HCC. In patients with cirrhosis and HCC, liver transplantation provides both replacement of hepatic function as well as complete surgical removal of the tumor. While early experience with OLT for cancer resulted in poor patient survival and high recurrence rates, methods of patient selection have been refined and results have improved dramatically [75].

The so-called Milan criteria were borne of a 1996 study by Mazzaferro et al., which reviewed radiologic and histologic results of patients with ESLD and HCC who received liver transplants [76]. They reported that in patients with a solitary tumor of $\leq$5 cm or no more than three tumors, each no larger than 3 cm, overall and recurrence-free survivals after transplant were 85% and 92%, respectively. Overall HCC recurrence was 8% at 4 years' follow-up. Patients who exceeded the criteria showed an actuarial survival of 50% and only 59% of surviving patients were free of recurrence. The Milan criteria are currently the standard by which the UNOS and Medicare guide selection of patients for cadaveric OLT in the US, with some variation established by regional review boards.

During the past decade, several studies have challenged the Milan criteria, reporting comparable outcomes after transplantation for more advanced stages of HCC. Yao et al. showed a 5-year survival of 70.2% in patients with HCC fulfilling so-called University of California, San Francisco (UCSF) criteria [77]. These criteria, based on explant pathology, allowed inclusion of patients with single tumors of $\leq$6.5 cm, or a maximum of three tumors of $\leq$4.5 cm, and a cumulative tumor size of $\leq$8 cm.

The Barcelona Clinic Liver Center has developed a system for treatment of HCC with OLT, based on tumor stage, liver function, physical status, and cancer-related symptoms [78]. Its emphasis is on dropout rates and intention-to-treat analyses, and its expanded criteria include one tumor of $<$7 cm, three tumors of $<$5 cm each, or five tumors of $<$3 cm each, or down-staging to conventional Milan criteria with pretransplant adjuvant therapies. Using this expanded approach, the Barcelona group has achieved 5-year post-transplant survival in excess of 50%, versus 20% for palliative treatment alone.

Studies that followed appear to support the UCSF and the Barcelona group criteria, and such observations led to the description of the so-called "metro ticket paradigm" by Mazzaferro et al. [76] using a decision analysis model. This model follows the concept that larger tumor diameter and/or increased number of tumors is related to a "higher price" in terms of HCC recurrence. Although this model has not been widely adopted, transplant physicians and surgeons recognize that both tumor size and number of tumors relate to recurrence and outcomes, and the decision to select patients for transplant in large part depends on these factors.

Cholangiocarcinoma

Today, available therapies for hilar cholangiocarcinomas (CCAs) are not satisfactory. These tumors are rare, but they bear high death rates in afflicted patients, rivaling those for pancreatic cancer. Surgical resection offers the only current hope for long-term survival, averaging only 20% in major series [79]. The reality is such that for the majority of patients, CCA is a diagnosis of despair.

Although surgery has offered the only hope of cure, one of the major limiting factors in achieving long-term survival following surgical resection of CCA is the technical ability to achieve negative resection margins. The presence of malignant cells at the surgical margins following resection is a major prognostic factor predicting recurrence and death [80]. Theoretically, the likelihood of achieving negative margins can be increased with total hepatectomy and OLT. Previous experience with OLT performed in isolation for hilar CCAs has shown that this practice has met with various success. However, a protocol developed at the Mayo Clinic demonstrated that long-term survival can occur in carefully selected patients by combining the benefits of radiotherapy, chemosensitization, and OLT [81]. With this strategy, patient survival rates of 92%, 82%, and 82% at 1, 3, and 5 years after transplantation, respectively, have been achieved.

Selection criteria used in consideration of liver transplantation for hilar CCA that is not amenable to surgical resection require accurate assessment of potential extrahepatic disease, including lymphatic spread. Contrast computed tomography (CT) is sensitive for detecting intrahepatic bile duct tumors, the level of biliary obstruction, and the presence of liver atrophy. In addition, CT may also permit visualization of the pertinent nodal basins [82], and dynamic CT may provide more information regarding resectability than magnetic resonance imaging (MRI). While both imaging methods have similar abilities to show tumor enhancement and biliary ductal dilatation, the relationship of the tumor to the vessels and surrounding organs is more easily evaluated using CT.

A significant number of patients have peritoneal implants or locoregional lymph node involvement that are not easily detected on preoperative imaging studies. At centers with expertise, endoscopic ultrasound may be useful to determine the local extent of the tumor and to detect local lymphatic involvement, especially for distal lesions. In addition, diagnostic laparoscopy helps identify many of these patients before committing them to a laparotomy [83]. A study that examined the role of laparoscopy in the staging of hepatobiliary and pancreatic neoplasms detected unknown metastases in 30% of patients [84]. In addition, laparoscopy offers the opportunity for intraoperative hepatic ultrasound, which may be useful for the detection of occult intrahepatic metastases. Ultimately, however, true resectability and/or candidacy for transplant cannot be determined until a complete abdominal exploration has been performed.

Liver transplantation for CCA is controversial and, because of the high recurrence rate published by most authors, many centers have abandoned this as an indication for liver transplantation. The most recent review of 207 patients who underwent liver transplantation for CCA reported 1-, 2-, and 5-year survival rates of 72%, 48%, and 23%, respectively, but $>$50% of patients had recurrence within 2 years [85]. A second review, with a 30% 3-year survival rate, reported that small tumor size and a single tumor focus are positive prognostic indicators [86].

Given these data, the use of liver transplantation for the treatment of CCA should be reserved for very select patients as a part of research protocols. As more effective adjuvant and neoadjuvant protocols are developed, transplantation may be a more useful treatment for this disease. This is suggested by the results from De Vreede et al. in which highly selected patients with stage I and II hilar CCA underwent neoadjuvant external beam radiation, systemic 5-fluorouracil (FU) therapy, and brachytherapy prior to liver transplantation [81]. Survival times of $>$36 months were reported for seven of 11 transplanted patients, and eight of 11 patients had no tumor recurrence with a median follow-up of 44 months. A similar study using neoadjuvant chemoradiation therapy for highly selected patients with stage I–IIIa hilar CCA reported a 45% survival rate (5 of 11) at a median follow-up of 7.5 years, but two patients died from tumor recurrence [87]. These studies

demonstrate that early-stage CCA may be an indication for liver transplantation as part of a research protocol.

Benign liver conditions

Not all patients presenting for liver transplantation will have hepatic dysfunction. In patients with benign conditions (those that are neither malignant or the result of chronic disease), the decision to consider liver transplantation depends on the probability that patient symptoms will be improved with transplant.

Budd–Chiari syndrome (BCS) results from obstruction of the hepatic venous outflow. This results in centrilobular congestion, necrosis, and fibrosis, and may result in cirrhosis [88]. The degree of liver damage and dysfunction in large part drives the decision regarding transplantation. Patients with acute BCS may have severe hepatic necrosis from which the liver may not recover. In addition, the chronic outflow obstruction may lead to fibrosis and cirrhosis and in these individuals with hepatic insufficiency liver transplantation may be considered [89]. The underlying etiology of thrombosis is rarely reversed with transplantation and long-term anticoagulation is required following transplant. Myeloproliferative disorders have been found to be a frequent cause of BCS in the US [90]. Consideration of patient candidacy for long-term anticoagulation would be an essential part of the selection of candidates with BCS for liver transplantation [91]. The 1-, 3-, and 5-year graft and patient survivals appear to be comparable in BCS patients and non-BCS patients undergoing OLT [92]. Therefore, liver transplantation should be considered in patients with BCS in whom alternate therapies, including medical, surgical and radiologic, have failed or in whom liver decompensation has occurred.

Liver transplantation remains the only curative therapeutic option for patients with polycystic liver disease (PCD). Indications for transplantation in this group depend primarily on symptoms [93]. In patients in whom pain, poor nutrition, dyspnea, fatigue, or recurrent cystic infection occurs, liver transplantation may be considered to improve overall function of the patient and quality of life [94]. Survival in this group of patients is excellent, with 5-year survival of approximately 92%. Consideration of combining liver transplantation with kidney transplantation should be made on the basis of overall symptoms related to liver size. Not all patients considered for kidney transplant will have liver involvement and only a select few of those with liver involvement will be symptomatic and expected to benefit from combined kidney and liver transplant [95].

Acute liver failure

Altered mental status (hepatic encephalopathy) and coagulopathy in the setting of acute hepatic decompensation without evidence of prior hepatic disease define ALF. The term "fulminant hepatic failure" (FHF) is generally applied to patients in whom hepatic encephalopathy develops within 8 weeks of the onset of illness, whereas "subfulminant hepatic failure" is used to describe a minority of patients in whom hepatic encephalopathy develops after a longer illness of up to 26 weeks in duration (also called late-onset hepatic failure) [96]. ALF is used as the designation to encompass all of these clinical presentations. It is a potentially devastating syndrome that may manifest with increasing cerebral edema, hemodynamic instability, renal failure, profound metabolic disturbances, and a particular susceptibility to fungal and bacterial infections, as well as coagulopathy.

ALF is uncommon but not rare; approximately 2000 cases occur annually in the US, with a mortality approaching 80%. It accounts for approximately 6% of transplants in the US [97].. In most instances, massive necrosis of hepatocytes occurs; however, hepatocellular failure without necrosis is characteristic of fatty liver of pregnancy and Reye's syndrome, suggesting that the actual death of cells is not a universal or essential feature. Regardless of the inciting event, the typical pathologic picture is that of coagulative necrosis throughout the hepatic lobule.

Viral hepatitis was previously reported to be the most common cause of ALF in the US, but acetaminophen overdose and idiosyncratic drug reactions have emerged as the most frequent causes in recent studies [96]. Malignancy is an uncommon cause of ALF, and thus imaging studies may not be useful in this setting, but liver biopsy may be beneficial in selected cases. Other less common causes of FHF include drug reactions (e.g. certain antibiotics and antiretroviral agents), autoimmune hepatitis, pregnancy-related disorders such as acute fatty liver of pregnancy, the HELLP (hemolysis, elevated liver enzymes, and low platelet) syndrome, BCS, Wilson's disease, and ingestion of certain toxins such as *Bacillus cereus* toxin or *Aminita phalloides* poisoning.

Liver transplantation has emerged as a life-saving procedure leading to marked improvement in survival rates. Although select patients with ALF may recover spontaneously, those with subfulminant hepatic failure have 100% mortality without transplantation [96]. Improved surgical techniques, immunosuppression, and comprehensive care have led to an overall 1-, 5- and 10-year survival rate of approximately 65%, 59%, and 54%, respectively, with liver transplant [1].. Those patients who survive beyond the first 3 months follow a survival curve comparable to that seen after elective OLT for cirrhosis [97]. Appropriate selection of FHF patients, determining which patients may or may not survive with medical support alone, remains perhaps the most daunting aspect of caring for this subpopulation of potential liver transplant patients. Further refinement of selection criteria, intensive care support, and perioperative care will hopefully continue to improve clinical results. An emerging technology for this condition is the use of hepatic assist devices. This is covered in Chapter 47.

Previous graft failure

Patients undergoing liver transplantation may have graft failure due to technical complications, primary non-function of the graft, or recurrent disease leading to decompensation. Approximately 10% of liver transplants are performed in the USs for this indication. Consideration of retransplantation must take into account the significant reduction in both patient and graft survival as compared with first transplant [1]. Recurrent disease due to hepatitis C or severe autoimmune disease may be an additional risk in patients being considered for repeat transplantation for these diagnoses [98]. Poor prognosis appears to be related to serum bilirubin, creatinine, Child–Pugh score of 10 or more, or MELD score of >25 [99,100].

Evaluation

The evaluation process for liver transplantation is designed to gather additional information regarding patients such that overall morbidity and mortality risk versus benefit may be estimated. This ensures that selected patients can be expected to have overall benefit from the procedure. The following are considered as part of the evaluation: lack of or failed alternative treatment for the underlying condition, anticipated survival following transplant equivalent to or better than in the current literature, and acceptable rate of recurrent

Table 29.2. Evaluation

Medical/surgical
Consultations
• Hepatology
• Transplant surgery
Laboratory
• Complete blood count with platelet
• Biochemical profile including electrolytes, blood urea nitrogen (BUN), creatinine and liver profile
• Prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio (INR)
• Tumor markers alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) (CA19-9 when appropriate)
• Anti-human immunodeficiency virus (HIV)1/HIV2
• Venereal Disease Research Laboratory (VDRL) test
• Viral serologies including anti-hepatitis C virus (HCV) antibody, HBs antigen, anti-HBc antibody, anti-HBs antibody, anti-hepatitis A virus (HAV) IgG, anti-herpes simplex virus (HSV) IgG, anti-cytomegalovirus (CMV) IgG, anti-Epstein–Barr virus nuclear antigen IgG, antitoxoplasma IgG, antiherpes zoster IgG (and as appropriate in patients with acute liver failure)
• Prostate-specific antigen (PSA) in men
• Iron, total iron-binding capacity (TIBC), ferritin
• Antinuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA)
• Serum protein electrophoresis (SPEP)
• Ceruloplasm
• Alpha-1 antitrypsin
Radiologic
• Doppler ultrasound of the liver
• Computed tomography (CT) or magnetic resonance imaging (MRI) of the liver
• Chest X-ray, posteroanterior and lateral
Cardiopulmonary
• Cardiology consultation
• Dobutamine echocardiography or other non-invasive cardiac stress test
• Arterial blood cas
• Electrocardiogram (ECG)
• Pulmonary consultation in select patients
Additional testing
• Mammogram in women
• Tuberculosis skin test (PPD)
• Colonoscopy (patients aged 50 years or older or with increased risk of colon cancer)
• Pulmonary function tests (in patients with a history of smoking, symptomatic patients, or those with increased risk of pulmonary disease)
• Bone density
• Coronary angiogram in select patients
Psychosocial
• Social worker evaluation
• Chemical dependency evaluation (in patients with a prior history of substance abuse)
• Psychological screening and evaluation
Financial
• Determination of benefit coverage for transplantation and medications, as well as financial resources of patients when coverage is less than adequate

disease in the graft [101]. Evaluation of patients requires a detailed assessment of the patient's liver disease, overall medical and psychological health, infectious risk, social support, and financial resources through a series of testing and evaluations by the transplant multidisciplinary team. The components for evaluation are listed in Table 29.2.

The evaluation process is designed to answer three simple questions:

1 Is the liver disease bad enough to consider transplantation as an option? In other words, in the absence of alternative treatments for the patient's liver disease, does the benefit of transplantation outweigh the risk of transplantation? The evaluation process in part is designed to better estimate the overall risk of death with transplantation as compared with the risk of death with continued medical and symptomatic support for the patient.
2 Is the patient a suitable candidate? The transplant community recognizes that there are numerous aspects of a patient's medical and social health to be weighed when determining his/her suitability for transplantation. Excess mortality risk or decreased anticipated benefit may be present depending on patient co-morbidities. Lack of social or financial support or the inability of patients to comply with medical recommendations will likely decrease the overall success of the transplant. These findings influence the overall assessment of risk versus benefit.
3 Is the atient and family willing to accept the risk if the transplant team recommends transplant as the best option? Patients can always refuse transplantation if recommended, but they cannot demand transplant if the transplant team determines the risk to outweigh the benefit.

The evaluation process involves consultation with transplant hepatology and surgery, testing for and assessment of co-morbidities, and a thorough psychosocial evaluation (see Table 29.2). Patients undergo a battery of tests designed primarily to identify significant co-morbidities that may affect outcome. The evaluation of insurance and prescription coverage is essential to inform both patients and caregivers of the resources available to the patient. Finally, patients and families are asked to participate in a family meeting, which serves to identify social support and resources, as well as to further educate the patient and caregivers on the demands and requirements of transplantation.

Cardiopulmonary evaluation

Evolutionary improvement in the outcomes of liver transplant over the past two decades has permitted an increased willingness to accept higher risk patients, and reflects the increased experience in anesthetic, operative, and critical care of liver transplant recipients. Previous "absolute" contraindications such as severe cardiovascular or pulmonary disease have become relative contraindications. Transplant centers have established basic criteria for pretransplant evaluation of patients with cardiac and pulmonary disease in the hope of maximizing opportunity for transplantation while minimizing the risk of untoward outcomes.

Preliver transplant assessment breaks down into two main objectives: (1) individual evaluation and stratification of the associated perioperative risk in patients with co-morbidities, and (2) estimation of the long-term prognosis considering other potential extrahepatic disorders in the hope of establishing a realistic risk-to-benefit ratio. For potential living liver donor recipients, a third objective is the assessment of the added risk of the donor operation in relation to the recipient's quality of life and estimated reasonable waiting period for cadaveric liver transplant. The inherent risk of liver transplantation and the shortage of donors mandate the selection of patients who are likely to obtain a significant survival benefit from transplantation.

Cardiac evaluation

Liver transplant recipients today are older and present with more co-morbidities than 20 or 30 years ago [102]. Early studies in liver transplant recipients seemed to suggest that ESLD somehow protected patients from coronary artery disease (CAD). However, recent data point to equal, if not increased, prevalence of CAD in these patients when compared to other surgical candidates [103,104]. Some evidence points to the possibility of a cardiomy-

opathy specific to cirrhosis, of any etiology, potentially related to severe cardiovascular events in this population [105].

Estimates of cardiac events around the time of liver transplant vary between 25% and 70% [106,107], and such events can significantly alter patient and graft survival. If recurrent disease and de-novo malignancies are excluded, cardiovascular disease represents the most common cause of death in long-term liver transplant survivors [108]. Thus, the cardiac preoperative risk assessment of candidates for liver transplant has garnered much scrutiny, and is an increasingly important clinical requirement, with significant impact on postoperative outcomes. The evaluation itself is complex and details are often center specific.

The liver transplant operation, compared to other transplant as well as abdominal surgeries, can provoke severe hemodynamic changes that may unmask CAD. Although cardiac morbidity and mortality have improved compared to older studies, due to improvements in selection and management of CAD as well as in perioperative management of liver transplants, they continue to be higher than in patients without CAD.

The optimal screening strategy for liver transplant candidates is a topic of debate due to conflicting data available for non-invasive stress testing and different algorithms used in several centers. Preoperative work-up must include a careful cardiovascular history to detect symptoms and risk factors. The limited functional capacity characteristic of the ESLD population may prevent common symptoms of CAD, such as chest pain or shortness of breath that are usually provoked by exercise. Thus, nearly all CAD patients with ESLD are asymptomatic, making it necessary to detect silent heart disease in many cases.

Recommendations from the American Heart Association/American College of Cardiology for perioperative evaluation for non-cardiac surgery are generally used and adapted to preliver tranplantation cardiac evaluation strategies [109]. The use of non-invasive stress testing in a set of selected patients (criteria differ by institution) is a general first step. Unfortunately, previous studies have failed to identify a clear risk factor profile in ESLD patients, and there is no optimal risk stratification strategy [110]. In a retrospective cohort analysis of liver transplantation candidates aged over 45 years without known CAD, coronary angiography showed a 26% prevalence of moderate-to-severe CAD [111]. A diagnosis of NASH independently increases the risk of CAD [112].

Dobutamine stress echocardiography (DSE) and myocardial perfusion scintigraphy (MPS) are the two most commonly used screening methods to detect CAD in patients being evaluated for transplantation. A positive test mandates further investigation with coronary angiography or, in some centers, cardiac CT or magnetic resonance angiography (MRA) to evaluate for CAD. While abnormalities seen on DSE and MPS testing correlate well with the presence of CAD in the general population [109], the reported sensitivities and specificities in patients with ESLD are more variable, ranging from 12–100% and 57–100%, respectively [101]. DSE is the preferred test in most liver transplant centers both because it seems to be more specific than MPS and also because it provides additional information regarding overall left ventricular function and valvular disease. In both screening methods, however, care must be taken to consider specific patient population concerns, such as the frequent use of beta-blockers in cirrhotics, as well as the overall vasodilated state, which may affect response to chronotropic agents such as dobutamine, or dilating agents such as adenosine or dipyridamole [113,114].

Recent advances in cardiac CT for the detection and quantification of coronary artery calcification have improved cardiovascular risk prediction in a general population; McAvoy et al. in a recent study demonstrated a strong relationship between coronary artery calcium score and a number of known cardiovascular risk factors in the setting of liver transplantation [115]. Although the utility of using calcium scores in predicting cardiovascular events around the time of liver transplantation requires further prospective evaluation, it seems to hold promise based on results in other surgical cohorts.

If significant obstructive CAD has been identified in a pretransplant candidate, management opinions vary widely among centers. Liver transplantation must be delayed to allow revascularization or percutaneous coronary intervention, and/or the titration of appropriate medical therapy such as statins or beta-blockers [116,117]. However, it is not clear whether revascularization influences outcome relative to transplantation status. In the general population, prophylactic revascularization in the absence of significant ischemia or symptoms has not been proven superior to conventional medical therapy [118]. On the other hand, this specific population undergoing liver transplantation has the aforementioned characteristics that frequently prevent or mask the manifestation of cardiac disease, and the transplant operation itself can be considerably more stressful than is modeled by either DSE or MPS. As always, individual patients and the overall picture of physical health, co-morbidities, and data obtained by various testing methods need to be carefully considered.

Pulmonary evaluation

While cardiac testing of liver transplant candidates generally receives more attention, pre-existing pulmonary disease also has a significant impact on perioperative risk. Chronic obstructive pulmonary disease (COPD) and emphysema due to smoking or other etiologies, primary or portopulmonary hypertension, hepatopulmonary syndrome, or hepatic hydrothorax, which may be identified in cirrhotic patients, can present multiple challenges in perioperative pulmonary management. In the liver transplantation context, it is critical to establish not only the diagnosis, but also the prognosis of the potential pulmonary co-existing disease. This section discusses the most common pulmonary problems observed in ESLD patients that may impact recipient selection.

COPD is common in cirrhotic patients who undergo transplant, and it is estimated that 18% of liver transplant candidates have COPD [119,120]. Although there are no consistent data, it seems plausible that COPD patients may have diminished long-term survival after liver transplant [121]. Postoperative intensive care unit and hospital length of stay, ventilator dependence, the need for post-discharge rehabilitation, and infectious risk all may be affected in patients with COPD. In addition, continuation or resumption of smoking post liver transplant may increase the risk of de-novo malignancy [122].

Forced expiratory volume in 1 s (FEV_1) is often used to grade the severity of COPD. The BODE (body mass index, airflow obstruction, dyspnea, and exercise capacity) index, a multidimensional grading system, is superior to simple pulmonary function testing at predicting the risk of death from any cause and from respiratory causes among patients with COPD [123,124]. Accurate respiratory evaluation, including walk tests, should be mandatory in liver transplant candidates with moderate-to-severe COPD.

Ascites and pleural effusions are common in cirrhosis, and can affect oxygenation and ventilation. Hepatic hydrothorax is defined

as the presence of pleural effusion in patients with cirrhosis in the absence of primary cardiopulmonary disease [125]. It manifests in 5–12% of patients with ascites. Ascites can cause increased intra-abdominal pressure with diaphragmatic rise and resulting decrease in lung volume and chest wall compliance. Pleural effusions in ESLD patients may be exacerbated by hypoalbuminemia and azygos vein hypertension [126].

Formation of hydrothoraces has been shown to be due to passage of ascitic fluid through defects in the diaphragm directly into the pleural space. Hepatic hydrothorax will accumulate when the peritoneal fluid accumulating in the pleural cavity exceeds the pleural space's absorptive capacity. For poorly understood reasons, this accumulation of fluid is much more common in the right hemithorax. Interestingly, up to 20% of cases of hepatic hydrothorax may occur in the absence of clinically detectable abdominal ascites [126].

Liver transplant candidates who present with hepatic hydrothorax require a work-up that includes chest X-ray and CT scan of the chest with contrast, to rule out any primary pulmonary pathology that would account for the pleural effusion. It is important to evaluate the absence of mediastinal lymphadenopathy that would signal the possibility of malignant effusion. Work-up usually also includes a diagnostic thoracentesis, to confirm a sterile transudate with low protein level and cell counts. Cultures and fungal and Gram stains, as well as acid-fast bacilli stains, should also be assessed, and cytology examined to rule out malignancy. Cases in which infection or malignancy must be absolutely ruled out may require thoracoscopy with pleural biopsy. Finally, patients with ascites should also undergo diagnostic paracentesis [127].

Hepatopulmonary syndrome defines a syndrome consisting of dyspnea, hypoxia, and increased pulmonary shunting in the presence of chronic liver disease. Several reports have suggested resolution of the syndrome following liver transplant [128]. Therefore, although it is important to diagnose and manage it prior to liver transplant, hepatopulmonary syndrome should not exclude patients from selection.

Pulmonary hypertension may be present in patients with cirrhosis and may first be diagnosed at the time of transplant evaluation due to lack of symptoms. Patients with increased pulmonary pressures have increased mortality as compared with cirrhotic patients with normal pulmonary pressures. In addition, perioperative mortality is significantly increased in patients with pulmonary hypertension and would be considered a contraindication to transplant [129,130]. Patients who respond to intravenous prostaglandins may be considered candidates for liver transplant [131] as their pulmonary pressures may reduce to normal following transplantation [132].

Psychosocial evaluation

Patients considered for liver transplant require extensive psychosocial evaluation in addition to a thorough medical evaluation to best determine the relative risk and benefit of transplantation. However, there are relatively few evidence-based guidelines for pretransplant psychological and social screening [133,134]. Although formal evaluation by both psychiatrists and social workers is common in most transplant centers, observation of adherence to medications and recommendations in the pretransplant phase is a strong predictor of 1-year outcomes following liver transplant [135]. Neroticism and limited social support may negatively impact on outcomes following liver transplant, whereas active coping skills may work to improve them [136].

For patients with a history of alcohol, the "6-month" rule has become a standard approach when evaluating patients for liver transplant [137]. A meta-analysis identified 6-month abstinence as one of three pretransplant predictors of alcohol relapse following transplantation [138], although the exact time period required for abstinence has been somewhat controversial [139]. The goal of evaluation is toward identifying patients likely to maintain abstinence following transplantation. This requires evaluation by an addictive disorders specialist [140].

Patients should be screened for depression and patients with a history of past depression should be further evaluated by a clinical psychologist or psychiatrist. Mental health issues are common among patients with advanced liver disease [141] and depression may affect post-transplant psychiatric morbidity and possibly adherence [142,143]. Patients would be considered for transplant if they were found to have a stable condition that was not anticipated to interfere with their recovery. Recommendations for psychiatric follow-up during the waiting period or following transplantation would be patient specific.

Although ongoing use of addictive drugs is considered a contraindication to liver transplant in the majority of transplant centers [144], a recent survey of transplant centers found that 56% of the responding programs accepted patients in methadone maintenance programs [145]. However, the same survey found 32% of programs required discontinuation of methadone prior to listing. Several studies have found that patients on methadone replacement can undergo successful liver transplant with similar outcomes to those patients not on methadone replacement for prior addiction [146]. Therefore, it is imperative that centers develop policies regarding the selection of patients with prior addiction.

Infectious disease evaluation

The pretransplant infectious disease evaluation of potential liver transplant candidates should include immunization history, history of prior peritonitis or cholangitis, and travel history.

Recommended studies include testing for tuberculosis, hepatitis, and prior exposure to certain viruses and other pathogens (see Table 29.2). This allows for appropriate vaccination prior to transplantation and identifies "at-risk" patients such that prophylaxis strategies may be employed.

Schaffner outlined four important aims of screening potential recipients of liver transplants [147]:

1. To determine the immune status of the recipient against common pathogens that can be transmitted through transplantation.
2. To permit the allocation of organs from donors infected with a certain pathogen to recipients who are already carriers of the pathogen.
3. To recognize and treat infections that can be expected to exacerbate or reactivate after immunosuppression.
4. To avoid transplantation in patients with a poor prognosis after transplantation.

Recommended screening laboratories reflect these aims and inform our selection and prophylaxis of patients before and following transplantation.

Selection of patients with acute liver failure

Selection criteria for OLT in the setting of FHF are not currently standardized. Work-up for transplant encompasses the same general requirements as that for patients with chronic liver disease who need transplantation, performed urgently. The most widely applied criteria were formulated at King's College Hospital in

Table 29.3. King's College criteria for acute liver failure

Acetaminophen overdose
Arterial pH <7.3 or all three of the following:
- Prothrombin time >100 s
- Creatinine >300 μmol/L
- Grade 3 or 4 encephalopathy

Non-acetaminophen cause
Prothrombin time >100 s or international normalized ratio (INR) >6.7 or any three of the following:
- Unfavorable cause (non-A, non-B hepatitis, halothane hepatitis, drug-induced hepatitis)
- Jaundice >7 days before encephalopathy
- Age <10 or >40 years
- Prothrombin time >50 s or INR >4
- Serum bilirubin >300 μmol/L

London based on a retrospective, multivariate analysis of possible prognostic factors in 588 patients treated medically between 1973 and 1985 [148] (Table 29.3). Other centers have noted that, while fulfillment of these criteria carries a poor prognosis for spontaneous survival, lack of fulfillment carries a less favorable outlook than originally suggested [149,150].

More recent analyses aim to consider which other factors currently not included in the King's College criteria have prognostic relevance. Several have been reviewed, including white blood cell count, hyperkalemia [150], and lactate levels [151]. Unfortunately, most additional risk factors for failure to recover without OLT occur late in the clinical course, and thus are of limited utility in determining timing or necessity of listing for transplant.

Liver transplantation has emerged as a life-saving procedure leading to marked improvement in survival rates. Improved surgical techniques, immunosuppression, and comprehensive care have led to an overall 1-, 5-, and 10-year survival rate of approximately 65%, 59%, and 54%, respectively, with liver transplant [1]. Those patients who survive beyond the first 3 months follow a survival curve comparable to that seen after elective OLT for cirrhosis. Appropriate selection of FHF patients, determining which patients may or may not survive with medical support alone, remains perhaps the most daunting aspect of caring for this subpopulation of potential liver transplant patients. Further refinement of selection criteria, intensive care support, and perioperative care will hopefully continue to improve clinical results.

Selection of patients with hepatocellular carcinoma

In the largest prospectively collected single-institution study of HCC in OLT to date, from the University of California, Los Angeles (UCLA), factors that predicted poor survival included increased tumor number, presence of lymphovascular invasion, and poor tumor differentiation. These findings echo the results of prior series and underscore the key principle that tumor biology, more than size or number, determines outcome after OLT for HCC [152]. This has led several researchers to surmise that tumors that on radiologic images are seen to respond favorably to pretransplant therapy, be it transcatheter arterial chemoembolization (TACE) or radiofrequency ablation (RFA), possess a more favorable biologic profile. More study needs to be done to elucidate this theory, especially with regard to the best means of assessing tumor biology pre transplant, be it by serologic or radiologic testing.

Within the framework of persistent organ shortage and high waitlist dropout rates due to HCC growth, pretransplant patient selection has become the determining factor in treating HCC in patients with ESLD, and pretransplant adjuvant therapies are a routine component of this process. Controlling tumor growth during the waitlist time may have several advantages, including preventing dropout, influencing HCC recurrence rates post transplant, and overall survival for this subgroup of patient.

While single treatment modalities are effective in slowing tumor progression in many patients with HCC, multimodality treatment may allow for increased rates of complete tumor necrosis, and thus better post-transplant recurrence-free survival. Several centers, and most notably that at UCSF, have shown in uncontrolled studies that multimodality approaches can offer a low dropout rate during the waiting time, favorable survival rates, and a low recurrence rate after transplantation [77]. Also, Freeman et al. showed similar results in a retrospective review of the SRTR data on liver transplantation in the US from 1997 to 2006 [153]. They observed a significant survival advantage at 3 years post transplant in patients with HCC exemptions and local ablative therapies during the waiting time.

Based on these findings, Duffy et al. from UCLA proposed that preoperative tumor staging is best accomplished with a CT or MRI scan within 6 months of the time of OLT, as well as liver biopsies to assess histologic grade and the absence or presence of lymphovascular invasion [154] . Because there are real concerns about liver biopsies in cirrhotic patients and the risks of sampling error, bleeding, or tumor dissemination, others have recommended using tumor biopsies only in cases of large tumors that approach 3 cm or more, or tumors that do not respond well to locoregional therapy.

Selection committee

Once patients have completed their medical, surgical, and psychosocial/financial evaluation, they may be discussed at the selection committee. This committee generally consists of members representing transplant hepatology and surgery, transplant nursing, psychiatry, chemical dependency, financial services, diet, and social work. During patient review, relevant problems can be identified, which may result in recommendations for further consultation or testing. This discussion allows full review of all data acquired during the evaluation such that relative risk-to-benefit can be determined and final recommendations regarding listing or monitoring can be made.

Summary

Patients should be evaluated and considered for liver transplantation when the overall benefit of transplant exceeds the risk. Consideration of overall medical and surgical risk, psychosocial support, and financial means should be made during the process of evaluation. Decisions regarding candidacy will necessitate the co-operative review of the transplant team, taking into account all aspects of the patient work-up. For patients with progressive liver failure, the need for transplantation may be influenced by the health consequences and mortality risk of the complications. For patients with liver cancer, the benefit of transplantation may be in the reduction of recurrent disease. For others, the overall need/benefit may be based on quality of life improvement. Regardless of the indication, the evaluation process for each individual is designed to better inform the transplant team as to the potential for benefit as weighed against the overall risk of the procedure and recovery.

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CHAPTER 30

Patient Selection and Indications for Heart Transplantation

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Introduction

Heart transplantation is performed in 2300 patients in the US each year, of whom approximately 1950 are adults over age 18. Despite broadening criteria for acceptable donors, this number has been constant over the past decade. It is estimated that 5000 heart transplants are performed each year worldwide [1]. The prevalence of heart failure is increasing, resulting in a widening gap between the numbers of patients listed versus transplanted each year. Additionally, left ventricular assist devices (LVADs) are now implanted as frequently as hearts are transplanted. LVADs are being used as a bridge to transplantation or as an alternative to transplantation in individuals not selected for transplant listing (destination therapy) [2]. Fortunately, for the growing heart failure population optimal therapy has improved in recent years, substantially changing the trajectory and in some cases the direction of severe myocardial dysfunction.

The general indications for heart transplantation (Table 30.1) have been stable over the past decades. However, heart failure therapy has evolved to include additional treatments prior to defining a patient as refractory [3]. Improved heart failure outcomes are the result of neurohomonal modulation with medications, reduction in sudden cardiac death with implantable defibrillators, and improved myocardial contraction with resynchronization pacing devices in patients with wide QRS on electrocardiogram [4]. Thus, the risk assessment for when a transplant is needed has changed, in an era where availability of organs has decreased. This chapter will focus on the selection of patients for heart transplantation with a discussion of allocation policy changes that have had a major impact on the characteristics of patients undergoing transplant in recent years. It serves as a companion to Chapter 39, which covers the management of patients on the waitlist for heart transplantation, and also complements Chapters 44, 48, and 49, which detail intensive care unit management of patients with heart failure, ventricular assist devices, and the emergence of the artificial heart, respectively.

Heart failure: A symptomatic and progressive syndrome

Heart failure is a common chronic problem in the US experienced by over 6 million people. The New Heart Association (NYHA) symptomatic classification has long been used to classify patients, ranging from those with asymptomatic or mild symptoms (class I–II) to those with marked or severe limitations (class III–IV). Symptoms usually respond to treatment of fluid congestion, but over time may become refractory. Therefore, it is helpful to think of a continuum of disease states that has been staged with important prognostic implications. Refractory end-stage heart failure is characterized as stage D [5]. Estimates vary, but as many as 150 000 people with heart failure in the US may have entered stage D. Of these, 75% are aged over 65 years and have associated co-morbidities, including diabetes mellitus, chronic kidney disease, and peripheral vascular disease. However, tens of thousands of stage D patients would live longer lives with heart transplantation if there suitable donors were available. Due to the mismatch of need and availability of donor hearts, heart transplant programs are forced to make decisions based not only on the individual's outcome, but also on ethical concerns for allocation of a scarce resource.

Heart transplant listing outcomes

Results of heart transplantation have improved in recent years with 1-year survival approaching 88–90%, compared to 80% in the 1990s (Figure 30.1). Five-year survival is approximately 75%. Of adult patients who survive 1 year, half can expect to live for 14 years and close to a third will live for 20 years [1]. According to the United Network of Organ Sharing (UNOS), of adults listed for heart transplantation in 2008, 60% underwent transplant within 12 months, 25% were still waiting, and 10% had died, while 5% were removed from the list either because of improvement or unfavorable reasons. By 36 months, 70% were transplanted, 8% were waiting, and 12% had died on list, while 10% had been removed. In recent years, approximately 3400 candidates are added to the list each year [6].

Notable is the fact that 77% of the heart transplant programs perform fewer than 20 transplants each year. The larger volume programs, those with 20 or more transplants each year, perform 52% of the total transplants (Figure 30.2) [1].

Left ventricular assist devices: Changing the landscape of stage D heart failure therapy

As discussed in depth in Chapter 48, mechanical circulatory assistance has been available for years, including LVADs, right ventricle assist devices (RVADs) or biventricular assist devices (BiVADs) to

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 30.1. General indications for heart transplantation

Common:
- Severe systolic dysfunction with advanced symptoms despite optimal therapy, and objective evidence supporting poor 1-year survival

Less common:
- Complex congenital heart disease with severe symptoms not amenable to palliative or corrective surgery
- Restrictive or hypertrophic cardiomyopathy with severe heart failure refractory to therapy
- Persistent life-threatening ventricular arrhythmias refractory to treatment
- Severe, disabling ischemia not amenable to revascularization

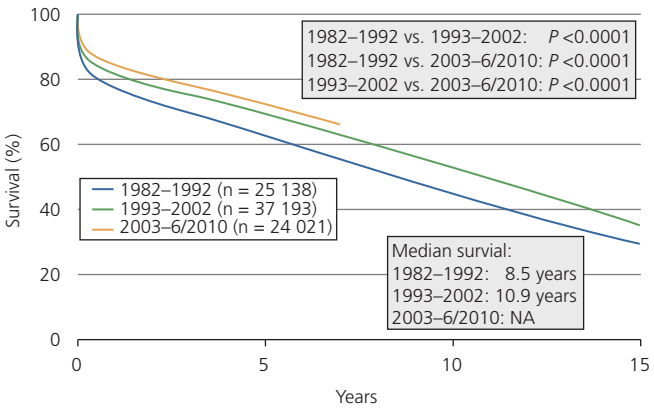

Figure 30.1. Adult heart transplant survival by era. NA, not available (Data from the ISHLT https://www.ishlt.org/registries)

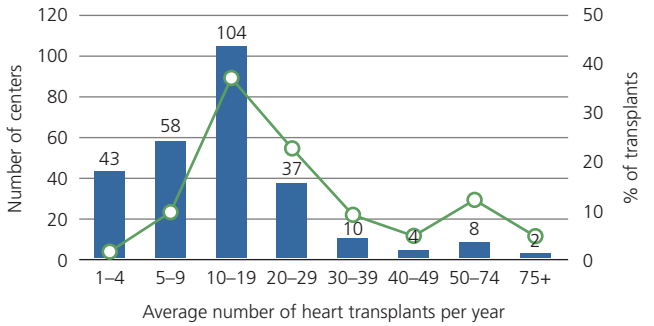

Figure 30.2. Number of heart programs performing specified numbers of transplants (columns) and percentage of total transplants done at these centers (line), Jan 2006–June 2011. (Data from https://www.ishlt.org/registries)

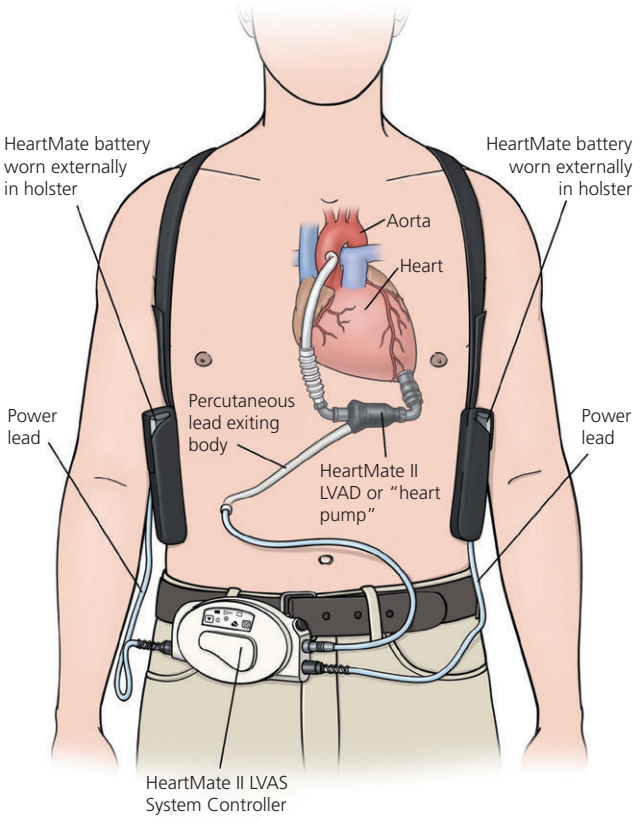

Figure 30.3. Thoratec HeartMate II left ventricular assist device (LVAD). Blood flows from the left ventricle through the impellar pump and into the aorta. (Reprinted with the permission of Thoratec Corporation)

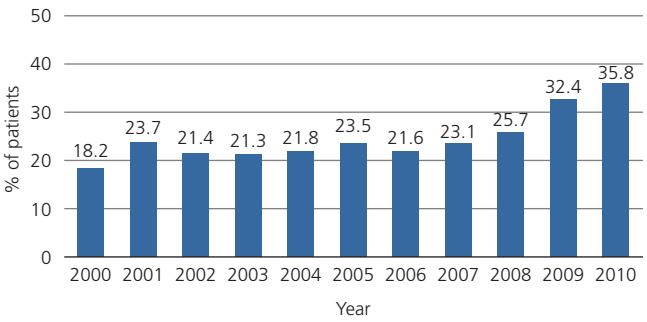

Figure 30.4. Percentage of patients bridged to transplant with left ventricular assist device, right ventricular assist device, or total artificial heart. (Data from the ISHLT https://www.ishlt.org/registries)

bridge critically ill patients to heart transplantation. A major change in the advanced heart failure environment has been the expanded use of continuous flow LVADs (Figure 30.3). These pumps are smaller and more reliable than previous generation devices and have resulted in improved outcomes, not only for patients awaiting transplant but also for patients as a permanent or "destination" therapy. Following LVAD implantation, patients can be discharged within 2 weeks and have a good ambulatory status within the first 2 months. The Interagency Registry for Mechanically Assisted Circulatory Support (Intermacs) reported approximately 1000 implants in 2009 and 1800 in 2011 in the US, approaching the number of heart transplants [2]. The indications for these implants were either as bridge to transplant (BTT) for patients deteriorating on continuous inotropic support; bridge to candidacy for transplant (BTC), i.e. support until a potentially modifiable contraindication is resolved; or as long-term, destination therapy (DT). In 2011 23% of implants were BTT, 37% were BTC, and 38% were DT [7]. There has been a marked increase in the use of LVADs as a BTT, with approximately one-third of patients transplanted in 2010 supported by these devices (Figure 30.4)

Improvement in LVAD technology has increased survival for patients with severe heart failure. Currently, 1-year survival for patients supported by continuous flow LVADs approaches 75% and 2-year survival 62%. The clinical results with LVADs are strongly linked to the severity of illness at the time of the implant. Patients in carcinogenic shock have substantially inferior results. If these

patients, as well as those with serious co-morbidities, are excluded from analysis, 2-year survival approaches 80%, which is similar to that for heart transplantation [7]. Longer-term data are limited.

Despite improvements with LVAD technology, infection, thrombosis, and bleeding remain problematic. Over 25% of patients who currently have LVADS are on antibiotics for bacterial infections, most commonly involving the skin where the driveline exits to the controller and battery source [8]. Patients require chronic anticoagulation with warfarin. Device thrombosis may result in stroke or device malfunction, which may require pump exchange. The need for chronic anticoagulation and the occurrence of acquired von Willebrand factor deficiency in these patients may result in gastrointestinal bleeding from arteriovenous malformations, with clinical bleeding occurring in up to 20% of patients [9]. Based on these complications, which impact long-term results, heart transplantation remains the favored therapy over destination LVAD therapy.

LVADs are being used increasingly to bridge unstable patients to transplantation. Overall results are comparable to non-device patients even though surgical complexity is increased. There is a downside to LVAD bridging in addition to the potential complications mentioned. Patients with LVADs are at risk of developing alloantibodies, manifest by an elevated panel reactive antibody (PRA) percentage (discussed in depth in Chapter 89). This has implications for the likelihood of finding a suitable donor. Elevated PRA may be due to transfusions needed for surgical or gastrointestinal bleeding; however, patients without transfusions have developed elevated PRA post implant. It appears that up-regulation of the immune system from the interaction of the body with biomaterials from the LVAD may be responsible, although not all antibodies measured in LVAD recipients are anti-HLA antibodies [10]. Some candidates for heart transplantation are at higher risk for complications with LVAD support. These candidates include patients with severe right ventricular failure, non-dilated cardiomyopathies, multiple prior sternotomies, or prior mechanical valve replacement, particularly aortic. Thus, patients should initially be evaluated for transplant and if a candidate, should be assessed for potential LVAD bridging candidacy in the event of deterioration. Patients not accepted for transplant should be assessed for suitability for DT.

Heart allocation in the US

Allocation of donor organs in the US is determined by UNOS policies [10]. The Thoracic Organ Transplantation Committee, composed of transplant physicians, nurse coordinators, transplant hospital representatives, organ procurement organization (OPO) representatives, and a patient representative, develops and monitors heart and lung allocation policies. Regional review boards provide additional oversight, including reviewing requests for high priority by exception. Heart allocation consists of three priority statuses designed with the intent of hearts going to candidates who are more critically ill (Table 30.2).

As indicated, the highest priority (1A) candidates may be critically ill at the transplant center hospital or may be out of the hospital with an LVAD under the 30-day rule or one that has serious complications such as thrombosis, device infection, or mechanical failure. Patients with LVADs who do not meet these criteria are 1B, as are patients on continuous inotropic support. Outpatients not meeting these criteria are status 2 and patients who are not immediately suitable to be transplanted can be temporarily inactivated to status 7. Waiting time accrued is only meaningful when based on level. For example, a patient who is listed 1A for 1 day will take priority over one who has been status 1B for 3 months or status 2 for 3 years. Regarding ABO typing, O hearts are prioritized to O or B recipients, A to A or AB recipients, and B to B or AB recipients.

In 2006, heart allocation was altered such that if a status 1A or 1B patient in the local OPO was not a match, instead of allocation to local status 2 candidate, first priority is given to 1A and 1B patients within a 500 mile radius of the donor surgery hospital. This geographic design was intended to reduce waitlist mortality while keeping organ ischemia time to <4 h so that transplant outcomes would not decline (Table 30.3) [11]. As a result, there has been a marked decrease in status 2 recipients being transplanted, but without an adverse impact on overall transplant outcomes [12].

In 2011, only 6% of heart transplants were status 2, with 62% status 1A and 32% status 1B (Table 30.4). In our current era of implantable cardiac-defibrillator therapy (ICD) and resultant decrease in sudden death, the outcome of status 2 patients on the waitlist is similar to the 1-year survival with heart transplantation. This fact, along with the low possibility that a patient will be transplanted as status 2, has led some to question the need for listing patients in this category [12,13]. However, a significant percentage of status 2 patients will deteriorate over a short period of time and for this reason there are advocates of the current listing system [14]. There is regional variability with regard to waiting times

Table 30.2. UNOS priority status for heart transplant

Status 1A – Admitted to the transplant hospital and one of the following:
- Hemodynamic monitoring and continuous infusion of high-dose inotrope or multiple inotropes
- Mechanical circulatory support with intra-aortic balloon pump, extracorporeal membrane oxygenator (ECMO), or total artificial heart
- Mechanical ventilator

Status 1A – Without requirement for hospitalization:
- Left (LVAD) and/or right ventricular assist device (RVAD) may be 1A for 30 days at any point determined by the program
- LVAD or RVAD with objective evidence of significant device-related complication or life-threatening ventricular arrhythmias

Status 1B:
- Continuous infusion of inotrope
- LVAD or RVAD

Status 2:
- All candidates not meeting 1A or 1B criteria

Based on guidelines from the Organ Procurement and Transplantation Network (OPTN), Organ Distribution: Allocation of Thoracic Organs. Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation. Available at: http://optn.transplant.hrsa.gov/PoliciesandBylaws2/policies/pdfs/policy_9.pdf. [accessed January 15, 2013]

Table 30.3. Priority for allocation

- **Local: status 1A, then 1B**
- **Zone A (500-mile radius): status 1A, then 1B**
- **Local: status 2**

Table 30.4. Percentage of heart transplants by status

Year	Status 1A	Status 1B	Status 2
2005	40	34	26
2011	62	32	6

Based on Organ Procurement and Transplantation Network (OPTN) Data. Available at http://optn.transplant.hrsa.gov/latestData/rptData.asp. [Accessed January 15, 2013]

for transplant and to numbers of status 2 patients transplanted. Waiting times are also impacted by body size, ABO type, and allosensitization. In some regions, a large male with O blood type might reasonably expect to receive a transplant based on 1A waiting time. Listing that patient as status 2 will not impact when he receives a heart.

It is obvious that organ availability limits the number of patients who benefit from heart transplantation. The process of surviving to a heart transplant is challenging to the patients and families. Patients with heart failure often experience prolonged time periods in the hospital, may require continuous home inotropic infusions, and more than one-third may require intra-aortic balloon pump or LVAD as a BTT. Patients undergoing LVAD placement will have a period of recovery followed by an uncertain period of waiting, often determined by highest priority listing when a serious complication occurs. Transplant centers educate patients about the hardships that they may endure on the waitlist, although the variability in waiting makes defining a path for an individual difficult.

The decision to list a patient for transplant is made by a multidisciplinary committee with the expectation that those listed will ultimately be transplanted with a good outcome. Favorable outcomes are needed to ensure proper use of a limited organ supply, to limit individual suffering, and to protect the continuation of the transplant program in a highly scrutinized regulatory environment. Thus, the selection of a patient for heart transplantation requires more than the possibility that that person's death might be delayed by the surgical implantation of a donated organ. Because of the differences in organs suitable for transplant, heart transplant programs have more stringent exclusion criteria than kidney and liver transplant programs.

Patient selection for listing for heart transplantation

The evaluation of patients for cardiac transplantation may occur urgently for the unstable patient in the hospital setting or be a stepwise process in the outpatient setting for ambulatory patients, including treatment adjustment, assessing response to therapy, and assessing adherence.

The following questions need to be considered prior to listing a patient for heart transplantation:

1. Does the patient have refractory end-stage heart disease? This includes a review of all reasonable therapeutic options, including optimization of medical therapy.
2. Does the patient have co-morbidities that will adversely impact survival and quality of life post transplantation?
3. Are there adequate support mechanisms to insure a successful outcome?
4. Does the patient have awareness of the commitment required to go through the waiting period, the transplant period, and the rest of his/her life?
5. Has the patient's autonomy to decide to undergo transplant been appropriately supported based on his/her expectations and desires?

Determination of end-stage heart disease

Over 95% of patients undergo heart transplantation for myocardial or "pump" failure, most commonly of chronic duration. Rarely, transplants are performed for intractable angina or for life-threatening arrhythmias in the absence of severe myocardial failure. According to the International Society for Heart & Lung Transplantation (ISHLT) registry, in the past 5 years non-ischemic dilated cardiomyopathy accounted for 54% of recipients and ischemic cardiomyopathy was the etiology in 37%. Congenital heart disease, valvular heart disease, and retransplantation each represented 3% of the total number of transplants [1].

The development of the cardiology subspecialty certification for Advanced Heart Failure and Cardiac Transplantation in part materialized due to the growth of surgical heart transplant programs in the 1980s. As referrals and waiting times grew, heart failure clinics were established to care for the population of patients with advanced chronic disease. The cardiologists in these centers worked in a multidisciplinary team to evaluate and chronically manage patients with severe left ventricular dysfunction. Patients who were previously thought to have intractable heart failure symptoms were noted to improve with the strategies employed by these programs.

Since that time numerous large clinical trials in chronic heart failure therapy have defined appropriate therapy for patients with systolic heart failure [4]. Optimal medical therapy now consists of titrated dosing of an angiotensin-converting enzyme (ACE) inhibitor, titration of beta-blocker, use of an aldosterone antagonist along with dose adjustment of loop diuretics to maintain appropriate volume status. African–American patients benefit from the addition of combination hydralazine/nitrate therapy. Appropriately selected patients with wide QRS duration benefit from cardiac resynchronization therapy (CRT). Beta-blockers and CRT therapy have both been shown to improve myocardial structure and function, and can reverse cardiac dilation. Patients with left ventricular ejection fractions (LVEFs) below 35% may have a survival benefit with ICD therapy. Guidelines have been developed with recommendations for these therapies as well as for referral of patients with refractory symptoms to a multidisciplinary disease management team [5]. The components of a heart failure disease management program [15] are listed in Table 30.5. These components can improve the lives of individuals with heart failure and may impact the need for transplantation.

It is important to recognize that while an individual seeks medical attention due to the symptoms of heart failure, there are a variety of disease states that may be the root cause of his/her problems. In some instances, treatment of these root causes can result in reversal of systolic dysfunction. At one end of the spectrum is the symptomatic patient with a LVEF of 15% due to tachycardia-mediated cardiomyopathy. Catheter ablation of the electrical focus in the atria or ventricles causing tachycardia may be curative [16]. Additionally, certain patients with dilated cardiomyopathy and left bundle branch block may be "super responders" to CRT with biventricular pacing systems [17]. At the irreversible end of the spectrum is the patient with a remote massive myocardial infarction who has deteriorated on medical therapy to a state of hypotension, tachycardia, and diuretic resistance. In between are patients with chronic severe left ventricular dysfunction who may be on inadequate doses of beta-blockers, and have inadequately treated hyper-

Table 30.5. Components of heart failure disease management

- Individualized education and counseling
- Promotion of self-care, including weight monitoring and diuretic adjustment
- Strategies to promote adherence
- Optimization of medical therapy
- Proactive interventions for symptomatic change
- Assistance with social and financial concerns
- Access to providers when needed

tension, chronic volume overload due to medication and diet adherence issues, untreated obstructive sleep apnea, or poor rate control of atrial fibrillation. These are patients who have a chronic disease state/co-morbidities that may result in NYHA class IV symptoms or if properly treated, may have mild or asymptomatic status.

Determination of need to evaluate for transplantation

The hospitalized patient

The majority of heart transplant evaluations can be performed on an outpatient basis; however, certain patients may need to be transferred to the transplant center for urgent evaluation. Patients with cardiogenic shock from suspected fulminant myocarditis may require mechanical support and transplant evaluation, particularly if their etiology is determined by biopsy to be giant cell myocarditis [18]. Included in this category are patients who are hemodynamically unstable after coronary interventions for acute myocardial infarction or those with refractory ventricular arrhythmias. In patients with chronic heart failure, the inability to wean inotropic support, the development of progressive renal or hepatic dysfunction, or the development of recurrent arrhythmias should prompt transfer to the transplant center.

The ambulatory patient with chronically treated heart failure

The majority of patients with severe left ventricular dysfunction are on complex medical regimens and generally have ICDs to prevent sudden cardiac death. Their chronic management is often through local cardiology outpatient settings. Many of these patients have favorable quality of life with occasional exacerbation of symptoms managed by dose adjustment of medications and lifestyle interventions. The challenge is to determine when prognosis has slipped to the point of necessitating advanced therapies.

Appropriate timing of transplant evaluation is done with the concept that patients may require time to meet requirements for listing, as well as to be engaged in shared decision-making about advanced therapies [19]. Although transplant testing results may be obtained rapidly, many patients require further evaluation, including routine health assessment with colonoscopy or specialty consultation for co-morbidities such as diabetes. Additionally, patients with numerous co-morbidities or psychosocial concerns may require a period of time in follow-up at the center to show that they are capable of maintaining a complex treatment program. Conversely, evaluation of patients too early may result in excessive testing and costs to the patient. Additionally, inappropriately early transplant evaluation may stress the resources of the center in attempting to manage the complexities of our current healthcare insurance and regulatory system. Once the transplant evaluation phase begins there will be many individuals involved and numerous communications with required documentation. Therefore, it is common for patients to initially be seen by a heart failure cardiologist in an advanced heart failure clinic prior to commencing the evaluation phase.

The heart failure clinical course is quite variable, making predictions for individual patients seen in clinical practice difficult. However, the clinician should heed certain warning signs that a patient's prognosis has deteriorated and that advanced therapies need consideration. The European Society of Cardiology has developed a definition of "advanced chronic heart failure" that should prompt the clinician to refer for transplant or LVAD consideration.

Table 30.6. European Society of Cardiology criteria for advanced chronic heart failure

1. Moderate-to-severe symptoms of dyspnea and/or fatigue at rest or with minimal exertion (NYHA functional class III or IV)
2. Episodes of fluid retention and/or reduced cardiac output
3. Objective evidence of severe cardiac dysfunction demonstrated by at least one of the following:
   - **(a)** Left ventricular ejection fraction <30%
   - **(b)** Pseudonormal or restrictive mitral inflow pattern by Doppler
   - **(c)** High left and/or right ventricular filling pressures, or
   - **(d)** Elevated B-type natriuretic peptide
4. Severe impairment of functional capacity as demonstrated by either inability to exercise, 6-min walk distance <300 m, or peak oxygen uptake <12–14 mL/g/min
5. History of at least one hospitalization in the past 6 months
6. Characteristics should be present despite optimal medical therapy

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All of the listed criteria should be present after optimal medical therapy has been implemented or attempted (Table 30.6) [20].

Additional factors that independently should raise a red flag are involuntary weight loss [21], the development of intolerance to ACE inhibitors or to beta-blockers due to symptomatic hypotension [22], or signs of the development of the cardiorenal syndrome [23].

Ideally, patients should be assessed objectively in the outpatient setting. In ambulatory patients, cardiopulmonary exercise testing can quantitate functional limitation, determine if the limitation is primarily heart failure related, and provide important prognostic information over the coming year. The peak oxygen consumption (peak VO_2), assuming adequate effort and achievement of anaerobic threshold, helps to stratify patients into risk categories. Peak VO_2 of <14 mL/kg/min had previously been used as an indication for heart transplantation [24]. However, studies in the era of beta-blockers, ICDs, and resynchronization devices have led to a lower cut-off point of <10–12 mL/kg/min [25,26]. Patients with clinical stability and favorable peak VO_2 results can be reassured that they have a favorable prognosis for at least 1 year.

Over 100 demographic, clinical, and laboratory variables have been associated with heart failure outcomes, including ischemic etiology, resting heart rate, prolonged QRS complex, low blood pressure, serum sodium, uric acid, hemoglobin, brain natriuretic peptide (BNP) level, ejection fraction, and left ventricular size among others. Multivariate risk scores have been developed to assess prognosis in populations of outpatients with heart failure.

The 20 variable Seattle Heart Failure model (SHFM) has been validated in a broad population of heart failure patients for providing a 1–5-year estimates of survival and average life expectancy (http://depts.wahington.edu/shfm/). It tends to underestimate risk in the very advanced stages [27]. The Heart Failure Survival Score (HFSS) was developed specifically for transplant selection, but prior to beta-blocker use and ICD therapy. It may add value in identifying the low-risk patient in whom transplantation may be safely deferred [28]. Neither of these scores should be used solely in determining transplant candidacy, but they do assist the clinician in determining which patients may require formal evaluation. Some centers include this information in the decision to list or to defer listing [25].

Heart transplant evaluation phase

After a thorough evaluation in the advanced heart failure clinic, patients assessed to be in need of and without absolute contraindications to heart transplantation are given the opportunity to

Table 30.7. Heart transplant evaluation

- Right heart catheterization with vasodilator challenge when indicated
- Cardiopulmonary exercise testing (CPX) – may be done pre-evaluation on an outpatient basis
- Electrocardiogram (ECG)
- Echocardiogram
- Pulmonary function test with bronchodilators and diffusion capacity for carbon monoxide (DLCO) (patients with history of nicotine use, asthma, or amiodarone therapy)
- Consultations with mental health social worker, social worker, financial coordinator, and nutritionist. Pharmacist is notified and performs a chart review; available for additional consultation as needed
- Hematologic profile with differential, platelet count, PT and PTT, comprehensive metabolic profile, TSH, PSA (if male patient >50 years old), lipid profile, Mg
- Serologic testing for HIV, hepatitis B and C,
- CMV, EBV, toxoplasma (post transplant implications)
- PRA antibody screen
- Comprehensive urine drug and nicotine screens
- Blood type and screen ×2
- 24-h urine collection for creatinine clearance and protein
- PPD skin test, pneumovax (if not previously received), hepatitis B vaccine
- Additional diagnostics or consults as indicated

PT, prothrombin time; PTT, partial thromboplastin time; TSH, thyroid stimulating hormone; PSA, prostate-specific antigen; HIV, human immunodeficiency virus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; PPD, purified protein derivative.

Table 30.8. Contraindications to heart transplantation

Absolute:
- Severe, irreversible pulmonary hypertension
- Active malignancy
- Systemic illness with poor prognosis
- Severe obstructive pulmonary disease

Relative:
- Advanced age (>70 years)
- Uncontrolled infection
- Active peptic ulcer disease
- Diabetes mellitus that is poorly controlled or with end-organ damage (neuropathy, nephropathy, or proliferative retinopathy)
- Severe obesity
- Cachexia
- Severe peripheral vascular disease
- Severe cerebrovascular disease
- Intrinsic renal disease with decreased estimated glomerular filtration rate (eGFR) <40 mL/min/1.73 m^2
- Hepatic dysfunction with bilirubin >2.5 mg/dL or transaminases 3× normal
- Cirrhosis
- Chronic viral infection (hepatitis B, hepatitis C, HIV)
- Amyloid
- Active systemic lupus
- Active sarcoid with multisystem involvement
- Recent pulmonary infarction (due to risk of abscess on immunosuppression)
- Poor functional status from stroke or muscular dystrophy
- Swallowing or vomiting disorders
- Poorly controlled hypertension
- Active or recent substance abuse (tobacco, alcohol, illegal drugs)
- Unstable mental illness
- Recent non-adherence to therapy
- Lack of social support structure
- Psychosocial or financial barriers to strict medication adherence
- Inadequate transportation for follow-up at transplant center

undergo the transplant evaluation phase. This is performed by a multidisciplinary team and includes appointments with a transplant nurse coordinator, a social worker, a nutritionist, a mental health specialist, and a financial coordinator, and a medication review by a pharmacist. Additionally, a variety of tests are performed to further assess the need for transplantation, contraindications to transplantation and for donor organ compatibility [ABO type and panel reactive antibody (PRA)]. A list of the tests and consults performed at our center is given in Table 30.7.

In the US the listing criteria for heart transplantation are determined at the local level of the transplant program, usually through a multidisciplinary selection committee that meets on a defined basis. Each heart transplant program determines absolute and relative contraindications that have been derived based on experience and registry results, with limited data from randomized clinical trials. Clinical guidelines have been published that offer a general approach [26,29]. Contraindications are considered based on surgical risk as well as long-term impact on longevity, quality of life, and the potential degree to interfere with or be exacerbated by post-transplant immunosuppressive therapy.

Absolute and relative contraindications are listed in Table 30.8. The relative contraindications listed are those that might be modifiable, may be determined based on degree, or may be considered acceptable at some but not all programs.

It is helpful to consider the relationship between a potential contraindication and the heart failure disease state:

1 Some conditions may be due to chronic or acute low output heart failure, elevated intracardiac pressures, and volume overload with tissue congestion, activated neurohormones, or the result of complications during hospital treatment. These may potentially be reversible with heart failure treatment and time.
2 Other contraindications may have contributed to the etiology of heart failure and if not modifiable, may result in ongoing health issues post transplant.
3 Some contraindications may be worsened by immunosuppressive therapy.

Pulmonary vascular resistance

Secondary pulmonary hypertension occurs in most patients with advanced heart failure and if irreversible, can result in acute right ventricular failure at the time of transplant. Twenty percent of early deaths after heart transplant are due to right ventricular failure [30]. The donor heart is often functionally abnormal following brain death and the ischemic time necessary for procurement and implantation. In this setting, a high pulmonary vascular resistance (PVR) may result in circulatory shock due to the inability of the right ventricle to pump enough blood volume to the left side. The ISHLT registry has consistently shown a linear relationship between PVR and post-transplant mortality [1]. Commonly accepted exclusion criteria include a PVR of >6 Wood units or a transpulmonary gradient of >15. However, pulmonary artery pressures can often be improved acutely with pulmonary vasodilator therapies; failure to show an acute response should not permanently exclude a patient from consideration. Chronic infusions of milrinone, dobutamine, or prostaglandin E_1 may result in improved pulmonary hemodynamics. Chronic sildenafil or bosentan therapy appears to be safe and has resulted in successful transplantation [30,31]. The reversibility of previously fixed pulmonary artery hypertension has been demonstrated with chronic LVAD support, a therapy that reduces left atrial pressure and restores cardiac output [32]. With the use of these therapies it appears that the majority of patients initially deemed to have unacceptable pulmonary resistance will ultimately not be excluded for this reason. Once listed for transplant, serial right heart catheterizations every 6–12 months should be considered to exclude the development of fixed pulmonary hypertension.

Malignancy

Active malignancies, other than some skin cancers, are a contraindication to transplantation due to the impact of immunosuppression on tumor growth and longevity. Additionally, it is difficult to manage chemotherapy and transplant immunosuppressive medications simultaneously. For candidates who are in remission, oncological consultation is indicated to define the risk of recurrence or second malignancy to determine if a specific cancer-free waiting period is required to assess prognosis. Our center will consider listing patients with a low likelihood of recurrence, which we consider to be at <10% recurrence.

The number of patients with heart disease from chemotherapy is increasing [33]. Anthracyclines have improved cancer survival for breast cancer, lymphoma, sarcoma, and leukemia. Newer agents, including trastuzumab and the tyrosine kinase inhibitors, have been implicated in cardiac toxicity and the development of chemotherapy-induced cardiomyopathy (CCMP). Two-thirds of patients undergoing heart transplantation for CCMP have been female, which is interesting given that approximately 75% of heart transplant recipients are male. Cancer recurrence has been low. When this group is compared to patients with dilated cardiomyopathy, the incidence of infection and skin cancer has been higher, but the incidence of rejection has been lower with overall comparable survival. Patients with CCMP requiring LVAD therapy may be at higher risk for right ventricular failure and have been more likely to require right ventricular assist device support [34].

Heart transplantation in patients with prior mediastinal radiation has emerged as a significant safety and efficacy concern due to increased surgical bleeding from intense scaring of mediastinal structures, as well as the risk of postoperative pulmonary complications. Patients with prior mediastinal radiation have often had prior cardiac surgery, which adds to this risk. Additionally, malignancy recurrence appears to be higher in these patients [35,36].

Age

The median age for heart transplant recipients is 54 years; over 25% of recipients are over the age of 60 years, while only 2% are older than 70 years [1]. Older candidates are more likely to have co-morbidities and are more prone to immunosuppressive complications such as renal dysfunction, osteoporosis, steroid-induced diabetes mellitus, and malignancy. Older recipients are less likely to have rejection and may require less intensive immunosuppression [37]. The 2006 ISHLT update stated that patients should be considered if they are ≤70 years of age [26]. Centers generally base decisions on physiologic parameters, with more restrictive criteria in patients above age 65 years [38,39]. The growing scarcity of donor hearts creates an ethical dilemma: offering a finite resource to an older person might preclude a younger recipient from being transplanted. Some centers have therefore created alternative lists using marginal donor hearts, although the improved results with LVADs may be a preferred option [40].

Diabetes mellitus

Diabetes mellitus is present in almost 25% of outpatients with systolic heart failure and in ~40% of those hospitalized [41]. In recent years, 25% of patients undergoing heart transplantation had diabetes mellitus [1]. Many centers consider the presence of end-organ damage, including nephropathy, neuropathy, or proliferative retinopathy, to be exclusion criteria. Patients with autonomic dysfunction are considered to be at particularly high risk for complications [26]. The most recent registry data found that diabetes had only a modest impact on 5-year survival [1]. This likely reflects selection of patients with a lower risk profile as well as improved glucose management in the surgical period. Immunosuppression with steroids as well as with tacrolimus (especially in African–Americans) may result in new onset or worsening of diabetes following transplantation. Close to 40% of patients will have diabetes within the first 5 years after transplantation [1].

Obesity

Pretransplant obesity with a body mass index (BMI) >30 kg/m^2 or percentage ideal body weight (PIBW) >140% has been associated with poor outcomes after heart transplantation. Obese patients have increased complications after heart surgery, including infection, venous thromboembolism, and pulmonary complications. Increased rejection has also been noted in heart and other solid organ transplant recipients [42,43]. Weight gain is common in all heart transplant recipients and obesity may be worsened by steroid immunosuppression, particularly in the first year. Almost 40% of heart recipients have a BMI of >30 kg/m^2 by 5 years post transplant. Many centers require weight loss to <140% of ideal body weight prior to transplant listing. Patients with severe compromise may undergo LVAD implantation to allow survival while weight loss is attempted. This can be successful; however, for severely obese patients it has been rare to achieve or maintain weight loss post LVAD placement and these patients remain on LVAD support long term [44,45].

Renal dysfunction

Renal dysfunction post heart transplantation is multifactorial and strongly linked to long-term outcomes. Over 16% of patients have a creatinine of >2.5 mg/dL by 5 years and patients with this degree of dysfunction have a four times greater risk of death [46,47]. Predicting which patients will have severe kidney disease post transplant is problematic. Renal dysfunction is common in patients with advanced heart failure and is often reversible with normalization of hemodynamics. However, conditions that cause heart disease, including hypertension, diabetes mellitus, and atherosclerosis are also major risk factors for intrinsic renal disease. Additionally, heart failure can cause permanent fibrosis of the kidneys through chronic neurohormonal activation as well as chronic hypoperfusion.

Renal function should be assessed by estimated glomerular filtration rate (eGFR) or creatinine clearance, preferably when the patient's status is stable. Renal ultrasound should be performed in those with abnormal results. The presence of severe echogenicity or of bilaterally small kidneys is indicative of serious intrinsic disease. However, markedly hypoperfused kidneys may appear abnormal on ultrasound. In these patients, renal biopsy to establish etiology as well as to assess severity of fibrosis may be helpful in candidate selection [48]. Some centers list irreversible renal dysfunction with creatinine clearance of <40 mL/min as a contraindication, but practices vary. Our center does not have an absolute cut-off for creatinine clearance, but decisions are based on chronicity of dysfunction, biopsy results, and other co-morbidities. Combined heart and kidney transplant can be performed in selected individuals with favorable results.

Amyloid

Amyloidosis is a clinical disorder caused by deposition of misfolded, insoluble proteins in tissues. There are many different types

of amyloidosis, with two common types warranting discussion in relation to heart transplantation. In light chain (AL) amyloidosis the protein is produced by a clonal population of bone marrow plasma cells. Infiltration of numerous organs over a period of months is common and up to 50% of patients may have signs of cardiac involvement. Only 5% of patients have disease that manifests isolated to the heart [49]. Amyloid initially impacts the interstitium and with progression causes myocardial necrosis. Clinically, there is restrictive physiology with thick myocardium and with disease progression, there is decline in the ejection fraction. Usual therapies for systolic dysfunction including ACE inhibitors and beta-blockers are generally not helpful and are poorly tolerated. Digoxin should be avoided. The literature reports fewer than 100 patients who have had heart transplants with 5-year follow-up for AL amyloid. In recent years, patients have been carefully screened for clinical extracardiac involvement and have received post-transplant chemotherapy, sometimes followed by hematopoietic stem cell transplant. Despite this, the 5-year survival is significantly less than in those without AL amyloid and there is often disease progression in other organs [50,51]. Many centers will not consider transplant in patients with AL amyloid. Unfortunately, cardiac amyloidosis often occurs in patients who were previously healthy, making this an emotionally difficult disease.

Transthyretin (TTR) amyloid also commonly infiltrates the heart. Unlike AL amyloid it is slowly progressive, but may result in advanced heart failure. The familial form is due to a mutated TTR that is produced in the liver. Liver transplantation is a potential option for those without significant heart involvement and there have been approximately 26 heart–liver transplants performed for severe heart disease. New small molecule pharmaceuticals may arrest the disease and make liver transplantation unnecessary [52]. A wild-type TTR is another form and the liver is not involved. Patients with TTR amyloid appear to have better results than those with AL amyloid, which is related to the slower nature of progression. However, data are limited.

The restrictive physiology that all types of amyloid cause makes managing these patients on a waitlist difficult: there is the concern that the new heart will become involved or that extracardiac disease will limit survival.

Chronic viral infections

A study from over a decade ago reported that patients with hepatitis B surface antigenemia pretransplant had significant hepatic complications, but without clear impact on survival [53]. In the current era, with well-tolerated antiviral therapy against hepatitis B, liver complications appear to be controlled with lamivudine therapy. Some patients have developed resistance, requiring alternative antivirals [54–56]. Hepatitis C is of greater concern for several reasons. Antiviral therapy is much less well tolerated. Additionally, a large retrospective study showed decreased post-transplant survival in patients with pretransplant hepatitis C virus positivity. Of note is the fact that the 5-, 10-, and 15-year survival in those with hepatitis C was 73%, 47%, and 25% compared to 77%, 67%, and 47% in the matched non-hepatitis C patients, indicating that long-term outcomes are substantially impacted [57].

Traditionally, human immunodeficiency virus (HIV) infection has been considered a contraindication to solid organ transplantation. The use of highly active antiretroviral therapy (HAART) has markedly improved outcomes of patients with HIV and transplantation has been investigated in kidney and liver populations [58]. In kidney transplantation, a non-randomized trial of 150 carefully selected patients showed acceptable graft survival at 1 and 3 years, although inferior to overall kidney transplant results [59]. Immunosuppression management is very complex in these patients due to drug interactions between protease inhibitors and calcineurin inhibitors. An unexpectedly high rejection rate was noted in these patients. There have been isolated reports of heart transplantation in patients with HIV suggesting favorable short-term outcomes, but experience appears to be limited to a few centers with fewer than 15 reported cases in the literature [58,60]. Because HAART therapy may result in coronary atherosclerosis, heart disease is likely to increase in this population of patients.

Psychosocial factors

While medical factors have been better defined and more thoroughly assessed with regard to outcomes in transplantation, psychosocial factors are recognized to be very important in relationship to rejection, graft function, infection, and survival [61]. Success after transplant is dependent on the ability to obtain medications that easily cost over $1000 per month and may involve 20% co-pays (i.e. for Medicare recipients), and the ability to follow complex medication schedules and to take medications that are needed to treat immunosuppressive side effects [62]. Early intervention for rejection and infection is dependent on patient and caregiver recognition of change in status as well as appropriate transportation to the medical center for urgent or routine follow-up care. Depressions, social isolation, prior history of non-adherence, and substance abuse have all been associated with worse outcomes [61]. The severe physical impairment that patients experience before and after heart transplantation may contribute to temporary mental impairment; thus, an identified support person is required by many heart transplant programs. Married patients appear to have better outcomes [63].

Selection committees involve a social worker, a mental health expert, and other team members in determining if the patient has a readiness level to manage illness, an appropriate support system, psychological stability, and appropriate lifestyle to ensure success. To date, assessment has not been standardized across programs, but attempts are being made to establish tools to identify patients who may have excessive risk and thus establish standardized criteria for recipient selection [64].

Complex congenital heart disease and heart transplant outcomes

Patients with complex congenital heart disease are a unique population. The success of pediatric cardiac surgery has allowed patients with congenital heart disease to live into adulthood. Unfortunately, debilitating heart failure can develop. Patients transplanted for congenital heart disease have inferior short-term outcomes due to complications from reoperation and bleeding from collateral circulation. Patients with single ventricle physiology appear to be at higher risk compared to those transplanted with two-ventricle physiology [65]. Chronic hepatic congestion may play a role. Despite the higher 1-year mortality, 10-year survival is the same as for those without congenital disease [66].

Assessment of potential candidates

Risk stratification of a potential heart transplant recipient involves consideration of a variety of characteristics, including etiology of heart failure, prior surgeries or treatments (i.e. radiation), concomitant chronic medical conditions, as well as degree of patient acuity resulting in multiorgan compromise. Psychiatric stability,

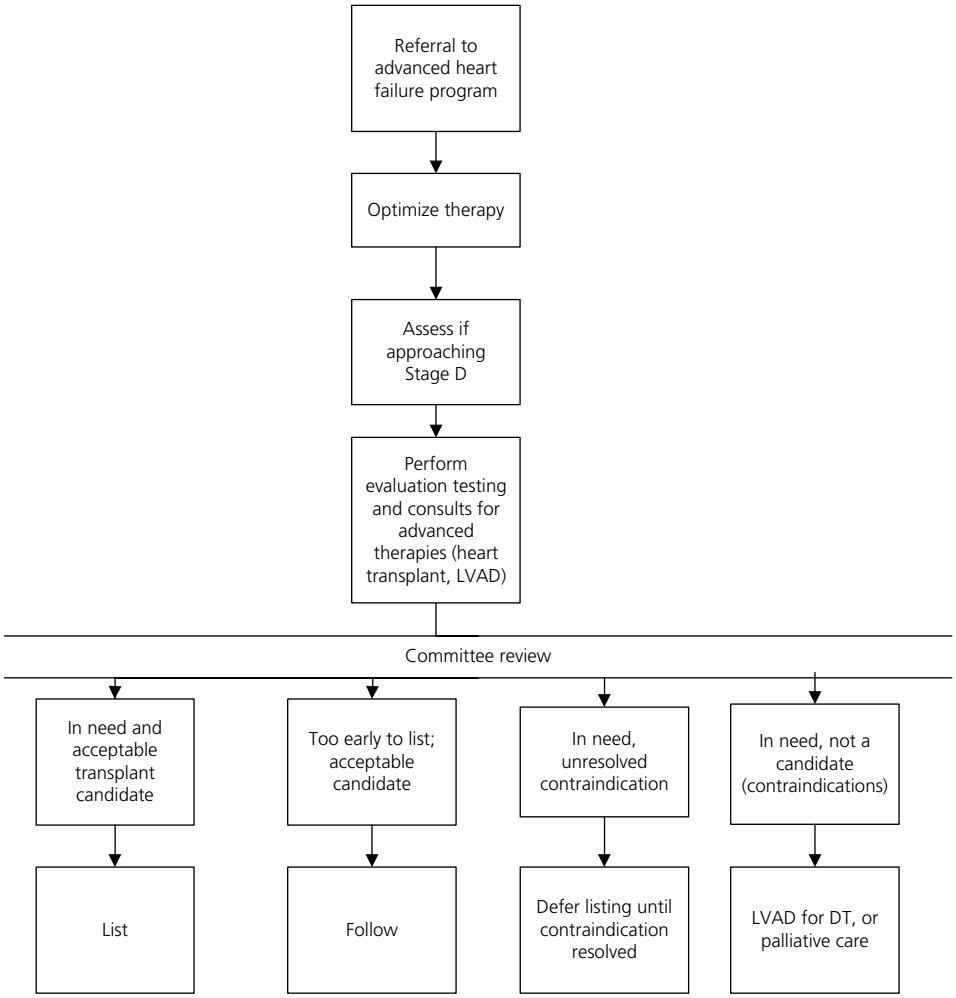

Figure 30.5. Approach to heart transplantation

rehabilitative potential, and social support are important but more difficult to quantitate. Each candidate's ability to overcome difficult pretransplant or surgical phase complications should be assessed and discussed in committee as well as with the patient and family. Patients with several characteristics that may decrease survival require careful consideration. Attempts are being made to develop risk stratification scores that identify patients who fall into unacceptably high risk for poor 1-year survival [67].

Decision regarding transplant listing

The approach to heart transplant evaluation at our program is reviewed in Figure 30.5. Following the evaluation testing and consultations the advanced heart failure therapeutics committee meets to determine candidacy for transplant and/or LVAD therapy. The committee uses exclusion criteria that are defined by our program and attempts to apply these criteria with consistency. Our criteria are reviewed on a regular basis and are changed by the committee. Much of the discussion at our meetings relates to concerns about potential problems with adherence to therapy. Patients who are excluded from transplant are informed verbally and in writing that they may seek an opinion at another center.

Centers do vary in their criteria and may need to adjust these based on the center's outcomes. In the US, detailed program-specific data are publicly reported through the U.S. Scientific Registry of Transplant Recipients (SRTR). Reports are updated every 6 months and include waitlist mortality, transplant rates, and survival, which is adjusted to give observed and expected outcomes. This information, easily accessed on the internet, is used by regulatory agencies, insurance providers, medical professionals, and patients. Centers may be at risk of losing certification if their results substantially deviate from expectations. Indeed, not only are transplant programs evaluating patients, but US programs are being highly evaluated through public reporting.

Heart transplant programs, which do low volumes compared to kidney and liver programs, are challenged by the need to be risk restrictive to assure favorable outcomes, not only to ensure the best use of an organ but from a practical standpoint to sustain the existence of the program. In has been argued that in a changing environment, it is challenging for the SRTR to adequately adjust outcomes for risk. A consensus report that related to all solid organ transplants stated that not appropriately adjusting for risk may cause programs to avoid performing transplants on suitable but surgically higher risk patients [68]. Many of the contraindications to transplant are relative and they are additionally dynamic as medical treatments for co-morbidities improve and immunosuppressive strategies change. Transplant programs are generally careful to test barriers gradually, rather than implementing sudden

changes to numerous criteria, given the relative lack of randomized clinical data.

Decision-making in advanced heart failure

The advanced heart failure therapeutics committee makes the determination of what treatment options are appropriate at its center for each individual. These options are reviewed with the patient and family, with the recognition that heart transplantation and LVAD therapy are disease-exchanging therapies that can improve longevity and quality of life, but with the need to adjust to the burden of surviving to transplant as well as the long-term maintenance of required therapies [69,70]. Ideally, all patients should be offered palliative and supportive care consultation in order to assist them and their families with disease burden. This care includes consideration of the physical, psychological, spiritual, and social needs of the patient and family. Some patients may not be candidates for or may elect not to undergo maximum therapies for heart failure progression. It is important for the advanced heart failure team to continue to offer care beyond the decision of the committee and the decision of the patient.

Summary

Heart transplantation is a highly effective treatment for end-stage heart failure, but its implementation remains severely limited by a shortage of donor hearts. This limited donor availability and the growing prevalence of heart failure has led to a widening gap between those in need of heart transplantation and those who will receive a transplant. Therefore, heart transplant candidacy is more restrictive than candidacy for other organs, and the consideration of candidacy involves not only establishing the patient's suitability for the procedure, but a pragmatic consideration of alternative therapies. Fortunately, therapies for heart failure are improving, which may prevent or delay the need for transplant. Additionally, improvements in chronic LVAD technology have resulted in better capability to bridge patients to transplants and to improved survival for patients who are not transplant candidates. Heart allocation policies based on severity of illness have made it uncommon for the patient without continuous inotropic therapy or LVAD to be called in from home for a transplant. Thus, an understanding of the priority system is important not only in managing the patient's illness, but also in managing his/her expectations. In all, candidacy selection is optimal when a patient whose condition uniquely requires a heart transplant is delivered to the waitlist in a timely manner and in a condition that is conducive to recovery from the procedure.

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CHAPTER 31

Patient Selection and Indications for Lung Transplantation

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Introduction

Lung transplantation is performed for end-stage lung disease of many causes, with chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and cystic fibrosis (CF) representing the majority of causes for transplants performed. To obtain a survival benefit, referral for transplant should take into account the prognosis for any given condition. Patient selection requires a careful assessment of pretransplant medical conditions that have a large impact on post-transplant survival. In this chapter, we discuss general indications and contraindications for lung transplant. We review recommended criteria for transplant for the most common disease processes and provide review of the most recent literature that may inform patient selection. This chapter complements Chapter 40, which covers list management and the lung allocation score in depth.

Indications

The 29th official lung and heart–lung transplant report of the Registry of the International Society for Heart and Lung Transplantation was released in 2012. Lung transplant has become an increasingly common procedure, with 3519 transplants performed in 2010; an increasing share of the transplants is bilateral [1,2]. COPD and IPF were the leading indications for transplant, with IPF gaining share from 16% in 2000 to 28.3% in 2010 (Table 31.1) [1].

In 2006, the International Society for Heart and Lung Transplantation (ISHLT) released the most recent guidelines for the selection of lung transplant candidates [3].

Given the current challenge of chronic rejection and high mortality rate relative to other solid organ transplants, as well as the limited availability of donor lungs, lung transplantation should be limited to those in whom a survival benefit is expected. Overall median survival in the most recent report is 5.5 years; those who survive at least 1 year had a median survival of 7.7 years [1]. Thus, selected patients should have end-stage lung disease having failed maximum medical therapy with a 50% expected survival of <2–3 years or with significant symptoms, which the ISHLT has defined using the New York Heart Association (NYHA) classes for functional status. NYHA functional class III (symptoms with minimal activity) and class IV (symptoms at rest) are considered indications for transplant [3]. In addition, given the rigors of transplant, patients must be in relatively good health.

Contraindications

Absolute contraindications

The ISHLT's 2006 guidelines included absolute contraindications (Table 31.2). Some of these contraindications are now being overcome in transplant.

Malignancy

While malignancy in general is a contraindication, certain subtypes of lung cancer have been proposed as appropriate indications for lung transplantation. The terms "adenocarcinoma in situ" and "minimally invasive adenocarcinoma" of the lung have been adopted in a recent interdisciplinary classification scheme to replace the term "bronchoalveolar cell carcinoma" [4]. Patients with bilateral or multifocal carcinoma of this sort have been treated with lung transplantation without short-term demonstration of recurrence [5,6]. However, several series of patients transplanted for advanced minimally invasive adenocarcinoma demonstrated a high rate of recurrence [7,8].

Patients with end-stage lung disease and stage I non–small-cell lung carcinoma have also been treated with lung transplant, though often unintentionally, with good success. One survey of ISHLT lung transplant centers reported 43 cases of incidentally found primary lung carcinoma in patients transplanted for other indications; those with stage I carcinoma and minimally invasive adenocarcinoma demonstrated low rates of recurrence or disseminated disease, while those with stage II or III non–small-cell lung cancer had high rates of death from recurrence of lung cancer [9]. The Cleveland Clinic reported a 2% (four cases) incidence of undetected lung carcinoma in 214 consecutive transplant patients between 1991 and 2000, with no evidence of recurrence or decreased survival in the three patients with stage I disease; of note, this review was prior to the age of routine computed tomography, and presumably some of those patients would currently be excluded from transplant [10].

Significant chest wall/spinal deformities

Due to mechanical difficulty with surgery and with postoperative mechanical ventilation and pulmonary toilet, thoracic transplantation in patients with significant deformities is rarely performed. There have been a handful of cases reported of patients transplanted with severe scoliosis or chest wall deformities [11–13] and in at least one patient, scoliosis contributed to recurrent airway stenosis requiring frequent bronchoscopy and intervention [14].

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 31.1. Indications for adult lung transplants performed from January 1995 to June 2011 [1]

Diagnosis	Single lung (n = 13721) Number (%)	Bilateral lung (n = 20831) Number (%)	Total (n = 34102) Number (%)
Chronic obstructive pulmonary disease/emphysema	6048 (45.6)	5549 (26.6)	11587 (34.0)
Pulmonary fibrosis			
Idiopathic	4420 (33.4)	3495 (16.8)	7925 (23.2)
Other	498 (3.8)	659 (3.2)	1157 (3.4)
Cystic fibrosis	219 (1.7)	5469 (26.3)	5688 (16.7)
α1-Antitrypsin deficiency	741 (5.6)	1332 (6.4)	2073 (6.1)
Pulmonary arterial hypertension	82 (0.6)	982 (4.7)	1064 (3.1)
Bronchiectasis	54 (0.4)	891 (4.3)	945 (2.8)
Sarcoidosis	251 (1.9)	614 (2.9)	865 (2.5)
Obliterative bronchiolitis	91 (0.7)	260 (1.2)	351 (1.0)
Retransplant			
Obliterative bronchiolitis	259 (2.0)	254 (1.2)	513 (1.5)
Non-obliterative bronchiolitis	166 (1.3)	191 (0.9)	357 (1.0)
Connective tissue disease	140 (1.1)	281 (1.3)	421 (1.2)
Lymphangioleiomyomatosis	122 (0.9)	241 (1.2)	363 (1.1)
Congenital heart disease	45 (0.3)	248 (1.2)	293 (0.9)
Cancer	6 (0.0)	28 (0.1)	34 (0.1)
Other	119 (0.9)	347 (1.7)	466 (1.4)

Table 31.2. Absolute contraindications to lung transplantation

1. Malignancy in the last 2 years, with the exception of cutaneous squamous and basal cell tumors. In general, a 5-year disease-free interval is prudent.
2. Untreatable advanced dysfunction of another major organ system (e.g. heart, liver, or kidney). Coronary artery disease not amenable to percutaneous intervention or bypass grafting, or associated with significant impairment of left ventricular function, is an absolute contraindication to lung transplantation, but heart–lung transplantation could be considered in highly selected cases
3. Non-curable chronic extrapulmonary infection, including chronic active viral hepatitis B, hepatitis C, and human immunodeficiency virus
4. Significant chest wall/spinal deformity
5. Documented non-adherence or inability to follow through with medical therapy or office follow-up, or both
6. Untreatable psychiatric or psychological condition associated with the inability to co-operate or comply with medical therapy
7. Absence of a consistent or reliable social support system
8. Substance addiction (e.g. alcohol, tobacco, or narcotics) that is either active or within the last 6 months

If performed, surgical approach and donor selection would need to be tailored to the thoracic size [13].

Non-curable extrapulmonary infection

Three decades of progress in human immunodeficiency virus (HIV) treatment have changed the prognosis to that of a chronic disease. One case of an HIV- and hepatitis B virus (HBV)- positive patient successfully transplanted for CF has been reported with excellent lung function and no unusual complications 2 years post transplant [15]. A review of 170 hepatitis C virus (HCV) seropositive patients who received lung transplant demonstrated no difference in survival, although the majority of patients were not viremic [16]. A small group of HCV-positive lung transplant recipients at Cleveland Clinic with no evidence of cirrhosis have shown no increased mortality [17].

Untreatable advanced dysfunction of another major organ system

In general, advanced dysfunction has been a contraindication to lung transplant. However, a combined organ transplant may be considered. Based on Organ Procurement and Transplantation Network (OPTN) data as of November 25, 2011, there have been 17 kidney–lung, 44 liver–lung, two heart–lung–kidney, two pancreas–lung, and one liver–pancreas–lung transplants performed in the US since 1988.

Lung transplant in combination with dialysis has been associated with increased 1-year mortality [1,2,18], but small numbers limit the data.

The largest experience is for liver–lung transplants, typically performed for CF complicated by cirrhosis or for portopulmonary hypertension or hepatopulmonary syndrome complicating cirrhosis. Available series have reported no difference in survival compared with single organ transplant patients [19–26].

Adherence and addiction

A careful assessment of social support and adherence by experienced transplant psychologists and social workers is essential. One available tool is the Transplant Evaluation Rating Scale, which has been used to assess patients prior to organ and bone marrow transplants [27].

Non-adherent behaviors to immunosuppression, lifestyle, and general medical prescriptions are common in recipients of solid organ transplants, estimated to affect nearly half of organ transplant patients, including those who live alone [28]. Pretransplant non-adherence is associated with late acute rejection [29]. Pretransplant non-adherence with medication taking, lower social support, lower personality trait scores for "conscientiousness," and higher educational level have been shown to be predictors of non-adherence with an immunosuppressive regimen at 1 year post transplant [29]. Rates of adherence among lung transplant recipients have varied in different studies and with different definitions of adherence [30,31]. Lung transplant patients may have more non-adherent behavior than other solid organ transplant patients, in part due to the higher medication needs [30].

Prior tobacco smoking is common in patients who have end-stage lung disease, so interest in smoking behavior is critical. One survey with self-reported rates of smoking showed 100% abstinence post transplant [32]. However, self-reported rates tend to underestimate smoking compared with biologic markers such as

urine cotinine [33], and one can imagine substantial desire among a lung transplant recipient to report total abstinence. We suggest biologic testing is performed in those patients considered high risk for smoking recidivism.

Relative contraindications

The relative contraindications to transplantation are, not surprisingly, an area of more clinical debate.

Age

While 2006 ISHLT guidelines recommend against transplantation in recipients over the age of 65 years [3], the practice has been controversial. Registry data indicate that older patients have been transplanted at increasing frequency, with 28 transplants in 2000 (1.6%) documented in patients aged 66 years or older. In contrast, in the first 6 months of 2011, 13.5% of transplants were done on recipients in this age group [1] despite increased mortality with advanced age. Results to support a precise upper age limit for transplant are lacking since studies use different definitions for older patients. In a matched cohort study from the Toronto group, 42 patients over the age of 60 years were compared with matched patients younger than 60 years. Survival at 1, 2, 3, 4, and 5 years was significantly worse in the older cohort, due to increased infectious causes in the early period post transplant followed by increased malignancy [34]. A later retrospective review of over 8000 patients included in the UNOS database from 1999 to 2006 stratified patients in age quartiles. Age over 60 years, the highest age quartile, was associated with a 37% increase in risk of death at 1–2 years, while short-term 30- and 60-day mortality was no different from younger patients. Within the quartile of patients over the age of 60 years, the oldest patients (age >70 years) had a substantially increased risk of short-term mortality. Unlike in previous studies, age was thought to be protective against infection and rejection [35]. In the current ISHLT report, patients older than 65 years had decreased median survival (3.6 years vs. 6.5 years) compared with patients aged 35–49 years, and decreased long-term survival (39% vs. 51–56% at 5 years) compared with patients younger than 60 years old [1].

Other studies, many of which report results with carefully selected recipients, have demonstrated that transplants in older patients may be performed with similar mortality to that in younger patients [36–38]. Criteria used to select older patients included body mass index (BMI) in the accepted range, and absence of obstructive coronary artery disease, peripheral or cerebrovascular disease, renal insufficiency, or debilitation [38]. A larger series comparing survival rates for patients aged under 60, 60–65, and older than 65 between January 2006 and May 2008 found no survival difference, but did find that complications differed by age group, with malignancy and drug toxicity more common in patients over the age of 65 years, and rejection more common in those aged 60–65 years [39].

Several series have suggested single lung transplant (SLT) may be tolerated better in the older population, leading some centers to only consider SLT in older patients. Meyer et al. reviewed 2260 recipients of lung transplant for COPD [1835 SLT and 425 bilateral transplants (BLT)] and found that a mortality benefit existed for BLT until the age of 60 years, after which SLT had improved mortality [40]. A similar review of transplanted patients with pulmonary fibrosis demonstrated no benefit for BLT over SLT [41]. However, there are some contradictory studies. Series have shown similar 30-day [42] and 1-, 2-, and 5-year survival for patients over 60 years undergoing BLT versus SLT [43], and a large review of UNOS data demonstrated no mortality effect of SLT versus BLT in patients over 60 years [44].

Nutritional status

Nutritional status is frequently abnormal in patients with chronic lung disease; in one study, only about half of patients were deemed to have a normal nutritional status, with the remainder being underweight or overweight [45]. The ISHLT guidelines suggest against transplanting patients with BMI of $\geq 30\,kg/m^2$, though they do not comment on underweight recipients [3].

A link between increased BMI and mortality is fairly consistent, supporting a BMI threshold for transplant. The Toronto program and others have shown similar deleterious effects of excess weight on outcomes [46–48] A Spanish study of 256 transplanted patients, including 38 obese patients, was one of the few to find no statistical difference in mortality between BMI groups, although there were trends towards increased mortality and longer mechanical ventilation in the obese [49]. Overweight and obesity have also been associated with increased incidence of primary graft dysfunction [50].

Underweight had been suspected to be a risk factor for death [51]. More recently, very large reviews of the lung transplant databases have shown effects of body mass index at both extremes, stratifying patients into underweight (BMI $<18.5\,kg/m^2$), normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25.0–29.9 kg/m^2), and obese (BMI $>30\,kg/m^2$) by World Health Organization categories. Lederer et al. retrospectively reviewed 5978 lung recipients in the UNOS registry with CF, COPD, or diffuse parenchymal lung disease to evaluate the effect of BMI. BMI other than normal was associated with increased risk of death overall and at 1 and 5 years, with hazard ratio for overall mortality of 1.15–1.16 for being the underweight, 1.14–1.17 for the overweight, and 1.20–1.25 for the obese [52]. In the largest evaluation, Allen et al. reviewed 11 411 lung transplants performed between 1998 and 2008 from the UNOS database and for whom adequate data were available to stratify them by BMI. There was a small but statistically significant increase in mortality for every BMI strata above or below normal BMI, with odds ratio for death for overweight of 1.06, for obese of 1.16, and for underweight of 1.14 in multivariate analysis. The increased risk of death appeared to be short term mortality (only seen within the first year) for patients who were overweight and obese, but manifested later (after 1 year) in underweight patients [53].

Other medical conditions that have not resulted in end-stage organ damage

Coronary artery disease

Underlying coronary artery disease (CAD) has been associated with increased mortality in the univariate analysis of a Brazilian transplant cohort [54]. Intervention for pre-existing CAD may be implemented prior to lung transplant or at the time of transplant. The UCLA group recently reported a 10-year experience during which 27 patients with discrete coronary lesions and preserved left ventricular ejection fraction underwent coronary revascularization prior to lung transplant with no difference in survival or causes of death [55]. This echoes previous series, which showed similar success [56,57]. Of note, all patients demonstrated preserved cardiac function and had discrete (i.e. not diffuse) coronary artery lesions.

Diabetes

Diabetes is a common co-morbidity in lung transplant patients, particularly patients with CF. Although the number of patients with diabetes in their series was small, Plantier et al. found that pretransplant diabetes was independently associated with death from all causes and from cardiovascular causes [58]. Likewise, a small retrospective review of 25 patients transplanted for CF demonstrated increased morbidity and mortality for those with pretransplant CF-related diabetes [59], though interestingly, a larger study suggested no impact of diabetes on survival in CF patients and improved 1-year survival for those patients with pre-existing diabetes [60]. There has been no systematic assessment of the effect of diabetes control on success post transplant, although Bradbury et al.'s experience suggests that uncontrolled diabetes played a role in some post-transplant readmissions [59]. We suggest careful selection of diabetic patients to include those with good control, and to engage expert consultation in management of blood sugar in the perioperative setting.

Gastroesophageal reflux disease

Gastroesophageal reflux has been recognized as a cause of allograft dysfunction after lung transplant with both increased rates of bronchiolitis obliterans syndrome and acute rejection episodes [61,62], as well as worse short-term forced expiratory volume in 1 s (FEV_1) and early survival compared to patients without reflux [63]. Gastroesophageal reflux is highly prevalent among candidates for lung transplant and may be asymptomatic, requiring invasive testing [64–66]; this condition also worsens post transplant [67], perhaps in part related to medication-induced gastroparesis [68].

Treatment may require surgical intervention. Proton pump inhibitor therapy alone may affect gastric acid, but does not prevent non-acid reflux and gastric aspiration [69]. Fundoplication after transplant has been associated with improvement in bronchiolitis obliterans [70]. Laparoscopic fundoplication has also been performed successfully in patients pre transplant [71] and may preserve lung function in patients pre and post transplant [72,73].

Thus, we suggest that invasive testing for esophageal reflux should be performed on patients prior to transplant and surgical management should be considered. In those patients with severe, symptomatic disease that is refractory to treatment, reflux may be a relative contraindication to transplant.

Critical or unstable condition

Use of mechanical ventilation, artificial lung surrogates, and hospitalization at the time of transplant are associated with increased 1-year risk for mortality. According to ISHLT data, being hospitalized in any setting at the time of transplant confers increased risk of death [1,2], and in European experience, urgent transplantation is associated with lower survival than traditional transplantation [74]. Transplantation in critically ill patients, including those supported with mechanical ventilation and artificial lung surrogates, is a burgeoning area.

The use of mechanical ventilation at the time of transplant is associated with increased early mortality. In the 2011 ISHLT report, the 390 patients transplanted on mechanical ventilation from January 1996 to June 2009 had a relative risk of death at 1 year of 1.57 in comparison to a COPD SLT. In US and European experience, patients on mechanical ventilation and extracorporeal membrane oxygenation support (ECMO) had significantly lower 1-year survival than non-urgent transplants [75–77], but with a substantial survival benefit compared with not transplanting [75,76]. In the UNOS data, with a cohort consisting mainly of younger patients with CF, those on mechanical ventilation had unadjusted survival at 1, 6, 12, and 24 months of 83%, 67%, 62%, and 57%; those on ECMO, 72%, 53%, 50%, and 45%; and those unsupported 93%, 85%, 79%, and 70%, respectively [75]. Smaller series have had mixed results [78–81], but current experience suggests that transplant for mechanically ventilated patients should be carefully restricted to those with single organ failure, no resistant airway colonization, and the ability to participate in physical therapy and rehabilitation [82,83].

Single center series have reported numbers of patients who have been transplanted while on ECMO, with patients demonstrating higher perioperative and short-term mortality, but acceptable mid-term survival, even equaling traditional transplant when contingent on 3-month survival [84]. Other smaller series have demonstrated some success with a Novalung venovenous device [85,86] or with devices for extracorporeal carbon dioxide removal in the absence of hypoxemia as bridges to transplant [87]. Devices placed to allow ambulation and physical therapy while on support have shown promise in early use as bridges to transplant [88–92]. Intriguingly, patients bridged with ECMO therapy who avoid mechanical ventilation appear to have improved survival compared with those bridged with mechanical ventilation [93–95]. There are no existing guidelines about when and how to utilize ECMO technology when bridging to transplant. The topic of artificial lungs and mechanical lung surrogates is covered in depth in Chapter 50.

Colonization with highly resistant or highly virulent bacteria, fungi, or mycobacteria

Active infection is a contraindication to transplant with the concern for causing sepsis-related deaths with intense immunosuppression. The suppurative lung diseases (CF and non-CF bronchiectasis) are characterized by colonization with microbes that may become resistant. The specific risk factors for transplant mortality in a non-CF population have not been well described. Airway colonization in CF is described separately in the disease-specific section of this chapter.

Severe or symptomatic osteoporosis

Osteoporosis and osteopenia are highly prevalent in lung transplant recipients [96–98]. Therapy with bisphosphonates and resistance training have been shown to be effective prophylaxis in lung transplant recipients [99,100], but those who have advanced disease prior to transplant are at risk for severe complications as osteoporosis routinely worsens after transplant [98,101,102]. Given the steroid therapy that accompanies lung transplant, those patients with very severe or symptomatic osteoporosis would be at risk for excessive complications post transplant.

Other considerations

Previous thoracic surgery

Previous thoracic surgery, particularly previous pleural procedures, is associated with increased bleeding in the perioperative period, but has not been associated with increased mortality [103–105] and should not in general be considered a contraindication to transplant. However, pleural adhesions are associated with increased technical challenge during transplant [106].

Clinical circumstances require adjustment of surgical technique based on surgeon preference as specific scenarios, e.g. with previous pneumonectomy, heart transplant, or unilateral pleural adhesions, may impart increased risk [107–109].

Obstructive lung disease

Chronic obstructive pulmonary disease

COPD remains the most common indication for lung transplantation to date [1,18]. Its heterogeneous course makes prognostication difficult and thus, raises challenges for selection of patients for transplantation.

Survival benefit

Hand in hand with the challenge in prognostication, survival benefit for transplant in COPD is less clear. In early experience, prior to the current lung allocation method, emphysema had an improved waitlist survival than other diseases [110]. Thabut et al. have created a statistical model that estimates that, of 8182 people on the lung transplant waitlist, 50.1% of those undergoing SLT and 63.7% of those undergoing BLT would have a survival benefit. This model has not yet been validated [111]. The analysis by Charman et al. also supports a survival benefit for transplantation in COPD [112].

Prognostication

Recent large population studies have demonstrated that progression of COPD is heterogeneous. An observational study of 2163 patients with COPD showed a highly variable decline in FEV_1 over 3 years, with 38% having a decline in FEV_1 of >40 mL/year; 31% a decline of 21–40 mL/year, 23% a range from −20 to +20 mL/year, and in 8% an increase of >20 mL/year. Patients with very severe COPD declined less than those with less severe disease [113]. Many characteristics have been found to be markers for mortality in COPD, including FEV_1, lung hyperinflation, weight loss with low BMI, anemia, exercise capacity, dyspnea, health-related quality of life and activity level, use of oxygen supplementation, distribution of emphysema, and exacerbations [114–117].

FEV_1

Dating back to earliest attempts to prognosticate, survival in COPD has been tracked by FEV_1, with Burrows et al. noting an overall 58% mortality for a group of 200 patients followed over 7 years. Patients with a FEV_1 of <0.75 L had substantially worse survival than those with a FEV_1 of 0.75–1.24 L, with the best survival for those with a FEV_1 of >1.24 L. It was also noted that FEV_1 dropped by a mean of 56 mL/year, but was highly variable [118].

A post-bronchodilator FEV_1 association with mortality was confirmed in observational studies of non-hypoxic patients with COPD followed for 3 years [119].

Exacerbations

Mortality following hospitalization for acute exacerbations of COPD has long been noted to be high, with 1-year mortality reported in various studies of 22–59%, and highest among those with a $PaCO_2$ of ≥50 mmHg (49%) [120] or admitted to an intensive care unit (59%) [120–123]. The link between repeated exacerbations requiring hospitalization and mortality was established by a prospective analysis of 304 patients with COPD followed over 5 years. Patients with three or more exacerbations per year had a hazard ratio for death of 4.13, and for one to two exacerbations a hazard ratio of 2.0 compared with patients with no exacerbations, a risk that was independent of lung function or age [115]. More recently, large population-based studies have confirmed that each exacerbation confers an increased risk of death regardless of other exacerbations and of baseline lung function [124].

Table 31.3. Variables and point values used for the computation of the Body Mass Index, Degree of Airflow Obstruction and Dyspnea, and Exercise Capacity (BODE) index

Variable	Points on BODE index			
	0	1	2	3
FEV_1 (% predicted)	≥65	50–64	36–49	≤35
Distance walked in 6 min (m)	≥350	250–349	150–249	≤149
Modified Medical Research Council dyspnea scale	0–1	2	3	4
Body mass index	>21	≤21		

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Pulmonary hypertension

Elevated pulmonary artery diastolic pressure has been associated with death on the lung transplant waitlist for patients with emphysema [125]

BODE index

Celli et al. in 2004 released a landmark prognostication score for COPD known as the BODE index, referring to BMI (B), degree of airflow obstruction (O), dyspnea (D), and exercise capacity (E) measured by the 6-min walk test (6MWT). Patients with higher BODE scores (Table 31.3) were at higher risk of death, with improved concordance compared with FEV_1 [126].

Extrapolation to a transplant population is limited as the score was validated in predominantly elderly patients as well as an almost entirely male cohort [126]. However, Lahzami et al. found that a BODE score of ≥7 may predict those patients expected to have a mortality benefit from transplant [127]. Further studies have supported the usefulness of the BODE index for prognosis, both as a single measurement and when tracking longitudinal change. Martinez et al., in a review of 609 patients with severe emphysema (FEV_1 ≤45%, residual volume ≥150%) enrolled in the National Emphysema Treatment Trial, found that increased mortality was independently associated with a higher modified BODE score [117]. An analysis of the same patient population found that changes in the modified BODE score at 6, 12, and 24 months were predictive of future mortality [128]. A recent large study prospectively followed both BODE score and FEV_1 over at least three measurements for 751 patients in the US and Spain from 1997 to 2008. The group with an increasing BODE score but stable FEV_1 (n = 69) demonstrated higher mortality [129].

A modification of the BODE score using cardiopulmonary exercise testing (CPET) with VO_{2max} [130] did not improve prognostication, and one replacing 6MWT with severe exacerbations performed equally well and may simplify scoring [131]. Of particular note is that the use of BODE, or any index, is not now part of the lung allocation score (LAS) used for priority rating, although it is recommended as a transplant referral criterion.

Other composite indices have been developed, including the HADO score (Health status, Physical Activity, Dyspnea scale, and Obstruction) [132] and a classification and regression tree (CART) analysis utilizing age, FEV_1, dyspnea, physical activity, general health, and number of hospital admissions in the previous 2 years [133]. These have not supplanted the BODE index to date and have not been routinely adopted in the transplant evaluation.

Lung volume reduction surgery

Lung volume reduction surgery (LVRS) should be considered as an alternative or a bridge to lung transplantation in eligible patients.

LVRS has been used to improve hyperinflation in patients with heterogeneous emphysema and has been demonstrated to improve physiologic parameters of obstruction, air trapping, oxygenation, and exercise tolerance [134]. In a randomized controlled trial, LVRS improved mortality in patients with upper lobe predominant disease and low exercise ability [135]. Surgically treated patients showed improvements in a modified BODE score associated with improved mortality [128]. Successful LVRS has been shown to extend time before lung transplant is needed without significantly impacting post-transplant morbidity and mortality [136–140], although patients with unsuccessful LVRS represented a group at increased risk for transplant [139,140]. In one series, 24 of 31 patients could be deactivated from the transplant list after LVRS [136]. Patients with a FEV_1 of <20% as well as a diffusion capacity for carbon monoxide (DLCO) of <20% or homogenous emphysema are at high risk of death from LVRS [141] and are not eligible for this procedure.

Procedure choice

While both SLT and BLT are performed for COPD, BLTs are gaining favor. A review of the ISHLT/UNOS registry from 1991 to 1997 looked at 2260 lung transplant recipients (1835 SLT, 425 BLT) with COPD and concluded that there was a survival benefit below the age of 60 years for BLT; above the age of 60 years, mortality favored SLT [40]. The Washington University group in 2002 published its experience with 306 patients from 1998 to 2000 transplanted for emphysema and found a significant 5-year survival benefit for BLT in both COPD and α-1 antitrypsin deficiency (AATD) patients. Five-year survival overall was unchanged between COPD and AATD, and was no different from other diagnoses, and even early hospital survival was unchanged between COPD and AATD [142].

Guidelines for transplant

Based on the above information, the ISHLT recommends referral for patients with a BODE index of >5, and transplantation for those with a BODE index of 7–10, or at least one of the following: history of hospitalization for acute exacerbation associated with acute hypercapnia, pulmonary hypertension and/or cor pulmonale, FEV_1 of <20% or a DLCO of <20% or homogenous emphysema [3].

Alpha-1 antitrypsin deficiency

Emphysema related to AATD, a genetic disorder with an autosomal co-dominant inheritance, is the fourth most common indication for lung transplants and accounted for 7% of the transplants in the most recent ISHLT registry [18]. It is an under-recognized contributor to obstructive lung disease [143] that causes accelerated lung disease in young persons [144], particularly those exposed to smoking [145]. The most common severe deficiency, with clinical manifestations that include emphysema and/or end-stage liver disease, is in patients homozygous for the ZZ allele [143]. US [146] and Danish registries [147] have confirmed that mortality in patients with AATD is frequently related to emphysema, in both smokers [148] and never smokers [149]. Transplant criteria used to select patients are the same as those for non-AATD emphysema, with prognostic data supporting the use of FEV_1 as part of the selection criteria. Mortality rates in AATD are significantly related to FEV_1, with highest mortality in those with a FEV_1 in the lowest tercile (<35–37.5% predicted) [145,150,151]. Additionally, DLCO and CT scan score have been shown to be associated with respiratory death [150,151]. Augmentation therapy with enzyme replacement should be considered in all patients as it may slow progression of FEV_1 decline and improves overall mortality [145].

Diffuse parenchymal lung disease

Diffuse parenchymal lung disease is a frequent indication for transplantation. Idiopathic pulmonary fibrosis (IPF) is now the second most common indication for transplant, representing an increasing proportion of lung transplants performed. Among the other diffuse parenchymal lung diseases, transplants are also performed for lymphangioleiomyomatosis, sarcoidosis, and at times for collagen vascular disease associated interstitial lung diseases (ILDs) [2,18].

Idiopathic pulmonary fibrosis and fibrotic non-specific interstitial pneumonia

Prognosis

The American Thoracic Society released an updated classification of the idiopathic interstitial pneumonias based primarily on histopathology [152]. IPF, with its pathological pattern of usual interstitial pneumonia (UIP), has a uniquely poor prognosis when compared to other histopathologic patterns, and thus it is crucial to diagnose it appropriately when considering a patient for transplant. In comparison with patients with desquamative interstitial pneumonia (DIP) and respiratory bronchiolitis interstitial lung disease (RBILD) with an 80% 5-year survival, and fibrotic nonspecific interstitial pneumonia (NSIP) with a 70% survival, patients with UIP in one series were found to have a 20% 5-year survival [153]. Similarly, a prospective analysis of 315 patients with diffuse parenchymal lung disease demonstrated substantially worse survival for IPF (2 year of 48.4%, 5 year of 35.4%) compared with the other diseases (2 year 74.9–100%, 5 year 69.5–91.6%) [154]. Survival benefit for patients with IPF who are transplanted has been established in several evaluations [112,155,156].

The timing of listing and transplant must be informed by the patient's prognosis, with the goal to identify those patients with high short-term mortality for immediate listing and to hold off on transplantation for those who may be predicted to have a more indolent course. This assessment has been made more challenging by the at times rapid and unpredictable decline of patients with IPF [157]. Among patients with mild to moderate disease followed in an observational study, death was often described as acute or abrupt or subacute with a sharp increase in dyspnea or A–a gradient shortly before death [158]. Median survival was previously thought to be 2–3 year;, however, there is increasing recognition that the clinical course is variable and may include some patients with much longer survival [157]. Recent registry data indicate a survival median of 55.6 months for those with mild disease, with a median of 27.4 months for those with severe disease by forced vital capacity (*FVC*) [156]. The 2006 ISHLT guidelines for the referral of patients with ILD include histologic or radiographic evidence of UIP irrespective of vital capacity, or histologic evidence of fibrotic NSIP [3]. Patients with a histologic diagnosis of both UIP and NSIP (discordant biopsies) have been shown to have similarly poor prognosis to those with UIP alone and may be considered for transplant with the same category [159].

Prognosis

Pulmonary function

Different parameters of pulmonary function have been associated with survival. Assessment of vital capacity at baseline has been associated with mortality, although the value at which increased

risk is conferred has varied between studies: ≤60% predicted [160], and with cut-offs ranging from *FVC* of 60% to 83% [161]. More recently, Nathan et al. noted that patients divided by *FVC* (mild ≥70%, moderate 55–69%, severe <55%) had correspondingly worse median survival of 55.6, 38.7, and 27.4 months, respectively [156]. Latsi et al. found that patients with high short-term (2 year) mortality had lower baseline *FVC* (62.0% vs. 78.7%) [162].

Longitudinal change in *FVC* has been noted to be associated with increased mortality in multiple studies [163–167], and even marginal changes in *FVC* (5–10%) over 6 months conferred higher mortality than in those with stable disease [164].

DLCO

DLCO has been correlated with survival, though like *FVC*, the value of importance differs in different studies, with ranges from 30% to 45%, and with other studies noting a continuous increase in mortality with decreasing DLCO below 50% predicted, and the worst outcome in those with a DLCO of <20% predicted [156,161,162,168,169]. In a group of 487 patients with IPF seen at the Mayo Clinic, decreased DLCO was associated with a relative risk of 1.4 for every 10 percentage point decrease [166]; Mogulkoc et al. also found that DLCO was significantly associated with survival on multivariate analysis with a hazard of death increasing by 4% for every 1% decrease in DLCO [169]. In longitudinal analysis, a decline in DLCO of >20% at 1 year was found to be significantly associated with mortality [167], though lower levels of change in DLCO were not significant in some analysis [164,170].

Alveolar–arterial oxygen gradient (A–a gradient)

Decline in A–a gradient has had mixed results, with some suggesting mortality association [165] and others finding none [170].

Pulmonary hypertension

Pulmonary hypertension has been associated with worsening outcomes in IPF [160,168,171] and combined pulmonary fibrosis and emphysema [172], with 5-year survival declining as mean pulmonary artery pressure (PAP) rose above 17 mmHg.

Exercise tolerance

Performance and longitudinal change on 6MWT provides useful prognostic information. In a population of 822 patients followed longitudinally, the risk of death was over four times greater for patients whose 6MWT distance decreased by more than 50 m, and was three times greater for patients whose walk distance declined by 26–50 m [173]. A similar value of 29–34 m was found in a previous analysis [174]. In an Israeli population awaiting lung transplant, distance on 6MWT has been inversely associated with risk of death while listed for transplant [175].

Likewise, abnormal heart rate recovery 1 and 2 min after 6MWT, desaturation, and lowest saturation on climbing or on 6MWT have also been associated with poorer prognosis and may be associated with pulmonary hypertension [176–178].

Radiology

High-resolution computed tomography (HRCT) characteristics have been reproducibly found to be predictive of mortality [169,179,180]. The extent of fibrosis was associated with increased risk of death, and patients with more fibrosis were also more likely to have a low DLCO and *FVC*, and an increased A–a gradient [180]. The risk of death increased by 106% for every unit increase in HRCT fibrosis score [169].

Mechanical ventilation

The mortality for patients with IPF who require mechanical ventilation for acute respiratory failure is exceedingly high. Traditionally, patients requiring mechanical ventilation have been excluded from consideration for transplant [161]. In one retrospective review of 34 patients with IPF presenting with respiratory failure, 15 were placed on invasive mechanical ventilation with 100% inhouse mortality; there was a 74% mortality for the 19 patients placed on non-invasive ventilation. Of the five surviving patients, four died within 6 months of discharge [181].

Guidelines for transplantation

Based on much of the above information, the 2006 ISHLT guidelines suggest referral for any patients with UIP or fibrotic NSIP, and transplantation for histologic or radiographic UIP with a DLCO of <39% predicted, ≥10% decrease in *FVC* over 6 months, desaturation <88% during 6MWT, or honeycombing on HRCT scan. Patients with NSIP are recommended for transplant if they have a DLCO of <35%, or a ≥10% decrease in *FVC* or a >15% decrease in DLCO over 6 months [3].

Given the variation in cut-offs for prognosis, an exact physiologic parameter may be controversial; for example, Flaherty et al. suggested listing patients with a *FVC* or a total lung capacity (*TLC*) of ≤65% predicted and a DLCO of ≤45% predicted [161]. Subjective variables such as progression in dyspnea scores and cough have been associated with mortality, but have not been incorporated into transplant guidelines [165,177,182]. Thus, a full assessment of a patient's clinical course and co-morbidities must be taken into account when making the decision to list.

Single versus bilateral lung transplant

Several analyses have found a survival benefit favoring BLT over SLT despite the initial challenge of a longer and more difficult surgery. A review of the UNOS database from 1987 to 2008 found a 1-year conditional survival benefit for BLT over SLT in younger patients [183]. UNOS data from 2005 to 2007 also demonstrated better 1-year survival for BLT in patients with the highest risk as measured by the highest lung allocation score (see Chapter 40) [184]. In another evaluation of IPF patients, post-transplant 6MWT and best FEV_1 were better for BLT recipients, as well as their having better 1-year, overall, and bronchiolitis obliterans syndrome-free survival compared to SLT; this benefit held despite significantly higher preoperative mean PAP for BLT recipients [185]. Other evaluations have shown an overall worse 30-day and 1-year survival for IPF patients compared to patients with other diagnoses, and no difference between SLT and BLT [41,186].

Other diffuse parenchymal diseases

Connective tissue disease with associated lung disease

Transplantation for pulmonary fibrosis associated with connective tissue disease represents only 0.8% of transplants [18], at least in part due to concerns over systemic disease complicating transplant as well as a better prognosis in comparison to IPF [187]. Diffuse lung disease has been associated with multiple connective tissue diseases, including systemic sclerosis, polymyositis/dermatomyositis, rheumatoid arthritis, mixed connective tissue disease, systemic lupus erythematosus, and Sjögren's syndrome [161]. Given limited experience, there are no specific recommendations for timing of referral or transplant.

Systemic sclerosis

Pulmonary disease is ubiquitous in patients with systemic sclerosis, but is progressive in a minority of patients [188]. Patients have been transplanted with success [189–192], with one center reporting 14 patients transplanted successfully with 1-year survival no different from that for IPF patients, albeit with more acute rejection seen [189]. The systemic disease associated with scleroderma can contraindicate transplant, including skin ulceration, chronic kidney disease, and esophageal dysmotility with aspiration [193]. Patients to be considered for transplant for scleroderma should have minimal systemic disease and progressive lung disease.

Idiopathic inflammatory myopathies

Polymyositis, dermatomyositis, clinically amyopathic dermatomyositis, and antisynthetase syndrome are associated with ILD in 35–40% of patients, with some experiencing progressive respiratory decline [194]. The associated muscle weakness may be a contraindication to transplant [161] as it would preclude rehabilitation and pulmonary clearance. Transplant may be considered in a patient with progressive lung disease as measured by serial pulmonary function tests (PFTs) and quiescent systemic disease.

Rheumatoid arthritis, mixed connective tissue disease, systemic lupus erythematosus, and Sjögren's disease

ILD is seen with varying frequency in rheumatoid arthritis, with debilitating pulmonary disease demonstrated in 1–4% of patients; severe inflammatory arthritis remains the most important contraindication to transplant in this group [161]. A UIP pattern on HRCT and on biopsy is seen more frequently in rheumatoid arthritis compared with other connective tissue disease-associated ILD, and correspondingly appears to be associated with a worse prognosis [195]. Pleural disease is common in systemic lupus erythematosus, but progression of overt fibrotic lung disease appears rare and the disease most likely progresses slowly; in a small series of 14 patients with interstitial disease and lupus only two died of progressive lung disease [196,197]. In one sequential series of 144 patients with mixed connective tissue disease (MTCD), 96 (66.6%) had evidence of active ILD, the majority of which resolved after therapy, although 13 patients remained with some fibrosis on HRCT and one with honeycombing [198]. In patients with MCTD, there is an association between esophageal dilatation and ILD [199], raising concern that this could be an additional contraindication to transplant. Finally, ILD complicates the course of Sjögren's disease for many patients, in one series affecting nine of 20 patients followed [200].

The approach to transplantation is similar to that of the other connective tissue diseases, in that patients with progressive pulmonary disease and minimal systemic disease should be referred to a transplant center.

Sarcoidosis

A multisystem disease, sarcoidosis may lead to significant other organ dysfunction (e.g. heart, liver) that can preclude transplantation. Sarcoidosis currently accounts for 2.6% of lung transplants performed [18]. Those patients with advanced pulmonary sarcoidosis have been shown to have a high mortality on a lung transplant list; of 43 patients with sarcoidosis listed for lung transplant at the University of Pennsylvania Medical Center, 23 (53%) died while listed. Listed patients had a 31% survival at 3 years, while the 12 transplanted patients had a 50% survival at 3 years [201]. The waitlist survival has been shown to be similar to that for IPF, although the patients with sarcoidosis had a higher burden of pulmonary hypertension [202].

Prognosis

Pulmonary hypertension has emerged as the most important risk factor for mortality in sarcoidosis [203]. In multivariate analysis, a right atrial pressure (RAP) of $\geq$15 mmHg was independently associated with increased risk of death [201]. In a review of 405 patients listed for transplant for sarcoidosis in the US between 1995 and 2000, risk of death was associated with increased pulmonary artery pressure, increased need for supplemental oxygen, and African–American race [204].

Guidelines for transplantation

Given these findings, the 2006 ISHLT guidelines have recommended referral for patients with NYHA functional class III or IV, and transplant for patients with NYHA functional class III or IV along with hypoxemia at rest, pulmonary hypertension, or RAP $>$15 mmHg [3].

Lymphangioleiomyomatosis

Lymphangioleiomyomatosis (LAM), which is the indication for 1.1% of the transplants in the most current ISHLT report [18], is a rare disease that had been considered to occur only in women of child-bearing age and to be nearly uniformly fatal within 10 years when it was first described [205,206]. With increased awareness, detection, and the creation of rare disease registries [207], the full spectrum of the disease is now understood to include older women and some who have a slow and indolent course with 10-year survival reported as high as 91% [208–211]. In a LAM registry that enrolled patients from 1998 until 2001, one-third of patients either received or were listed for lung transplant for LAM [212].

Transplant concerns

Although survival post transplant has been comparable to other conditions, disease-specific complications after transplant have included recurrent pneumothorax and chylothorax requiring intervention, and surgery may be complicated by previous pleural procedures leading to increased bleeding [104,213–215]. Transplantation for LAM has also rarely been complicated by recurrence of disease [214–216]. These risks must be adequately conveyed to a patient during the process of transplant evaluation.

Prognosis

Decreased maximum oxygen consumption corresponded to disease severity by FEV_1 and DLCO, and the patients undergoing transplant in one study had an average VO_{2max} of 41.7% predicted [217]. Disease progression has typically been defined by a decrease in FEV_1 and/or DLCO, both of which have been correlated with CT scan extent of disease and histology score [218,219].

Guidelines for transplantation

Decline of FEV_1 and DLCO are highly variable, with yearly decline in FEV_1 anywhere from none to 285 mL/year in rapidly progressive disease [218], with one study showing an average loss of 110 mL/year of FEV_1 [208]. Only one treatment, sirolimus, has proven to have some efficacy in slowing decline of lung function [220]; its effect on progression to lung transplant for LAM remains to be seen.

Serial assessment of lung function and functional capacity remains the best way to determine those patients heading for

decline who may require transplant. The 2006 ISHLT guidelines recommended referral for NYHA functional class III or IV, and transplantation for severe impairment in lung function and exercise capacity or hypoxemia at rest [3]. If serial lung function measurements demonstrate rapid decline despite therapy, earlier referral should be considered.

Pulmonary Langerhans cell histiocytosis (eosinophilic granuloma)

Transplantation

Pulmonary Langerhans cell histiocytosis (PLCH) is a rare diagnosis for lung transplant, reflecting that the disease for many patients may stabilize or regress [221].

A retrospective review of 39 patients transplanted throughout France for PLCH demonstrated survival of 76.9% at 1 year, 63.6% at 2 years, 57.2% at 5 years, and 53.7% at 10 years [222]. Median survival in two retrospective studies has been similar at 12.5 and 13 years [223,224]. Advanced disease is very commonly associated with severe pulmonary hypertension unrelated to the degree of pulmonary function impairment, with the disease directly causing a pulmonary vasculopathy [221,222,225–227]. Pulmonary hypertension is nearly ubiquitous among those transplanted [222].

Timing for transplantation

As in LAM, serial assessment of lung function and functional capacity is required as there are no precise prognostic tools for this rare disease. Attention to the severity and progression of pulmonary hypertension should be considered when listing. The 2006 ISHLT guidelines recommended referral for NYHA functional class III or IV, and transplantation for severe impairment in lung function and exercise capacity or hypoxemia at rest [3].

Cystic fibrosis and other causes of bronchiectasis

Cystic fibrosis (CF) is a major indication for lung transplant, accounting for 16% of transplants between 1995 and 2010 [2]. CF is a multisystem disease affecting younger patients than the typical transplant diagnoses. Survival benefit for patients undergoing transplant for CF has been consistently demonstrated [110,112,228,229], and in some analyses has been the best among transplant diagnoses [228,229].

Bronchiectasis not associated with CF may have a variety of causes, among them congenital, related to autoimmune disease or malignancy, related to previous infection, and related to immune deficiency [230]. These disorders have been treated similarly to CF with regard to lung transplant [3].

Guidelines for transplantation

The 2006 ISHLT guidelines suggested that patients be referred if they demonstrate a FEV_1 of <30% or a rapid decline in FEV_1, particularly in young female patients; exacerbation requiring ICU stay, increasing frequency of exacerbations requiring antibiotics, refractory or recurrent pneumothorax, or recurrent hemoptysis not controlled by embolization [3]. Transplantation was recommended for oxygen-dependent respiratory failure, hypercapnia, or pulmonary hypertension [3].

Prognosis

Prognosis in CF has improved steadily over the years; according to the Cystic Fibrosis Foundation Registry annual data report for 2010, median survival has improved from age 27 years in 1986 to age 38.3 years in 2010. Respiratory failure remains the most common cause of death for patients with CF [231]. Among the factors associated with survival, FEV_1, gender, age, pulmonary hemodynamics, supplemental oxygen requirement, hypercapnia, and frequency of exacerbations were incorporated into the ISHLT guidelines in 2006 [3]; other factors that may impart increased mortality include diabetes, 6MWT, and nutritional status [232–235].

FEV_1

The traditional transplant referral basis of a FEV_1 of 30% predicted came from an early study by Kerem et al. in 1992. In a retrospective study of 673 patients between 1977 and 1989, patients having an FEV_1 of <30% predicted, $Pao2$ of <55 mmHg, and Pco of >50 mmHg had a 2-year mortality rate of over 50% [236]. This mortality rate implied an appropriate time for transplant to confer survival benefit. Worsening FEV_1 has also been associated with death while listed for transplant; in one series, patients who died awaiting transplant had a significantly lower FEV_1 (15% predicted) compared to those who were successfully transplanted (21% predicted), as well as a lower 6MWT distance [237]. FEV_1% predicted has been included in several predictive models for survival for CF [233,238,239].

As CF survival has improved, more recent studies have suggested that FEV_1 cut-off of 30% is insufficient as an absolute cut-off for transplant. Augarten et al. evaluated 40 patients with a FEV_1 of <30% predicted, and found that there was a trend for patients who were not transplanted to have improved survival [240].

The mortality rate of 50% at 2 years described by Kerem et al. has not been reproduced in follow-up evaluation of patients with severely decreased lung function, suggesting that evolution in CF treatment has impacted survival. One retrospective review was performed of 61 patients with a consistent FEV_1 of <30% at the University of Minnesota. Of 49 deceased patients with a FEV_1 of <30% predicted, only one-third had reached that level within 2 years of death. The remainder lived with this severe reduction in FEV_1 from a range of 2 to 14 years. Survival analysis indicated that the median life span for patients with a FEV_1 of <30% predicted was 3.9 years [241]. Another series found that 178 patients with a FEV_1 of <30% predicted had a median survival of 4.6 years, with 25% living >9 years in the pretransplant era (1986–1990) [242]. George et al. followed 276 patients with a FEV_1 of <30% predicted identified between 1990 and 2003 and followed until 2007 at one center in the UK, finding that median survival improved from 1.2 years in 1990 to 5.3 years in 2003. The largest gain happened between 1994 and 1997, co-incident with the availability of DNAse in the UK as well as improved nutritional status for the cohort [243].

Rate of decline of FEV_1 also has been indicated to be a useful parameter to predict mortality and timing for transplant [244]. For patients who survived for less than the median, the rate of FEV_1 decline was higher (1.8% predicted per year) compared to patients who survived for longer than the median (0.73% predicted per year), with the rate of decline in FEV_1 was a significant predictor of death. There was an increase in the relative risk of death of 1.3 for every 1% predicted per year increase in the rate of decline[241]. Augarten et al. also found that rate of decline of FEV_1 contributed independently to mortality [240].

Gender

Female gender has been associated with increased risk of death in many studies [231,233,236,245–248], though a handful have also

evaluated for gender and not found an effect [240,241,249]. The mechanism of a gender disparity is unclear.

Age

Younger age has been implicated in increased mortality. Age younger than 18 years has been associated with shorter survival [235,236,241]; in the review by Kerem et al., younger patients (<18 years) had a relative risk of death of 2.0 [236].

Exacerbation frequency

Acute exacerbations are associated with worse outcome. Number of admissions has been correlated with mortality [248,250] and more severe admissions requiring intensive care are associated with worse outcomes [235,251].

Pulmonary hemodynamics

Pulmonary hypertension is associated with death and thus has been incorporated into transplant referral criteria. Patients dying while awaiting transplant were more likely to have severe pulmonary hypertension, with patients dying on the list having worse mean pulmonary artery pressure, P_{aCO_2}, and systemic vascular resistance compared with patients either receiving transplant or alive and waiting [252]. The severity of pulmonary hypertension was also associated with death in a small series of 27 patients [248], and another review of 146 patients listed for lung transplant for CF at Barnes Jewish Hospital showed that higher pulmonary artery pressure was an independent risk factor for death while awaiting transplant [232].

Predictive model

One predictive model for 5-year survivorship was proposed in 2001 and was created and validated using data for patients in the Cystic Fibrosis Foundation Patient Registry. The model included age, FEV_1, gender, weight-for-age z score, pancreatic sufficiency, diabetes, *Staphylococcus aureus* infection, *Burkholderia* cepacia infection, and annual number of acute pulmonary exacerbations [233]. The same researchers found that using their model to identify patients with a <30% 5-year survival resulted in transplant survival benefit for that group, while using only the criterion of FEV_1 <30% predicted resulted in equivocal survival benefit. Groups with higher expected survival by predictive model had either equivocal or negative survival effect from transplant [253]. Despite these findings, the predictive model has not been formally incorporated into transplant referral.

Infection risk

Airway microbial colonization and infection are a chronic problem for patients with CF. For this reason, virtually all lung transplants performed for cystic fibrosis are bilateral.

The microbial milieu for an individual patient has been evaluated for both mortality and risk for transplant. The presence of *B. cepacia* infection has been associated with an odds ratio for death of 4.12 in the predictive model of Liou et al. [233]. Likewise, *Burkholdaria cepacia* infection has been associated with an increased risk of infectious death after transplantation [254,255] and at many centers is considered a contraindication to transplant. Recent analysis has agreed that the increased risk seems to be particular to *B. cenocepacia* [255–257], which in one series of 75 transplant patients at Duke was associated with substantially reduced survival compared with both those infected with other *B. cepacia* and those not so infected [256].

Other microbial colonization aside from *Burkholderia* has not demonstrated a significant impact. Liou et al. reported a survival benefit to *S. aureus* colonization in their predictive model [233]. Patients with pan-resistant bacterial infection aside from *B. cepacia* were found to have decreased survival at one center compared with patients not so infected, though their survival compared favorably to UNOS data. Microbial colonization in that cohort was mostly with resistant *Pseudomonas aeruginosa*, *Stenotrophomonas*, and *Achromobacter* [258].

Liver disease

Significant liver disease, in many patients evolving to cirrhosis, is common in CF; cumulative incidence of hepatic involvement is thought to be 27–35%, most frequently focal biliary cirrhosis which in a subset of patients progresses to multilobular cirrhosis and complications of end-stage liver disease [259]. Liver disease has been seen to be an independent risk factor for death or lung transplantation [260]. Successful lung transplant has been performed in patients with well-compensated cystic fibrosis liver disease who had preserved hepatic synthetic function with no adverse effect on outcomes [261,262]. Those with advanced liver and lung disease may be considered for a combined lung–liver transplant.

Critical illness and mechanical ventilation

Some small series have reported success in transplant of patients with CF who require mechanical ventilation [78–80]. Transplant should be limited to those patients who remain with single organ failure, no systemic infection, and able to participate in physical therapy [263].

Pulmonary arterial hypertension

Pulmonary hypertension, a disorder of many different etiologies [264], has long been an indication for lung transplantation. Idiopathic pulmonary arterial hypertension has represented a decreasing share of the total number of transplants performed, now representing 3.1% of the total transplants since 1995 [1]. Patients with idiopathic pulmonary arterial hypertension (IPAH) demonstrate high short-term mortality, but good long-term survival compared with other transplant patients, second only to those with CF [1,2]. The decreasing percentage of transplants for IPAH has been influenced by modern therapy leading to improved prognosis.

Prognosis

Prior to the current age of therapy, prognosis was grim for patients with pulmonary arterial hypertension; the survival for a group of 57 patients with pulmonary hypertension who did not receive transplants was reported at 35% at 3 years [265]. Median survival was estimated at 2.8 years from the NIH registry, and was negatively impacted by mean PAP ($\geq$85 mmHg vs. $\leq$55 mmHg), mean RAP (<10 mmHg vs. $\geq$20 mmHg), and mean cardiac index (<2 L/min/m^2 vs. $\geq$4 L/min/m^2) [266]. Subsequent evaluations have confirmed that right heart failure and RAP of $\geq$12 mmHg were associated with poor survival at baseline [267].

The substantial mortality benefit obtained from long-term prostacyclin infusion [266,268,269] has resulted in decreased referrals for transplant. A lack of response to therapy is predictive of mortality and influences transplant referral. Patients presenting with NYHA functional class IV have been shown to have worse survival compared with patients in NYHA class III, and patients who did

not have improvement in functional class or a 30% fall in pulmonary resistance had significantly increased mortality [267].

6MWT has been independently related to mortality, with survival decreased in patients able to walk less than 332 m in 6 min [270]. Patients enrolled in the randomized controlled trial of epoprostonol who died had significantly lower 6-min walk distances as well [268].

Referral

Based on the above prognostic data, the ISHLT recommended referral for NYHA functional class III or IV or rapidly progressive disease, and transplantation for persistent NYHA class III or IV despite therapy, <350 m or declining 6MWT, failing therapy with prostacyclin, cardiac index of <2 L/min/m^2, or RAP >15 mmHg [3].

Despite the effectiveness of medical therapy, survival for progressive disease remains better with lung transplantation for patients with severe disease [271], although waitlist mortality has not been improved by the recently implemented lung allocation score (discussed in Chapter 40) [272]. Serial assessment remains important to identify those patients with a declining course who will require surgical therapy [273].

Summary

Lung transplantation remains a complex procedure that is reserved for individuals likely to derive sustained benefit from its successful conduct. Although many causes of end-stage lung disease are appropriate for referral—most commonly, COPD, IPF, and cystic fibrosis—a thorough assessment of the patient's co-morbidities, anatomical considerations, and social situations is required to make an appropriate referral. A partnership between the referring physician and the transplant service, aiming to optimize the patient's condition and suitability for transplant, is required to achieve the best outcomes.

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CHAPTER 32

Patient Selection and Indications for Pancreas and Islet Transplantation

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Introduction

Type 1 diabetes mellitus (T1DM) is a disease characterized by the inability to regulate blood glucose as a result of autoimmune destruction of the β cells of the pancreas. The β cells comprise approximately 1–2% of the pancreas. T1DM affects over 1 million people in the US and is a multisystem disease that may result in nephropathy, neuropathy, gastropathy, cardiovascular disease, peripheral vascular disease, and retinopathy [1,2]. In the past, one in five people with TIDM died and one in four developed end-stage renal disease (ESRD) within 20 years of diagnosis. Prognosis improved with the era of intensive insulin therapy, originally described in the landmark publication of The Diabetes Control and Complications Trial in 1993 [3]. Today the long-term survival with T1DM has improved dramatically, and the rate of development of ESRD has leveled off. Intensive glucose management delays the onset and progression of diabetic nephropathy, retinopathy, and neuropathy compared to conventional therapy [3]. Longitudinal studies have shown a marked improvement in serious diabetic complications among patients receiving intensive insulin therapy compared to conventional therapy: the incidence of nephropathy is reported as 9% compared to 25% for conventionally treated cohorts, retinopathy incidence has improved to 21% from 47% in the conventional group, and the incidence of ESRD has improved to 1% from 2% [4,5]. However, intensive insulin therapy also results in more frequent hypoglycemic events, up to three times that experienced on conventional therapy [3]. Although intensive insulin therapy has its merits, it has not completely eliminated the secondary complications of diabetes. In addition, patients are required to perform frequent daily blood glucose measurements and insulin injections, and follow a meticulous diet, all of which may contribute to a negative impact on quality of life and significant healthcare costs.

Solid organ pancreas transplantation is a surgical option for the treatment of T1DM. The most immediate benefit to patients undergoing pancreas transplantation is improved quality of life as a result of less intensive blood glucose management [6,7]. Simultaneous pancreas–kidney transplantation (SPK) is currently the "gold standard" for patients with T1DM and ESRD, resulting in normalization of hemoglobin A1c (HbA1c) and elimination of hypoglycemic unawareness. SPK is the most commonly performed solid organ pancreas transplant, accounting for 65% of pancreas transplants performed in the US in 2007, and is the most durable with current median graft survival of 12–16 years.

Pancreas after kidney (PAK) transplant is another option for patients with T1DM who have already received either a deceased or living donor kidney transplant. Pancreas transplant alone (PTA) and islet cell transplantation are options for patients who have failed medical management with symptoms of hypoglycemic unawareness and labile blood glucose management with preserved renal function. Transplantation of the islets of Langerhans is a minimally invasive technique that eliminates transplantation of the exocrine portion of the gland. Islet transplantation is also associated with fewer periprocedural complications than whole organ pancreas transplant [8]. This chapter will cover the general factors involved in selecting patients who are likely to benefit from pancreas or pancreatic islet transplantation and distinguishing them from those most likely to benefit from medical management of their disease. It also will cover factors influencing the timing of a pancreas transplant relative to a kidney transplant for patients with diabetic nephropathy. It complements Chapter 41, which covers waitlist management of patients awaiting pancreas transplantation.

History

The first pancreas transplant was performed in 1966 at the University of Minnesota as a simultaneous duct-ligated segmental pancreas with a kidney from a deceased donor (Table 32.1). The patient remained insulin-independent for 6 days but experienced multiple complications due to duct ligation [9]. The rationale for pancreas transplantation at the time was to prevent recurrence of diabetic nephropathy in the transplanted kidney. The success of pancreas transplantation has since been associated with advances in surgical technique and management of immunosuppression. Management of the pancreatic duct was the principal surgical challenge in the evolution of pancreas transplantation. The second SPK transplant in 1967 involved drainage of exocrine secretions via a duodenal–cutaneous fistula. This graft functioned for 2 months before failing due to rejection [9]. Subsequent variations included duct obstruction with acrylate glue and neoprene, which led to pancreatitis and exocrine fibrosis [10,11], and duct drainage to the peritoneum, which led to chemical peritonitis [12]. Ductal drainage to

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 32.1. Evolution of pancreas transplantation. The principal challenge was management of the pancreatic duct. With improved technique and immunosuppression, enteric duct drainage is the most common technique in use today.

Year	Development	Outcome	Institution
1966	First pancreas transplant Segmental gland duct ligated	Insulin – independent 6 days, failure due to pancreatitis	University of Minnesota
1967	Duct drainage by duodenal-cutaneous fistula	Rejection at 2 months	University of Minnesota
1973	Duct drainage to ureter	Urinary stones, acidosis, dysuria	Albert Einstein/ Montefiore
1977	Duct obstruction by acrylate glue	Pancreatitis, exocrine fibrosis	University of Sydney
1979	Duct drainage to peritoneum	Chemical peritonitis	University of Miami/University of Wisconsin
1983	Duct drainage to bladder	Urinary stones, acidosis, dysuria; easier management of leak and detection of rejection	University of Wisconsin
1996	Duct – enteric drainage (side–side proximal jejunum)	Physiologic, difficult to manage leak	Karolinska Institute, Stockholm

the recipient ureter was described in 1973 [13], which was modified as drainage to the urinary bladder via pancreaticocystostomy [14]. This was later modified to duodenocystostomy [15]. Enteric drainage using a Roux-en-Y limb was described concurrently [16].

Early attempts at whole pancreas transplantation yielded high mortality and complication rates, with rare insulin independence. In 1980 the International Pancreas Transplant Registry (IPTR) reported a 1-year graft survival of 21% with a 1-year mortality of 39% [17]. The 1970s–1990s brought significant advances in surgical technique, organ preservation, and immunosuppression, resulting in dramatic improvement in outcomes. With the advent of cyclosporine and refinement of the duodenocystostomy technique, Sollinger reported an 80% 1-year graft survival in 1983 [18]. The majority of pancreas transplants were performed using this technique for the next decade, as bladder drainage had the advantages of easier management of anastomotic leaks (via a Foley catheter) and easier detection of rejection (via a decline in urinary amylase). However, postoperative urologic complications occurred with an incidence of 15–30%, necessitating the conversion to enteric drainage. Bladder drainage was also associated with dehydration and metabolic acidosis due to the loss of pancreatic exocrine secretions [19]. With improvement in operative technique and the development of more potent immunosuppressive agents such as tacrolimus in the early 1990s, enteric drainage became the most common technique. More than 80% of pancreas transplants reported to the IPTR since 2003 have been performed using enteric drainage [18]. Several studies have shown that graft survival and incidence of technical complications are equivalent between the two techniques [20–24]. Additional discussion of the history of the pancreas transplant procedure can be found in Chapter 60.

Patient selection

The criteria for candidacy and placement on the waitlist vary for each transplant center. For SPK, absence of endogenous insulin production and a documented glomerular filtration rate (GFR) of <40 mL/min/1.73 m^2 are absolute requirements for listing eligibility. Age, insulin requirements, body mass index (BMI), cardiovascular status, peripheral vascular disease, and smoking status are also important considerations. Pancreas transplantation is associated with a 2% higher absolute mortality than kidney transplant alone within the first 90 days postoperatively [25]. This increase in absolute mortality between pancreas transplant and kidney transplant alone is equilibrated after 12–24 months post transplant. Compared to remaining on the waitlist, the relative risk of death is similarly increased within the first 90 days post transplant, and thereafter the risk of death is higher for those remaining on the waitlist. Gruessner et al. compared patient mortality of waitlisted versus transplanted patients as reported to the United Network for Organ Sharing (UNOS)/IPTR database from 1995 to 2003 [26]. Within the first 90 days post transplant, the mortality hazard ratio was 1.95 for SPK and 4.64 for PAK ($P < 0.0001$). After 90 days the mortality hazard ratio was <1. Patients were most likely to die from cardiovascular or cerebrovascular causes with a functioning transplant in the early postoperative period. Mortality was independently associated with a recipient age older than 45 years and pretransplant dialysis.

In summary, in order to derive a benefit from pancreas transplantation, patients must survive the operation, as early postoperative mortality is significantly higher than with kidney transplantation alone. Pancreas recipients are therefore selected on their relative fitness in an attempt to optimize survival benefit.

Simultaneous kidney and pancreas transplantation

SPK is currently the "gold standard" for patients with T1DM and ESRD. The most immediate benefit to patients undergoing pancreas transplantation is improved quality of life: freedom from insulin injections, frequent glucose monitoring, and dialysis [6,7]. Although pancreas transplantation has been shown to attenuate the development of diabetic nephropathy, retinopathy, and cardiovascular disease, it has been unclear whether this translates into a survival benefit for patients compared to kidney transplantation alone [27]. Recent studies have been divided on a survival benefit in SPK recipients, with some showing benefit after 10 years with a functioning kidney transplant compared with a living donor renal transplant alone [28–30] and others showing no survival benefit associated with SPK when compared to recipients of living donor kidney transplants at 10 years [31–34]. Recipients of SPK transplants have significantly improved graft and patient survival compared to recipients of deceased donor kidney transplants. However, Rayhill et al. examined patient survival after SPK, living related and unrelated renal transplant (LRT), and deceased donor renal transplant (DDK) in 600 patients from a single center from 1986 to 1996. The 10-year patient survival was similar for SPK and LRT transplanted patients: 75% and 80% respectively. Recipients of deceased donor transplants had a significantly worse 10-year patient survival of 62% ($P < 0.01$). Additionally, renal allograft survival was similar between SPK and living related donor (LRD) groups at 10 years and significantly higher in these groups compared to recipients of DDK [34]. A registry analysis of 13 000 patients with T1DM enrolled on the pancreas transplant waitlist from 1988 to 1997 confirmed these findings. The adjusted 10-year survival was 67% for SPK recipients, 65% for LRT recipients, and 46% for DDK recipients (P 0.05) [33].

Equivalent survival at 10 years after SPK and living donor renal transplant likely reflects the high quality of organs selected for pancreas transplantation; donors are typically young with a low

BMI (<35 kg/m^2), without cardiovascular disease or hemodynamic instability prior to donation. Minimized organ preservation times for donor pancreata and the simultaneously transplanted kidney may also contribute to the improved long-term survival rates in this population [35–37]. These factors have been shown to correlate with a lower incidence of pancreas graft thrombosis and improved graft survival [36]. SPK recipients are also generally younger and in better health than kidney-only recipients, further confounding comparisons between recipients of SPK, DDK, and LRT. A recent study examined recipient outcomes after SPK and DDK with organs from the same donor to eliminate donor-specific variables between the two groups. There was no difference in adjusted renal allograft and patient survival up to 9 years post transplant [38]. This premise notwithstanding, SPK organ quality may more closely approximate kidney transplantation from a living donor rather than a deceased donor [37,39].

However, there is a survival benefit associated with SPK versus LRT beyond 10 years post transplant. This is attributed to fewer cardiovascular deaths, reflecting the long-term systemic effects of normoglycemia. Morath et al. examined 11 000 patients after SPK, LRT, and DDK reported to the Collaborative Transplant Study (CTS) from 1984 to 2000. All were patients with T1DM under the age of 45 years with ESRD. In those transplanted between 1984 and 1990, 18-year patient survival was significantly improved in recipients of SPK versus LRD with a functioning renal allograft at 10 years post transplant. Patient survival was 74% at 18 years for recipients of SPK, 60% after LRT, and 40% after DDK ($P \leq 0.001$). SPK patients with a functioning pancreas at 10 years post transplant had significantly improved long-term survival when compared to patients without a functioning pancreas (80% vs. 50%; $P < 0.0001$). Superior SPK survival versus LRT was corroborated by Cox regression analysis, which considered pretransplant cardiac risk (hazard ratio 0.55; $P = 0.0005$). Multivariate analysis confirmed that the risk of cardiovascular death was significantly lower in recipients of SPK versus LRT (relative risk 0.56; $P = 0.007$) and DDK (relative risk 0.77; $P = 0.049$). The long-term patient survival after SPK is superior compared to LRT or DDK in patients with a functioning kidney transplant at 10 years post transplant; and in this group, patients with a functioning pancreas had superior survival compared to those without a functioning pancreas. Death was more commonly cardiovascular in patients without pancreas transplant, which may reflect the significant positive cardiovascular impact of long-term normoglycemia [28].

In summary, SPK is the gold standard for the treatment of patients with T1DM and ESRD. The most immediate benefit to patients after SPK compared to those receiving kidney transplant alone is improved quality of life. There is evidence that SPK diminishes the development of diabetic nephropathy, retinopathy, and cardiovascular disease. A survival benefit for recipients of SPK is evident after 10 years with a functioning pancreas and renal allografts when compared to T1DM after living related renal transplants alone. SPK recipients had fewer cardiovascular events than recipients of LRT, reflecting the long-term systemic effects of normoglycemia [28].

Pancreas after kidney transplantation

PAK is considered for patients with T1DM who have already received a living donor or deceased donor kidney transplant for ESRD. Given the high mortality of waitlisted patients for SPK (46% at 4 years) and the proven benefit of kidney transplant for patients with T1DM [40], pursuing kidney transplant prior to pancreas transplant is a viable option for patients in regions with long wait times for SPK or those who have a matched living donor for kidney transplantation.

The role of PAK has historically been controversial. Renal transplantation confers a survival advantage compared to dialysis, and jeopardizing a functioning kidney allograft for a pancreas transplant with an unclear survival benefit may not be advantageous [40,41]. Earlier studies have demonstrated conflicting results; one study showed a long-term survival disadvantage with PAK [compared to kidney transplant alone (KTA)] and another demonstrated a survival advantage evident after 4 years with a functioning pancreas allograft [26,42]. With improved surgical technique and immunosuppression, the recent literature has supported increased or equivalent survival in patients who receive a pancreas after living donor kidney (PALK) versus living donor KTA, and an improved GFR in patients after PALK versus living donor KTA up to 10 years post transplant [43,44].

PAK has been shown to worsen GFR post transplant compared to SPK [45]. As a result, identifying the patient population that will benefit most has been the subject of recent investigation. In particular, the decline in GFR is thought to be a result of the combination of increased immunosuppression and higher doses of nephrotoxic calcineurin inhibitors (CNIs), the impact of surgery on the renal transplant itself, and immunologic factors, such as a new set of alloantigens, which may lead to an increase in rejection episodes [44]. In an analysis of 126 patients 3 years post PAK, risk factors for kidney graft loss included a pre-PAK GFR of <45 mL/min/1.73 m^2, pre-PAK acute rejection episode, pre-PAK proteinuria, and pancreas transplant interval of >1 year after kidney transplant [46]. A larger registry analysis of 2776 patients demonstrated improved overall kidney graft survival in PAK patients when compared to young T1DM kidney transplant patients, after controlling for selection bias. Stratifying PAK kidney allograft survival by GFR, improved 10-year allograft survival was noted with pretransplant GFR of 40–49, 50–59, and 60–69 mL/min/1.73 m^2. Pretransplant GFR of 30–39 mL/min was not associated with a survival advantage over KTA, although the 10-year graft survival for these patients was still 69%. Pancreas allograft failure was a significant risk factor for kidney allograft failure. This study found no relationship between timing of PAK and kidney graft failure [47].

In summary, PAK is associated with improved long-term patient survival and improved kidney allograft survival for recipients with a pretransplant GFR of >40 mL/min/1.73 m^2. Patients with preoperative episodes of kidney rejection, and patients at high immunologic risk or marginal kidney allograft function may not benefit from PAK [44]. Like all forms of pancreas transplantation, patients must be fit enough to benefit from the surgical procedure [48].

SPK versus PAK

Although PAK outcomes have improved, median pancreas graft survival for SPK remains superior. Median graft survival is 12–16 years for SPK and 7 years for PAK [49]. Diminished graft survival in PAK is related to increased rates of immunologic graft loss, presumably due to a separate antigenic stimulus or difficulty in monitoring the pancreas allograft for acute rejection [25]. From 2004 to 2008 the rate of immunologic pancreas graft loss was 14% for PAK and 5% for SPK at 3 years in a registry analysis of all US and international pancreas transplants [25]. A comparison of SPK versus PALK in T1DM recipients from 2000 to 2007 in the Organ

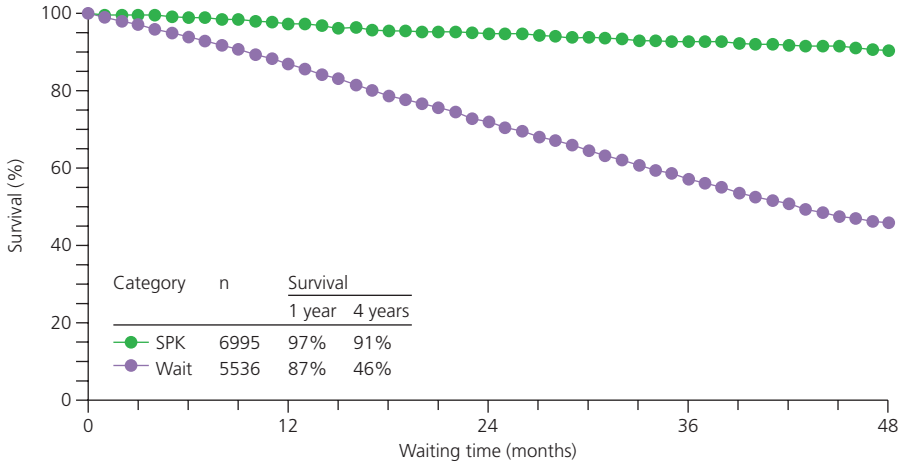

Figure 32.1. Patient survival at 4 years after simultaneous pancreas–kidney transplantation (SPK) compared to those remaining on the waitlist. Longer wait times are associated with increased mortality in patients listed for SPK, with reported 46% mortality after 4 years on the waitlist in a registry analysis of 12 478 patients listed for SPK from 1995 to 2003.
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Procurement and Transplantation Network (OPTN)/UNOS database confirmed these findings. Death-censored pancreas allograft survival was significantly and independently associated with SPK versus PALK; 5-year survival was 80% for SPK and 64% for PALK ($P < 0.01$). Overall patient survival between the two groups 5 years after pancreas transplantation was similar. Notably, the rates of rejection 1 year post transplant were significantly higher in PALK as compared to SPK (16.88 vs. 10.97%; $P < 0.01$) [50].

Advising patients to pursue SPK versus PALK ultimately depends on the waitlist time for SPK. All things considered equal, SPK is the gold standard for patients with T1DM requiring renal transplant. Length of time on the waitlist must be balanced against time spent on dialysis and availability of living donors. SPK waitlist time varies by blood type and region. In 2007 the US national median time to SPK transplant was 498, 341, 337, and 165 days for O, A, B, and AB blood types, respectively, according to the Scientific Registry of Transplant Recipients (SRTR) database [48]. Longer wait times are associated with increased mortality in patients listed for SPK, with reported 46% mortality after 4 years on the waitlist in a registry analysis of 12 478 patients listed for SPK from 1995 to 2003 (Figure 32.1). Patients listed for PAK had 18% mortality over the same amount of time, highlighting the potential impact of ESRD on patient mortality [26]. The decision to proceed with SPK versus PALK should therefore be made on an individual/center basis. Patients on dialysis facing a long wait time for SPK should proceed with living donor kidney transplantation (if available), whereas patients who are predialysis should be listed for SPK (Figure 32.2) [48].

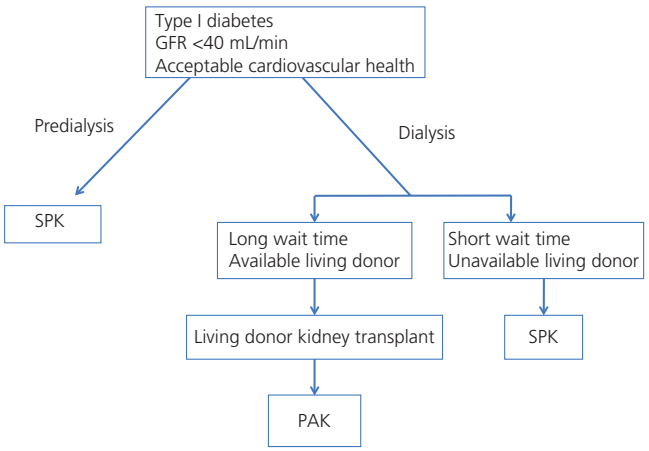

Figure 32.2. Algorithm for simultaneous pancreas–kidney transplantation (SPK) versus pancreas after kidney transplant (PAK). SPK is the gold standard of treatment for type 1 diabetic patients requiring renal transplant who are fit enough to survive the operation. Given the high mortality of patients on the waitlist for SPK (Figure 32.1), time on the waitlist must be balanced against availability of living donors for renal transplant. Patients on dialysis facing a long wait time for SPK should proceed with living donor renal transplant (if available) and then pursue PAK transplantation. Patients on dialysis in regions with short SPK wait times should be listed for SPK. Patients who are predialysis should be listed for SPK.
Data from [26,48].

Indications for pancreas transplantation alone and islet cell transplantation

Pancreas transplantation alone (PTA) accounts for 10% of all pancreas transplants performed in the US. Islet transplantation (IT) is currently experimental and is only available under research protocols. Indications for PTA and islet cell transplant are similar and include frequent acute and debilitating metabolic complications such as hypoglycemia, hyperglycemia, and ketoacidosis; incapacitating aversion to and/or clinical challenges with exogenous insulin therapy; and consistent failure of exogenous insulin therapy to mitigate these complications [8]. The benefits of addressing these complications with PTA or IT should outweigh the risks associated with lifelong immunosuppression, such as serious infection and cancers. Candidates include patients with preserved renal function able to withstand the nephrotoxic effects of CNIs: GFR >80 mL/min/1.73 m^2 and/or serum creatinine <1.5 mg/dL pretransplant (Figure 32.3). If these criteria are met, recipients are less likely to develop renal failure requiring dialysis or future kidney transplantation [51]. The overall incidence of kidney failure reported in two

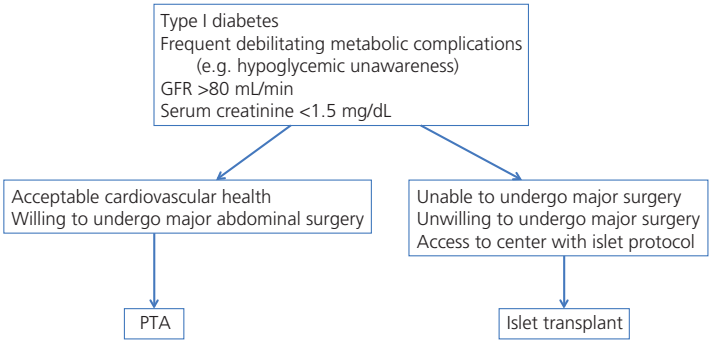

Figure 32.3. Algorithm for pancreas transplant along (PTA) versus islet transplant. PTA and islet transplantation are considered for type 1 diabetic patients experiencing potentially fatal metabolic complications such as hypoglycemic unawareness. Patients must have sufficiently preserved renal function able to withstand the nephrotoxic effects of calcineurin inhibitors, the mainstay of post-transplant immunosuppression. Patients willing and able to undergo major abdominal surgery should proceed with PTA as this is the most durable way to achieve insulin independence. Islet transplantation is currently experimental and offered at only a few transplant centers. It is an option for patients who would not otherwise qualify for or desire major abdominal surgery.

large single-center series after PTA was 10–15% at 5 years [51,52]. Patients selected for PTA must be fit enough to benefit from the operation, considering its associated morbidities and mortality. IT is associated with significantly less procedural morbidity and mortality and will be discussed in detail below. The procedural aspects of IT are covered in depth in Chapter 61.

Pancreas transplant alone

Patients with T1DM experiencing metabolic complications such as blood glucose lability and frequent episodes of hypoglycemia and hypoglycemic unawareness have a severely compromised quality of life. Additionally, hypoglycemic unawareness has the potential to be fatal [2,53], and frequent episodes of hypoglycemia have been shown to adversely affect cognition in children and adults [2]. For this subset of patients with T1DM, PTA may be indicated. PTA is less durable than SPK or PAK with a graft half-life of 5 years [49].

The rationale for PTA has been debated in the literature, as a direct result of the lack of data regarding mortality associated with episodes of hypoglycemic unawareness. Patients selected for PTA do not have renal failure and are thus not necessarily considered to have an immediately life-threatening condition, making it difficult to utilize waitlist mortality as an outcome measure. Venstrom et al. analyzed 672 patients waitlisted for PTA between 1995 and 2000 and found that the relative risk of death was higher for patients after PTA versus those waitlisted (RR 1.57; $P = 0.06$) [42]. Gruessner et al. analyzed the same population but eliminated patients with multiple transplant center listings, such that each patient was counted only once. Additionally, they accounted for patients who had been removed from the waitlist to undergo KTA. After these modifications, the overall hazard ratio for mortality after PTA was 0.66 ($P = 0.12$), a trend toward favoring transplantation. Importantly, the hazard ratio after day 366 was 0.15 ($P < 0.0001$) [26]. Thus, for this selected group of patients, survival is at least equivalent to patients remaining on the waitlist and at best improved for recipients with >1 year of survival with a functioning pancreas allograft. The mortality advantages of PTA are not as striking as for recipients of SPK and PAK, however, and this procedure should be reserved for a subset of patients experiencing debilitating metabolic complications [26].

Patients selected for PTA must have sufficiently preserved renal function to withstand the nephrotoxic effects of CNIs, the mainstay of immunosuppression after pancreas transplantation. PTA is an independent risk factor for post-transplant renal failure reported in a single center review of 131 patients. Mean GFR decreased by 33 mL/min/1.73 m^2 at 4 years post transplant ($P < 0.0001$) [52]. A total of 10% of patients developed renal dysfunction requiring dialysis. Of patients who suffered a >40 mL/min/1.73 m^2 drop in GFR, the only significant risk factor by multivariate analysis was BMI; 70% of patients with a BMI >30 kg/m^2 suffered a decline in GFR of >40 mL/min/1.73 m^2 post transplant [52].

The largest published series of patients undergoing PTA is from the University of Minnesota (n = 513) and demonstrated that 12% of those treated with tacrolimus (n = 239) developed renal failure. The authors identified creatinine >1.5 mg/dL and age younger than 30 years as significant risk factors for post-transplant renal failure by multivariate analysis ($P < 0.0001$ and <0.07, respectively). They noted a trend toward renal failure in patients treated for two or more rejection episodes and concluded that, as younger patients had more episodes of treated rejection, their risk of renal failure was higher [51].

Although renal failure is a risk of PTA, there is evidence that the long-term metabolic control provided by pancreas transplantation has a beneficial effect on diabetic nephropathy. Solitary pancreas transplantation has been shown to improve the diabetic lesions associated with nephropathy after 10 years of normoglycemia [54]. Fioretto et al. examined the biopsies from eight patients pretransplant at 5- and 10-year intervals after transplant and found a statistically significant decrease in glomerular and tubular basement membrane thickness at 10 years post transplant ($P < 0.0001$). There was no improvement in GFR pre and post transplant, although there was a trend toward decrease in urinary albumin excretion [54]. Similarly, Coppelli et al. documented the disappearance of nephrotic syndrome in six patients after PTA with a 36-month follow-up, albeit without a significant change in creatinine clearance [55].

In summary, PTA is recommended for a subset of T1DM patients with poor metabolic control causing debilitating quality of life or potentially fatal complications. Patients selected for PTA should have excellent renal function, with a creatinine of <1.5 mg/dL and a GFR of >80 mL/min/1.73 m^2 predictive of patients who will not go on to develop renal failure after CNI exposure [51]. Other risk factors for renal failure include a BMI of >30 kg/m^2 and age younger

than 30 years, which should be taken into consideration when selecting patients for the operation [51,52]. Patients should also be in excellent cardiovascular health to optimize their perioperative outcome.

Islet cell transplantation

Intensive insulin therapy has markedly improved the incidence and progression of secondary diabetic complications at the cost of increasingly frequent episodes of severe hypoglycemia. IT is a more physiologic means of obtaining glucose control than injected exogenous insulin and has been shown to achieve insulin independence in selected patients with T1DM over the past decade.

IT represents a minimally invasive alternative to PTA. The indications for the two procedures are the same, namely persons with T1DM experiencing hypoglycemic unawareness and/or extreme metabolic lability, negatively impacting quality of life [8]. IT is currently experimental and is performed at fewer than a dozen centers worldwide [56].

The first successful alloislet transplantation was performed in 1990 at Washington University in St. Louis, Missouri; the patient achieved insulin independence lasting up to 25 days post transplant [57]. Long-term insulin independence after IT proved elusive, however, until the landmark publication describing the Edmonton Protocol in 2000 [58]. The Edmonton Protocol consists of refined techniques for islet processing, number of islets infused, and a steroid-free immunosuppressive regimen including tacrolimus, sirolimus, and dacluzimab. All seven patients who received IT on this protocol were insulin-free at 1 year post transplant [58]. The Edmonton Protocol was reproducible at other centers. At three such centers, the insulin independence rate was 90% at 1 year [59]. The first multicenter trial using the Edmonton Protocol was reported in 2006 [60]. Of 36 patients enrolled in the trial, 44% were insulin independent at 1 year, and 13% were insulin independent at 2 years. Results varied by site, as centers with the most familiarity with IT were able to achieve the best results: 67% insulin independence at 1 year. Complete graft loss was experienced by 28% of patients by 1 year post transplant, defined as a decrease in C-peptide to <0.3 ng/mL (C-peptide is produced by islets in amounts equal to insulin and is a more reliable indicator of endogenous insulin production than insulin levels). The remaining 28% of patients had partial graft function (C-peptide >0.3 ng/mL and an exogenous insulin requirement for adequate glycemic control). Patients with partial graft function experienced improved glycemic control and protection from severe episodes of hypoglycemia, however, with reductions in pretransplant HbA1c, insulin requirement, and fasting blood glucose. The authors concluded that, though long-term insulin independence was difficult to achieve, IT provided considerable metabolic benefits in the majority of patients who experienced partial graft function [60].

Although the IT procedure is associated with substantially less morbidity and mortality than PTA, the results are significantly less durable than whole organ transplantation. At 5 years post transplant, 10% of the original Edmonton islet recipients remained insulin independent, compared to 50% of PTA recipients [49,51,61]. Although few patients remained insulin independent, 80% demonstrated C-peptide positivity and improved glycemic control without hypoglycemic unawareness [61]. Of all alloislet transplants reported to the Collaborative Islet Transplant Registry (CITR) from 1999 to 2007, 23% of IT recipients remained insulin independent at 3 years post transplant, and 29% were insulin dependent with positive C-peptide levels and marked improvements in glycemic control and hypoglycemic unawareness. Most of these patients reported to the CITR received islets from multiple donors [62].

Like PTA, IT involves a risk-to-benefit ratio that must be determined on an individual patient basis. IT has been shown to improve episodes of severe hypoglycemia and hypoglycemic unawareness as long as some residual graft function is present [63,64]. Patients with functioning islet grafts report improved quality of life as it relates to fear of hypoglycemia [65–67]. These benefits must be balanced against the risks of lifelong immunosuppression. The original immunosuppressive drugs used in the Edmonton protocol, tacrolimus and sirolimus, may have deleterious effects. Tacrolimus is known to adversely affect renal function [68], and both drugs have been shown to impair insulin sensitivity and islet vascularization [69,70]. A progressive decline in GFR after islet transplantation was demonstrated in patients participating in the original Edmonton trial: GFR declined by 0.39 mL/min/1.73 m^2 per month [71]. GFR deterioration was also noted in the subsequent multicenter trial of the Edmonton Protocol: average GFR declined by 0.45 mL/min/1.73 m^2 per month [60].

Compared to persons with T1DM receiving intensive medical therapy, however, Warnock et al. noted no significant difference in decline in GFR. This study compared IT recipients maintained on tacrolimus or sirolimus to patients with T1DM managed with best medical therapy for a mean 3-year follow-up [72]. Additionally they noted improvements in HbA1c and in retinopathy progression in IT recipients compared to the control population [72]. Still, some argue that the benefits of IT are not outweighed by the risks of immunosuppression [73]. Single-center studies have subsequently successfully employed CNI-free protocols or CNI-minimizing protocols in ITs from a single donor in an attempt to minimize these risks [74,75].

Several recent studies have demonstrated beneficial effects of IT on diabetic micro/macroangiopathy, cardiovascular function, and renal allograft survival in the context of kidney transplantation, a population that is already immunosuppressed [76–78]. Fiorina et al. found an improvement in renal allograft survival 6 years after IT compared to those receiving KTA [78]. Gerber et al. reported comparable glucose control in patients 5 years after SPK and those who received a kidney allograft with simultaneous IT [79].

The majority of ITs to date have required more than one donor to gain sufficient islet mass to achieve insulin independence, potentially putting patients at risk for HLA sensitization [62]. Campbell et al. reported 34% of patients had developed de-novo anti-HLA antibodies after failed IT, the majority of whom had received islets from two donors. Of recipients who had discontinued immunosuppression, 70% had a panel-reactive antibody (PRA) of $>50\%$. This degree of sensitization would make subsequent kidney or pancreas transplantation more difficult. The authors acknowledged HLA matching for islet recipients was not feasible, as the pool of donors was extremely limited. Transplanting islets from a single donor would decrease the number of HLA mismatches, but broad sensitization could still occur if there were many mismatched epitopes from the single donor [80]. Recent trials using alternative immunosuppression based on the biologic agents efaluzimab (anti-LFA-1) and abatacept (CTLA4-Ig) have shown no allosensitization compared to Edmonton protocol-treated controls [75]. New immunosuppressive regimens along with the ability to list unacceptable HLA antigens at the time of transplant may favorably impact the development of anti-HLA antibodies in this population.

In summary, IT is experimental and is best performed at experienced clinical research centers. Although long-term insulin

independence is difficult to achieve, the majority of patients at 5 years post transplant have at least partial graft function that protects them from extreme hypoglycemia or hypoglycemic awareness, the main indications for transplantation [61].

For persons with T1DM experiencing hypoglycemic unawareness and debilitating metabolic complications, PTA remains the "gold standard" of treatment. Patients selected for PTA must have excellent renal function and should be healthy enough to survive the operation. Like PTA candidates, IT candidates should have excellent renal function able to withstand the nephrotoxic effects of CNIs. While still an experimental therapy, IT is an alternative to PTA in patients who would not otherwise qualify for or choose to undergo major abdominal surgery. The risks and benefits of both procedures should be weighed on an individual patient basis.

Summary

Pancreas transplantation in all its forms remains a reasonable alternative for patients with T1DM who have progressive secondary complications and are physiologically capable of enduring the substantial surgical procedure that it entails. Its success has improved over time, but remains associated with not insignificant morbidity and as such, should not be considered solely as a means of eliminating the need for exogenous insulin. IT remains less successful than whole organ pancreas transplantation, but is a less morbid procedure. Thus, IT remains an investigational alternative primarily for patients with severe hypoglycemic unawareness. Both are conscribed by a relative shortage of suitable donor organs and thus, the allocation of this resource should be carefully considered and reserved for those patients most likely to benefit.

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CHAPTER 33

Patient Selection and Indications for Intestinal and Multivisceral Organ Transplantation

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Introduction

Intestinal transplantation (ITx) and multivisceral transplantation (MTx) have continued to evolve over the past two decades from an experimental procedure to one that is routinely performed at several specialized medical centers throughout the world. Indeed, one of the major steps forward for this area was the first description of indications [1]. Since that time, there have been several other reports specific to this topic [2–6]. Yet, until recently, these indications have changed very little. Regardless, it is arguable that patient selection is one of the most important issues impelling a successful transplant and post-transplant course. In this chapter we will describe the indications for ITx including those for the isolated intestine and multivisceral types of transplants. This information is useful in understanding the content in Chapters 42, 62, and 63, which cover waitlist management for patients who have been listed for and are awaiting ITx/MTx, and the procedures themselves, respectively.

Intestinal failure

Irreversible intestinal failure is the basis for most but not all indications for ITx and prompts the inclusion of the intestine in grafts performed in the face of other complicated problems that require the consideration of a MTx. It is defined as the inability to sustain one's fluid, electrolyte, caloric, micro- and macro-nutrient requirements through the gastrointestinal tract [7]. Clinically it is synonymous with the need for parenteral nutrition (PN) for >60–90 days, and in children it can contribute to the inability to grow and develop appropriately. Like other solid organ failures, there are both reversible and irreversible conditions leading to intestinal failure. Etiologies are commonly classified broadly into mucosal disorders, motility disorders, and surgical/anatomical disorders. The latter are commonly referred to as short bowel syndromes.

Patients with reversible intestinal failure should *not* be considered as candidates for ITx or intestine-containing MTx. Conversely, those with irreversible intestinal failure should. Several prognostic factors that are considered when deciding on the rehabilitative potential of intestinal failure are shown in Table 33.1 [8–10]. Note, these factors apply mainly to the subset of intestinal failure etiologies that are surgical/anatomical.

Intestinal adaptation is the process whereby the failed intestine recovers full function and enteral autonomy. It is characterized by elongation of the villi, deepening of crypts, decreased gastrointestinal motility, as well as dilation and elongation of the intestine. This process is energy dependent, requiring sufficient calories and micronutrients and can take months to years. For this reason, PN has been instrumental in optimizing the adaptive potential in patients with intestinal failure.

Not surprisingly, younger patients have a better adaptive potential. Further, most studies support that the adaptive process is maximized at approximately 24–36 months after the onset of intestinal failure, although there are case reports of late adaptation. The length and location of remnant small intestine is crucial for adaptation. The longer the remaining intestine, the better the adaptive potential. The ileum is more adaptive than the jejunum. The role of the ileocecal valve is controversial. Some consider its positive prognostic value lies in the fact that the presence of an ileocecal valve is almost always accompanied by ileum. Likewise, it may be a surrogate marker for the presence of colon. Others feel that it is important in itself to prevent colonic backwash and bacterial overgrowth. Regardless, patients with an ileocecal valve do better than those without. The presence of colon is also important for adaptation. In these cases, not only does the colon absorb excess fluid from the succus entericus but it also, through commensural bacteria, can digest and absorb some calories. Therefore, restoration of enteric continuity through the surgical takedown of enterostoma and fistulas is extremely important for adaptation. Lastly, the health and function of the remnant intestine is crucial. Remnant bowel that has suffered ischemic insults or has active disease processes such as inflammatory bowel diseases tends not to adapt well, regardless of the above factors.

Parenteral nutrition-related complications

In general, irreversible intestinal failure alone is not a sufficient indication for ITx and similarly, does not impel one to consider an intestinal component to a MTx. ITx is intended to target those patients with permanent intestinal failure who are at risk for death with prolonged TPN administration. This is because the survival of patients with permanent intestinal failure on uncomplicated home TPN is quite good and in many cases equals or exceeds that seen after ITx [11]. Exceptions do exist, as discussed below.

In contrast, patients with irreversible intestinal failure who develop one or more major parenteral nutrition-associated

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complications should be considered for ITx. In these instances, outcomes after transplantation exceed those seen with complicated long-term TPN administration.

The most concerning TPN-associated complication is liver disease. Variously named TPN cholestasis, parenteral nutrition associated liver disease (PNALD), or intestinal failure-associated liver disease (IFALD), it remains a leading cause of morbidity and mortality in this patient population. The exact etiology of liver disease in this setting is unclear. Several theories exist, including a lack of enterally stimulated bile flow, and components or deficiencies of parenteral nutrition; discussion of these is beyond the scope of this chapter [12]. Risk factors for IFALD include: prematurity, low birth weight, lack of enteral nutrition, central venous catheter infections, small bowel bacterial overgrowth, excessive parenteral carbohydrate, protein or lipid administration. In children, IFALD usually presents as a cholestatic liver injury that can progress to biliary cirrhosis. In adults, the disease is usually more of a steatotic liver injury with or without inflammatory hepatitis. It falls into the non-alcoholic steatohepatitis (NASH) category. Once liver disease becomes evident, it is difficult to reverse, with progression to end-stage liver disease.

Typically, irreversible intestinal failure with liver disease is a major indication for ITx. There are groups that advocate proceeding with isolated ITx at the early onset of liver disease to prevent the progression to end-stage liver disease. This is a rational approach as it prevents the candidate from prolonged waiting time on the liver transplant list—a proposition plagued with a high mortality rate. However, with newer preventive strategies for early IFLAD, discussed below, this practice is being reconsidered. Once advanced liver disease with cirrhosis is present, combined liver–intestinal or multivisceral transplantation is required. When decompensation occurs in the cirrhotic IFALD patient, death typically follows rapidly.

A second common indication for ITx relates to central venous access. Patients who develop thrombosis or occlusion of the major central veins need to be considered for transplantation, especially if two or more central veins or the vena cava is involved. There are interventional radiology techniques wherein chronically thrombosed or stenotic central veins can be dilated, stented, or thrombolysed [13]. However, these techniques should not be considered a good long-term solution, but rather a means to improve access to allow for safe perioperative management with transplantation.

The other major central venous catheter-related complication that is a driving force for referral to an intestinal transplant center is bloodstream infection (BSI). BSIs can be common in this patient population, with the best series reporting instances of 0.5 BSI per year on TPN [14]. There are subsets of patients who have a much higher incidence of BSI and commonly lose catheters to infection. High rates of BSI are an indication for ITx. Other related indications besides incidence are organism types with resistant bacterial or fungal BSI (very concerning); metastatic foci of BSI such as endocarditis or abscesses; and systemic inflammatory response conditions requiring critical care admission, pressors and/or mechanical ventilator support. Table 33.2 summarizes the common indications, and their relationship to the above-mentioned co-morbidities that have been reported in the recent literature.

Table 33.1. Prognostic factors influencing the outcome of intestinal failure/adaptation

- Patient age
- Time since onset of intestinal failure
- Length of remnant small bowel
- Anatomical portion of remnant small bowel
- Presence of ileocecal valve
- Length of remnant colon
- Presence of enterostoma
- Presence of underlying intestinal disease/disorder

Other indications not meeting the standard criteria

Unresectable desmoids or gastrointestinal stromal tumors

Low-grade malignancy or the potential for the development of low-grade malignancies can be an indication for ITx and, based on the degree of tumor extension, can require resections that necessitate a MTx [15]. It is important to state that these are lesions that are unresectable by conventional surgical means. They typically are either desmoid-type tumors or gastrointestinal stromal tumors that involve the root of the mesentery. The spectrum of familial adenomatous polyposis syndromes (Gardner's syndrome) [16] can also be an indication for ITx. With each indication, complete tumor

Table 33.2. Indications and their associated conditions leading to intestinal transplantation

Author	Irreversible IF	Liver disease	BSI	Vascular access	Other	OTHER
Kaufman et al. [1]	Yes	Yes	Yes (metastatic foci; SIRS; Tx resistance)	Yes (2/4 infant; 3/6 child)	Ultra-SGS, gastrocolonc discontinuity; congenital mucosal disorders; untreatable fluid/electrolyte disorders; chronic pain	NONE
AGA technical review [2]	Yes	Yes	Yes (2-year SIRS 2/2 bacteria; 1-year SIRS 2/2 fungus)	Yes (2/6 adult)	Frequent severe dehydration	NONE
CMS [3]	Yes	Yes	Yes (2-year SIRS 2/2 bacteria; 1-year SIRS 2/2 fungus)	Yes (2/6 adult)	Frequent severe dehydration	NONE
Pironi et al. [4]	Yes	Yes	Yes (>1/1000 days; metastatic foci)	Yes (2/4 infant; 3/6 child/adult)	Ultra-SGS, gastrocolonc discontinuity; congenital mucosal disorders; tumor requiring massive resection; mesenteric vascular ischemia 2/2 thrombophilia	POOR QOL
Avitzur & Grant [5]	Yes	Yes	Yes (2-year SIRS 2/2 bacteria; 1-year SIRS 2/2 fungus; 1-year shock; 1-year ARDS)	Yes (2/6 all)	Ultra-SGS, gastrocolonc discontinuity; frequent severe dehydration; congenital mucosal disorders; tumor requiring massive resection	POOR QOL

IF, intestinal failure; BSI, bloodstream infection; Tx, treatment; ARDS, acute respiratory distress syndrome; SGS, short gut syndrome; SIRS, systemic inflammatory response syndrome.

resection with negative margins is imperative. Replacement of removed organs using modified multivisceral or multivisceral grafts is most common. However, newer techniques involving ex-vivo tumor resection with autotransplantation have recently been successfully applied to this patient population, potentially altering the transplant indications [17].

Porto-spleno-mesenteric vein thrombosis

The standard approach to patients with thromboses involving the portal, mesenteric, and/or splenic vein involves endoscopic and medical therapies followed by surgical shunt therapies. However, there is a group of patient that requires transplantation, usually due to underlying liver disease. In the vast majority of cases, isolated liver transplantation with portal venous revascularization using thrombectomy techniques, venous conduits, or portocaval transposition is standard [18]. However, there is a small subset of these patient in whom there is no suitable venous inflow option for isolated liver transplantation. These patients should be considered for MTx. The experience in general is limited to a few cases each year at specialized transplant centers [19].

Non-reconstructible gastrointestinal tract/ultrashort gut syndrome

A non-reconstructible gastrointestinal tract is one in which the entire midgut is surgically absent, rendering restoration of fore- and hind-gut continuity surgically impossible or impractical. These patients frequently have a blind end duodenum with tube gastrostomies draining foregut secretions. Ultrashort gut syndrome patients have less than 10 cm of remnant jejunoileum ending in an ostomy or surgically anastomosed to the colon. In either case, the prognosis for adaption is poor and parenteral nutrition complications are frequent. In addition, these patients represent challenges for medical management with frequent episodes of dehydration and electrolyte disorders. These issues have resulted in an aggressive approach to transplantation with successful outcome [20].

Potential evolution in indications

The traditionally accepted indications for ITx have been obscured by new preventive medical therapies. Specifically, the minimization of intravenous lipids, and the introduction of intravenous omega-3–based lipid solutions have reduced the prevalence of IFALD, thus altering the landscape of intestinal failure management. Lipid sparing techniques ($\leq$1 g/kg/day) have become a relatively common preventive and therapeutic approach for patients with IFALD. In small single-center experiences, this approach has successfully normalized cholestasis [21], providing clinicians with the opportunity to continue to work towards intestinal adaptation. More recently, omega-3 fatty acid (O3FA) emulsions (Omegaven; Fresenius Kabi, Bad Homburg, Germany), which are readily available throughout Europe and Asia, and in the US through research-based and compassionate use protocols, have shown the ability to prevent and reverse cholestasis in the majority of children with IFALD [22,23,24]. O3FA solutions lack phytosterols, contain higher amounts of vitamin E, and tend to be dosed at 1 g/kg/day. Newer emulsions containing soybean oil, medium chain triglycerides, olive oil, and fish oil have been touted as potentially more physiologically balanced and effective in preventing IFALD [25]. While the novel lipid therapies have demonstrated an excellent safety profile and are clearly effective at reversing cholestasis, the long-term efficacy in high-risk intestinal failure patients unable to come off parenteral nutrition is unknown.

Summary

ITx and MTx have evolved dramatically over the past two and a half decades and are now established modalities used in the treatment of patients with intestinal failure, or selected locally aggressive tumors requiring intestinal resection for cure. As experience has been gained, specific indications for transplantation have been clearly delineated. Further expansion beyond these criteria awaits further improvement in outcomes. Newer developments in the management of patients with intestinal failure will potentially impact the traditional indications for ITx and MTx. The full extent of this is yet to be seen.

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CHAPTER 34

Role of Primary Care Provider in the Referral and Care of Abdominal Organ Transplant Patients

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Introduction

By the end of 2007, 183 222 persons were recorded in the Organ Procurement and Transplantation Network (OPTN) as living with a functioning solid organ transplant [1], and 27 000 patients undergo organ transplantation annually in the US. Patient survival rates have improved for all solid organ transplant types, with the exception of pancreas transplant alone, pancreas after kidney, and intestine. Five-year survival rates are >70% for most organ transplant recipients [1]. As a result of the current success of transplantation, a growing number of patients are expected to survive to leave the hospital and ultimately return to their primary care physicians (PCPs) for continued care.

As more transplant recipients survive beyond the initial months after surgery, they can be expected to develop more chronic medical conditions that are associated with their immunosuppressive therapy. These conditions include hypertension, diabetes, hyperlipidemia, obesity, cardiovascular and cerebrovascular disease, chronic kidney disease, and osteoporosis. The PCP specializes in the primary and secondary prevention of chronic medical conditions and thus is well suited to provide care for patients after transplantation. In a survey of liver transplant centers in the US, 40% expressed the expectation that the PCP should be the principal medical provider 1 year after transplantation. An additional 40% employed a combination of subspecialists and PCPs to manage their transplant recipients [2]. PCPs should be prepared to manage the primary care of transplant patients.

The purpose of this chapter is to assist PCPs in the management of chronic medical conditions occurring after abdominal transplantation, as well as to provide advice regarding specific disease prevention through screening, immunization, and contraception. A companion chapter covering similar topics for thoracic transplantation follows in Chapter 35.

Coordination of care

Coordination of care between PCPs and subspecialty and surgical practitioners is critical to optimize health outcomes of transplant patients, as well as to minimize duplication of tests and avoid errors. A meta-analysis reporting on the coordination of care between PCPs and subspecialty physicians showed that interactive communication between practitioners can improve patient outcomes in the areas of mental health and diabetes care [3]. Though coordination of care has proven benefits, optimal strategies of collaboration have yet to be developed. While systems to employ health information technology and reports on performance are well established components of current primary care practices, methods to improve communication between practitioners are underdeveloped [4]. In a survey of transplant hepatologists [5], over 60% indicated that chronic medical conditions after transplant should be managed by PCPs. However, less than 40% of hepatologists were satisfied with the level of involvement of the PCPs in the care of their patients. While 70% of respondents indicated that they had appropriate levels of communications with PCPs, the majority of respondents were dissatisfied with the amount of communication that they received from PCPs. Ultimately, transplant hepatologists felt that their patients were receiving inadequate control of their chronic medical conditions after transplantation.

One collaborative care model for transplant recipients could involve inclusion of PCPs within the transplant team to assist with optimization of post-transplant care. Other models could include shared electronic medical records, email-based messaging regarding complex medical decision-making, or communications among physicians through shared interdisciplinary team members.

Before the development of the electronic medical record, PCPs communicated with subspecialty colleagues by telephone. During this call, relevant background information and questions for the consultant could be communicated. With the onset of shared electronic medical record systems, an initial phone call is often not made. The Associates of Specialty Professors Workforce Committee developed guidelines for communication between generalists and specialists in the co-management of patients with chronic medical conditions [6]. Included in these guidelines are the following recommendations:

1. The generalist should send an initial note to the specialist that includes the reason for consultation, the question to be answered, and the background data that have been collected by the generalist.
2. The generalist should send copies of clinic notes after the referral has been made.
3. The specialist should consider using "disease-specific templates" to communicate guidelines and recommendations to the generalist.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Other models of pretransplant care include the establishment of multidisciplinary teams that include advanced practice nurses [7] and the use of telemedicine to provide pretransplant care to patients located in a rural setting [8].

Prior to transplantation PCPs, play a critical role that can affect the long-term prognosis of their patients after transplantation. The establishment of open lines of communication with subspecialists, appropriate timing of referrals, identification and treatment of modifiable risk factors, and working with patients to improve adherence to treatment plans are important goals for pretransplant care.

Timing of referral to subspecialists

PCPs can optimize post-transplantation prognosis, reducing mortality and morbidity, by achieving guideline-directed targets of other chronic medical conditions. In a cohort of 5978 adults with severe lung disease, Cox models were employed to determine an association between body mass index (BMI) prior to transplant and the risk of death after transplant. This study revealed that after a mean follow-up period of 4.2 years, death rates for underweight and overweight patients were 15% higher compared with normal weight transplant recipients [9]. Other chronic medical conditions, including pretransplantation chronic kidney disease (CKD), have been shown to increase post-transplant morbidity [10,11].

Research on the appropriate timing of referral to subspecialists has not been developed for most chronic medical conditions, with the exception of CKD. The Generalist Subspecialist Collaborative has developed initial guidelines for the co-management of cardiovascular diseases, diabetes, CKD, asthma and chronic obstructive pulmonary disease, osteoarthritis and polymyalgia rheumatica, irritable bowel syndrome, and inflammatory bowel disease [6]. The authors acknowledge that this is an area that is in need of more research to guide generalists and specialists in the identification of their individual and shared roles.

Late referral to specialists of patients with CKD has been shown to result in an increase in the morbidity, mortality, and cost of medical care [12–16]. Cass et al. have shown that CKD patients who are referred to subspecialists within 3 months of the initiation of renal replacement therapy [reliant renal care (RRC)] were less likely to be put on the transplant waitlist, and less likely to receive a transplant up to 2 years after the initiation of RRC. The authors postulate that the reasons for this lack of access to transplantation are that patients are sicker at the start of RRC and that there is not enough time to go through the pretransplant evaluation to allow patients to bypass dialysis and undergo pre-emptive transplantation [13]. PCPs can improve their patients' access to appropriate care by referring to a nephrologist well before RRC is needed.

Optimizing adherence

Medication adherence and proactive patient self-care are key components to a successful outcome after transplantation. Several studies have shown that even minor deviations in the extent of adherence with immunosuppression are correlated with graft rejection. In addition, clinical event-free survival has been shown to be shorter for patients who deviate from adherence to medicines [17–19]. In a study of immunosuppressant adherence after transplantation, Dew et al. showed that patients identified the quality of care given as an important determinant of adherence [20]. PCPs usually have a long-standing relationship with their patients prior to transplantation. As a result, PCPs are well suited to the task of identifying patient risk factors for non-adherence and assisting their patients with strategies to improve adherence to complex medical regimens. Patient factors that have been shown to be associated with a reduced adherence are male gender, a sense of lower self-efficacy, anxiety and depression, personality disorders, neurocognitive disorders, and a lack of social support. Patient pretransplant behaviors that predict post-transplant non-adherence include pretransplant non-compliance with medicines, alcohol abuse, and non-compliance with monitoring vital signs [21,22]. PCPs can assist in the pretransplantation assessment of adherence and can counsel their patients on the importance of medications and lifestyle adherence as a means to improve both pre- and post-transplant quality of life as well as the likelihood of obtaining a transplant. PCPs can improve post-transplant outcomes by diagnosing and treating anxiety and depression, by providing pill boxes and through guideline-supported interventions on tobacco use, alcohol abuse, and dependence, and arranging for improved social support systems for their patients. Many of the milestones developed by Bell et al. for adolescents to achieve prior to transition to adult care can be adapted to the pretransplant patient to improve post-transplant adherence [23] (Table 34.1).

Table 34.1. Recommended milestones for patients to achieve before transplantation

- Understanding of and ability to describe the original cause of their organ failure and need for transplantation
- Ownership of their medical information in a concise portable accessible summary
- Awareness of the long- and short-term implications of the transplant condition on their overall health and other aspects of their life (e.g. infection prevention, cancer surveillance)
- Comprehension of the effect of their illness on their sexuality and reproductive health, including:
  - Effect of pregnancy on their own well-being
  - Effect of their medications on fertility
  - Any potential teratogenicity of their medications
  - Role of genetic counseling and genetic risk of their disease recurrence in future offspring, if pertinent to their condition
  - Their own increased susceptibility for sexually transmitted disease
- Demonstration of a sense of responsibility for their own healthcare:
  - Knowledge of the names (and shapes/colors), indications, and dosages of their transplant and ancillary medications (or carry that information in wallet/purse)
  - Call for their own prescription refills and renewals
  - Prepare their own medication dose boxes, if not done by their pharmacist
  - Independently communicate their healthcare needs to their providers
  - Know when and how to seek urgent medical attention, including health emergency telephone number(s)
  - Ability to make, keep a calendar of, and follow through with their own healthcare appointments
  - Understanding of their medical insurance coverage and eligibility requirements
- Capacity to provide most self-care independently

Adapted from Bell et al. [23]; with permission from John Wiley and Sons [192].

Chronic medical conditions post transplantation

Hypertension

Cardiovascular diseases are the most common cause of death in transplant recipients [24,25]. Hypertension, as defined by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure VIII, is a systolic blood pressure of >140 mmHg and a diastolic blood pressure of >90 mmHg, or the need for hypertensive therapy [26]. In transplant recipients, donor characteristics, host characteristics, and immunosuppressive

agents contribute to the development of hypertension [27,28]. Host characteristics that increase the risk of hypertension include pre-transplant high blood pressure, high BMI, CKD, and post-transplant hyperthyroidism. Donor characteristics that can lead to hypertension in the recipient include older age, male gender, and hypertension [29–31]. No large prospective studies exist as to the appropriate targets for blood pressure control in transplant recipients, but current guidelines state that the target blood pressure for liver and kidney transplant recipients is <130/80 mmHg [32,33].

The diagnosis of hypertension in transplant recipients can be challenging. Blood pressure readings in the office compared with those done before and after office visits show the presence of a significant white coat hypertensive effect [34,35]. Self-measurements of blood pressure have been shown to be significantly higher in transplant recipients [36]. One study of 200 renal transplant recipients [37] showed that continuous 24 h ambulatory recordings have the greatest sensitivity in diagnosing hypertension. Nocturnal hypertension with an absence of nocturnal dipping was found in this population [37].

Treatment of hypertension in the general population traditionally includes weight loss, sodium avoidance, and regular exercise. Little data suggesting the efficacy of these interventions exist for transplant recipients. When urine sodium excretion was used as a measure of dietary salt intake in a population of renal transplant recipients, no association between sodium excretion and blood pressure could be found [38]. Nevertheless, guidelines for the management of hypertension in renal transplant recipients still include sodium restriction to <2.4 g/day, achievement of normal weight, and regular exercise [39].

Several classes of antihypertensive agents, including calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers, beta-blockers, and diuretics have been shown to be effective in transplant recipients [40–51]. One large prospective trial comparing the efficacy of nifedipine versus lisinopril revealed that renal transplant function was superior with nifedipine [52]. In a retrospective study of liver transplant recipients [53], hypertension did not occur more often in patients treated with cyclosporine compared to tacrolimus. However, careful reductions in doses of calcineurin inhibitors (CNIs) after transplantation have been associated with improved blood pressure control [54].

In general, transplantation patients have hypertension that is difficult to control [55,56], and two or three agents are often required concurrently. Better control has been shown to reduce morbidity and cardiovascular mortality in the renal transplant population [57]. CNI levels are increased by the addition of dihydropyridine calcium channel blockers; thus, these agents are often used so that lower doses of expensive CNIs can be used. Additional special considerations in the transplant population are that CNIs (particularly tacrolimus) may contribute to hyperkalemia, and thus, the use of ACE inhibitors, angiotensin-receptor blockers, and potassium-sparing diuretics should be used with careful follow-up of potassium levels after initiation of these agents. Diuretics would be expected to counteract the sodium- and fluid-retaining effect of CNIs and prednisone. For patients with concurrent myocardial infarctions, proteinuria, and gout, beta-blockers, ACE inhibitors, and valsartan should be considered for the co-management of these conditions, respectively.

Hyperlipidemia

Hyperlipidemia is common after solid organ transplantation. One year after transplant up to 66% of liver and 60% of renal transplant recipients have developed an abnormal lipid profile [58–66]. The causes of hyperlipidemia in the transplant population are multifactorial and include post-transplantation obesity, diabetes, and hypothyroidism. In addition, the choices of antihypertensive agents and of immunosuppressive agents have also been shown to affect lipid levels [62,64–66].

Guidelines for lipid targets in renal and liver transplant recipients have been published [67,68]. The National Kidney Foundation Kidney Diseases Outcomes Quality Initiative recommends that renal transplantation patients be placed in a cardiac risk equivalent group of the National Cholesterol Education Panel (NCEP) Adult Treatment Panel (ATP) III [67]. Thus, the low density lipoprotein (LDL) cholesterol goal is set at <100 mg/dL, and the level at which drug therapy should be initiated is >130 mg/dL (and should be considered at levels >100 mg/dL). Two large randomized controlled trials (RCTs) have shown successful reductions in cardiovascular or overall mortality for transplant recipients who were treated for hyperlipidemia [69,70].

Initial treatment of hyperlipidemia should focus on weight reduction, dietary adjustment, and encouragement of exercise. A diet consisting of 25% energy from lipids, <10% from saturated fats, and <500 mg of cholesterol per day [71] was shown to produce significant reductions in LDL cholesterol for renal transplant recipients, but not low enough to achieve LDL targets of <100 mg/dL. The American Phase 3 diet has also been shown to reduce lipid levels in the renal transplant population [72]. Though no studies have shown that physical exercise has effectively improved lipid panels and reduced mortality in the transplant population, levels of physical activity have been shown to be low after transplantation [73] and studies have shown that transplant recipients do tolerate exercise programs and can reach levels of exercise that are similar to the non-transplant population [74]. If diet and exercise are insufficient to achieve LDL targets, PCPs should consider, in conjunction with transplant physicians, whether or not an adjustment in immunosuppressive therapy is feasible. Immunosuppressive regimens that reduce exposure to cyclosporine and to corticosteroids have been shown to improve lipid abnormalities [75–78].

For patients who do not achieve targets for LDL cholesterol with changes in diet and exercise, drug therapy is recommended. All classes of lipid lowering agents, including statins, bile acid resins, cholesterol absorption inhibitors, fibrates, nicotinic acid, and omega-3 fatty acids have been studied in the transplant population [69,70,72–107].

Statins are considered first-line therapy for transplant recipients [67]. Fluvastatin and pravastatin are not metabolized by the CYP450 3A4 system and thus have a lower likelihood of affecting levels of CNIs. PCPs should be aware of an increased risk of statin related myopathy, and levels of creatinine phosphokinase (CPK) should be checked along with transaminases 4–6 weeks after initiation of therapy or adjustment of the doses. Statins that are metabolized through the 3A4 pathway, such as simvastatin and atorvastatin, should be started at lower doses and should be associated with frequent checks of CNI levels.

In general, bile acid-binding resins are less well tolerated than statins [84], but may be considered for patients who have experienced severe side-effects from statin therapy. Their usefulness is debated in transplant recipients because of the controversy surrounding their impact on the absorption of immunosuppressive agents [108,109].

Only one cholesterol absorption inhibitor, ezetimibe, has been studied in transplant recipients. Combinations of statins with

ezetimibe have been shown to lead to further safe reductions in lipids [99–103]. However, no long-term study has demonstrated significant benefits in terms of reduction in cardiovascular morbidity and mortality.

The role of fibrates in the treatment of hyperlipidemia should be limited in transplant recipients. Only a few small studies of the impact of fibrate therapy have shown an effect [110], while the risk for myopathy in the transplant population is of concern. Fibrate use should be reserved for patients with severe elevations in triglycerides who are intolerant of statin therapy.

Nicotinic acid has not been studied significantly in the transplant population, possibly because of its known gastrointestinal, renal, hepatic, and flushing side-effects. One study to date [91] has reported niacin to be safe and effective at improving lipid panels.

Omega-3 fatty acids have been shown to represent an alternative to fibrates in the treatment of severe hypertriglyceridemia. A meta-analysis of omega-3 fatty acids in renal transplant patients showed significant reductions in triglycerides, without any change in glomerular filtration rate (GFR), graft survival, or incidence of rejection [107].

Diabetes

In 2005, new-onset diabetes after transplant (NODAT) was defined as a fasting plasma glucose of 126 mg/dL (7.0 mmol/L) or above. Patients with a fasting plasma glucose (FPG) of 110–125 mg/dL (6.1–6.9 mmol/L) were defined as being glucose intolerant and those with a FPG of <110 mg/dL (6.1 mmol/L) were defined as having a normal glucose. These definitions were based on the definition for diabetes of the World Health Organization [111]. Since these definitions were published, the American Diabetes Association has altered the cut-off points for the definitions of glucose intolerance to a FPG of 100–125 mg/dL (5.6–6.9 mg/dL) [112]. In a systematic review of the literature, Montori et al. showed that the incidence of new-onset diabetes mellitus after solid organ transplantation ranged from 2% to 50% [113]. A more recent study from a single center in the US showed that 74% of renal transplant recipients developed NODAT [114]. These studies were done prior to the most recent American Diabetes Association approved criteria for the diagnosis of diabetes, which would be expected to increase the percentage of transplant recipients diagnosed with diabetes.

Risk factors for the development of diabetes are similar to those for renal and liver recipients. Non-modifiable risks for diabetes include age, non-white ethnicity, male gender, family history of diabetes, hepatitis C, and polycystic kidney disease. Modifiable risk factors include pretransplant weight, steroid use, CNIs (particularly tacrolimus), and pretransplant degree of glucose intolerance [115–122].

To diagnose NODAT recent published guidelines suggest that post-transplant testing of FPG should begin weekly for the first 4 weeks, then at 3, 6, and 12 months, then annually after transplantation [123]. Hemoglobin A1cs (HbA1cs)should not be performed in the immediate post-transplant period, since many transplant recipients receive transfusions of packed red blood cells, which can alter the results. However, once patients are diagnosed with NODAT, HbA1cs should be checked on a routine basis [123].

Very little research exists on the efficacy and safety of diabetes treatments in the transplant population. Consensus conference guidelines have been published for renal transplant recipients [123,124]. Reynolds et al. have presented a simplified algorithm for the management of NODAT. For patients with a FPG of >250 mg/dL, initial therapy should include diet, exercise, and insulin therapy. To improve control and decrease tissue resistance, the addition of metformin, thiazolidinediones, or pramlintide is recommended. For patients with a FPG of <250 mg/dL at diagnosis, the first-line therapy should be diet, exercise, and metformin. If the HbA1c is >7% after 3 months, then the addition of a sulfonylurea or glinide, thiazolidinedione, exenatide, or sitagliptin should be initiated. If after 3 months of therapy the HbA1c remains >8%, then insulin is recommended. If the HbA1c ranges from 7% to 8%, then a thiazolidine, exenatide, or sitagliptin could be considered as an alternative to insulin [125]. New data on the association of thiazolidenediones and heart failure make these agents a less desirable choice for transplant recipients [126].

Osteoporosis

The prevalence of osteoporosis in a population of patients with end-stage solid organ disease is 31% among patients with liver failure and 24% among patients with kidney failure [127]. After transplantation, exposure to immunosuppressive agents including steroids, sirolimus, cyclosporine, and mycophenolate increases the likelihood of reduced bone mineral density (BMD) [128–130]. Within the first years after transplantation, further losses in BMD have been shown to occur in the renal transplant population [131]. In addition to immunosuppression, the presence of diabetes [132], decreased weight, and increased post-transplantation creatinine [130] has been shown to increase the risk of osteoporosis in this population. Correlation between low T scores on the dual X-ray absorptiometry test and vertebral fractures was shown in one study of renal transplant recipients [133]. In this study, a Cox proportional hazard analysis showed that the risk of fractures was increased in patients with osteoporosis by 3.5 times (95% CI 1.8–6.4) and in patients with osteopenia by 2.7 times (95% CI 1.6–4.6) [133]. A retrospective registry of 33 479 renal transplant recipients revealed an adjusted incidence ratio for fractures of 4.59 (95% CI 3.29–6.31). When hospitalized for hip fracture, this population had a hazard ratio for mortality of 1.60 (95% CI 1.13–2.26) [134]. Since bone loss progresses rapidly within the first year post transplantation, bone density testing should be performed soon after transplant and prophylaxis considered for patients who meet the criteria for osteopenia.

While no study of osteoporosis treatment has demonstrated reduced fracture rates for transplant patients, several treatments have been shown to slow the reduction in post-transplant BMD. One study of 57 simultaneous kidney–pancreas transplant recipients was able to demonstrate increased T scores at the lumbar spine and femoral neck during a follow-up period of 4 years [129]. The success of this study was attributed to the steroid-sparing immunosuppressive regimen employed in this population, coupled with an aggressive treatment protocol [129]. Other treatments with proven benefits on BMD have included vitamin D [135], and calcitonin [136]. A meta-analysis of five RCTs of bisphosphonate therapy in a population of 180 renal transplant recipients showed that this treatment resulted in a reduced decline in the BMD of the lumbar spine within 6–12 months after transplant. No major side-effects of bisphosphonate therapy were detected in this population [137].

Palmer et al. performed a meta-analysis of 24 RCTs (1299 patients) in renal transplant recipients and showed that bisphosphonates, vitamin D, and calcitonin were effective in improving BMD of the hip. None of these interventions showed a reduction in fractures [138].

A protocol for detection, treatment, and monitoring osteoporosis based upon published guidelines for the transplant population

improved screening and prevention practices for patients with kidney and pancreas transplants [139].

In addition to minimization of steroid dosing, transplant recipients should be encouraged to prevent osteoporosis through regular weight-bearing exercise and alcohol and smoking cessation. Adequate calcium intake (1000 mg/day for men, 1200 mg/day for premenopausal women, 1500 mg/day for postmenopausal women) and vitamin D intake (400–1000 IU/day) are also currently recommended; however, recent studies of a possible association between calcium and vitamin D supplements and cardiovascular mortality in the non-transplant population may result in a modification of these recommendations [140]. Bisphosponate therapy should be considered for patients with T scores of <-2.5. Prevention of osteoporosis with bisphosponates should be considered on an individual patient basis, recognizing that there has not been any proof that this treatment prevents fractures. No studies have been published to date that address the safety or efficacy of strontium, selective estrogen receptor modulators, cyclical parathyroid hormone, or the RANKL inhibitors (denosumab) in the transplant population.

Fertility and contraception

Impairment in normal menstrual function that is present before transplantation has been shown to resolve after transplantation in women who receive kidney and liver transplants [141,142]. Similarly, normal fertility has been reported after renal transplantation in men [143–145]. In contrast, male renal transplant recipients who received immunosuppression with sirolimus were shown to have a significant reduction in total sperm count, decreased proportion of motile spermatozoa, and a decreased fathered pregnancy rate compared with transplant recipients who did not receive sirolimus [146].

The National Transplantation Registry Database now reports that 919 transplant recipients have had 1418 pregnancies and 1451 live births, including twins and triplets [147]. Because of the return to fertility, PCPs should counsel their patients before and after transplantation regarding the use of contraception.

Studies of contraceptive efficacy have been reported in the transplant population for hormonal combination contraception [148–150]. One study of renal transplant recipients demonstrated that the intrauterine device had reduced efficacy in the renal transplant population [151]. No studies to date have reported on the efficacy of progestin-only oral contraception, depomedroxyprogesterone acetate (DMPA), barrier contraception, or emergency contraception in this population. Most experts consider barrier contraception to be the safest form of contraception in the transplant population.

The following issues are of particular concern to the transplant population. Combination oral contraceptive agents have been shown to inhibit the cytochrome P450 3A4 pathway [152] and thus may cause increased levels of CNIs. They also can cause decreased clearance of steroids such as prednisolone [153] and thus increase the risk of steroid-related side-effects. Patients on chronic CNIs are at an increased risk for atherosclerosis. Thus, any transplant patient with known coronary or cerebrovascular disease has an absolute contraindication to combination oral contraception. Patients who have hypertension, diabetes, or hyperlipidemia have a relative contraindication. The primary concern about DMPA in transplant recipients is the increased risk of osteoporosis associated with the use of this agent [154]. In addition to efficacy concerns, the intrauterine device also poses additional problems for the transplant recipient because of the increased risk of infection associated with immunosuppression.

Table 34.2. Contraindicated and safe vaccines in transplant recipients

Contraindicated live virus vaccines	Safe killed virus vaccines
• Varicella	• Human papilloma virus
• Herpes zoster	• Hepatitis A
• Intranasal live attenuated influenza virus	• Hepatitis B
• Measles, mumps, rubella	• Influenza (injectable form)
• Oral polio	• Diphtheria toxoid
• Oral live attenuated typhoid	• Tetanus toxoid
• Calmett–Guerin bacillus (BCG)	• Pertussis
• Yellow fever	• Hemophilus influenza type B (HIb)
	• Inactivated polio
	• Pneumococcal
	• Meningococcal
	• Rabies
	• Typhoid
	• Japanese encephalitis virus

Immunization

The potent immunosuppressive regimens for transplant recipients make them candidates for appropriate immunization protocols. Though killed virus- and toxin-derived vaccines are safe in the transplant recipients, many transplant recipients (up to 89%) are not adequately informed about immunizations [155]. Antibody responses to inactivated virus immunizations have been shown to be protective for influenza [156–159], pneumococcus [160,161], and hepatitis B virus [162,163]. A rapid decline in titers has been demonstrated for hepatitis A virus [164] when patients are immunized after transplantation.

Because of the rapid decline of antibody response to immunizations in the transplant population, studies are underway on the effects of booster immunizations. A booster influenza immunization strategy has been shown to increase therapeutic titers in the liver transplant population [165].

PCPs should advise patients to update all immunizations before transplantation and should counsel their patients to avoid family members who have received live virus vaccines for 2 weeks. Though preliminary studies have shown that live virus vaccines with measles, mumps, rubella and varicella are safe in selected pediatric liver transplant recipients [166], PCPs should continue to council adult transplant recipients to avoid live virus immunizations. Table 34.2 shows vaccines that are considered to be safe and those that are contraindicated in transplant recipients.

Smoking in transplant recipients

The Center for Disease Control (CDC) reports that smokers comprise 25.5% of the population in the US. It would thus not be surprising that tobacco use is common among transplant recipients. Studies of liver, kidney, and simultaneous kidney–pancreas transplant recipients have shown independent risks of increased morbidity and mortality associated with smoking. Among liver transplant recipients, smoking has been demonstrated to increase mortality [167], malignancy rates [168,169], biliary complications [170], and post-transplant length of stay and resource utilization [171]. Kidney transplant recipients with a history of smoking also have reduced survival [172–174] and an increased risk of graft failure [173,175], major cardiovascular disease, and malignancy [173].

In a retrospective study of 997 live donor kidney recipients, Nogueira et al. showed that a history of ever smoking conferred a greater risk of death when compared with never smokers (adjusted HR 1.47; 95% CI 1.08–1.99; $P = 0.01$) [172], and even among those who were not actively smoking at the time of their transplant. Kasiske et al. demonstrated in a retrospective chart review of 1228 renal transplant recipients that there was a dose effect of tobacco on mortality rates, and that if recipients had quit smoking over 5 years prior to transplant, the mortality rates were reduced [173]. Smoking has also been shown to cause progression of macroangiopathy and mortality in type 1 diabetic patients who have undergone simultaneous kidney–pancreas transplantation [176].

Banas et al. reported that a significant proportion (27.6%) of renal transplant patients were able to quit smoking [177]. In a cohort of patients undergoing liver transplantation for alcohol-related liver disease, DiMartini et al. showed that 3 months after transplantation 50% had returned to smoking [178].

Despite the negative impact of tobacco on transplant recipients, there are no data on the efficacy of tobacco cessation assessment and counseling or pharmacologic interventions in this group. Tobacco cessation techniques that have been shown to be successful in the general population, such as motivational interviewing techniques, nicotine replacement therapy, and bupropion could be considered in the transplant population. While varenicline is successful in the general population, it should be used with caution in transplant recipients because of the increased risk for anxiety and depression associated with the use of the drug [179,180]. At present the guidelines for the care of the renal transplant recipient state: "Active measures against tobacco smoking are recommended" [181].

Cancer screening

Immunosuppressive therapy is associated with an increased risk of malignancy through the reduction of cancer immunosurveillance, the increased risk of cancer promoting viral infections, and the possible direct carcinogenic effects of immunosuppressants. As a result, transplant recipients have been shown to be at three- to four-fold increased risk of developing cancer compared to the general population [182]. Increased standardized incidence ratios, defined as the ratio of the observed number of cancers over the expected number of cancers, have been shown to occur for non-Hodgkin's lymphoma, cancers of the cervix, vulva, kidney and thyroid, Kaposi's sarcoma, and non-melanoma skin cancer [183]. Independent risk factors for the development of cancer in the transplant population have been shown to include the duration and intensity of immunosuppression [184], hepatitis C virus infection, smoking, alcoholism, sun exposure [185], male gender, and age older than 45 years [184].

Despite the fact that malignancy is one of the most common causes of post-transplant mortality [186,187], transplant recipients are often unaware of this increased risk [188]. In a self-administered survey of 338 transplant recipients, Kauffman et al. demonstrated that 35.5% report knowing "nothing" or "not much" about the warning signs of cancer. Less than half reported knowing "a lot" or "all" about important cancer risk factors. Only 30.3% reported that they had learned "enough" about cancer prevention. It is therefore not surprising to learn that a telephone survey of 60 liver transplant recipients in one academic transplant program revealed that yearly screening for skin or oral cancer occurred in only 40% of patients. Breast cancer screening took place in 38% of the women, and 11% of women had received cervical cancer screening in the past 2 years [189].

PCPs should be aware of this increased risk among their transplant recipients and follow current guidelines for screening and cancer surveillance published by the United States Preventive Services Task Force, the Canadian Medical Association, and the American Cancer Society [190–192]. While these publications do not include guidelines for screening in transplant populations, recent guidelines for cancer screening have been published for liver [68] and renal [39] transplant recipients. In addition, it is important for PCPs to counsel their patients regarding the increased risk for malignancy before undergoing transplantation, as well as the importance of taking steps to prevent cancer, such as smoking and alcohol cessation, prevention of obesity, eating a healthy diet daily, the use of sun block with an SPF of at least 30, and human papillomavirus (HPV) immunization prior to transplantation.

Drug interactions

Drug interactions are commonly reported with immunosuppressive agents and can manifest as pharmacokinetic or pharmacodynamic interactions. Pharmacokinetic interactions arise from alterations in absorption, metabolism, distribution, and elimination, and can lead to increases or decreases in drug concentrations. Pharmacodynamic interactions arise from additive toxicities of two drugs. Table 34.3 summarizes drug interactions for patients taking immunosuppressive agents.

Table 34.3 Common drug interactions with immunosuppressive agents

Drug	Interaction	Comments
Calcineurin inhibitors (CNIs): cyclosporine (CSA); tacrolimus (TAC)		
Azole antifungals:	Increase CNI concentrations	Decrease CNI dose and monitor levels twice weekly
Fluconazole	Increase by 30–40%	
Itraconazole	Increase by 50%	
Ketoconazole	Increase by up to 80%	
Posaconazole	Increase CSA by 25%; increase TAC by 66%	
Voriconazole	Increase by 40–50%	
Dihidropyridine CCBs:	Increase CNI concentrations:	Monitor CNI levels weekly or twice weekly
Diltiazem	Increase by 25–50%	
Verapamil	Increase by ≤25%	
Macrolide antibiotics:	Increase CNI concentrations:	Azithromycin is best alternative with less potential for interaction
Clarithromycin	Increase by 25–50%	
Erythromycin	Increase by 50%	

(*Continued*)

Table 34.3. (*Continued*)

Drug	Interaction	Comments
Other antimicrobials:		
Amphotericin B	Additive nephrotoxicity	Use lipid products or alternative antifungal, if possible
Aminoglycosides	Additive nephrotoxicity	Use alternative antimicrobial, if possible
Anidulafungin	Increased anidulafungin concentrations (CSA only)	No dose adjustment needed
Caspofungin	Increased caspofungin concentrations	Avoid with CSA; monitor liver function tests with TAC
Rifabutin	Decreased CNI concentrations	Monitor CNI levels weekly or twice weekly
Rifampin	Decreased CNI concentrations	Monitor CNI levels weekly or twice weekly
HMG Co-A reductase inhibitors (statins):	Increased statin concentrations (CSA > TAC)	Start statin at lower dose and titrate upward slowly; monitor closely for rhabdomyolysis
Atorvastatin		Avoid lovastatin with CSA and TAC
Fluvastatin		Fluvastatin and pravastatin may have less potential for interaction
Lovastatin		Avoid simvastatin with CSA
Pravastatin		
Rosuvastatin		
Simvastatin		
Bile acid resins:	Decreased CNI absorption	Separate dose of CNI from bile acid resin by 4 h
Cholestyramine		
Colsevelam		
Colestipol		
SSRIs:	Increased CNI concentration	Use alternative SSRI or antidepressant if possible
Fluvoxamine	Increased concentrations at high doses only	
Fluoxetine		
Paroxetine		
Other antidepressants:		
Nefazodone	Increased CNI concentrations	Avoid combination; use alterative antidepressant agent
St. John's wort	Increased CNI concentrations	Avoid combination; rejection reported with CSA
Anticonvulsants:	Decreased CNI concentrations	Monitor CNI levels weekly or twice weekly
Carbamazepine		Use alternative agent when possible (i.e. for neuropathy)
Phenobarbital		
Phenytoin		
Protease inhibitors:	Increase CNI concentrations (>100% for ritonavir-based regimens)	For ritonavir-based regimens: use single low-dose CNI (CSA 25 mg or TAC 0.5 mg) and monitor CNI level daily before redosing; CNI often dosed as single dose weekly
Amprenavir		
Atazanavir		
Fosamprenavir		Less effect from fosamprenavir; unknown magnitude for atazanavir and tipranavir (may use lower CNI dose twice daily and monitor CNI level)
Indinavir		
Lopinavir		
Nelfinavir		
Ritonavir		
Saquinavir		
Tipranavir		
NNRTIs:	Altered CNI concentrations:	Monitor CNI levels and adjust dose accordingly
Delavirdine	Increased CNI concentrations	
Efavirenz	Decreased CNI concentrations	
Etravirine	Increased CNI concentrations	
Nevirapine	Decreased CNI concentrations	
Antacids	Decrease TAC absorption (only)	Separate doses from TAC by at least 2 h
Miscellaneous agents:		
Cimetidine	Increase CNI concentrations	Use alternative H_2 blocker
Mycophenolate mofetil (MMF); mycophenolic acid (MPA)		
Antacids	Decreased MMF/MPA absorption	Separate doses from MMF/MAP by at least 2 hours
Antivirals:	Additive bone marrow suppression:	Monitor CBC for evidence of bone marrow suppression
Acyclovir	*Renal failure*:	Decrease MMF/MPA dose accordingly
Ganciclovir	Increase MPA concentrations	Decrease antiviral dose accordingly
Valacyclovir	Increase antiviral concentrations	
Valganciclovir		
Bile acid resins:	Decreased MMF/MPA absorption	Separate dose of MMF/MPA from bile acid resin by 4 h
Cholestyramine		
Colsevelam		
Colestipol		
Proton pump inhibitors:	Decreased MMF/MPA absorption	Use H_2 blocker or increase MMF/MPA dose
Lansoprazole		
Omeprazole		
Pantoprazole		
Rabeprazole		
Azathioprine		
Bile acid resins:	Decreased azathioprine absorption	Separate dose of azathioprine from bile acid resin by 4 h
Cholestyramine		
Colsevelam		
Colestipol		
Xanthine oxidase inhibitors:	Increased azathioprine concentrations	Reduce dose of azathioprine by 50–75%
Allopurinol		
Febuxostat		
Proliferation stimulation inhibitors: sirolimus; everolimus		
Same interactions as with calcineurin inhibitors		

CCB, calcium channel blocker; SSRI, serotonin reuptake inhibitor; H_2 blocker, histamine-2 blocker; NNRTI, non-nucleotide reverse transcriptase inhibitor.

Table 34.4. Summary of recommendations for the primary care of transplant patients

Coordination of care	Primary cphysicians (PCPs) and specialists should assure that they collaborate closely on transplant patients; establishing the division of labor between specialists and generalists would optimize care and reduce redundancies
Timing of referral	Early referral of patients with chronic kidney disease (CKD) increases the likelihood of pre-emptive transplantation and can reduce morbidity, mortality, and the cost of care
Optimizing adherence	PCPs should work to improve adherence by diagnosing and treating depression, anxiety, and substance abuse
Hypertension	• Blood pressure target is <130/<80 mmHg for liver and renal transplant recipients and for all transplant recipients with CKD or other cardiac risk equivalents • All classes of blood pressure agents have been shown to be effective in the transplant population
Hyperlipidemia	• The target LDL is <100 mg/dL for renal transplant recipients • HMG-CoA reductase inhibitors that are not metabolized by the CYP450 3A4 system should be considered as first-line therapy for hyperlipidemia (fluvastatin, pravastatin) • Transplant recipients on calcineurin inhibitors (CNIs) are at increased risk of statin myopathy, and thus CPK levels should be checked along with transaminases during follow-up
Diabetes	• Transplant recipients should be carefully screened for new-onset diabetes after transplant • Recommendations of the American Diabetes Association should be applied to the transplant population
Osteoporosis	• Transplant recipients are at increased risk of developing osteoporosis • Vitamin D supplements, calcitonin, and bisphosphonates have been shown to reduce the decline in bone mineral density over time
Fertility and contraception	• Women of child-bearing age should be counseled about the need for contraception after transplant • Barrier contraception is considered the safest form of contraception in the transplant population • Combination hormonal contraception has been shown to be effective in this population, but may cause an increase in CNI levels
Immunization (see Table 34.2)	• Killed virus and toxin-derived vaccines are safe in the transplant recipient • Transplant recipients should avoid live virus vaccines and family members who have received live virus vaccines for 2 weeks
Smoking	• Active measures including motivational interviewing and nicotine replacement therapy are recommended
Cancer screening	• Transplant recipients are at 3–4 fold increased risk for developing cancer compared with the general population • PCPs should follow guidelines on cancer screening that are published for liver and renal transplant recipients

The CNIs, cyclosporine and tacrolimus, are substrates for cytochrome P450 3A4 (CYP 3A4) enzymes, a common pathway for the metabolism of many drugs. As a result, many pharmacokinetic drug interactions are reported with the CNIs. The proliferation signal inhibitors (PSIs), sirolimus and everolimus, are also substrates for CYP 3A4 enzymes. There are fewer reported drug interactions with the PSIs, but this is mainly related to the fact that these agents are not used as extensively as the CNIs. It is expected that drugs that interact with the CNIs will also alter PSI levels in the same way. Drugs that inhibit CYP 3A4 activity will decrease the metabolism of CNIs and PSIs, thereby increasing serum concentrations of the immunosuppressive agents. These interactions are generally mediated by competitive inhibition of the CYP enzymes; therefore, serum concentrations of the immunosuppressive agents will be expected to increase almost immediately, within 24 h, of starting the interacting agent. If the magnitude of the interaction is known, it is wise to empirically decrease the dose of the immunosuppressive agent correspondingly at the time the interacting drug is started. Alternatively, drugs that induce the activity CYP 3A4 enzymes will be expected to increase metabolism of CNIs and PSIs, thereby reducing serum concentrations of the immunosuppressive agents. Unlike inhibition, induction of CYP activity requires an up-regulation of enzyme production, which generally occurs over the course of several days. Therefore, serum concentrations of the immunosuppressive agents will not be expected to fall for several days and will not reach the steady-state nadir for nearly a week after starting the interacting drug. When starting a drug that is known to induce CYP enzyme activity, the interaction is best managed by monitoring serum concentrations of the immunosuppressive agents more frequently and adjusting the dose once serum concentrations begin to fall.

Drugs and herbal products known to stimulate the immune system should be avoided when possible to prevent potential rejection. Herbal products known to stimulate the immune system that should be avoided include, but are not limited to: echinacea, alfalfa, and black cohosh.

Summary

PCPs have the opportunity to affect dramatically the burden of chronic medical conditions that occur as a result of immunosuppressive therapy after solid organ transplantation. Table 34.4 provides summary recommendations on the primary care of the transplant recipient.

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CHAPTER 35

Role of Primary Care Provider in the Referral and Care of Thoracic Organ Transplant Patients

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Introduction

Since the initial successful heart transplantation in 1967 by Christiaan Barnard and combined heart and lung transplantation by Bruce Reitz and Norman Shumway in 1981, the field of thoracic transplantation has evolved with major advances in the selection of candidates, operative techniques, critical care management, immunosuppression, long-term screening, and follow-up. Although there are no data on the prevalence of lung failure requiring lung transplantation, 10% of heart failure patients are estimated to be in an advanced stage (ACC/AHA stage D) [1]. Transplantation has become an increasingly viable option for such patients who remain symptomatic despite optimal medical treatment and other surgical interventions. By the end of 2011, approximately 100 000 heart, 38 000 lung, and 4200 combined heart–lung transplants were reported to the International Society of Heart and Lung Transplantation (ISHLT) [2]. While the number of lung transplants performed annually in the US has gradually increased, that of heart transplants has fluctuated over the past decade [3], with 1670 adults undergoing lung and 1853 undergoing heart transplantation in 2009 [4]. With improving survival rates over the years, the number of patients living with heart or lung transplants has increased gradually to 20 369 and 7425, respectively, in 2009 [4]. Accordingly, more patients now are referred for transplant evaluation and co-managed by the transplant center and non-transplant physicians while listed and waiting for organ availability than in any previous year on record. As post-transplant patients live longer, they are expected to develop new chronic medical conditions, some related to their original illness, and some as sequela of immunosuppressive therapy. Moreover, as years pass from transplantation, surveillance for rejection and follow-up become less vigilant and patients, particularly stable ones, may return back to their primary care physicians (PCPs) for all general medical care unrelated to the transplant. Partnership between transplant and non-transplant physicians is required to provide comprehensive care in the transplant candidates for timely referral, evaluation, timing of transplantation, optimization of comorbidities, and preparation for transplantation; and in the recipients for long-term care and management of pretransplant and new comorbid conditions. PCPs can manage most common comorbidities arising in the thoracic transplant patient, including hypertension, diabetes, dyslipidemia, osteoporosis, and infections; help patients maintain a healthy lifestyle, diet, and exercise; and are on the front line to screen for malignancy (Table 35.1).

The purpose of this chapter is to assist PCPs in the management of chronic medical conditions occurring after thoracic transplantation, as well as to provide advice regarding specific disease prevention through screening, immunization, sexual and reproductive health, and health maintenance. Chapter 34 is a companion chapter covering similar topics for abdominal transplantation.

Coordination of care

Although it is recognized that collaboration between specialists and community physicians leads to improved outcomes [5] and elimination of errors by shared information, standards of coordination of care are not established for lung or heart transplantation. Communication between the transplant team and generalist is the primary feature improving care for transplant recipients. Poor communication between the providers can impair patient safety, decrease patient satisfaction, and increase economic burden of care. Direct voice or written communication has been the mainstay of information exchange, and has well-recognized limitations. In the future, broader utilization of health information systems is expected to improve coordination of care with better communication and collaboration between specialties. Initial consultation requests should include the background data collected and the question to be answered. Copies of clinic notes should be shared between the providers in a timely manner even when there is no or little change in status [6]. In general, given their proximity and relationship to the patient, non-transplant physicians can best achieve continuous management of health maintenance, preventive care, and common chronic comorbidities [6]. The generalist's office is tailored to provide these services with appropriate facilities, electronic reminders, support staff, and proper patient education materials.

The medical care of heart and/or lung transplant patients is unique because of the possibility of acute or chronic rejection; immunosuppression with its associated risk for toxicity, infection,

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Table 35.1. Major domains for contribution of care by non-transplant healthcare providers

Pretransplant phase
- Identification of candidates for transplantation
- Timely referral
- Optimization of treatment of chronic respiratory or heart failure:
  - Medical treatment
  - Pulmonary or cardiac rehabilitation
  - Adherence
- Treatment of pre-existing comorbidities
  - Hypertension
  - Diabetes mellitus
  - Dyslipidemia
  - Osteoporosis/osteopenia
  - Mood disorders (anxiety, depression)
- Avoid sensitization by limiting transfusion of blood products
- Health maintenance
  - Preventive care and age-appropriate cancer screening
  - Counseling against substance use
  - Exercise, cardiac or pulmonary rehabilitation
  - Weight optimization
  - Immunization

Post-transplant phase
- High index of suspicion for rejection and infection
- Avoid drug interactions
- Contact transplant center as indicated (Table 35.2)
- Treatment of pre-existing and de-novo comorbidities (as above)
- Health maintenance (as above)
- Adherence

Table 35.2. Indications for non-transplant physician to contact the transplant center

General
- Hospitalization
- Change in medication (addition or deletion)
- Hypotension or unexplained drop in systolic pressure of 20 mmHg from baseline
- Increase in resting heart rate of >10 bpm over baseline
- Fever ≥101 °F or any unexplained fever ≥100.5 °F for ≥48 h
- 2 lb or more weight gain in 1 week
- Unexplained weight loss of >5 lb
- Elective surgery

Cardiopulmonary
- Increased shortness of breath
- Pneumonia
- Any respiratory infection in a lung transplant patient
- Decline of >10% in FEV_1 in lung transplant patients
- Syncope
- Chest pain other than musculoskeletal
- Myocardial infarction, arrhythmia, change in ejection fraction

Gastrointestinal
- Abdominal pain other than constipation or gas
- Nausea, vomiting, or diarrhea
- Major abdominal disease

Neurologic
- Cerebral vascular event
- Seizure
- Mental status changes

Other
- New-onset renal failure
- Malignancy
- Intolerance to oral medications
- Non-compliance

Modified from the American Society of Transplantation guidelines [7].

and malignancy; the modification of presenting signs and symptoms by antirejection medications; and the toxicities and drug interactions of antirejection medications. Moreover, there are allograft-specific issues such as painless ischemia in heart recipients and airway strictures and hyperexpansion of the native lung in lung transplant recipients. Because of the unique management issues, the American Society of Transplantation recommends that major changes in the clinical condition of a transplant recipient are communicated to the transplant center in a timely manner (Table 35.2) [7]. Such communication is also important for listed patients who are usually provided with instructions to directly report and access the transplant team for any clinical changes. Based on Organ Procurement and Transplantation Network (OPTN) data, approximately 700–800 patients on a waitlist for a heart or lung transplant die annually [8]. Worsening heart failure, as indicated by need for inotropic support, could potentially need escalation of interventions that finally can lead to an upgrade in the listing status. Changes in functional capacity, worsening oxygenation with increased supplemental oxygen requirements, need for continuous mechanical ventilation, pulmonary hypertension, changes in spirometry, creatinine or arterial blood gases in lung transplant candidates should be communicated to the transplant center as they affect the listing status for organ allocation. Hospitalizations and illnesses like infections, pulmonary emboli, and strokes, should also be communicated to the listing center to be translated into changes in transplant candidacy.

Pretransplant phase

Early identification and referral of patients with end-stage heart or lung disease for advanced therapies is an important step in the care of chronic heart or respiratory failure; and is discussed in detail in Chapters 30 and 31. Transplantation should be discussed with the candidate early in the course of a progressive decline in health, long before the patient needs to confront it as an immediate personal option. Timely referral allows thorough evaluation, discovery, and mitigation of previously unknown comorbidities, improved nutritional and functional status, and preparation of the patient and his/her family for the life-changing events of transplantation. Although the average wait from listing to transplantation has substantially improved in the US after the introduction of lung allocation scoring system in 2005, wait times for some subgroups, such as patients with antibodies against human leukocyte antigens (HLAs), may be as long as 2 years. Disease-specific candidate selection for transplantation usually follows the published international guidelines and is discussed in detail in Chapters 30 and 31 [9–13]

Optimization of medical treatment

With advances in critical care and immunosuppression, and as detailed in Chapters 79 and 106, there has been improvement in short and long-term survival after thoracic transplantation in the past decade. However, thoracic transplant outcomes trail those of liver or kidney transplantation. Efforts to physically, medically, emotionally, and psychosocially optimize the patient and prepare him/her for surgery may reduce the risk of complications and improve outcomes.

Pulmonary and cardiac rehabilitation

Exercise performance is reduced in chronic respiratory or heart failure. The functional impairment results from reduced aerobic capacity and decreased maximal oxygen consumption, and correlates with dyspnea, muscle strength, pulmonary function, or central and peripheral circulatory abnormalities. Skeletal muscle

dysfunction is well documented in both chronic obstructive pulmonary disease (COPD) and congestive heart failure (CHF), but is also present in other forms of chronic respiratory failure [14–17].

Measures of functional capacity (maximum oxygen consumption during cardiopulmonary exercise testing, 6-min walk test, clinical staging of functional class, etc.) are important prognostic factors in patients with chronic cardiac and respiratory failure. Lower exercise capacity is associated with lower survival in patients with these conditions; therefore, it may have an impact on survival to transplantation and beyond [18–23].

Both lung and heart transplantation are major surgical procedures that carry substantial risk of perioperative complications and mortality. Although randomized controlled trials of the benefits of pulmonary rehabilitation prior to lung transplantation are lacking, patients with maximum preservation of exercise tolerance and undergoing postoperative rehabilitation have faster recovery and better outcomes in thoracic surgical procedures. Physical training is safe and effective with improvements in functional capacity, dyspnea, and health-related quality of life in COPD, interstitial lung disease, cystic fibrosis, pulmonary hypertension, and heart failure [24–29]. Exercise training was associated with modest significant reductions for hospitalizations, all-cause mortality, and cardiovascular mortality in a large cohort of patients with chronic heart failure [30].

Preoperative pulmonary or cardiac rehabilitation may identify persons who are non-adherent, too debilitated, or lack adequate social support, and therefore who may be poor or suboptimal candidates for transplantation [31].

Despite increasing validation of the role exercise training plays in the modification of exercise intolerance, challenges remain in its routine therapeutic application including acceptance, limited insurance coverage, tailoring of exercise programs to best address the needs of subgroups of patients, and improved short and long-term adherence to exercise training and a physically active lifestyle [32]. Referring physicians are encouraged to prescribe exercise training in the form of cardiac or pulmonary rehabilitation and daily exercise programs as an adjunct intervention in the management of the patient with chronic heart or respiratory failure, starting with the diagnosis and continuing after the transplantation.

Optimizing weight and nutritional status

Pretransplant nutritional status varies according to the underlying lung disease and may lead to underweight, overweight, or obesity. The cause of malnutrition in individuals with end-stage lung or heart disease is multifactorial, including decreased dietary intake, increased energy expenditure, release of inflammatory cytokines, malabsorption, and frequent hospitalizations. Body mass index (BMI) is commonly used to classify underweight (BMI $<18.5\,kg/m^2$), overweight (BMI 25–29.9 kg/m^2), obesity (BMI $\geq 30\,kg/m^2$), and extreme obesity (BMI $\geq 40\,kg/m^2$). Obesity is a worldwide epidemic with increasing prevalence in the recent decades. Results from the 2007–2008 National Health and Nutrition Examination Survey (NHANES) indicate that an estimated 73.9% of US adults have a BMI of $\geq 25\,kg/m^2$, with 34.2% overweight, 33.8% obese, and 5.7% extremely obese [33].

Obesity is listed as a relative contraindication in the international guidelines for selection of lung transplant candidates [13]. The United Network for Organ Sharing (UNOS) data show that >15% of lung transplantations in the US were performed on obese patients. Given that 73.9% of US adults are overweight or obese, this percentage is likely to rise. Overweight and obese patients are considered at risk for developing associated morbidities such as hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, malignancy, and other diseases, and therefore are at increased risk for both perioperative and long-term mortality and morbidity. Moreover, obesity is an independent risk factor with obese patients showing a two-fold increased risk of primary graft dysfunction after adjusting for center, diagnosis, cardiopulmonary bypass, and transplant procedure [34]. Single-center retrospective studies suggest obese individuals have a mortality risk more than three times greater than that for non-obese individuals [35,36]. In a large retrospective multi-institutional data analysis, the increase in mortality risk was 15% for obese patients and 14% for underweight (BMI $<18.5\,kg/m^2$) patients after lung transplantation [37]. In another retrospective analysis of a US cohort of recipients with COPD, cystic fibrosis, and diffuse parenchymal lung disease, the three most common diagnostic groups for lung transplantation, the adjusted odds of death were greater for any patient outside of normal BMI for either 1 year or 5 years, with 1-year mortality of 27%, 40%, and 34% greater among overweight, obese, and underweight patients, respectively, compared with normal weight recipients [38]. The increased risk of death is mediated by primary graft dysfunction, respiratory failure, infection, cardiac disease, and cancer in overweight and obese patients. Deaths in underweight patients can be attributable to infection, diaphragmatic weakness, respiratory failure related to poor nutritional status, and chronic allograft dysfunction [34,37,38]. Nutritional depletion and underweight are also risk factors for death while on the waitlist [39,40].

There is strong evidence that weight loss decreases the associated morbidity and mortality of transplantation [41]. Because the majority of lung transplant recipients fall outside the normal BMI range, there is a significant opportunity to improve survival outcomes. Primary providers are encouraged to follow available guidelines to optimize nutritional status of transplant candidates with appropriate corticosteroid withdrawal and avoidance, nutritional counseling and dietary modification (including enteral nutritional support in the underweight), exercise and lifestyle changes for all patients, and surgical and other treatments in select patients [42,43]. Bupropion and the selective serotonin reuptake inhibitor (SSRI) fluoxetine at high doses (60 mg/day) have both appetite suppressant and mood altering properties. Early mortality rates following bariatric surgery are 1% or less in large published controlled trials. In case reports for lung transplant candidates, bariatric surgery was helpful not only to achieve sustained weight loss, but also lead to a reduction of comorbid conditions [44,45].

Overweight and obese lung transplant recipients have lower fat-free mass, and lower levels of physical activity and resting energy expenditure compared to normal weight recipients. Moreover, substantial weight gain occurs in the first year after lung transplantation with increased risk of late mortality attributable to abnormal BMI [38,46,47]. Therefore, lung transplant recipients should continue to be mindful about their dietary intake and participate in pulmonary rehabilitation and exercise programs after lung transplantation in order to prevent the development of overweight or obesity.

For the cardiac transplant candidate, BMI is an important predictor of survival on the waitlist and post-transplant outcomes, including perioperative morbidity, post-transplant cardiovascular comorbidities, long-term complications, and mortality [48]. ISHLT

guidelines recommend that candidates should achieve a BMI of $<30\,kg/m^2$ (or percentage ideal body weight of <140%) before being listed for cardiac transplantation [12]. Obese individuals are underrepresented in the demographics of orthotopic heart transplantation (OHT), wait substantially longer for organs, and are less likely to receive OHT once on the waiting list. Moreover, metabolic syndrome pre heart transplant and/or its development in the early post heart transplant period negatively impacts long-term prognosis [49]. Analysis of 19593 OHT recipients in the UNOS database found that a BMI of $18.5–34.9\,kg/m^2$ does not have a significant impact on mortality, perioperative morbidity, post-transplantation cardiovascular comorbidities, and the long-term complications of transplantation [50]. Therefore, the presence of metabolic syndrome or obesity with a BMI of $>30–34.9\,kg/m^2$ pre transplant is not necessarily a contraindication for heart transplantation; instead, the patient requires close monitoring and alternative treatment regimens. However, the relationship between BMI and post-transplant outcomes is U-shaped with the extremes [i.e. the underweight (BMI $<18.5\,kg/m^2$) and obese (BMI $>35\,kg/m^2$)] experiencing worse outcomes. The diminished survival in the underweight patients results from excess morbidity in the first year post transplantation, whereas the diminished survival in those with a BMI of $>35\,kg/m^2$ appears to be due to increasing mortality beyond the first postoperative year [50]. Hence, many centers consider a BMI of $>35\,kg/m^2$ as a contraindication for OHT due to concerns of postsurgical and long-term decreased survival [50]. Because nutritional status is a potentially modifiable risk factor, the development of strategies designed to optimize nutritional status in end-stage heart failure patients may prolong survival in the absence of transplantation and lessen the short-term risks in the post-transplant period, thus expanding the benefit of transplantation [51–54].

Optimizing adherence

The treatment regimen of a transplant patient consists of lifelong medications, including immunosuppressants; monitoring for signs and symptoms of rejection, infection or other complications; avoidance of risk factors for cardiovascular disease and cancer (i.e. diet and exercise prescriptions, non-smoking); avoidance of abuse/dependence on alcohol or illegal drugs; as well as attending regular clinical check-ups and therapeutic drug monitoring [55]. Non-adherence to treatment regimens is widespread and varies from 7.5% to 37% in heart or lung transplantation [55–61].

Although psychological factors and medical adherence are recognized as being important in the transplant outcomes (and are covered specifically in Chapter 120), no standard approach to psychological assessment currently exists for heart or lung transplant candidates [62–64]. Non-adherence is more common in adolescents and patients with anxiety and depression, personality disorders, and neurocognitive disorders. It is worse in patients who live alone, are unemployed or lack social support [65]. Thoracic transplant candidates experience high levels of psychological distress while awaiting transplant and both pretransplant and post-transplant psychological functioning have been found to predict post-transplant quality of life, adherence to treatment, and medical outcomes [66]. Because of their desire to win acceptance from the transplant team, some patients may be guarded and refrain from sharing their concerns with transplant center professionals [66]. Referring physicians who have a long-term relationship with the patients, are uniquely qualified to identify patients who are non-adherent or who may benefit from adherence-enhancing interventions. No single intervention proved to be superior in increasing medication adherence in organ transplantation, but a combination of interventions including patient-focused cognitive, educational, behavioral counseling and psychologic interventions in a team approach may be effective in the long term [67,68]. PCPs can also help improve adherence by lifestyle interventions (exercise, tobacco, alcohol, and substance use), diagnosing and treating anxiety and depression, and addressing social support systems for their patients.

Avoid transfusion of blood products

The presence of anti-HLA antibodies in the sera of transplant recipients, or "humoral sensitization," has been associated with an increased frequency of acute rejection and decreased survival. Patients who are not sensitized and listed for transplant can become sensitized due to various causes, of which exposure to foreign antigens via transfusion of blood products is an important potentially avoidable etiology. The benefit of transfusion of any blood products needs to outweigh the risk of sensitization in transplant candidates.

Post-transplant phase

The important principles to consider while caring for a thoracic transplant recipient are the altered immune system, denervated graft (heart or lung), and a high potential for drug–drug interactions.

Allograft rejection

Rejection after the first weeks of transplantation can be classified into acute cellular rejection (T-cell mediated), acute humoral rejection (antibody and hence B-cell mediated), and chronic rejection. Acute rejection in the lungs manifests as acute vascular rejection or airway inflammation. Chronic rejection is mostly in the form of coronary allograft vasculopathy for the heart and chronic airway rejection (obliterative bronchiolitis) for the lung graft. Chronic vascular rejection is seldom recognized in the lung as it requires open lung biopsy. The complexities and controversies of rejection are beyond the scope of this chapter but are discussed in Chapters 71 and 72. There is a significant variation between centers in techniques used to detect, define, and treat the different kinds of rejection.

While acute cellular rejection is most common in the first 6 months to a year after heart transplantation, with a significant decline in its contribution to mortality beyond 3 years, primary graft failure (loss of cardiac function with no detectable rejection) causes 15–20% of deaths beyond 1 year post transplantation [3]. Irrespective of cause, any compromise in cardiac graft function changes the clinical outcome; hence, high index of suspicion and early recognition of rejection or graft failure is important to intervene with higher level of immunosuppression. Most centers perform surveillance endomyocardial biopsies frequently in the first post-transplant year. Gene expression profiling of the peripheral blood mononuclear cells (Allomap®) may also be used as a non-invasive surveillance tool. In either strategy, surveillance represents only a cross-sectional sampling and cannot reliably predict future episodes with significant residual risk for rejection. Healthcare providers need to be wary of atypical and subtle signs of heart failure as a manifestation of graft dysfunction. Due to denervation of the heart, patients might not have chest discomfort due to

ischemia or a high left ventricular filling pressure. General malaise, early satiety, nausea, and vomiting due to gut congestion are a few atypical presentations.

Rejection is more frequently seen in lung transplant recipients than other commonly performed solid organ transplants. About 35% of lung recipients are treated for allograft rejection in the first year of transplant [2]. After the first year, risk of acute rejection substantially decreases, but is never eliminated. The overall median survival for adult lung recipients is 5.5 years, with mortality mostly due to chronic rejection, the incidence of which increases after the first several months and reaches 50% at 5 years [2]. Rejection episodes can be asymptomatic or manifest with dyspnea, cough, sputum production, fever, hypoxia, rales, wheezing, or rhonchi. Radiographic abnormalities if present are non-specific. Spirometry may show a decrease in forced expiratory volume in 1 s (FEV_1) and forced vital capacity (*FVC*) in up to 60% of rejection episodes [69], but is unable to differentiate between rejection and other causes of allograft dysfunction such as infection, airway complications (stenosis in particular), or pleural effusion. Centers using home spirometric monitoring confirm any decline of >10% with formal spirometry. As clinical presentation, laboratory, and radiographic findings are non-specific and unable to differentiate between the various etiologies of graft dysfunction, including infection, rejection, malignancy, and airway stenosis, etc., non-transplant physicians are encouraged to discuss the findings with the transplant center and arrange for definitive diagnostic studies.

Cardiac allograft vasculopathy (CAV) involves the entire extent of the coronary vasculature and is characterized by intimal thickening due to smooth muscle cell proliferation and extracellular matrix deposition with varying degrees of inflammatory cells; calcification and atheroma formation are uncommon [70]. CAV causes long-term morbidity and mortality with >50% of recipients affected by 10 years post transplant and 10% of patients dying within 1 year of diagnosis. Coronary angiogram is commonly used to screen for CAV. Intravascular coronary ultrasound (IVUS) is more sensitive, but is not universally available. Computed tomogram (CT) angiography is also emerging as a new technology to identify vasculopathy and graft dysfunction [71,72]. Dobutamine stress echocardiogram not only detects significant CAV, but also is a good prognostic marker for mortality [73].

Though immune mechanisms play a predominant role in the pathogenesis of CAV, many metabolic risk factors, including insulin resistance and hypercholesterolemia, contribute to the onset and progression [74–76]. In a recent study using an IVUS definition of CAV, metabolic syndrome was associated with an eight-fold increased incidence of CAV [77]. Classic risk factors of coronary atherosclerosis, such as age and hypertension, were not consistently associated with CAV [78–80]. Currently there is no consensus on the goals of therapy for any of the metabolic risk factors. However, control of blood pressure, lipids, weight optimization, and smoking cessation should be targeted for the heart transplant recipient just as it is for a non-transplant individual. The role of the non-transplant physician is paramount in controlling such risk factors and possibly reducing the burden of CAV.

There is no effective therapy to significantly impact the outcomes once CAV sets in. Small studies have suggested calcium channel blockers and angiotensin-converting enzyme (ACE) inhibitors to have a protective effect on the development of CAV [81,82]. The proliferation inhibitors sirolimus and everolimus reduce the rate of progression and probably decrease mortality, as shown in small studies, but the side-effect profile of these drugs has precluded their widespread use. All heart transplant recipients should be prescribed a statin irrespective of their cholesterol profile as these delay the onset and progression of CAV [83,84]. Beyond lipid lowering, this class of medications inhibits antigen induction on endothelial cells, monocytes, and macrophages [85]. Early postoperative initiation has been shown to be beneficial, impacting both mortality and rejection [86]. Interactions with calcineurin inhibitors (CNIs) and mTOR inhibitors can lead to higher risk of myopathy and rhabdomyolysis. Hence, pravastatin and fluvastatin are the preferred agents due to their minimal drug interactions. The maximum allowable dosage in patients receiving these immunosuppressants is 80 mg/day. With close clinical and muscle enzyme monitoring, atorvastatin may be considered for moderate-to-severe hypercholesterolemia, despite some metabolism by the P450 system.

Infection

Thoracic transplant recipients are at high risk for various infections due to intense immunosuppression, particularly in the first 12 months and at times of augmentation for treatment of episodes of rejection. Other predisposing factors in lung recipients include loss of cough reflex due to denervation, ineffective mucociliary clearance, and loss of mucosal barrier postoperatively due to cell death resulting from lack of bronchial artery vascularization and ineffective lymphatic drainage. Moreover, the transplanted lower respiratory system remains open to the environment with continued exposure to irritants and micro-organisms. Specific coverage of the infectious issues in long-term transplant recipients is discussed in Chapter 94.

Transplant patients receive extensive education on lifestyle changes to avoid infections, as discussed in Chapter 94 [87]. Prophylactic antimicrobial regimens vary by the thoracic transplant center and commonly include medications for *Pneumocystis jiroveci*, *Toxoplasma gondi*, Candida infections, and cytomegalovirus (CMV). Some lung transplant programs use prophylaxis against aspergillus species. Infective endocarditis is uncommon in OHT but has a high mortality risk of 80%; therefore, prophylaxis is a reasonable option during dental procedures [88,89].

Due to immunosuppressant-induced changes in innate and adaptive immunity, transplant recipients may not always manifest typical signs and symptoms of infection. Vague and generalized symptoms of malaise and fatigue could suggest significant bacteremia and septicemia even in the absence of systemic inflammatory response syndrome. In addition, non-infectious causes of fever such as rejection occur commonly in thoracic transplant recipients. Otherwise uncommon atypical and rare bacterial, mycobacterial, fungal, and viral organisms can cause significant infections in the immunosuppressed thoracic organ recipient. Epidemiologic exposure (nosocomial, community, donor- or recipient-derived infections), travel, and pet history can provide clues to the most likely etiology.

The infection timeline of a transplant patient (not specific to thoracic transplant) can be divided into the first month; the first 1–6 months; and beyond 6 months after transplant [90]. Between 1 and 6 months is the period for the immunomodulating viruses (cytomegalovirus, Epstein–Barr virus, human herpesvirus-6, and the hepatitis viruses B and C) to cause clinical disease; and in combination with a higher immunosuppressant level can lead to opportunistic infections such as *Listeria monocytogenes, Aspergillus fumigatus*, and *Pneumocystis jiroveci*, even without an intense exposure. Beyond 6 months, most patients have a preserved graft

function are at risk mostly for community acquired respiratory infections including viruses (influenza, parainfluenza, and respiratory syncytial virus), while those with any graft dysfunction and or rejection are chronically maintained on higher doses of immunosuppressants and hence at higher risk for opportunistic infections with such organisms as *Pneumocystis jiroveci, Listeria monocytogenes, Cryptococcus neoformans*, and *Nocardia asteroides*.

Early postoperative screening, prophylaxis, and pre-emptive treatment regimens have decreased the incidence of infections. These strategies, while changing the timeline, also lead to the emergence of infections due to organisms with antimicrobial resistance. Early and specific microbiologic diagnosis is essential for guiding treatment and minimizing non-essential drug therapy [91]. Invasive diagnostic procedures are often required for accurate and timely diagnosis. Diagnosis of both infection and rejection require a high index of clinical suspicion as the signs and symptoms are non-specific. Non-transplant physicians are encouraged to contact the transplant center when suspicion of infection or rejection arises, to coordinate the diagnostic studies and appropriate and timely treatment, particularly when the immunosuppression is most intense to circumvent the high risk of rejection in the first post-transplant year.

Hypertension

Hypertension is highly prevalent after heart or lung transplantation, occurring in 50–90% of the recipients [2,92]. Patients often have an abnormal circadian blood pressure profile with nocturnal and diastolic hypertension, making self-monitoring crucial in the identification and treatment of hypertension to goal blood pressure [93,94]. Classic pretransplant risk factors such as high BMI, chronic kidney disease, and hyperthyroidism contribute to hypertension post transplant. In addition, arterial rigidity [95], CNIs (systemic vasoconstriction, renal vasoconstriction and hypersympathetic state, imbalance between prostaglandins and thromboxane and endothelin) [96,97], abnormal baroreceptors [98], and salt sensitivity [99] are mechanisms that have been implicated in development of hypertension post-transplant. The incidence of hypertension and renal dysfunction are comparable in both tacrolimus- and cyclosporine-treated patients [100]. High blood pressure along with other risk factors is suspected to contribute to the occurrence and worsening of CAV in heart transplant recipients [80].

Assessment of hypertension, treatment goals, and lifestyle changes, including weight loss, sodium avoidance, and regular exercise are similar to the general population [101]. When choosing an antihypertensive, comorbid conditions and drug interactions should be considered. Calcium channel blockers might have a physiological advantage to counteract CNI-mediated hypertension. Diltiazem has a significant interaction with CNIs (increases serum levels), while amlodipine does not and hence could be a safe first choice, followed by ACE inhibitors or angiotensin receptor blockers (ARBs). In the presence of proteinuria or diabetes, ACE inhibitors or ARBs are preferred, with close monitoring of serum potassium and renal function [102]. In the presence of azathioprine, ACE inhibitors may cause myelosuppression and should be avoided. Diuretics help reduce concomitant volume overload. Diltiazem has also been shown to reduce the progression of CAV in heart recipients. Beta-blockers are safe for lung transplant recipients, but should be avoided in OHT, especially early after the surgery as the denervated heart is more prone to bradycardia.

Diabetes mellitus

In the ISHLT registry, the prevalence of diabetes at 1 and 5 years is 26.2% and 39.6% for lung transplant recipients; and 28% and 40% for heart transplant recipients, respectively [2,103]. A higher prevalence up to 65% was reported in patients with cystic fibrosis [104]. New-onset diabetes mellitus after lung transplant (NODAT) occurred in 33.4% of the 2991 lung recipients in the UNOS database during the median follow-up of 2 years [105]. Pre-existing diabetes in about one-fifth of recipients can worsen due to steroids and CNIs. Glucocorticoids induce insulin resistance, stimulate lipolysis, and increase hepatic glucose production, while CNIs inhibit insulin secretion through interaction with transcription factors and alteration of mitochondrial function [106,107].

Although post-transplant diabetes mellitus (DM) is recognized as an independent risk factor for cardiovascular events, infections, and graft failure, the effect of DM after lung transplantation has not been studied adequately, with conflicting results on its impact on outcomes [104,108,109]. The use of steroids, CNIs (particularly tacrolimus), weight gain with ensuing insulin resistance, postoperative stress, and infections increase the risk of diabetes or pose challenges for diabetic control [100,110,111]. Other risk factors for NODAT include male gender, recipient age >50 years, African–American ethnicity, and cystic fibrosis [105].

Diagnostic criteria are similar to those for non-transplant patients, except that the hemoglobin A1c (HbA1c) should not be used in the immediate post-transplant period as transfusion of packed red blood cells can alter the results. The international consensus guidelines on NODAT recommend fasting plasma glucose monitoring weekly during the first month, then every 3 months for 1 year, and annually thereafter. A glucose tolerance test is also recommended in patients with impaired fasting glucose concentration in order to increase diagnostic yield [112].

The management of DM after transplantation is similar to that for non-transplant patients, with consideration given to drug toxicities or interactions. In patients with normal or adequate renal, hepatic, and cardiac function, most of the available classes of medications (including insulin sensitizers, insulin secretagogues, and incretins) can be tried before proceeding to insulin. Choice of agent should be tailored to the individual patient. Use of a combination of two or three glucose-lowering agents could be considered. Metformin has been used; however, patients with an unstable glomerular filtration rate may be at increased risk of developing lactic acidosis. High-dose corticosteroids used for induction of immunosuppression or treatment of acute rejection may require temporary insulin therapy for glycemic control. Insulin can be in the form of a single injection of intermediate- or long-acting insulin at bedtime. Better glucose control may be achieved with lower insulin dose plus an oral agent. If the desired level of control is not achieved with initial therapy, then a more aggressive approach will be needed, such as an insulin pump [112]. After hospital discharge, close monitoring of blood glucose during the first year is recommended.

Longstanding poor glycemic control may require conversion of tacrolimus to cyclosporine. Some transplant centers attempt to minimize corticosteroid exposure. Corticosteroid dose reduction needs to weigh the risk of rejection and hence only be done in select patients by the transplant center. Lifestyle modifications such as exercise and weight optimization, and a heart healthy diet with reduced saturated fat and cholesterol, increased complex carbohydrates, and fiber are recommended. In addition to hyperglycemia, appropriate surveillance for complications and the control of comorbidities such as dyslipidemia and hypertension need to be

optimized [113]. Annual screening is performed for diabetic complications as in the general population.

Dyslipidemia

One and 5-year prevalence of hyperlipidemia in lung transplant recipients is 24.7% and 57.5%, respectively [2]. Approximately 66% of the OHT recipients have at least one dyslipidemia despite universal statin usage [114]. Risk factors include personal or family history, inactivity, diet, obesity, hypothyroidism, diabetes, and immunosuppressive medications, including corticosteroids, sirolimus, and CNIs. In one study of 212 heart transplant patients, older age, everolimus use, and lack of statin and/or tacrolimus use were associated with increased low density lipoprotein (LDL) levels, while male gender, pretransplant CAD diagnosis, and lower ejection fraction were associated with low high density lipoprotein (HDL) levels in multivariate analysis [114]. Only higher BMI correlated with a high triglyceride levels. The association of dyslipidemia and specific lipid parameters with CAV or mortality has not been proven, but association of hyperlipidemia with the development of atherosclerotic CAD was shown in larger studies [115,116]. Optimal lipid level goals are not known. In the absence of specific guidelines for lung or heart transplant recipients, guidelines for kidney transplant recipients and the National Cholesterol Education Program's (NCEP) most recently updated recommendations (LDL cholesterol goal of <100 mg/dL) can be used [117,118]. Drug therapy is initiated for a LDL of >130 mg/dL (and should be considered at levels of >100 mg/dL) after 3 months of lifestyle interventions consisting of diet and exercise. Initial drug therapy of hypercholesterolemia should be a statin ([119,120]). Statin use was associated with improved function and survival of lung allografts by prevention of acute and chronic rejection [121]; and delayed onset and slow progression of CAV in heart transplant recipients [83,84]. Ezetimibe may be added to the statin if the response is suboptimal [122,123]. Although side-effects of statins are uncommon, careful monitoring for these, including diabetes, memory loss, confusion, muscle weakness and pain for myopathy and rhabdomyolysis, is warranted. Of the statins, fluvastatin and pravastatin have lower risk of rhabdomyolysis when used in combination with CNIs. Non-transplant physicians should be aware of the increased risk of rhabdomyolysis with a statin and CNI combination; muscle enzymes such as creatinine phosphokinase and aldolase should be followed after initiation of therapy or dose changes. Other potential therapeutic drugs include gemfibrozil, nicotinic acid, and fish oil supplements at high doses [124,125]. Combination of fibrates along with statins requires caution and close follow-up and should be limited to patients with severe elevation of triglycerides. Small studies have shown beneficial reduction of triglycerides with omega-3 fatty acids in patients using m-TOR inhibitors [125]. Bile acid-binding resins may decrease the absorption of immunosuppressive medications, particularly immunosuppressive agents that bind to lipids [118]. Therefore, it is prudent to avoid administering a bile acid sequestrant from 1 h before to 4 h after the dose of CNI and to monitor therapeutic drug levels.

Bone health

With risk factors such as tobacco, inactivity, vitamin D deficiency, low BMI, and prior corticosteroid use, the prevalence of osteoporosis is reported to be up to 61% in lung transplant candidates [126,127]. Other risk factors such as malabsorption and hypercatabolic state may be contributory in patients with cystic fibrosis who have lower bone mineral density (BMD) than those with other causes of end-stage chronic respiratory failure [128–130]. Osteopenia or osteoporosis was observed in more than half of the pretransplant patients with severe CHF [131]. Due to the high prevalence of osteopenia and osteoporosis, vitamin D levels and BMD should routinely be included in the evaluation of lung or heart transplant candidates. Referring physicians and transplant centers should proactively start treatment for abnormal BMD and hypovitaminosis D before the transplant. BMD at the lumbar spine and femoral neck has a significant decrease in the first year after heart or lung transplantation due to high doses of corticosteroids and immunosuppressive therapy [132–136]. Atraumatic fracture rates of 18–38% and a loss of BMD of 4–12% were reported in the first year following lung transplant [130,135–137]. Biochemical markers of bone resorption were significantly higher in patients who sustained bone loss and/or fractures [135].

For prevention and treatment of osteopenia and osteoporosis, physical activity including regular weight-bearing exercises, and avoidance of tobacco and alcohol should be encouraged [138–141]. All patients should receive the recommended daily allowance for calcium (1000–1500 mg/day) and vitamin D (400–800 IU/day). Any vitamin D deficiency should be treated to keep serum 25-hydroxyvitamin D level above 30 ng/mL. Replacement doses of vitamin D and calcium do not reliably prevent clinically significant bone loss after transplantation [134–136]. Calcitriol was shown to decrease the rate of bone loss in the first year after lung or heart transplant, but close monitoring of serum and urinary calcium is required [142,143]. Calcitonin has shown no benefit in preventing early bone loss after transplantation and hence should not be used [144,145].

Controlled randomized and non-randomized studies of heart and lung recipients and ISHLT guidelines suggest that the most effective choice to prevent the increased bone resorption and rapid bone loss early after transplantation is a bisphosphonate [146–150]. Both intravenous (pamidronate, zoledronic acid, ibandronate) and oral (alendronate, risedronate) formulations are available. Bisphosphonates slightly increase the risk of atypical femur fractures. It is not clear if oral bisphosphonate therapy is associated with an increased risk of esophageal cancer. Oral bisphosphonates should be taken first thing in the morning after awakening, with a full glass of plain water. Patients should remain in an upright position for at least 30–60 min after the dose. Recombinant human parathyroid hormone (teriparatide) is effective for treatment of glucocorticoid-induced osteoporosis, but was not shown to improve BMD early after kidney transplantation [151,152]. Due to cost, it should be reserved for those with severe osteoporosis or esophageal dysfunction (stricture, achalasia, scleroderma esophagus, etc.) or intolerance to bisphosphonates.

For follow-up, proximal femur and lumbar spine BMD should be assessed by DEXA or equivalent scan in all adult transplant patients at 1 year and annually thereafter if they remain on corticosteroids [149]. Follow-up testing can range from 2 to 3 years based on the presence of osteopenia or normal range of BMD.

Psychological issues

The psychological stress of receiving a thoracic organ transplant is quite often under-recognized by transplant professionals [153]. In a study from Italy, 41% of heart transplant patients were found to have major depression, while 12% had transplant-related post-traumatic stress disorder [154]. Personality disorders, dysfunctional coping strategies, complications, and side-effects of

immunosuppressant medications, stressful events, limitations in job performance, sexual dysfunction, and lack of psychosocial support contribute to depression [155]. Onset of complications including chronic rejection can cause a decline of functional status associated with a dramatic increase in depressive symptoms. Psychological illness negatively influences the recipient's ability to cope with the new organ and could lead to decreased quality of life, unmet expectations, and non-adherence to rehabilitation and pharmacologic therapy. Effective and reliable psychotherapeutic or psychopharmacologic interventions for depression and anxiety may improve adherence and health-related quality of life. SSRIs are well-tolerated and efficacious for depression, panic disorder, and post-traumatic stress disorder, and replace tricyclic antidepressants as the first-line intervention. Among them, citalopram and escitalopram appear to have the least risk of drug–drug interactions [156]. Adjustments in dosage are required when renal or hepatic impairment is present. Although benzodiazepines are often the drugs of choice for the management of anxiety and insomnia associated with depressive symptoms, caution is suggested with the use of these agents in lung transplant candidates and recipients because of the risk of dependence, the reduction in upper airway muscle tone, and blunting of the arousal response to hypercapnia [157]. Mirtazapine can be effective in anxiety disorders and for depression accompanied by anorexia and insomnia. Complementary therapies and interpersonal, group, family, supportive, or cognitive psychotherapy may help the recipient cope with the psychological adaptation to transplantation [62,155]. A high-intensity exercise program was found to improve exercise capacity, self-perceived health, anxiety, and depression in heart transplant recipients [158].

Self-medication for depression with St. John's wort should be discouraged as there are serious problems including toxicity; contamination with heavy metals, micro-organisms, and pesticides; adulteration and drug–drug interaction leading to lowering of cyclosporine levels [155].

Depression in thoracic transplant candidates or recipients is often regarded as a normal, understandable, and predictable reaction to the physical illness, and remains underdiagnosed and undertreated [159]. Moreover, depressive symptoms in this population may be harder to detect and as there may be atypical features such as irritability, cognitive disturbance, frustration, headache, gastrointestinal complaints, fatigue, sleep problems, decreased appetite, or vague somatic aches [160]. Non-transplant primary providers could make a significant contribution to the health and survival of transplant patients by being vigilant about the psychological stressors, recognizing the associated illnesses by utilizing standard instruments to screen for and treat the common mood disorders, with the caveats discussed above. Referral to psychiatrists with special interest in transplantation may be required for treatment and monitoring in difficult cases.

Return to work, recreation, and life

Transplant patients look and feel their usual self soon after transplant and many wish to resume their pretransplant healthy lifestyle. In the US and other countries, post-transplant employment rates vary greatly from 22% to 86% with follow-up from 1 to 12 years; higher rates are found when the definition of employment is broad, including any paid work or return to school [161–165]. Many patients face issues of coping with maintaining normalcy with a transplanted organ compounded by employer fears of higher health insurance burden and more sick days off work. In spite of the complexities of the task of reintegration of these patients into society, they should be encouraged to be active and productive in order to achieve better physical and mental outcomes. The only restrictions to be enforced are those to avoid activities that could potentially expose them to infections. Recreational activities with increased risk of infection should be avoided. Public pools are a source of infections and hence should be avoided particularly when crowded. Consumption of raw animal protein and handling of raw meat during activities (cooking or hunting) should be avoided.

There are no specific studies addressing limitations in return to driving and operating a vehicle in heart or lung transplant patients. With a 3–10% incidence of syncope in heart transplant recipients, restrictions related to such an occurrence are applicable. State laws differ, but most require a 6-month syncope-free period prior to returning to operating a vehicle. Every effort should be made to identify and correct the cause of syncope. Symptomatic bradycardia should warrant a permanent pacemaker placement. Sinus node dysfunction is declining with many centers adopting a bicaval surgical technique instead of the older biatrial method in heart transplantation [166]. Although one small study showed an incidence of 18%, the true incidence of sudden cardiac death is unknown due to lack of autopsy studies [167].

Sexual and reproductive health

Normal fertility, which most patients have after heart or lung transplantation, poses complex medical, psychosocial, and ethical problems. This complex issue is discussed in depth in Chapter 97. Uncertainties exist, including the risks that pregnancy presents to the graft, the patient herself, and the long-term risks to the fetus [168]. The National Transplantation Pregnancy Registry (NTPR) has acquired experience with 2000 pregnancy outcomes in female transplant recipients: 71–76% of the pregnancies of female kidney recipients produced a livebirth and for the other organs, the live-birth likelihood ranged from 50% to 86% [169]. The incidence of birth defects in the liveborn appears similar to the general population, except for pregnancies with mycophenolic acid exposure that have a 23% incidence of birth defects [169,170]. Long-term follow-up of the offspring of transplant recipients has provided reassurance [169]. The American Society of Transplantation Consensus Conference on Reproductive Issues recommended that individual factors should be considered when offering advice for timing of pregnancy, including risk of acute rejection and infection, concomitant therapy with potentially toxic and teratogenic medications, and adequacy of graft function [168]. Although the patients are at risk of certain maternal, fetal, and neonatal complications, including hypertension, pre-eclampsia, infection, preterm birth, and low birth weight, successful pregnancy outcomes have been reported following heart or lung transplantation [171]. Heart transplant recipients planning to conceive need a baseline graft evaluation with an angiogram if not performed in the last 6 months to document absence of CAV and intact graft function. In the lung recipient, the baseline pulmonary function test should show stable findings. With intact graft function, the physiological changes of pregnancy are usually well-tolerated by heart transplant patients.

Although there is very limited information on pregnancy after lung transplantation, the risk of allograft rejection during and after pregnancy appears significant. Pregnancy in cystic fibrosis lung transplant recipients is feasible, but should still be regarded as a risky undertaking as there is an increased risk of acute rejection in half and progressive allograft dysfunction in all patients after delivery [172].

With the availability of newer agents for which pregnancy safety has not been established, it is unclear how to best modify immunosuppressive agents or treat rejection during pregnancy. To minimize the risk of graft rejection, it is important to maintain an adequate level of immunosuppression with close monitoring throughout pregnancy and in the postpartum period, keeping in mind the risk of fluctuations related to changing body volume and glomerular function. With recent developments in assisted reproductive technologies, controlled ovarian hyperstimulation and gestational surrogacy may be a safer option for patients with a thoracic transplant who wish to have a genetic child [173,174].

Genetic counseling is crucial for patients with hereditary diseases, including congenital heart disease, familial cardiomyopathy, heritable pulmonary hypertension, cystic fibrosis, familial pulmonary fibrosis, and alpha-1 antitrypsin deficiency as their primary transplant etiology. Women who underwent cardiac transplantation for peripartum cardiomyopathy should be advised to avoid pregnancy due to the high risk of recurrence.

The timing of conception after a heart transplant is an area of debate. Most recommend waiting for 2 years before becoming pregnant. Patients need to be counseled regarding the relatively limited long-term survival of thoracic transplant recipients and the impact of pregnancy on long-term outcomes that further confound the ability to participate in raising the child.

Available contraception options are similar to the non-transplanted patient. Intrauterine devices are mostly avoided for fear of infections. Barrier precautions, personal or partner sterilization in adults, and hormonal contraception in adolescents are effective options. Depo-medroxyprogesterone acetate intramuscular injection every 3 months is effective, but is associated with decreased bone density. Combination oral contraceptives can cause decreased clearance of steroids and inhibit the cytochrome P450 3A4 pathway, leading to increased levels of CNIs. If combination hormonal contraception is considered, a thorough screening for hypercoagulable state and monitoring of immunosuppressant drug levels is therefore necessary. Hormonal contraception should not be used in patients with significant hypertension, vasculopathy, liver disease, and estrogen-sensitive cancers.

Risk of infection is significant and extreme aseptic precautions need to be practiced during routine vaginal examination. Suspected infections need to be treated while awaiting cultures. Routine antibiotic prophylaxis is also recommended during delivery. Individuals with multiple sexual partners should be counseled on safe sex practices and screened for sexually transmitted infections.

Although the numbers are small, available data suggest that paternity by cardiac transplant recipients may be safe [175]. Erectile and sexual dysfunction is common after heart transplantation with an impact on perceived quality of life [176,177]. Specific psychological causes need to be addressed prior to embarking on therapy. Phosphodiesterase inhibitors are safe as first-line treatment modalities [178]. Treatment with the m-TOR inhibitors sirolimus and everolimus may cause a decrease in testosterone level, and disruption of spermatogenesis with decreased counts and motility [179].

Immunization

The incidence of vaccine-preventable diseases is high, with approximately 45 000 adult deaths annually in the US from such causes, the majority from influenza. PCPs and patients are advised to complete all routine vaccination per national guidelines before transplantation. A survey of the pediatric lung transplant programs showed that the vaccines were more commonly provided by the PCP pre-transplant (69%) rather than post-transplant (38%) [180]. Immunization schedules are periodically updated [181,182]. The US Centers for Disease Control and Prevention (CDC) National Center for Immunization and Respiratory Diseases website provides direct access to vaccination recommendations and links to other vaccination-related websites (http://www.cdc.gov/vaccines).

Solid organ transplant recipients are assumed to have altered immunocompetence and live, attenuated vaccines should not be administered. Pretransplant patients on high-dose steroids (equivalent to either $\geq$2 mg/kg of body weight or $\geq$20 mg/day of prednisone administered for $\geq$14 days) or immunosuppressive medications should also avoid live attenuated vaccines, including intranasal live attenuated influenza virus, varicella, herpes zoster, measles, mumps, and rubella [183]. As there are no alternatives to the last five of the aforementioned, it is of particular importance that vaccine series for these are completed pretransplant as indicated. Patients are advised to avoid family members who receive live attenuated vaccines for at least 2 weeks.

The CDC considers heart or lung transplant candidates or recipients to have high-risk medical conditions and recommends they receive influenza and pneumococcal vaccinations regularly.

Cancer screening

Malignancy is a major cause of late deaths in both heart and lung transplant survivors [184]. The general topic is discussed in Chapter 95. In addition to the usual risk factors in the general population (such as exposure to tobacco and alcohol, age, genetics), immunosuppression-induced reduction of cancer immunosurveillance and higher risk of oncogenic viral infections contribute to the increased incidence [185]. Immunosuppressed organ allograft recipients have a three- to four-fold increased risk of developing tumors, but the risk of developing certain cancers is increased several 100-fold [186]. With the exception of breast and prostate cancer, the incidence of most of the common malignancies seen in the general population is increased [187]. Moreover, there is a higher frequency of some relatively rare tumors, including post-transplant lymphomas and lymphoproliferative disorders (PTLDs), Kaposi's sarcoma (KS), renal carcinomas, in-situ carcinomas of the uterine cervix, hepatobiliary carcinomas, anogenital carcinomas, and various sarcomas.

Incidence of lung cancer is increased after heart or lung transplantation [188–191]. In single lung recipients, it occurs in the native lung, particularly in patients with primary COPD or idiopathic pulmonary fibrosis, and a history of smoking. The cancer is usually diagnosed at an advanced stage with poor outcome [192]. Efforts to improve screening are recommended, as aggressive management and treatment may be beneficial for earlier stage disease [188]. Patients with cystic fibrosis had a significant incidence of colon cancer (5.7%) with onset ranging from 246 days to 9.3 years post transplant [193]. Colonoscopic screening may identify patients with premalignant colonic lesions and prevent progression to colonic malignancy.

Skin cancer, especially squamous cell and basal cell carcinoma, is the most frequent malignancy in organ transplant recipients. Other skin cancers, including melanoma, Merkel cell carcinoma, and KS, are also more common than in the non-transplant population. Voriconazole, an azole antifungal commonly used in lung transplantation, induces photosensitivity and increases the risk of skin cancer post transplantation [194].

The majority of malignant lymphoproliferative disorders occurring following solid organ transplantation are B-cell origin PTLDs [195]. Preventative measures include reducing immunosuppression to the lowest level compatible with good allograft function and prophylactic measures against certain virus infections.

Maintenance immunosuppression with the m-TOR inhibitors sirolimus and everolimus, is associated with a significantly reduced risk of developing any post-transplant de-novo malignancy and non-skin solid malignancy [196].

Cancer screening and prevention in thoracic transplant recipients is not thoroughly studied. Computed tomography of the chest, abdomen, and pelvis was not useful in a single-center observational study of cardiac transplant patients [197]. For screening and cancer surveillance of thoracic transplant recipients, PCPs may follow current published guidelines from the American Society of Transplantation, the United States Preventive Services Task Force, and the American Cancer Society for cancer screening in renal transplant patients [198,199]. Other preventive measures include appropriate antiviral prophylaxis and vaccination, and patient behavioral education and modification (avoidance of tobacco and alcohol, healthy diet, etc.), regular use of sun block; monthly self-examinations, and annual dermatologic examinations [200–202].

Preparing for surgical procedures

Thoracic transplant recipients can safely undergo surgical procedures with caution to certain aspects of care. Individuals on sirolimus and everolimus may have impairment in wound healing; the risks and benefits of stopping these agents for major surgeries should be discussed. Significant corticosteroid exposure necessitates stress-dose steroids for risk of adrenal insufficiency during major surgery. Conversion of CNIs and cell cycle inhibitors to intravenous or sublingual formulations while the patient is nil per mouth should be done in consultation with a transplant physician, and therapeutic drug levels must be monitored. Although leukoreduction may decrease sensitization, unnecessary transfusion of blood products is best avoided. Cytomegalovirus (CMV)-negative blood products are needed for CMV-naïve recipients.

Drug interactions

The immunosuppressants commonly used in transplant medicine include corticosteroids, CNIs (cyclosporine, tacrolimus), mTOR inhibitors (sirolimus, everolimus), and antimetabolite cell cycle blockers [azathioprine and mycophenolate mofetil (MMF) or derivatives]. These medications, covered in depth in Chapter 98, have a narrow therapeutic index and relatively small changes in dose or concentration lead to serious therapeutic failures or serious adverse drug reactions. Such consequences may be persistent, irreversible, slowly reversible or life threatening, and may result in hospitalization, persistent or significant disability, graft loss, or incapacity and death [203]. Therapeutic drug monitoring is routinely done for m-TOR inhibitors and CNIs. Target serum level varies based on time from transplant and individual patient rejection burden. CNI and mTOR inhibitors are metabolized through the cytochrome P450 system and have significant interactions with many other commonly used medications leading to subtherapeutic or toxic levels (Table 35.3). Initiation or cessation of therapy with these medications warrants consultation of the literature and coordination with the transplant center and frequent (at times daily) therapeutic drug monitoring. Co-administration of sirolimus and azole antifungals is contraindicated by the manufacturer. However, two case series found no clinically significant toxicity and stable trough concentrations when the doses of voriconazole and posaconazole were in advance decreased by 90% and 33–50%,, respectively [204,205].

Table 35.3. Significant drug interactions for the cytochrome P450 system

Inducers (risk of rejection)	Inhibitors (risk of toxicity)
Carbamazepine	Amiodarone
Nafcillin	Azole antifungals:
Phenytoin	Fluconazole
Phenobarbital	Itraconazole
Pioglitazone	Ketoconazole
Rifabutin	Posaconazole
Rifampin	Voriconazole
Troglitazone	Calcium channel blockers:
St. John's wort	Diltiazem
	Nicardipine
	Verapamil
	Cimetidine
	Ciprofloxacin
	Macrolides:
	Clarithromycin
	Erythromycin
	Glucocorticoids
	Indinavir
	Grapefruit juice

Concomitant use of the following medications with azathioprine may lead to severe bone marrow suppression and cytopenias: allopurinol, captopril, and other ACE inhibitors [206,207]. Concurrent use of MMF and proton pump inhibitors may result in reduced exposure to the active metabolite of MMF, leading to increased risk of rejection.

All changes in immunosuppressant medications should only be made by the transplant center [7]. If any other medications are to be added or eliminated, the literature and drug interaction tables should be consulted. If any change in medications, including generic drug substitution, occurs, the transplant center should be informed so that heightened close therapeutic drug monitoring can be implemented until a steady-state level is achieved.

Summary

Thoracic transplantation has become an increasingly commonplace component of medical care and its improving survival rates have introduced thousands of patients back into the community who are able to conduct productive lives, but who also require heightened scrutiny by medical professionals. In general, the issues facing long-surviving transplant recipients are similar to those facing the general community, although their presentation is altered by immunosuppressive drugs. Partnership between transplant and non-transplant physicians is required to provide comprehensive care for transplant candidates for timely referral, evaluation, optimization of comorbidities, and preparation for transplantation; and for the recipients for management of common medical comorbidities, immunization, screening for malignancy, and counseling for healthy lifestyle. The single most important factor in this process is ongoing communication between the primary care and transplant teams.

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CHAPTER 36

Alloantibodies, Sensitization and Virtual Crossmatching

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Introduction

In 1969, Patel and Terasaki reported that a positive pretransplant cross-match between recipient serum and donor lymphocytes was associated with a high risk of hyperacute renal allograft rejection (see Chapter 3) [1]. Subsequently, the risk of rejection was shown to be strongest when positive cross-matches were due to pre-existing antibodies directed to donor HLA antigens, particularly when those antibodies fixed complement. The association between pretransplant donor-directed HLA antibodies and allograft rejection was so compelling that Patel and Terasaki insinuated that it was malpractice to transplant patients without performing a pre-transplant cross-match. Such sentiments meant that the histocompatibility laboratories that supported transplant programs needed to be operational 24/7/365 in order to perform prospective lymphocyte cross-matches. The paradigm was uncomplicated: simply avoid transplanting patients possessing HLA antibodies (i.e. sensitized recipients) with donor organs expressing one or more of the corresponding HLA antigens. Early studies [2,3] suggested that with detailed prescreening of patient sera to identify HLA antibodies, it was possible to predict which HLA antigens to avoid before a physical cross-match was even performed. This first iteration of what today is called the virtual cross-match (vXM) was completely rational, but its utility was severely hampered by the limitations of the technology at that time. Furthermore, the underlying assumptions, that all positive reactions were the result of clinically relevant HLA antibodies and that all negative reactions assured long-term graft survival, were incorrect. In part, this was because the early assays used to identify HLA antibodies and perform cross-matches were neither sufficiently specific nor sensitive enough to identify all relevant HLA antibodies [4]. Thus, although avoiding donor-directed HLA antibodies was conceptually appealing, the methods to do so were not up to the task. Adding to these serious issues was that the serologic HLA typing assays used to assign HLA antigens were frequently subjective and incomplete, which meant that HLA antigens were not always correctly or completely identified.

In recent years, the development of solid phase testing platforms using purified HLA antigens (class I or class II) permitted the identification of HLA antibodies with a specificity and sensitivity not previously attainable [5,6]. Similarly, implementation of molecular typing methods led to the fine resolution of HLA antigens and characterization/definition of individual alleles [7,8]. Collectively, these developments led to objective identification of HLA antigens expressed by potential organ donors and an exact assessment of the HLA antibodies present in the sera of sensitized recipients. The concept of virtual cross-matching was ready to move from theory to practice. This chapter will summarize the development of the fundamental assays used in modern transplant histocompatibility testing and principles governing their proper deployment in the clinic. The chapter is complemented by Chapter 6 outlining the fundamental mechanisms of antibody-mediated rejection, Chapter 4 outlining the structure and function of histocompatibility molecules, and Chapter 89, detailing the use of antibody testing as a postoperative biomarker.

Assays

Serologic antibody testing

Initially, complement-dependent cytotoxicity (CDC) assays were used prospectively to determine whether patients awaiting transplantation were sensitized to HLA antigens [9–12]. Briefly, HLA-typed cells from at least 30 donors were incubated with patient serum and complement. Target cell death was interpreted to mean that the panel member expressed at least one HLA antigen to which the patient serum had a corresponding antibody. The logic behind performing this test was twofold. First, it provided valuable information to the patient, namely his/her likelihood of being cross-match compatible (and therefore transplantable) with a random donor. Second, it was possible to define HLA specificities, which could then be used to predict compatibility with specific donors. In its simplest form, the percentage of panel cells reacting with a patient's serum was considered to correspond to the breadth of his/her sensitization. For example, if 10 of 30 panel cells reacted with serum from a patient, that patient was considered to have panel reactive antibody (PRA) activity of 33%. This patient would then

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Table 36.1. The two tables represent an HLA typed (class I only) cell panel. In this example, there are 24 typed panel members being tested with a patient serum. Twelve of 24 cells (darkly shaded) reacted with the patient serum, corresponding to a panel reactive antibody activity (PRA) of 50%. All 12 cells express HLA-A2, meaning the antibody specificity is to that antigen. Note, however, that four of the HLA-A2-positive cells also expressed HLA-B44. Since HLA-B44 was not present except in the context of HLA-A2, a second antibody to HLA-B44 may be present

A		B		C	
2	11	35	75	8	7
2	23	53	81	4	6
2	24	7	44	5	8
2	24	35	37	4	6
2	24	44	35	4	6
2	24	35	50	2	6
2	26	39	46	1	7
2	29	44	62	4	5
2	29	7	44	2	9
2	30	45	56	1	7
2	31	35	47	4	6
2	32	27	63	2	5

A		B		C	
1	3	35	63	4	7
1	11	37	52	4	6
1	25	35	52	5	7
1	26	27	57	2	6
1	26	38	73	2	8
1	32	42	50	5	6
1	33	8	62	7	10
1	34	35	57	6	7
1	66	50	58	4	7
1	74	8	72	2	7
1	11	13	55	1	7
1	11	13	46	1	6

be informed that he/she would be cross-match compatible with two of every three random donors. However, the validity of this interpretation was predicated on whether the frequency of HLA antigens in the cell panel reflected the frequency of those antigens in the donor population. Initially, cell panels were assembled at the transplant center using randomly selected healthy volunteer donors or based on their HLA phenotypes. Subsequently, frozen cell panels became commercially available from several vendors. Unfortunately, there was no standard approach to select donors to be panel members. Thus, the HLA phenotype composition of panel members did not necessarily reflect the frequency of those HLA antigens among the deceased donor population. Accordingly, if the serum sample referred to above contained an antibody to HLA-A01 and was tested on three different cell panels, where none, half, or all of the target cells expressed HLA-A*01, the PRA would be correctly interpreted as 0%, 50%, and 100%, respectively. Which of those results most accurately reflects the distribution of HLA-A*01 in the population of individuals who become organ donors? In reality, the frequency of HLA-A01* in the US donor population is 23% [13], meaning none of the PRA values from the above example is correct.

Another far-reaching complication of using PRA values derived from cell panels composed of random HLA phenotypes is the impact on deceased donor renal allocation when all antibodies are not identified. It is well-recognized that highly sensitized patients (currently defined as patients with a PRA of $\geq$80%) are significantly disadvantaged compared to non-sensitized patients with regard to finding compatible donors [14]. In the US, renal transplant recipients are prioritized for transplantation based on a point system. The more points a patient accumulates, the higher up they move on the waitlist managed by the United Network for Organ Sharing (UNOS) [15]. When a patient's point value puts him/her at the top of the waitlist, he/she is afforded the opportunity to be cross-matched with blood-type compatible donors. Points are awarded to recipients for the degree of HLA-DR matching with the prospective donor (two points for a two HLA-DRB1* match, one point for a one HLA-DRB1* match) and wait time (one point per year of waiting). In an attempt to reduce the disparity of transplantation between highly sensitized and non-sensitized patients, UNOS awards four additional points to highly sensitized (PRA >80%) patients. With nearly 100 000 patients currently awaiting a deceased donor kidney [16], the point system has been considered an equitable approach to deceased donor renal allocation. It is not unusual for highly sensitized patients to have also accrued several points for waiting time (they are, after all, difficult to match) and have four additional points for PRA; such patients routinely are at the top of the list. However, being at the top of the list does not guarantee that a patient will be transplanted. The patient must still be cross-match compatible with his/her donor. Historically, preliminary cross-matches were performed as a triage maneuver, filtering out patients with a positive cross-match and permitting those whose cross-matches were negative to move on to the next (or "final") phase where a current sample from the patient was cross-matched with cells from the intended donor. This, so-called "final cross-match" would have to be compatible (i.e. negative) in order for the patient to proceed to transplant.

Until recently, cross-matches with sera from highly sensitized patients were frequently positive [17]. At least part of the reason for this was due to incomplete assignment of unacceptable antigens using HLA-typed cell panels [18]. For example, in Table 36.1 all of the cells reacting with the patient serum express the HLA-A*02 antigen, while none of the non-reactive cells expressed HLA-A*02. This reaction pattern suggests the patient has a single HLA antibody directed to the HLA-A*02 antigen. Therefore, only HLA-A*02 would be considered to be an unacceptable antigen for the patient. Donors possessing all other HLA antigens would be considered provisionally compatible with the patient (i.e. vXM negative). However, closer examination of Table 36.1 reveals that all cells expressing HLA-B*44 also express HLA-A*02. If the patient also had antibodies to HLA-B*44, their detection would be masked by the HLA-A*02 antibodies. In this example, if HLA-A*02 were considered the only unacceptable HLA antigen, a donor expressing HLA-B*44 (but not HLA-A*02) would be considered acceptable; the final cross-match, though, would be unexpectedly positive. In addition to incomplete identification due to masked antibodies, cell panels were not the ideal targets to identify the entire repertoire of antibodies among broadly sensitized (i.e. $\geq$80% PRA) patients. As such, their sera were routinely cross-matched with any blood group-compatible donor. If the cross-match was positive, the donor was considered incompatible with that patient. Complicating matters further was the sensitivity of the cross-match that was performed (reviewed in [19]). When first implemented, cross-matches were performed using a complement dependent cytotoxicity (CDC) assay to identify clinically relevant antibodies. However, it soon became apparent that patients without CDC-detectable

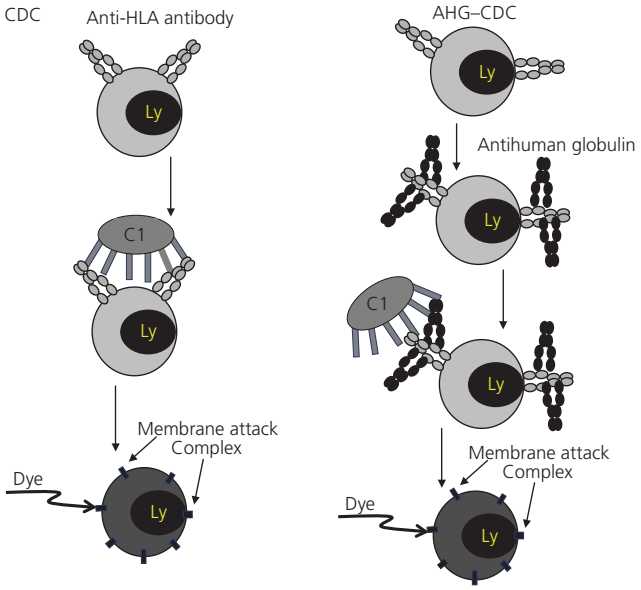

Figure 36.1. Complement-dependent and antihuman globulin augmented complement-dependent cytotoxicity assays to detect HLA antibodies. Donor lymphocytes are incubated with patient serum and complement (CDC assay) or patient serum, antihuman globulin, and complement (AHG-CDC). Antibody by itself or augmented with AHG activates complement and the damage to lymphocyte membranes is detectable by the uptake of a vital dye.

antibodies still experienced early antibody-mediated graft rejection/graft loss. This led to modifications of the assay, including extended incubations, extra washes, and enhancement with an antihuman globulin (AHG) reagent [10], the latter eventually becoming the laboratory gold standard for many years (Table 36.1). However, even with this augmented technique to identify antibodies, there was still a group of patients who experienced early episodes of rejection or graft loss attributable to donor-directed antibodies. Furthermore, cytotoxicity testing usually identified only class I HLA antibodies. As the field of organ transplantation matured, it became evident that it would be prudent to also identify whether patients had antibodies against class II specificities (e.g., HLA-DR, HLA-DQ) prior to transplant [20].

The development and implementation of the flow cytometric cross-match (FCXM) provided the transplant community with an extremely sensitive new assay, one that allowed simultaneous evaluation of T and B lymphocytes, meaning an assay was now available to look at targets (B cells) that expressed class II histocompatibility antigens. At the time, published data suggested there was little, if any, clinical relevance of class II HLA antibodies and most transplant centers proceeded to transplant as long as the T-cell cross-match was negative. However, those early studies were of questionable reliability because they were typically based on low numbers of B cells that had to be physically separated from T cells. Additionally, there was a failure to recognize that B cells, like T cells, express class I antigens. Multiple studies demonstrated that T-cell–negative, B-cell–positive cross-matches resulting from low titer antibodies to donor class I antigens were associated with early graft loss (1-year graft survival of 50–67%; reviewed in [19]).

Another feature of the FCXM was that it identified donor-directed antibodies that did not fix complement and whose clinical impact, though questionable, was nonetheless worrisome. Indeed, antibodies detected only by flow cytometry were associated with increased episodes of rejection and graft loss [21–23]. However, other studies reported that the FCXM offered no additional benefit over cytotoxic cross-matches [24]. Although apparently contradictory, one explanation for these differences was that some positive FCXMs were not due to HLA antibodies. Apparent false-positive cross-matches could occur as a consequence of autoantibodies, non-specific binding of immune complexes (frequent in patients with autoimmune diseases such as systemic lupus erythematosus) and/or Fc binding of IgG molecules to Fc receptors expressed on lymphocytes [25,26]. B cells were particularly susceptible to these non-specific binding issues. One study reported that up to 75% of positive B-cell cross-matches were due to non-HLA antibodies and, hence, clinically irrelevant [27]. However, this same study also showed that when the positive B-cell cross-matches were due to HLA antibodies (specifically, class II antibodies), graft survival was significantly lowered. This study underscored the challenge of using B cells as targets and the need to consider additional explanations when a B-cell cross-match was unexpectedly positive.

Tipping point

In the early 1990s, advances in molecular biology (specifically the development of the polymerase chain reaction) enabled DNA-based HLA antigen testing to be performed. By the mid-1990s, the introduction of multiplexing microparticle technology and cytometry-based instrumentation permitted the detailed assessment of HLA antibodies and the accurate determination of an individual's HLA type. Currently, multiplexing fluorescence-based solid phase assay technology is the most commonly used platform in the clinical HLA laboratory. This technology has the ability to simultaneously distinguish and analyze up to 100 uniquely coated microparticles [4,28]; see following section. By documenting whether patient sera possess donor-reactive HLA antibodies, a lymphocyte cross-match (positive or negative) can now be more reliably interpreted. Indeed, with solid phase data, a positive lymphocyte cross-match can be categorized as clinically irrelevant versus relevant, and, if relevant, the positive cross-match could be interpreted as a risk factor for rejection or a definitive contraindication to renal transplantation (reviewed in [19]). Thus, in a manner similar to how the introduction of calcineurin inhibitors as immunosuppressants revolutionized transplant medicine in the early 1980s, multiplexing technology revolutionized clinical histocompatibility testing.

Solid phase revolution

Since T and B lymphocytes express a multitude of membrane proteins in addition to HLA, the assignment of HLA antibody specificity using membrane-dependent assays was daunting. The development of solid-phase assays utilizing purified HLA proteins devoid of all other cell membrane components helped make antibody identification more manageable. The first iteration of solid phase testing was an enzyme-linked immunosorbant assay (ELISA) using affinity purified HLA class I isolated from platelets as target antigens to detect HLA class I antibodies in patient sera [29]. Although this approach discriminated HLA from non-HLA antibodies, the specificity of positive sera was not readily identifiable. Although ELISAs were an improvement over membrane-dependent assays, they were cumbersome and not as sensitive as flow cytometric-based methods. Furthermore, even after the implementation of ELISAs to identify antibodies to class II HLA antigens,

separate tests were needed to distinguish class I from class II antibodies.

The next breakthrough in HLA antibody testing came when Pei et al. described a methodology wherein purified HLA class I and class II proteins isolated from Epstein–Barr virus (EBV)-transformed cells lines were attached to microparticles and analyzed by flow cytometry [5]. In contrast to ELISA, the FlowPRA® (One Lambda, Inc, Canoga Park, CA, USA) permitted simultaneous identification of antibodies to both HLA class I and class II antigens on a standard flow cytometer. Additional advantages of solid phase analysis of antibodies included objective and semi-quantifiable analysis of data, no requirement for viable target cells, and a consistent and renewable source of HLA antigens for coating microparticles. Microparticle-based assays have gone through several configurations since their inception. The FlowPRA® was a simple and straightforward assay to determine whether HLA antibodies were present. Briefly, it combined 60 separate microparticles, 30 coated with class I antigens and 30 coated with class II antigens (Figure 36.2a). Each individual microparticle in the mix was coated with a different class I (A, B, C) or class II (DR, DQ,DP) phenotype derived from a single EBV-transformed cell line. Class I-coated particles were discernable from class II-coated microparticles based on two parameters, size and fluorescence. Class I-coated microparticles are larger and non-fluorescent compared to class II-coated microparticles, which are smaller and internally stained with a red fluorescent dye. Beads exhibiting increased fluorescence when stained with fluoresceinated antihuman IgG compared to background controls are considered positive. Positive reactions are determined by the number and fluorescence intensity of the microparticles. Since an individual class I or class II microparticle represents 1 in 30 (~3%) of the total number of events, each positive reaction must demonstrate a distinct peak of fluorescence above that of the negative control and contain at least 3% of the total gated events to be considered.

Although the FlowPRA® assay identified HLA antibodies and was significantly more sensitive and specific than cell-based assays or the ELISA, the assay could not determine the HLA specificities to which the antibodies were directed and another test was developed, namely a flow-based specificity assay. In this assay, the cluster of HLA phenotypes (class I or class II) isolated from the EBV cell lines were attached to individual bead populations just as described for the FlowPRA® screening test; however, each individual microparticle was internally stained with a specific amount of red fluorescent dye. Up to 11 different beads could be uniquely identified on a single fluorescence axis and using such a panel of targets, individual antibody specificities could be assessed (Figure 36.2b) [6].

While FlowPRA® paved the way to identify the HLA antibody specificities, there were still two stumbling blocks:

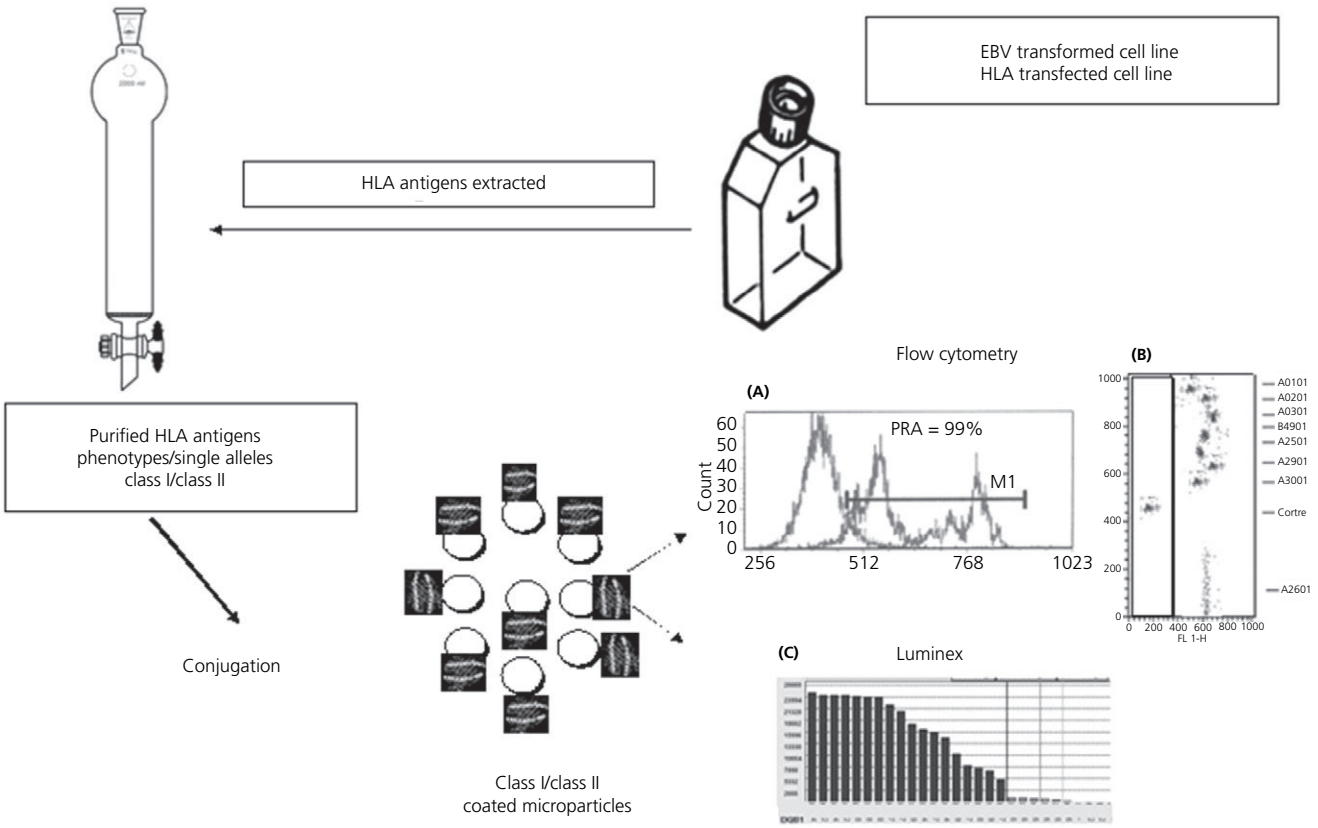

Figure 36.2. HLA class I and class II antigens (entire clusters of phenotypes or single alleles) are extracted and purified from cell lines and coated onto microparticles. Individual microparticles (n = 30) are combined to create a screening pool of HLA antigens (A) that are analyzed on a flow cytometer. The percentage bead reactivity allows for the calculation of a percentage of panel reactive antibody (PRA). EBV, Epstein–Barr virus. Alternatively, individual microparticles are assayed by flow cytometry (B) or Luminex (C) to evaluate antibody specificity The percentage bead reactivity allows for the calculation of a percentage of PRA.

1 the extensive polymorphism of the HLA complex which hampered design of the target array;
2 only 11 beads could be tested at one time (which reduced technical efficiency).

The development of another flow cytometric-based instrument (Luminex) was a major improvement in the identification of HLA antibodies because this platform permitted up to 100 individual microparticles to be evaluated simultaneously (a so-called multiplex assay) [28]. Each microparticle had a unique fluorescence signature that was derived by incorporating the microparticle with varying amounts of two fluorescent tags. For each fluorescent tag there were ten different shades, meaning one could establish a 10 × 10 matrix yielding a set of 100 uniquely identifiable microparticles. The fluorescence read-out or "reporter" molecule was of a third and distinct color that could be measured on each bead in the matrix. Then, each microparticle was conjugated with a different HLA antigen or nucleotide sequence, allowing positive immunoassay results to be automatically correlated to a unique HLA specificity (Figure 36.2c). In theory, a third and fourth set of dyes could be included, leading to the ability to detect 1000 and 10 000 analytes at the same time, respectively. Another approach to increasing the number of analytes tested would be to use a set of microparticles of a different size from the original set. The more analytes that can be simultaneously analyzed, the greater the efficiency of the test.

A significant proportion of patients awaiting transplantation possess such broadly reactive HLA antibodies that 100% of the HLA antigen-coated microparticles will be positive [30]. However, patients never actually have 100% PRA, but instead possess multiple antibodies that each react with several common HLA specificities [31,32]. With the implementation of recombinant technology, single HLA proteins could be purified and attached to microparticles. These so-called single antigen bead panels were then used to clearly delineate antibody reactivity.

At the same time that antibody detection and identification improved with new technology, so too did the ability to type HLA antigens. During the past 20 years, typing for HLA antigens shifted from serologic to molecular-based methodologies, allowing a simple test to discern between two different antigens differing by as little as a single amino acid (e.g. HLA-B*44:02 vs. HLA-B*44:03) [33]. While serologic typing distinguished a limited number of HLA specificities, molecular-based methods can identify all HLA alleles. From knowing the unique allele that is coated on to the microparticle, it was soon discovered that patients had the ability to produce allele-specific antibodies, i.e. an antibody that reacted with one allele of an antigen family. Allelic antibodies, in fact, were not uncommon and this recognition began to underscore the possibility of typing donors at the allele rather than antigen level, at least for those antigens where the frequency of alternative alleles was sufficiently high and alleic antibodies had been identified. Clearly, the vXM would not be possible without comprehensive molecular typing of donors. The era of serologic HLA typing has passed. The technologies for performing molecular-based HLA typing are reviewed in Chapter 3 [34].

Clinical application of the virtual cross-match

Although the concept of virtual cross-matching is nearly 30 years old [2], reliable implementation of this process did not become a clinical reality until 2006. Then, using solid phase antibody assays to identify HLA class I and class II antibodies with accuracy and sensitivity, Emory University and Duke University published their successful experiences using the virtual cross-match to predict compatibility among highly sensitized patients receiving deceased donor kidney and lung transplants, respectively [35,36]. Subsequently, a national study tested the ability of the virtual cross-match to predict positive cross-matches among highly sensitized patients on the UNOS waitlist and reported a predictive value exceeding 90% [37]. However, in that study, the ability to predict a negative cross-match was poor (~50%). Subsequent studies suggested that at least part of the inability to predict negative cross-matches was because of incomplete information about all the antibodies these highly sensitized patients possessed, specifically antibodies to HLA-C, HLA-DQ, and HLA-DP antigens [38]. Even though these antibodies may have been identified, they were not routinely listed as unacceptable, in part because UNOS only mandated that antigens of the HLA-A, HLA-B, and HLA-DRB1 loci be tested and reported for deceased donor organs, the implication being that antibodies to other HLA loci were not clinically relevant. This is no longer the working model at UNOS. Several studies have now been published that document that antibodies to HLA-C, DQB1, and DPB1 are associated with episodes of rejection and graft loss when patients with those antibodies are transplanted with organs possessing the corresponding antigens [39–42]. In June 2011, UNOS mandated that HLA molecular typing data be reported for the HLA-A, B, C, DRB1, and DQB1 loci. Currently, there is no requirement to mandatorily type for HLA-DPB1 or HLA-DQ alpha antigens, even though the frequency of those antibodies among potential recipient populations is on the rise. It would not be surprising if typing for these loci eventually becomes mandatory.

Since those early reports, the vXM has become widely accepted not only for deceased donors [43,44] but also for living donors, particularly those involved in paired donor exchanges [45]. For thoracic transplantation, the vXM has decreased deaths on the waitlist and permitted organs to be imported from centers up to 500 miles away, which, previously, would have been impossible because there would not be enough time to perform a prospective physical cross-match [46]. The vXM has effectively eliminated the need for costly and time-consuming preliminary cross-match trays in essentially all laboratories in the US. In Europe, Claas et al. adopted an international program of acceptable mismatching that significantly increased the number of deceased donor kidneys transplanted into highly sensitized recipients [47]. The UK also prioritizes broadly sensitized patients nationally for transplantation based on a vXM [48]. Late in 2014, UNOS will implement an allocation scheme designed to broaden access to extremely sensitized patients. Specifically, mandatory sharing of organs at the local, regional and national levels will be implemented for patients with cPRA of 98%, 99% and 100% cPRA, respectively.

The use of solid phase HLA antibody detection assays has the ability to place patients into at least three categories:
1 no HLA antibodies;
2 HLA antibodies without donor specificity;
3 donor-specific HLA antibodies (DSA).

Patients in the first category are predicted to have a negative cross-match. In fact, if the physical cross-match for a patient in this category is positive, it is generally not considered a contraindication to transplant. Patients in the second category are also vXM negative. When transplanted, these patients have a low risk of antibody-mediated rejection/graft loss and experience excellent long-term graft survival [35,49–51]. For patients in the third

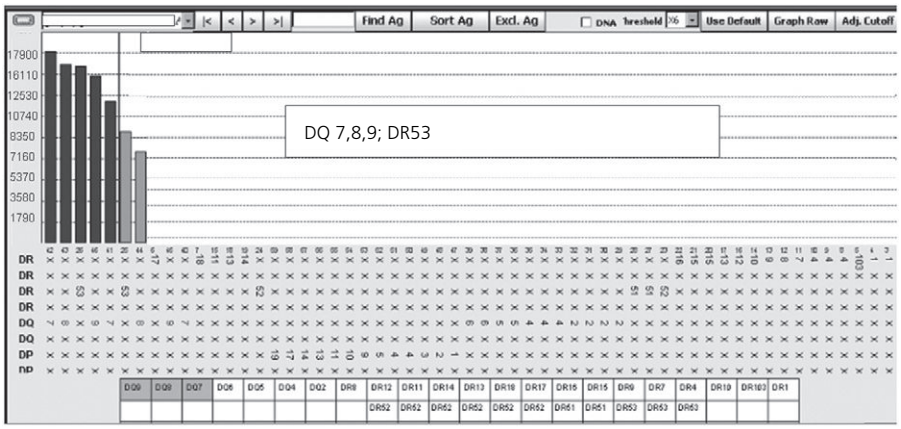

Figure 36.3. The serum in the histogram has antibodies to HLA-DQB1*03:01, 03:02, 03:03 (DQ7, DQ8 and DQ 9) and to DRB4* (DR53). The antigens corresponding to these antibodies would be listed as "unacceptable." The donor on the left would be considered unacceptable because the antibodies would be considered donor-specific antibodies (DSA) and the virtual cross-match is positive. In contrast, for the donor on the right, the same antibodies are present but they are not DSA. The virtual cross-match with this donor is negative.

category, the vXM will be positive. It is important to recognize that, for patients who have antibodies, the same antibodies may have no DSA for some donors and the vXM will be negative, but for other donors, one or more of the antibodies will be donor directed and the vXM will be positive (Figure 36.3). Recent data indicate that the physical cross-match in patients with DSA is not always positive [52–54], leading to the question of what should be the determining factor in proceeding to transplant: a DSA or a positive cross-match?

Paradigm shift

As mentioned above, the make-up of an HLA-typed panel (cells or microparticles) could easily impact whether a sensitized patient reaches the 80% threshold where four points are awarded. Until recently, four points were awarded to any patient whose reported PRA was ≥80%, even if only one unacceptable antigen was identified [55]. This approach was not particularly effective because these broadly sensitized patients who already had long waiting times received four additional points and routinely appeared at the top of each match run for each blood group-compatible donor. Positive final cross-matches were common among these broadly sensitized patients [17] and they were being transplanted at a rate far below their representation on the list of candidates awaiting transplantation. The recognition of this issue led to some sweeping changes in national organ allocation. In October 2009, UNOS implemented a new policy mandating that a patient would be awarded four sensitization points if and only if his/her percentage of unacceptable HLA antigens was ≥80%. Assessment of HLA antigen frequencies were determined from a UNOS database of >12 000 HLA-typed deceased donors. Under these new rules, PRA values are calculated (cPRA), not assigned. Patients who are awarded the four sensitization points will not be considered as recipients for donors who express one or more of the HLA antigens listed as unacceptable. The new cPRA policy was designed to establish both accountability and uniformity in reporting sensitization and to increase the efficiency of kidney allocation by eliminating offers that would be incompatible, thereby reducing the number of positive cross-matches among patients who had reached the top of the waiting list. In effect, the highly sensitized patients at the top of the list, as determined by cPRA, would be much more likely to be compatible with their donors [17]. Put simply, the numbers of positive cross-matches among the top-ranked patients were predicted to substantially diminish. This change in policy fundamentally changed how sensitized renal candidates were ranked for kidney offers. A subsequent review of national data demonstrated that within 2 years following the implementation of the new cPRA policy, the number of offers refused because patients had positive cross-matches with their prospective donors was reduced by >90% compared to the pre-cPRA era [56]. Additionally, highly sensitized patients appear to have particularly benefited from the cPRA policy based on the observed increase in the percentage of patients with >80% cPRA who were transplanted during the pre and post cPRA era (7.2% and 15.8%, respectively). These data are consistent with the tenet that, even at the national level, careful identification of unacceptable HLA antigens among highly sensitized candidates at the time of listing (and keeping the information up to date on the UNOS database) increases the probability that these patients will have negative cross-matches with donors who filter beyond the triage process.

When should an HLA antigen be considered unacceptable?

As a group, patients transplanted with DSA directed against donor HLA antigens are at higher risk for antibody-mediated damage to the graft than patients without DSA [57]. Is there a threshold level below which DSA is clinically irrelevant? The most common solid phase antibody detection systems today utilize Luminex technology, which is based on immunofluorescence reactivity that is reported as an arbitrary unit of mean fluorescence intensity (MFI). The higher the MFI value, the stronger the florescence signal

indicating the greater the quantity of antibody binding. Values range from 0 to 20 000 MFI, and as a general (but by no means universal) rule, MFI values from 300 to 2000 are considered weak, >2000 and <5000 moderate, >5000–10 000 intermediate, and >10 000 strong. The actual threshold at which to consider an antigen as unacceptable is a center-to-center decision and is based on each center's philosophy and risk aversion. Intermediate to strong antibodies generally result in a positive flow (and often cytotoxic) cross-match, while flow cytometric cross-matches with weak to moderate antibodies may be positive or negative. It is the latter type of antibodies that are the current challenge to the transplant community. If the cross-match is negative, is the DSA clinically irrelevant? It should be obvious that if thresholds to assign an antibody unacceptable are set too low, there will be patients who are denied the opportunity to be transplanted with what would be a cross-match compatible organ [58]. In contrast, by setting the threshold too high, patients could experience antibody-mediated rejection that can reduce the functional life of the allograft [59]. The literature is divided, with some groups reporting that antibodies detected solely by solid phase have no impact on graft outcome, while others report that such antibodies (even "weak" ones) are associated with increased risk for antibody-mediated rejection and graft loss.

It is likely that the discrepancies between a DSA detectable on microparticles but not cells are a combination of technical and biological variables. For example, the HLA antibody detected on the microparticle is against an allele of a given antigen, e.g. HLA-B*44:02, and the target expresses HLA-B*44:03. Even though there is only a single amino acid difference between 44:02 and 44:03, this is sufficient for a B*44:02 antibody to give a positive cross-match with a B*44:02 cell but not a B*44:03 cell. Another explanation is that the donor cell has reduced HLA expression that can occur after viral infection or in the context of certain drugs [60,61]. Other possibilities include differential avidity for antibodies binding to microparticles versus cells and expression of degraded or cryptic HLA antigens/epitopes on microparticles compared to native antigen expression on cells. Sera from some patients (even those with no known sensitizing events) bind to denatured but not native HLA antigens (and thus bind to microparticles and not lymphocytes). What those antibodies are directed to and what stimulated their production is unknown. In recent studies, one heart–lung and two renal patients displaying antibodies to denatured antigens were transplanted with donor organs expressing the relevant native antigens [52,62]. One patient (a renal recipient) has progressed to 5 years post transplant without experiencing an episode of rejection. While the other two patients are only 4 months and 1-year post transplant, neither has shown any signs of rejection to this point. There are also examples wherein patients appear to have no HLA antibodies yet present with a positive cross-match. The most logical interpretation is that those cross-matches are due to non-HLA antibodies. However, this is not always true. A simple example would be that the repertoire of HLA class I/II antigens present on the donor is incompletely represented on the microparticles used to screen for/identify HLA antibodies. Another explanation would be higher HLA antigen density on the target cell compared to the microparticles. If the donor expresses two or more antigens to which the recipient has low-level antibodies (below the threshold level considered to be positive), the cross-match could be unexpectedly positive. With increased antigen expression, more antibodies can be deposited on the cell surface, thereby yielding a higher fluorescent signal. Additionally, an antibody directed against a single epitope may be spread out over multiple microparticles that all express that epitope (e.g., an antibody to Bw4 would be present on ~60% of a target panel) and the antibody may be diluted over multiple beads, with no bead displaying MFI values above the threshold. Collectively, these data indicate that cut-off values alone may not be an appropriate approach to assigning an antibody as positive. Several other factors must be taken into account, including the sensitization history of the patient and/or whether beads below a cut-off threshold (but not negative) exhibit any pattern of antibody specificity. Otherwise, false-positive and false-negative results will not be uncommon and lead to discordant results between a vXM and a physical cross-match. Studies by Tambur et al. indicate discordant results occur in approximately 10% of patients [38]

Summary

The field of histocompatibility continues to evolve. Over the past 20 years, HLA typing has moved from serologic to molecular technology, and HLA antibody detection/analysis has transitioned from a cell-based approach to assays that rely on solid phase matrices. These advances led to a major change in the organ allocation system in the US by making the virtual cross-match a reality. The identification of unacceptable antigens with sensitive solid phase assays can effectively be used to triage sensitized recipients, such that time or resources are not needlessly expended on individuals incompatible with their prospective donors. Ischemia time is reduced and highly sensitized patients are much more likely to be transplanted then at any time in the past. Screening for HLA antibodies will determine whether a patient can go directly to transplant (e.g. no HLA antibodies) or needs further studies to identify class I and class II antibodies. If the latter, unacceptable antigens can then be identified and entered into a national computer system such that donors who possess one or more of the unacceptable antigens are not considered for allocation to the corresponding recipients (Figure 36.4). Clearly, the application of the vXM has played a substantial role in the identification of compatible donor–recipient pairs.

Unfortunately, the vXM is not perfect. One of the more controversial topics at this time is how unacceptable antigens are defined. Are thresholds being set too high or too low for any given patient? The challenge is to discriminate acceptable DSA from unacceptable DSA. Even if pretransplant antibodies were the sole risk factor for allograft outcome, more than simple quantifications of antibody levels are necessary to determine their long-term consequences. Factors such as the number and specificities of memory B cells and plasma cells, antibody affinity, complement fixing attributes, antibody titers, and antibody isotypes may all need to be evaluated. Currently, there are no routine tests that reliably distinguish patients who will do well from those who will do poorly when they have identical specificities and apparent strength of DSA pre transplant. Whether to proceed towards transplant is a philosophical decision that remains center dependent [63]. What is acceptable at one transplant center may be unacceptable at another center. On the horizon is the recognition that non-HLA alloantibodies (e.g. antiendothelial cell antibodies) are risk factors to graft survival and monitoring for such antibodies is increasing. Eventually, such antibodies and their corresponding antigens will be incorporated into the vXM of the future. The tools now available used to detect and identify HLA antibodies, while not perfect, represent the bleeding

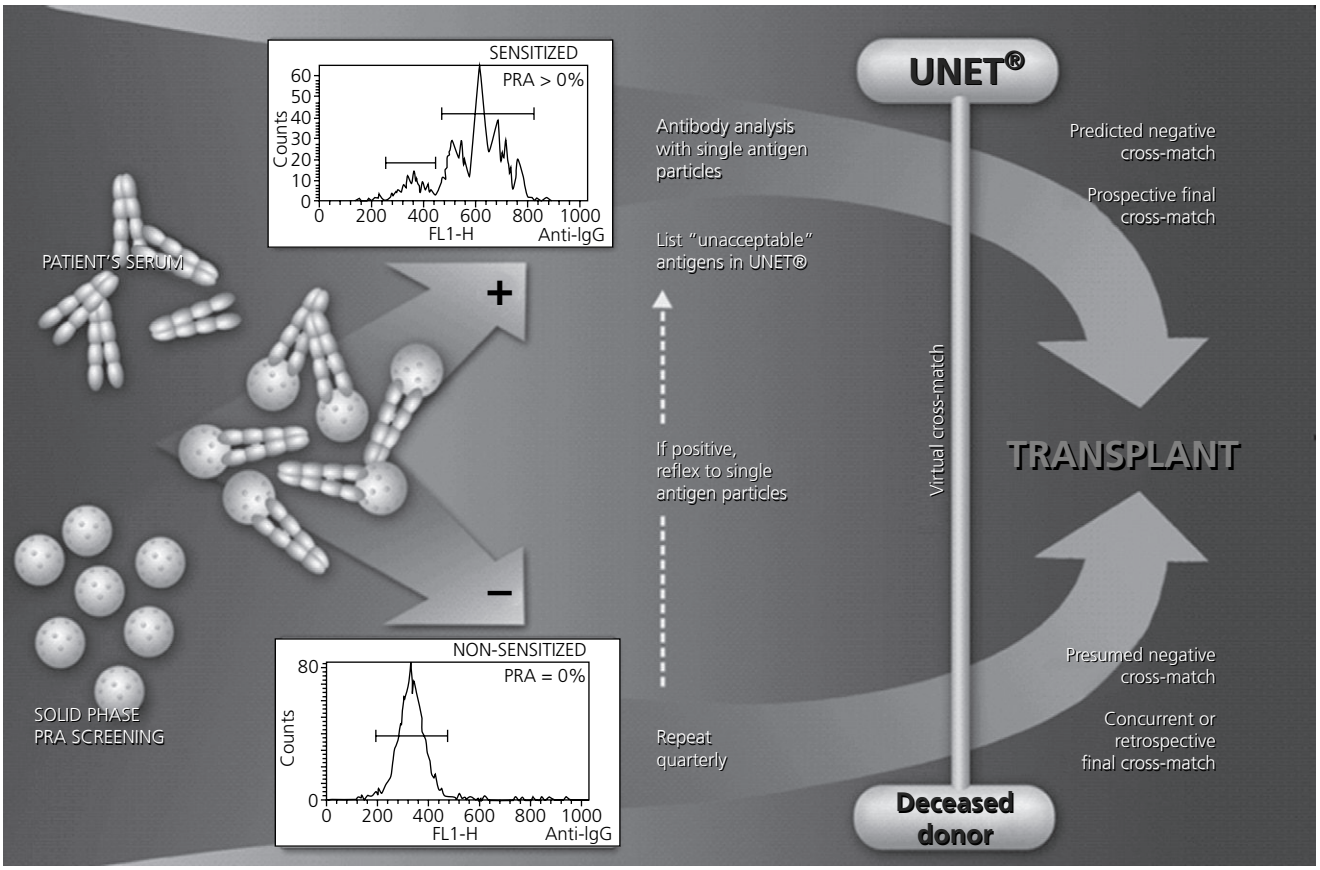

Figure 36.4. Virtual crossmatch (vXM) algorithm. Sera from potential transplant recipients are screened for the presence/absence of HLA class I and class II antibodies. Patients whose serum is class I and class II antibody negative can proceed directly to transplant and a physical cross-match can be performed retrospectively. Sera "positive" for class I and/or class II antibodies undergo detailed antibody specificity analysis with HLA antigen-coated microparticles. The HLA antigen specificities corresponding to the antibodies identified are listed as "unacceptable" antigens in the UNOS database. When a donor organ becomes available for these patients, their unacceptable antigens are used to triage incompatible combinations. Only combinations that are vXM negative will proceed to a final cross-match and (if negative) to transplant.

edge of technology. As this technology evolves, so too will our ability to apply it to the benefit of our patients.

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CHAPTER 37

Waiting List Management for Kidney Transplantation

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Introduction

The number of deceased donor kidneys has remained far below the growing need, leading to a steady increase in patients on the transplant waiting lists, longer waiting times, and increased waitlist deaths. The Organ Procurement Transplantation Network/United Network for Organ Sharing (OPTN/UNOS) database revealed that over the past decade, approximately 22 000–34 000 kidney transplant candidates of all ages were added to the list annually. During the same time period, approximately 4300–6700 patients were removed from the list annually due to death or medical conditions that preclude transplantation. Currently, it is estimated that approximately half of the deaths on the waiting list occur among patients who are placed on the "inactive status" list. Hence, managing the waiting list patients has been one of the greatest problems facing transplant centers. This chapter discusses the categorization of waiting list status set forth by the OPTN/UNOS, factors that may have an impact on kidney waiting times, suggested guidelines for the management of adult kidney transplant candidates on the active waiting list as well as those on hold for various medical and psychosocial reasons. It complements Chapter 28, which details the indications for kidney transplantation.

United Network of Organ Sharing (UNOS) waiting list status

Kidney transplant candidates on the deceased donor waiting list can be listed in one of two status categories, status 1 or status 7. Patients are listed status 1 when they are deemed medically and psychosocially ready for transplantation at all times. In contrast, status 7 listing represents an "on hold" status; this is an inactive status and a patient with this status cannot be offered an organ due to the development of complications that temporarily precludes him/her from kidney allocation. The UNOS/OPTN policy required that such a change in medical status be reported to the OPTN. Previously, accrual of waiting time stopped 1 month after the registrant was placed on hold and the waiting time point could only be restarted when resolution of the medical complication was reported to the OPTN. However, such a policy was thought to serve as a disincentive for transplant programs to accurately report the status of their registrants to the OPTN and could result in delays in organ placement. In November 2003, an important change was implemented whereby candidates placed on status 7 for any acute issue continue waiting time accrual, thereby reducing the disincentive for accurate reporting.

Patients can be made status 7 for various medical or psychosocial reasons or both. The former may include intercurrent illnesses or hospitalizations, cancer-free waiting times, weight loss to achieve an acceptable body mass index, or cardiac or neurological rehabilitation among others. The latter may include medical non-adherence, active substance abuse or psychiatric illnesses, or lack of psychosocial support. While on the waiting list, patients can be moved from status 1 to status 7 or vice versa according to interim events. However, those who develop irreversible complications that contraindicate transplantation, such as metastatic cancer, severe coronary artery disease, or massive stroke, are removed from the waiting list. The algorithm for the UNOS listing and delisting process is shown in Figure 37.1.

Waiting times for listed transplant candidates

The waiting time for a deceased donor kidney may vary greatly based on factors such as ABO blood type, ethnic background, sensitization (peak panel reactive antibodies), and different Organ Procurement Organization (OPO) regions within the US. Other factors may include willingness to accept expanded criteria donor (ECD) kidneys (or dual ECD kidney transplants when ECD kidneys are deemed unsuitable for single use), donor after circulatory death (DCD) kidneys, Public Health Service (PHS) high-risk donor kidneys (formerly CDC high-risk donor kidney), or hepatitis C virus (HCV) donor kidneys in candidates with documented chronic hepatitis C infection.

Knowledge of the primary factors that may affect waiting times allows clinicians and transplant coordinators to formulate practical plans to ensure that candidates at the top of the waiting list are indeed suitable and ready for transplantation to minimize last minute cancellation, and candidates with suboptimal risk management are not be inappropriately transplanted.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

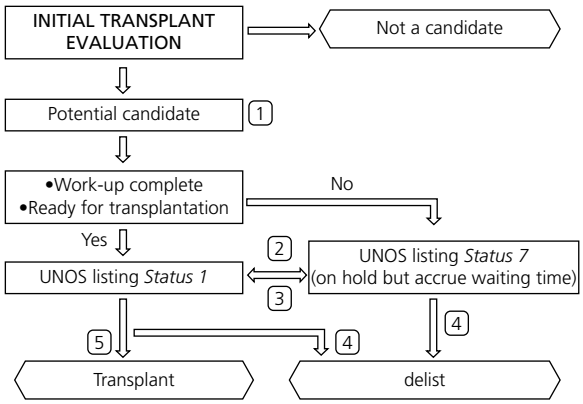

Figure 37.1. Algorithm for United Network for Organ Sharing (UNOS) listing and delisting process.

1 Discuss options (see text): Living kidney donation, ECD, dual transplant of ECD kidneys, DCD, PHS high risk, kidneys from donors with hepatitis C.
2 New medical and/or psychological issues (Status 1 → Status 7).
3 Work-up complete. All issues have been resolved (Status 7 → Status 1).
4 Development of irreversible complications that contraindicate transplantation.
5 Ongoing health maintenance and screening (see text).

UNOS, United Network for Organ Sharing; ECD, expanded criteria donor; DCD, donor after circulatory death; PHS, Public Health Service.

Effect of patient's intrinsic factors on waiting time

ABO blood types

The OPTN/UNOS database revealed that the Kaplan–Meier median waiting time for registrants listed between 1999 and 2004 ranged between 1763 and 1852 days for O blood type, 1085 and 1208 days for A blood type, 1967 and 2033 days for B blood types, and 597 to 855 days for AB blood type [1]. Hence, waiting time is longest for blood groups O and B.

Ethnic background

In the US, the ratio of Caucasian to African–American listed registrants has remained unchanged over the last decade. African–Americans account for approximately 35% of listed registrants, similar to their representation in the chronic dialysis population and approximately threefold higher than their frequency in the general population [2]. The percentage of newly listed African–American registrants is lower than their existing waiting list counterparts (30% vs. 35%), suggesting their slower rate of transplantation. In contrast, the percentage of newly-listed Caucasian registrants is higher than their existing waiting list counterparts (64% vs. 53%), reflecting their higher rate of transplantation [2]. Hispanics and Latinos account for approximately 17–18% of both listed registrants and new registrants [2]. A national registry study of non-elderly patients (18–64 years of age) initiating maintenance dialysis between 1995 and 2006 similarly demonstrated lower relative rates of deceased donor kidney transplantation among non-white compared with white patients [3]. The reduced rates of deceased donor transplantation among American Indians, Alaska Natives, and blacks reflected both lower rates of waitlisting and lower rates of transplantation among those waitlisted. However, among Asians, Pacific Islanders, and Hispanics, access to the waiting list was similar or better than for whites, but time to transplantation among those waitlisted was longer.

The OPTN/UNOS database revealed that the Kaplan–Meier median waiting times for white registrants listed between 1999 and 2004 were approximately 30–35% less than for blacks, Hispanics, Asians, and non-Hispanics, and approximately 15% less than for American Indians. Pacific Islanders had the longest waiting time among all ethnicities [1].

The discrepancies in the rates of transplantation and waiting times among different ethnicities are thought to be due to multiple factors, including personal and cultural beliefs in organ transplantation as well as organ donation, various frequencies of HLA mismatching, socioeconomic status and education levels, and variability in access to transplantation by ABO blood group and geographic location among others.

Highly sensitized transplant candidates

Approximately 40% of waitlisted transplant candidates in the US have high levels of preformed anti-HLA antibodies, also known as high panel reactive antibodies (PRAs). High PRA levels are generally thought to result from previous exposure to non-self HLA antigens, such as from pregnancies, blood transfusions, and/or prior failed transplants. The waiting times for a compatible cross-match among candidates with a high PRA may be considerably prolonged. The OPTN/UNOS database revealed that between 2001 and 2002, the Kaplan–Meier median waiting times for registered transplant candidates with PRA levels of 0–9%, 10–79%, and $\geq$80% were 1329 days ($\sim$3.6 years), 1920 days ($\sim$5.3 years), and 3649 days ($\sim$9.9 years), respectively [1]. Over the last half decade, various desensitization protocols (covered in depth in Chapter 68) have been developed to allow successful transplantation of highly sensitized patients, including intravenous immunoglobulins, plasma exchange or immunoadsorption, and rituximab (an anti CD-20 monoclonal antibody). More recently, bortezomib (a proteasome inhibitor) and eculizumab (an anticomplement C5 monoclonal antibody) have been introduced to various desensitization protocols. Currently, some centers offer desensitization programs to broadly sensitized candidates who are at the top of the deceased donor waiting list. Presently, at the University of California, Los Angeles, the top 30 candidates in each blood group are identified based on dialysis start date. Highly sensitized candidates whose identities appear on the match runs are seen in clinic and given the opportunity to participate in desensitization protocols. Generally, one patient in each blood group (except for the AB blood group) is being desensitized at the same time.

Effect of patient's location of residence on waiting time

The US is divided into 11 OPOs regions. Waiting times vary greatly by OPO region [4]. This has led some prospective transplant candidates to pursue listing at multiple centers (multilisting) served by different OPO agencies in an attempt to increase their likelihood of undergoing transplantation.

The UNOS registry data revealed that multiple listing provided an 88% greater rate of access to kidney transplantation, while reducing waiting times by nearly 50% (median waiting time: single- vs. multi-listed: 156 vs. 83 weeks, respectively, for candidates registered between January 7 1995 to 30 June 2000) [5]. Notably, multilisting resulted in a higher transplant rate in nearly all demographic, biological, and socioeconomic subgroups, including those known to have significantly less access to transplantation, such as

those of African–American ethnicity, female gender, and patients in the O and B blood groups. Multilisting did not appear to benefit patients who had health maintenance organization (HMO) coverage for their transplant. It is suggested that multilisting tends to reduce waiting time disparities among OPOs because secondary listing OPOs generally have shorter waiting times than primary listing OPOs. Nonetheless, it should be noted that while patients awaiting transplantation at centers with long waiting times might benefit from multilisting at centers with shorter average waiting times, secondary listing at transplant centers with large waiting lists was shown to be an expensive and futile process [6].

Specific options to reduce waiting time

Candidates with chronic hepatitis C

The number of listed hepatitis C antibody-positive transplant candidates who have consented to receive a hepatitis C donor kidney is generally small (<20 at the authors' institution at the time of this writing). The waiting time for a hepatitis C deceased donor kidney is relatively short (currently 2–3 years at our institution). Hence, HCV-consented patients should be followed closely to ensure that upon listing they are medically and psychosocially ready for transplantation at all times.

Public Health Service high-risk donor kidneys

The PHS has implemented specific criteria that determine a donor as high risk for transmission of viral disease, including human immunodeficiency virus (HIV) and hepatitis C. These risk factors, within 1 year of donation, include:

1. male homosexual contact;
2. non-medical intravenous, intramuscular, or subcutaneous use of drugs;
3. promiscuous sexual relations in exchange for money or drugs;
4. individuals who have had sexual contact in the preceding 12 months with any person described in 1 through 3 above *or* with known or suspected to have HIV infection through percutaneous inoculation or through contact with an open wound, non-intact skin or mucous membrane;
5. persons with known or suspected sexually transmitted disease;
6. inmates of correctional systems
7. persons who cannot be tested for HIV infection because of refusal, inadequate blood samples (e.g. hemodilution that could result in false-negative tests), or any other reasons;
8. persons with a repeatedly reactive screening assay for HIV 1 or HIV 2 antibody regardless of the results of supplemental assays;
9. persons whose history, physical examination, medical records, or autopsy reports reveal other evidence of HIV infection or high-risk behavior (e.g. diagnosis of AIDS, unexplained weight loss, night sweats, skin or mucous membrane lesions typical of Kaposi's sarcoma, unexplained lymphadenopathy lasting >1 month, unexplained fever >38.6 °C) for >10 days, unexplained persistent cough and shortness of breath, opportunistic infections, unexplained persistent diarrhea, or needle tracks or other signs of parenteral abuse).

The risk of transmission of viral disease from a PHS high-risk donor kidney is not known but has been estimated to range from 1 in 300 to 1 in 10 000. Nonetheless, in selected cases where there is anticipated prolonged waiting time or exhaustion of dialysis access sites, transplantation with a donor kidney of this category may confer a survival advantage over remaining on dialysis. Of note, a high-risk donor kidney as defined by the PHS does not include the risk of transmission of undetected malignancy from donor organ to recipient. The complete PHS guidelines can be found on http://www.publichealthreports.org/issuecontents.cfm?Volume=128&Issue=4.

Table 37.1. Factors that determine expanded criteria donors (as defined by UNOS)

Donor condition	Donor age categories (years)	
	50–59	≥60
CVA + HTN + creatinine >1.5 mg/dL	X	X
CVA + HTN	X	X
CVA + creatinine >1.5 mg/dL	X	X
HTN + creatinine >1.5 mg/dL	X	X
CVA		X
HTN		X
Creatinine >1.5 mg/dL		X
None of the above		X

X, expanded criteria donor; CVA, cerebrovascular accident was cause of death; HTN, history of hypertension at any time.
Adapted from UNOS.

Expanded criteria donor kidney

Currently, the waiting time for a deceased donor transplant is such that many waitlisted older transplant candidates die while awaiting transplantation from a standard deceased donor kidney. Furthermore, the duration of pretransplant dialysis has been shown to be associated with a significant and progressive increased risk of death-censored graft loss and risk for patient death after transplantation [7]. It is the practice at our center to offer the ECD program to all candidates aged 50 years or older. ECD kidneys are defined by donor characteristics that are associated with a 70% greater risk of kidney graft failure when compared to a reference group of non-hypertensive donors aged 10 through 39 years whose cause of death was not cerebrovascular accident (CVA) and whose terminal creatinine was ≤1.5 mg/dL. The donor factors associated with this increased relative rate of graft failure include age 60 years or older, or ages 50–59 years, with at least two co-morbid factors. The latter may include CVA as a cause of death, hypertension, and/or terminal creatinine >1.5 mg/dL (Table 37.1). Patients should be informed that candidates for ECD kidneys are simultaneously listed for a standard and ECD kidney. In selected cases, dual transplant of ECD kidneys has been offered to older recipients with excellent short- and intermediate-term allograft outcomes. Analysis of the OPTN/UNOS database consisting of 625 dual kidney transplants (DKT) and 7686 single ECD transplants demonstrated comparable 3-year overall graft survival between the two groups of transplant recipients (79.8% vs. 78.3%, respectively) [8]. It has been suggested that the salutary effect of DKT is due to greater viable nephron mass.

Donor after circulatory death kidney

One approach to expanding the pool of deceased donor organ supply has been to use organs from donors after circulatory death. A DCD kidney is by definition susceptible to variable degrees of warm ischemic damage and its use inevitably increases the incidence of post-transplantation delayed graft function (DGF) or primary non-function. The U.S. Renal Data System database demonstrated that recipients of DCD donor organs experienced nearly twice the incidence of DGF compared with heart-beating donors (42.3% vs. 23.3%, respectively). Nonetheless, DCD donor transplants experienced comparable allograft survival when compared with heart-beating deceased donor transplants at 6-year

follow-up (73.2% vs. 72.5%, respectively, P = NS) [9]. Interestingly, there was a trend for better patient survival at 6 years for DCD compared with heart-beating donor renal transplant recipients (80.9% vs. 77.8%, respectively, P = NS). Significant risk factors for allograft loss for DCD donor organ recipients include repeat transplant, DGF, donor age older than 35 years, and head trauma as a cause of initial injury. To optimize the utilization of DCD kidneys, machine perfusion parameters and viability testing have been used by a number of centers to assess the extent of kidney damage and to predict graft function. At the authors' institution, DCD kidneys are offered to preidentified and consented waitlisted candidates.

There should be a detailed discussion about the options of HCV, ECD, dual transplant of ECD kidneys, DCD, and PHS high-risk deceased donor kidneys with the potential transplant candidate at the time of initial evaluation and the options raised again during follow-up visits. The selection of these options is considered specifically in Chapter 21, 22, and 53 Living kidney donation should also be addressed (discussed in depth with regard to its conduct and ethics in Chapters 23 and 138, respectively).

Development of a new kidney allocation system: time on dialysis

At the National Conference on the Wait List for Kidney Transplantation held in Philadelphia in March 2002, a panel of experts consisting of representatives from nine US organizations proposed that time waiting for a transplant should be calculated from the point at which a patient begins maintenance dialysis rather than from completion of transplant evaluation [10]. The proposal was designed to eliminate the advantage of patients with early access to the list and provide equitable access to all suitable renal transplant candidates, particularly among ethnic minorities and economically disadvantaged patients. A pilot study to assess the impact of beginning the waiting time for a kidney transplant at the time a candidate first begins dialysis or meets a standard medical definition of kidney failure was first approved by the OPTN/UNOS Board of Directors in November 2003.

Early reports from two OPOs [One Legacy (CAOP) and Gift of Life (MIOP)] that adopted the dialysis waiting time allocation system demonstrated a trend towards an increase in the average number of years on dialysis and a decrease in the average number of years on the waiting list for patients receiving deceased donor transplants in the 18-month period after policy implementation. The apparent paradoxical increase in average number of years on dialysis prior to transplantation and a concomitant reduction in waiting time presumably reflects a higher number of transplantations among the high number of late referrals of long-time dialysis-dependent patients to transplant centers. This trend was not observed in the OPOs where this allocation policy was not implemented [11]. In the 18-month period prior to (on or before April 28 2004) or after the policy change (on or after April 29 2004), the average number of years on dialysis for deceased donor transplant recipients in the CAOP was 3.9 versus 4.8 years, respectively. In contrast, a decrease in the average number of years on the waiting list was observed after the policy change (2.3 years after vs. 2.7 years before, respectively). This increase in the average number of years on dialysis and decrease in the years on the waiting list was consistent across all ethnicities in the CAOP and MIOP (except for Hispanics and Asians in the MIOP). A slight increase in transplant rates for African–Americans was observed in some states but not in others. It is speculated that the effects may not yet be apparent in regions where waiting times may be substantial and longer follow-up is needed. Overall, there appeared to be a decrease in the average number of years on the waiting list across all ethnicities.

Based on preliminary reports, the OPTN Kidney Transplantation Committee has recently recommended that time on dialysis should be incorporated into the national allocation policy. Dialysis waiting time and an estimated glomerular filtration rate (eGFR) of $<$20 cc/min will be implemented in the national allocation algorithm in 2014.

Maintaining and monitoring waitlisted patients

The allocation policies require that all status 1-listed patients should be ready for transplantation at all times. However, guidelines for maintaining and monitoring the health of active waitlisted patients are lacking. The health status of patients, particularly cardiovascular status, may deteriorate with time spent on dialysis. The following section discusses suggested guidelines for managing waitlisted patients, including frequency of follow-up visits, storage sera for pretransplant cross-match, and general health maintenance. The latter includes cardiovascular and valvular heart disease, malignancy, and infectious disease screening. It should be noted that practices vary widely among transplant centers.

Follow-up frequency

With the ever growing number of patients placed on the waiting list and the disparity between donor organ supply and demand, regular or annual follow-up of all waitlisted transplant candidates has become impractical, particularly for large volume transplant centers where the number of waitlisted patients generally exceeds 1500/year. Hence, formulating an algorithm to update patient data remains a challenge for transplant physicians, coordinators, and social workers, particularly in regions where waiting times regularly exceed 6–7 years.

At the University Of California, Los Angeles, over the last half decade, $>$1500–2000 patients were on the waiting list each year. It is our current practice to see the top 30 patients in each ABO blood group and those whose identities appear on the "match run" annually to reassess their overall health and any demographic issues. In the US the match run is done via a UNetSM centralized computer network whereby medical information about the deceased donor is matched against transplant candidates. This match run automatically creates a ranked list of suitable candidates for each organ based on predefined factors, including tissue match between donor and transplant candidates, ABO blood type, PRA levels, time spent on the waiting list, pediatric candidates, and geographic distance between the potential recipient and the donor.

Sera storage for pretransplant cross-match

When sera from waitlisted patients are collected at a predetermined interval and are available in the HLA laboratory, a final cross-match can usually be performed without obtaining a fresh sample from the patient. At the authors' institution, sera from non-sensitized transplant candidates who are in the top 25% of the list are obtained quarterly, whereas for the remaining non-sensitized patients sera can be obtained semi-annually. For highly sensitized patients, screening tray sets should be prepared monthly. A preliminary cross-match is performed by testing donor cells on the appropriate tray set at the time of donor HLA typing.

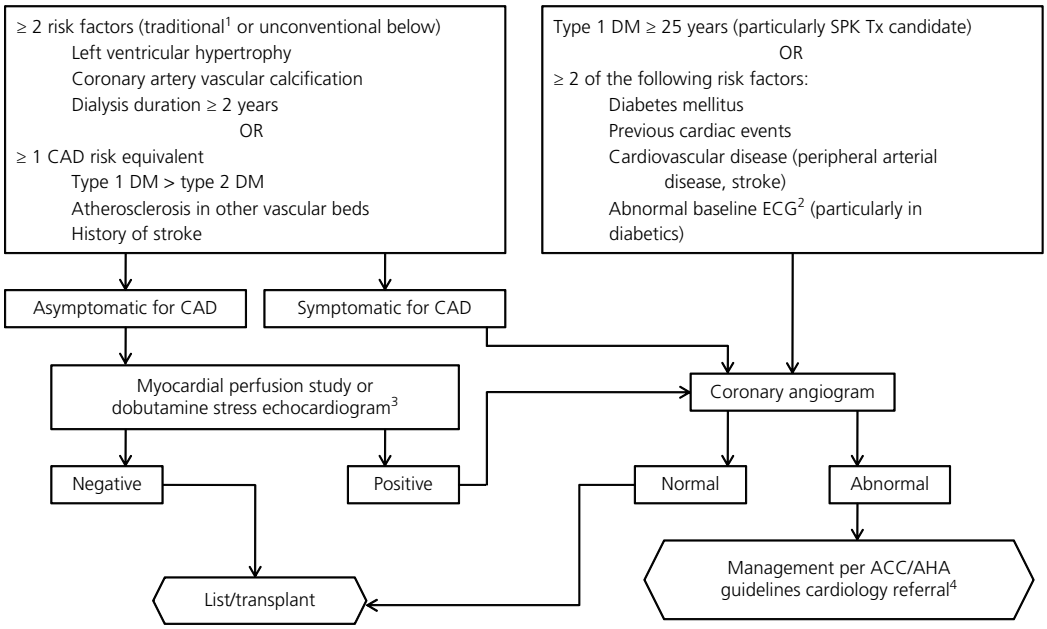

Figure 37.2. Proposed algorithm for pretransplant cardiac evaluation. (Reproduced from Pham et al. [19] with permission from John Wiley and Sons Ltd.)

1 Refer to Table 37.2 footnote.

2 ECG findings suggestive of a previous myocardial infarction, ST–T wave changes.

3 Center specific.

4 If necessary, PCI or CABG and cardiac rehabilitation should be performed prior to listing or transplantation.

DM, diabetes mellitus; SPK, simultaneous pancreas–kidney; Tx, transplant; ECG, electrocardiogram; CAD, coronary artery disease; ACC/AHA, American College of Cardiology/American Heart Association; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft.

Health maintenance

Cardiovascular and valvular heart disease

Cardiovascular screening

Cardiovascular screening is considered by most transplant centers as an essential component of the transplant evaluation process because of the high prevalence of clinically silent coronary heart disease among end-stage kidney disease (ESKD) patients. Furthermore, cardiovascular disease (CVD) is the leading cause of death after renal transplantation. Deaths with a functioning graft occurring within 30 days after transplantation are due to ischemic heart disease in nearly half of the cases. A detailed cardiovascular history not only predicts the operative risk but also helps in postoperative cardiac management to improve short- and long-term cardiac outcomes. However, controversies exist regarding the best strategy for pretransplant assessment and management of coronary artery disease (CAD) to prevent adverse perioperative cardiac events. Furthermore, there has been no consensus on the selection of candidates who should undergo repeated testing or the determination of the type or frequency of testing during the prolonged waiting period. The American Society of Transplantation recommends a cardiac stress test in high-risk renal transplant candidates, defined as patients with diabetes, prior history of ischemic heart disease, abnormal electrocardiogram (ECG), or age of 50 years or older. Coronary angiography should be considered only in patients with a positive stress test [12]. However, over the years this strategy has been challenged due to the now recognized reduced sensitivity and specificity of non-invasive testing in renal transplant patients compared with those of the general population. Abnormalities on myocardial perfusion study have been suggested to correlate well with the presence of CAD in the general population with mean weighted sensitivity of 88% and specificity of 74% [13], whereas in ESKD patients, sensitivities and specificities ranging from 37% to 90% and 40% to 90%, respectively, have been reported [14]. A number of transplant centers recommend direct coronary angiography in high-risk patients.

The authors' proposed algorithm for initial pretransplant cardiac evaluation and continued cardiac surveillance for waitlisted patients are shown in Figure 37.2 and Table 37.2, respectively. The authors' suggested guidelines were based in part on Lee' s cardiac risk assessment model in the general population [15], the 2007 American College of Cardiology and American Heart Association (ACC/AHA) surgical risk stratification [16], the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATPIII) guidelines [17], and the K/DOQI Clinical Practice Guidelines for Cardiovascular Disease in Dialysis Patients [18]. A detailed discussion of cardiac risk assessment in renal transplant candidates is beyond the scope of this chapter. Interested readers are referred to Pham et al. [19].

Valvular heart disease

Patients with ESKD on maintenance dialysis commonly develop valvular heart disease (VHD) as a result of secondary hyperparathyroidism, elevated calcium–phosphorus product, hypercalcemia, and hyperphosphatemia. Other suggested risk factors include hypertension, diabetes mellitus, hyperlipidemia, left ventricular

Table 37.2. Suggested cardiac surveillance for waitlisted transplant candidates

No known CAD or initial evaluation negative	
(a) Diabetic ESRD	Annually
(b) Non-diabetic + any of the following: ≥2 traditional[1] or unconventional[2] risk factors or ≥1 CAD risk equivalents[3]	Biannually
(c) "Lower risk"[4]	Every 3 years
Established CAD	
Medical management per ACC/AHA guidelines	Annually
Successful prior PCI	Annually
History of successful CABG	3 years post CABG then annually
Asymptomatic aortic stenosis[5]	
Mild	Echocardiogram every 3–5 years
Moderate[6]	Echocardiogram annually

[1] *Traditional risk factors:* age >45 years in men and >55 years in women, diabetes mellitus, hypertension, dyslipidemia, obesity, history of angina pectoris, congestive heart failure, previous cardiac events, smoking, family history.
[2] *Unconventional risk factors:* left ventricular hypertrophy (LVH), coronary artery vascular calcification, dialysis duration ≥2 years.
[3] *CAD risk equivalent:* type 1 diabetes mellitus (DM) > type 2 DM, atherosclerosis in other vascular beds, history of stroke.
[4] *Lower risk:* defined as not meeting criteria (a) or (b) above.
[5] Clinical evaluation annually.
[6] Cardiology consultation advisable.
ESRD, end-stage renal disease; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; ACC, American College of Cardiology; AHA, American Heart Association.

Table 37.3. Categorization of cancers in the end-stage kidney disease (ESKD) population

ESKD related
- Kidney
- Urinary tract
- Thyroid
- Myeloma

Immune deficiency related
- Hodgkin's lymphoma
- Non-Hodgkin's lymphoma
- Leukemia
- Melanoma of skin
- Kaposi sarcoma
- Carcinoma of:
  - Lip
  - Mouth, tongue, tonsil, oropharynx
  - Esophagus
  - Stomach
  - Anus
  - Liver
  - Larynx
  - Lung
  - Cervix, uteri, vagina, vulva
  - Penis
  - Eye, squamous cell carcinoma only

Not related to immune deficiency
- Rectum
- Breast
- Ovary
- Prostate

Of uncertain status
- All other cancers

Data from Steward et al. [22].

hypertrophy, uremic milieu, and anemia among others [20]. The most common abnormalities include valvular and annular thickening, and calcification of any heart valves resulting in regurgitation and stenosis. Aortic and mitral valve thickening or sclerosis occur in 55–69% and 40–60% of patients, respectively [20]. Valvular sclerosis was previously thought to have no clinical significance, but has now been recognized as a potential cause for progressive stenosis and cardiovascular mortality. Mitral annular calcification occurs in 10–50% of patients, and aortic valve calcification in 25–55% of hemodialysis patients. Calcifications of the tricuspid and pulmonic valves are rare [20]. Currently, the K/DOQI Clinical Practice Guidelines for Cardiovascular Disease in Dialysis Patients [18] recommend following the ACC/AHA guidelines to evaluate dialysis patients for the presence of VHD. Similarly, follow-up of VHD in dialysis patients should follow the guidelines for the general population, except for the frequency of follow-up for aortic stenosis (discussed further below) [18].

The optimal frequency of visits by patients with VHD has not been clearly defined, but most clinicians advocate annual history and physical examination in asymptomatic patients. Additionally, the ACC/AHA guidelines recommend an annual echocardiogram in patients with asymptomatic severe aortic stenosis (AS), every 1–2 years in those with moderate AS, and every 3–5 years in those with mild AS (class I). However, an echocardiogram should be performed annually in asymptomatic waitlisted transplant candidates with moderate or more severe AS (or in any other dialysis patients who are suitable candidates for aortic valve replacement). It has been suggested that AS progresses faster in dialysis patients than in the general population. An echocardiogram should also be performed in any patients with changing symptoms (class I). Asymptomatic patients may be considered for exercise testing to elicit symptoms and to evaluate hemodynamic responses if their clinical status is equivocal due to sedentary lifestyles (class IIb) [21]. Similar guidelines are generally recommended for waitlisted dialysis patients. Of note, the K/DOQI Clinical Practice Guidelines recommend optimization of dry weight prior to testing [18]. No specific recommendations were made for other VHDs.

Malignancy screening

Studies in ESKD patients treated by dialysis or transplantation, and in patients with HIV/AIDS, suggest that cancers can be categorized into ESKD-related, immune deficiency-related, not related to immune deficiency, or of uncertain status (Table 37.3) [22]. ESKD-related cancers include kidney, urinary tract, thyroid, and multiple myeloma. Although there has been no consensus on the type and frequency of malignancy screening in waitlisted renal transplant candidates, all patients should adhere to standard age-appropriate cancer surveillance. Suggested guidelines for cancer screening are shown in Table 37.4. In addition, screening for malignancy in adult kidney transplant candidates should focus on the kidney and urinary tract, particularly in dialysis-dependent ESKD patients (discussed further below). Serum immunofixation electrophoresis (IFE) should be performed in all transplant candidates older than 60 years of age. Chronic hepatitis B virus- and C virus-infected individuals should be screened for liver cancer. Although thyroid carcinoma has been observed at increased frequency in dialysis patients compared with the general population, thyroid ultrasound is not part of routine pretransplant screening. It has been suggested that regular thyroid ultrasound is justified in dialysis patients, although there have been no studies to confirm or refute this recommendation [23]. Hence, screening prospective renal transplant candidates for thyroid cancer should be done at the discretion of the clinicians.

Table 37.4. Preventive care recommendations for cancer surveillance in waitlisted transplant candidates

Screening for	Starting at age (years)	Early detection and preventive care	Screening frequency
Colorectal cancer	Average risk: 50	Early detection and prevention (preferred)	Colonoscopy: every 10 years, or Flex sig every 5 years, or CT colonography every 5 years
		Early detection only	FOBT annually, or Fecal immunochemical test annually
	Increased risk: 40	Colonoscopy	Every 5 years if a first-degree relative had colorectal cancer at <60 years of age @ 10 years younger than the youngest family member with diagnosis of colorectal cancer Every 10 years if the relative was 60 years of age with diagnosis of colorectal cancer Consider referral to medical genetics if two or more first-degree relatives had colorectal cancer
Females			
Breast cancer	50–75	Breast exam and screening mammography	Every 1 or 2 years
	40–49	Breast exam and screening mammography	Every 1 or 2 years (no evidence for or against for this age group)
	Before age 30 (if mother or sister had breast cancer)		
Cervical cancer[1]	3 years after onset of sexual intercourse or by age 21 whichever comes first	Pap smear and pelvic exam	Annual for conventional cytology Every 2 years for liquid-based cytology
Males			
Prostate cancer[2]	50	PSA testing (with or without digital rectal exam)	Annual if @ screening PSA is >2.5 ng/mL Every 2 years if @ screening PSA is <2.5 ng/mL
	40–45[3]	PSA testing (with or without digital rectal exam)	

[1] For age >30 years, every 2 years after three normal consecutive smears and no increased risk.
[2] Recommended by the American Cancer Society.
[3] African–Americans, relative with prostate cancer <65 years of age, or men with *BRCA1* and/or *BRCA2* mutation.
Flex sig, flexible sigmoidoscopy; FOBT, fecal occult blood testing; PSA, prostate-specific antigen.

Acquired cystic kidney disease and renal cell carcinoma screening

Acquired cystic kidney disease (ACKD) is defined as having more than three to five macroscopic cysts in each kidney in a patient who does not have a hereditary cause of cystic disease. The potential for malignant transformation of ACKD has a reported incidence of <1–7% among ACKD patients or up to a 40-fold increased risk of renal cell carcinoma (RCC) in ACKD patients compared with the general population. RCC accounts for approximately 80% of renal cell neoplasms observed in uremic patients, of which nearly 85% are asymptomatic [23]. Suggested risk factors include male gender (male-to-female ratio, 7:1), African–American ethnicity, dialysis duration, and severe ACKD with marked organ enlargement [23].

There has been no consensus on the frequency of screening for renal neoplasms in waitlisted patients. However, all suitable renal transplant candidates should have a baseline renal ultrasound. The frequency of screening should follow the guidelines set forth for dialysis patients. If there is no evidence of ACKD at initial screening, repeat ultrasound can be done annually or biannually [23]. Annual screening in patients who have been on dialysis for 3–5 years has been advocated [24]. The presence of benign cysts (Bosniak I and II) requires follow-up ultrasound every 6 or 12 months and additional contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) for progressive lesions. In those with moderately complex cysts (Bosniak IIF), follow-up ultrasound every 3 months and contrast-enhanced CT or MRI every 12 months is advisable. The presence of an indeterminate cystic mass (Bosniak III) or clearly malignant cystic mass (Bosniak IV) mandates further evaluation with contrast-enhanced CT or MRI and urology referral [23]. RCC may require tumor-free waiting periods prior to transplantation depending on the stage or extent of the disease (see Chapter 28). A suggested screening algorithm for ACKD and renal neoplasms is shown in Figure 37.3.

It is noteworthy that native renal cysts (NRCs) and dialysis duration have also been suggested to be risk factors for RCC post transplant [25]. In a single-center study consisting of 1036 renal transplant recipients who underwent routine ultrasound screening of their native and transplant kidneys (within 1 month post transplant, then every 5 years for those without cysts, and every 2 years for those with cysts), RCCs were diagnosed in ten asymptomatic transplant recipients at a median of 5.8 years post transplant. Based on the presence and timing of NRCs, transplant recipients were grouped into those with no NRCs (No-NRC, n = 392), new NRCs (New-NRC, n = 166), pre-existing NRCs (Pre-NRC, n = 274), and time-indeterminate NRCs (TI-NRC, n = 204). During the study period, RCC developed in ten Pre- and TI-NRC patients who had significantly longer dialysis duration than the No-NRC or New-NRC groups (6.7 ± 3.9 and 3.3 ± 3.2 years vs. 2.7 ± 3.1 and 2.6 ± 2.4 years, respectively). None of the patients from the No-NRC or New-NRC groups developed RCC. The results of the study suggest that NRC and increased dialysis duration are risk factors for RCC post transplant [25].

Screening for infectious diseases

Although practice differs among transplant centers, generally accepted standard screening includes hepatitis serologies (hepatitis B surface antigen, hepatitis B core antibody IgM and IgG, hepatitis B surface antibody, and hepatitis C antibody), HIV 1 and 2 antibody, cytomegalovirus IgG antibody, rapid plasma reagin (RPR) or *Treponema pallidum* particle agglutination (TP-PA) test for syphilis, Epstein–Barr virus (EBV) antibody panel, herpes simplex virus (HSV) and varicella-zoster virus (VZV) IgG antibodies, and PPD

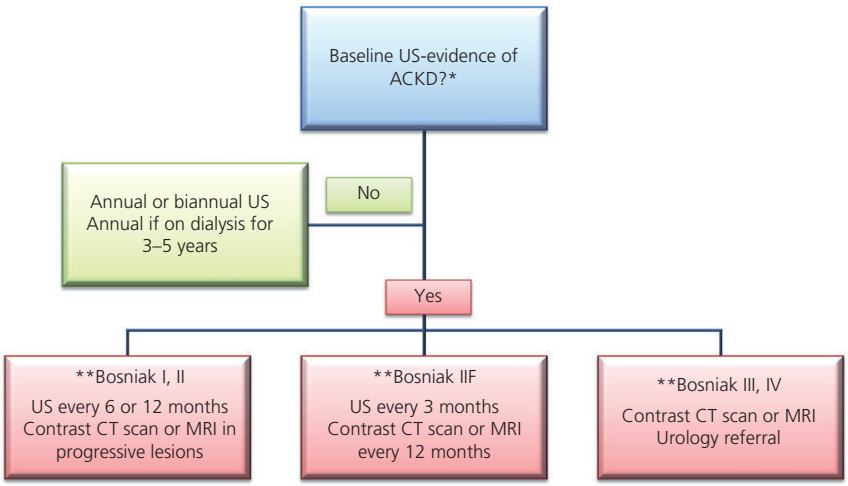

Figure 37.3. Suggested screening algorithm for acquired cystic kidney disease (ACKD) and renal neoplasms.
*ACKD: >3–5 cysts in each kidney.
**Bosniak I, II, benign cysts; Bosniak IIF, moderately complex cysts; Bosniak III, indeterminate cystic mass; Bosniak IV, clearly malignant cystic mass.
US, ultrasound.

Table 37.5. Infectious disease surveillance for waitlisted transplant candidates

Human immunodeficiency virus (HIV)	Annual screening for high-risk individuals
Hepatitis C virus (HCV)	Annual screening for ELISA-negative patients Consider PCR testing for ELISA-negative patients with abnormal transaminase levels HCV-positive patients require ongoing care, histologically advanced disease may preclude transplantation
Hepatitis B virus (HBV)	Previously unexposed patients should undergo immunization, with annual testing of antibody levels and appropriate booster doses if indicated
Tuberculosis*	PPD-positive patients should receive standard chemoprophylaxis and annual chest X-ray
Strongyloides	Annual screening in endemic areas
Epstein–Barr virus, toxoplasmosis, cytomegalovirus, herpes simplex	Annual screening not indicated

*In recent years, the PPD skin test has gradually been replaced by the QuantiFeron TB Gold test. The latter is useful in BCG-vaccinated patients to distinguish between BCG versus TB reaction on PPD test.

skin test or quantiFeron TB Gold test. In recent years, the traditional PPD skin test has gradually been replaced by the QuantiFeron TB Gold test. The latter is useful in BCG-vaccinated patients to distinguish between BCG reaction versus TB reaction on PPD test. All prospective transplant candidates are required to undergo testing at the time of initial transplant evaluation. Special serologic testing based on epidemiologic risks or exposure history include coccidioides IgM and IgG antibody, histoplasma immunodiffusion antibody or urine antigen, human T-cell lymphotrophic virus (HTLV-I/II) antibody, *Strongyloides* antibody, and *Trypanosoma cruzi* antibody [26].

Repeated serologic testing for hepatitis and HIV while awaiting a transplant are generally performed at the discretion of the referring nephrologists. At a National Consensus Conference on the management of waitlisted patients held in Philadelphia in 2002, a panel of experts set forth guidelines for periodic surveillance of certain infectious diseases among waitlisted patients (Table 37.5) [27].

Listing status awareness

A single-center survey study using the Dialysis Patient Transplantation Questionnaire (DPTQ) revealed that >50% of patients (18 of 34) who were undergoing pretransplantation work-up were unaware of their true listing status [28]. Of these patients, 88.9% (16 of 18) mistakenly thought they were listed and 11% were unsure of their listing status. Among 32 waitlisted patients, 81.3% correctly identified themselves as listed, 6.2% mistakenly thought they were not listed, and 12.5% were unsure. Notably, all of the waitlisted patients who were not aware of their listing status were status 7, indicating that they required additional testing or developed intercurrent illnesses. Nearly two-thirds of the surveyed patients had completed high school and 11% had some level of college education. Although the study population consisted predominantly of black/African–Americans (82.8%) and the results have yet to be validated in patients of other ethnic backgrounds, the finding of a substantial lack of listing status awareness among mostly high-school graduates merits attention because it suggests a lack of communication between the transplant program, patients, and referring nephrologists. It has been proposed that collecting self-reported data using the DPTQ and comparing self-reported listing status with the UNOS listing database is a quick and inexpensive screen for a correctable problem.

At the authors' institution, all listed transplant candidates are informed of their UNOS waiting list status via mail annually. Patients are advised to notify their assigned transplant coordinators (or other transplant team members) if there has been a change in demographic issues or health status, such as relocation, transfer to another dialysis center, change in contact numbers, intercurrent illnesses, or hospitalization. It is also our policy to send a list of all waitlisted transplant candidates and their current status to their corresponding dialysis unit social workers quarterly.

Management of the deceased donor waiting list: national survey

A national survey consisting of 192 UNOS-certified programs in the US revealed that 71% of programs advocate routine follow-up monitoring of all waitlisted transplant candidates via either scheduled appointments or program-initiated telephone contacts [27]. The frequency of follow-up and the requirement for adherence to

Table 37.6. General guidelines for the management of waitlisted transplant candidates

1 Each transplant program should uphold the following responsibilities:
- **(a)** Develop a well-structured system to maintain ongoing contact with its waitlisted patients
- **(b)** Develop clear lines of communication with the referring nephrologists and dialysis staff members [28]
- **(c)** Define clear expectations to patients, their nephrologists, and dialysis staff members (e.g. keeping transplant programs informed of any medical or psychosocial issues in a timely manner, ensuring that patients' blood samples are sent to the HLA laboratory at a predetermined time interval. This will allow the HLA laboratory to perform a final cross-match without obtaining a fresh sample from the patient when a kidney becomes available)
- **(d)** Closely monitor patients at high risk for cardiac events. A proposed algorithm for continued cardiac surveillance for waitlisted patients is shown in Table 37.2 [19]
- **(e)** If feasible, designate cardiologist(s) to assist in the care plan of waitlisted candidates
- **(f)** Develop standardized criteria for placing patients on hold or removing them from the waitlist to avoid allocation of kidneys to patients who are not medically ready for transplantation
- **(g)** Conduct regular multidisciplinary team meetings to address patients' active medical, surgical, and psychosocial issues relevant to their transplant candidacy. Based on the consensus decision-making, patients can be placed into one of the following categories: *List*; *Hold*; *Deferred*; *Delist*; or *Activate*
- **(h)** Identify the top 10–20 candidates in each ABO blood group based on "match run" and reassess their overall health and demographic issues to ensure their readiness for transplantation at all times
- **(i)** Develop a system to inform all listed transplant candidates of their UNOS waitlist status annually (or at regular intervals established by individual transplant programs)

2 Other aspects of the pretransplantation process should also be considered:
- **(a)** The cost implications, and medical, nursing, and administrative requirements for repeated patient evaluations and cardiac testing should be taken into account by government agencies, third-party insurers, and hospital administrations [28]
- **(b)** Meticulous attention should be paid to the health of waitlisted transplant candidates, particularly with respect to their cardiac risks, to optimize the eventual benefits and survival advantage of transplantation over dialysis [28]
- **(c)** The options of expanded criteria donor (ECD), dual transplant of ECD kidneys, and PHS high-risk deceased donor kidneys should be discussed with patients at the time of initial evaluation, and options should be reiterated during follow-up visits to facilitate the prompt and appropriate placement of these organs to preconsented patients
- **(d)** More frequent follow-up of high cardiac risk candidates and those awaiting pancreas transplants should be considered

standard cancer prevention screening (breast, cervical, and colon) are affected in part by transplant program size. Smaller programs are more likely to conform to the standard cancer screening recommendations compared with larger programs (72% of programs with $<$500 patients compared with 54% of programs with $>$500 patients; $P<0.05$). Periodic serologic testing for viral hepatitis is required by 80% of the programs, and screening for other infectious diseases is required by 52%. Regular prostate-specific antigen testing is required by 79% of programs. Asymptomatic high cardiac risk candidates, defined as those with established CAD, diabetes mellitus, advanced age, or obesity are required to undergo annual cardiac screening. Most programs performed non-invasive cardiac testing and $>$50% of transplant programs have a designated cardiologist or group of cardiologists.

Although regular follow-up and continuous updating of patients' medical, psychosocial, and demographic issues impose an enormous workload to transplant programs, the lack of such monitoring can increase the likelihood that a patient cannot be located because of demographic changes, frequency of cancellation of the transplant surgical procedure because of unanticipated or unrecognized new medical problems, and perioperative morbidity and mortality if such problems are overlooked. The frequency of the designated recipient being sent home because of unexpected medical problems was lower in smaller compared with larger programs. Such events affected $<$2% of the intended recipients in 61% of programs (80% of small programs; $P<0.001$ compared with larger programs). Seven programs indicated that $>$10% of patients were sent home because of unrecognized and/or unforeseen medical issues.

With the ever-growing number of patients placed on the deceased donor waitlist in general, and of older transplant candidates in particular, the management of waitlisted transplant candidates has become increasingly challenging for transplant programs. During the prolonged waiting period, patient's health and functional status may deteriorate or new medical or psychosocial issues may arise. These changes may render the patient unsuitable for transplantation or require additional diagnostic studies and/or management. While there have been no specific recommendations on how individuals programs should address these problems, general guidelines can be formulated and are outlined in Table 37.6.

Waiting list removals

Waiting list removals upon death, transplant, development of irremediable contraindications to transplantation, or removals for any other reasons should be reported to the OPTN within 24 h of removal.

Future studies

The need for the following future studies can be identified:

- The definition of "high risk" for cardiac events must be carefully studied and validated in prospective trials to avoid the increased cost and inconvenience of repeated testing [28].
- Prospective trials should be planned and implemented to assess the usefulness of non-invasive cardiac imaging for waitlisted patients and to determine the optimal and minimal requirements for imaging repetition [28].
- Randomized controlled trials to compare the effects of routine screening versus no screening on major adverse cardiac events are needed [29].

Summary

Waiting list management is an active process that entails ongoing communication between the dialysis units, patients, transplant coordinators, and transplant programs. Most transplant programs attempt to see transplant candidates on an annual basis. However, with the ever-growing number of patients placed on the waiting list, regular or annual follow-up of all waitlisted transplant candidates has become impractical. Knowledge of the factors that may affect waiting time may allow clinicians and transplant coordinators to formulate practical steps to ensure that patients who are at the top of the waiting list are suitable for transplantation at all times. At the

University of California, Los Angeles, it is our current practice to see the top 30 patients in each ABO blood group and those whose identities appear on the "match run" annually to reassess their overall health and demographic issues. During the follow-up visit, routine health maintenance status and cancer screening appropriate for age and gender, such as prostate-specific antigen, mammography, Pap smear, and colonoscopy are also reviewed. Although recommendations for cardiac surveillance of waitlisted patients vary among transplant centers, most advocate annual cardiac screening in diabetic transplant candidates.

In addition to reassessing patients' medical and psychosocial status, the availability of living donors should be readdressed. Currently, in an effort to maximize the utilization of living kidney donors, our program has implemented an algorithm to evaluate cross-match–positive and ABO-incompatible donor–recipient pairs. Patients are advised of living donor options, including paired exchange transplantation, positive cross-match and ABO incompatible transplantation through desensitization protocols, and living donor kidney exchange for both ABO-incompatible and cross-match–positive donor–recipient combinations. For older transplant candidates, the advantages and disadvantages of ECD kidney transplantation should be addressed.

Finally, effective communication between patients' primary nephrologists and transplant centers is invaluable in permitting waitlisted transplant candidates to be at their optimal medical health when a deceased donor kidney becomes available. Although practice guidelines vary among transplant centers, waiting list management will also need to be attuned to the changing kidney allocation system. Incorporation of dialysis waiting time and eGFR of $\leq$20 cc/min into the allocation system allows transplant centers to better predict which candidates are most likely to be offered an organ within a defined period of time. Hence, transplant centers should ensure that unsensitized patients in each blood group who have the longest waiting time are medically and psychosocially suitable for transplantation at all times.

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CHAPTER 38

Waiting List Management for Liver Transplantation

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Introduction

Patients placed on the waiting list for liver transplantation have by implication a condition that will require active management up to the point the transplant actually occurs. The profile of patients on the waiting list can be broadly divided into four categories: chronic liver disease, hepatocellular carcinoma, acute liver failure, and a group of miscellaneous conditions. The majority of patients have chronic liver disease and some of the complications of liver failure that indicated listing for liver transplantation. Hepatocellular carcinoma (HCC) can complicate cirrhosis in the full spectrum of its manifestations, ranging from fully compensated disease to a degree of liver failure that independently warrants liver transplantation. This has implications for how the malignant component of the disease is managed whilst awaiting transplantation. Some other indications for transplantation are also independent of the severity of the cirrhosis, e.g. hepatopulmonary syndrome (HPS) which correlates more with the severity of portal hypertension than the associated cirrhosis. Acute liver failure is managed by emergency transplantation and, while the time spent on the waiting list is measured in days, this is a period of intensive clinical activity.

Occasionally patients listed for transplantation recompensate to an extent that it is possible to remove them from the waiting list (if minimal listing criteria apply) or they do not accrue sufficient model for end-stage liver disease (MELD) points to be allocated an organ. This scenario is most typically observed in patients with alcohol or chronic viral hepatitis as the underlying aetiologies and is mediated by ongoing abstention from alcohol and response to antiviral therapy, respectively. However, the more typical clinical course is of deterioration and about 20–30% of patients placed on the waiting list with chronic liver disease do not receive transplants either because they die, become too ill to transplant, or have tumour growth beyond transplant criteria. These failings to maintain the patient in a condition suitable for transplantation illustrate the scale of the challenges in managing patients on the transplant waiting list. Similarly, a proportion of patients with acute liver failure deteriorate to a degree that they do not receive transplants or will survive without undergoing transplantation.

This review of the management of liver disease is not intended to be comprehensive in terms of treatment protocols and options. Instead, the emphasis is on how standard approaches to care are modified or applied in the context of the expectation that the patient will proceed to liver transplantation. It serves as a companion to Chapter 29, which covers indications for liver transplantation, and also to Chapter 43, which details the intensive care management of individuals with hepatic failure.

Waiting list

In the US, the waiting list-to-transplant ratio is in the order of 2.5 : 1, while the figure in the UK is around 1.5 : 1. The most complete data regarding the composition of a waiting list come from the US. Scientific Registry of Transplant Recipients (SRTR) data for the period 1997–2006 show that at the end of that period there were about 12 000 registrants, with 72% aged over 50 years, 72% white, and 61% male [1]. All but 3% were awaiting their first transplant. The blood group distribution was 50% O, 38% A, 11% B, and 2% AB. At the end of 2006, 64% had been waiting >1 year and 47% >2 years, but the median time to transplantation was 306 days. Mortality rates, which were steadily declining, ranged from 9% to 14% depending on ethnicity. Mortality rates for different clinical situations are shown in Table 38.1. Almost half of patients with MELD scores of >30 were transplanted within 30 days bur one-fifth died within that period. Lower waiting list mortality rates have been achieved in some areas, notably parts of Spain and France with rates below 5% [2].

Factors influencing waiting times

The considerable variation in the length of time patients wait for transplantation depends on a number of issues (Table 38.2). MELD-based and related allocation systems promote the concept of the sickest patients taking priority irrespective of the length of time spent on the waiting list. In the US system, the only role for the time spent waiting is as a tiebreaker for patients with identical MELD scores. However, about 25% of patients listed for transplantation will have low and stable MELD scores, including patients with HCC or variant indications (e.g. HPS, familial amyloid polyneuropathy) and additional points need to be allocated if they are to receive transplants. There are numerous approaches to this, but one of the more sophisticated is the French individualized system that adds priority points related to 13 clinical situations [2]. The waiting time may also be influenced by the degree to which a favourable donor–recipient match is needed. This may be considered formally by using the donor-risk index (DRI) or less formally by aiming to avoid unfavourable pairings, e.g. the use of a steatotic liver from an elderly donor in a patient with hepatitis C virus (HCV)-related disease.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 38.1. Waiting list mortality by clinical circumstance – SRTR data 2006

Circumstance	Mortality
Overall	11.7%
MELD 6–10	3.4%
MELD 21–30	6.5%
MELD >30	36.7%
Acute liver failure	18.7%
Metabolic disorders	14.3%
Malignant disease	12.3%
Cirrhosis (excluding cholestatic disorders)	10.9%
Cholestatic disorders	7.7%
Exceptions for hepatocellular carcinoma	11%
Other exceptions	15.0%

Table 38.2. Factors that influence waiting times and organ allocation

Parameter	Comment
Primary determinants	
"Raw" MELD score	Most widely used determinant of need
Adjusted MELD score	Compensates for patients whose need is not reflected by "raw" MELD
Time spent on waiting list	Significant factor in the UK and some parts of Europe
Secondary determinants	
Blood group	Blood group O patients tend to wait longer, effect on other groups vary with ethnicity
Donor-risk index (DRI)	Objective to find favourable match to optimize outcome
Degree of steatosis	Same concept as DRI
Size	Problem for small adults who compete with paediatric patients
Donor age	May influence allocation in patients with hepatitis C virus (HCV), although practice tends to reflect aspiration to reduce impact of disease recurrence
Viral history	Donors with evidence of viral exposure to HBV, HCV or HIV may be matched to patients with respective viruses
Little or no impact	
Histocompatability	Rarely used in liver allocation
Donor age	In absence of concerns about disease recurrence

Cirrhosis and related complications

Routine monitoring

The frequency of clinical and laboratory monitoring will reflect the severity of the liver disease and the relative stability or otherwise of the patient. Outpatient monitoring at intervals of 1–2 months is fairly typical for this population. Organ allocation systems that are based on MELD or related systems may mandate how often the laboratory results are updated. Monitoring is aimed at documenting change in the severity of the liver disease as well in electrolyte profiles or renal function that may be amenable to therapy.

Surveillance programmes for portal hypertension and HCC are standard practice for patients with cirrhosis. The details of the surveillance protocols may vary between institutions or countries, but these should be maintained after patients are placed on the waiting list for liver transplantation. Ultrasound of the liver at 4–6-month intervals addresses two components of the surveillance requirement as it typically is part of the screening process for HCC (frequently combined with alpha-fetoprotein estimations) and also assesses portal vein patency. A de-novo HCC may be detected on ultrasound, which will require staging using local protocol, most often with computed tomography (CT) and/or magnetic resonance imaging (MRI). A small proportion of these HCCs are found to be beyond transplant criteria and such patients should be removed from the waiting list. More typically, new diagnoses of HCC trigger consideration of bridging strategies while the patient remains active on the waiting list.

The development of portal vein thrombosis is relevant to the transplant surgeon as very extensive thrombosis may alter a patient's suitability for liver transplantation. Although anticoagulation is not standard management for portal vein thrombosis complicating cirrhosis, its role needs to be assessed on an individual basis in candidates for liver transplantation. The potential benefit of preventing extension of the thrombus and the impact this might have on the complexity of surgery are considered against the patient's risk of bleeding. New portal vein thrombosis results in a sudden increase in portal pressure, which could significantly alter the risk of variceal bleeding. Endoscopic re-evaluation of oesophageal and gastric varices is, therefore, indicated.

Table 38.3. Management of ascites

Intervention	Comment
Restrict salt intake	Do not compromise nutrition
Restrict fluid intake	Prioritize maintaining serum sodium >130 mmol/L
Diuretics	Titrate to safety rather than efficacy when these conflict
Paracentesis	Albumin replacement probably also beneficial
Transvenous intrahepatic portovenous shunt (TIPS)	Avoid if possible
Antibiotics	Consider prophylaxis against spontaneous bacterial peritonitis

Ascites

Intractable ascites can be the primary indication for liver transplantation and in this situation there are, by definition, few therapeutic options to consider. The emphasis in this scenario is limiting the side-effects of therapy that could impact on the transplant itself. Diuretic therapy can cause or aggravate hyponatraemia and impair renal function. Renal function is consistently identified as a correlate of outcome after liver transplantation and it is logical to minimize negative stresses on renal function. For these two reasons, it could be argued that diuretic regimens should be tailored on a safety rather than an efficacy basis, particularly as the patient is getting close to the time when there is a realistic chance of being allocated an organ. In MELD-based allocation systems, rising creatinine levels may require reduction or cessation of diuretic usage so as not to obtain advantage in respect to organ allocation. Regular or programmed paracentesis may be the only option for patients with intractable ascites, but each episode carries the risks of bleeding or infection.

Alternatively, ascites may develop or worsen in patients listed with an alternative lead indication for liver transplantation. The initial management is along standard lines, subject to the caveats outlined above (Table 38.3). These include salt restriction, limitation of fluid intake in the range of 800–1500 mL/day, diuretics and paracentesis. The normal paradigm of managing ascites would extend to consideration of transvenous intrahepatic portovenous shunt (TIPS) in selected patients with well-maintained synthetic function and no previous history of hepatic encephalopathy. However, patients with this profile are likely to be on the transplant waiting list and therefore would not be suitable candidates for TIPS. In the absence of HCC, concern regarding the possibility of jeopardizing liver transplantation with malposition of the TIPS limits the appeal of this therapeutic option.

Spontaneous bacterial peritonitis (SBP) is potentially a serious complication in patients awaiting liver transplantation. Antibiotic prophylaxis against SBP, typically with a quinolone (e.g. norfloxacin 400 mg/day), may be either used routinely in all patients with ascites or in patients considered to be at increased risk based on a previous episode of SBP or a very low albumen level in ascites. When the former policy is used, consideration should be given to extending prophylaxis to all patients on the transplant waiting list. Patients who develop repeated episodes of SBP can develop adhesions between the intestinal loops that in their most advanced form take on the appearance of a cocoon. Cocoons increase the complexity of surgery at the time of transplantation and may contribute to nutritional impairment post transplant.

Hyponatraemia

Hyponatraemia occurs in about 30% of patients with cirrhosis. It is often associated with diuretic therapy and may improve with adjustment in the use of these drugs. However, it also develops independently and then is more difficult to correct and is an indication of a deteriorating prognosis. Consequently, serum sodium levels are included in some scoring systems, including MELD-Na and United Kingdom End-stage Liver Disease (UKELD). Fluid restriction is the only currently available strategy to routinely manage spontaneous hyponatraemia. If serum sodium levels are below 125 mEq/L when an organ is allocated, corrective action with ultrafiltration is required before proceeding with the transplant as the rapid shifts in serum sodium levels that occur perioperatively and can lead to central pontine myeloinolysis.

Hepatorenal syndrome

Hepatorenal syndrome (HRS) is the most critical of the complications of cirrhosis with respect to liver transplant candidacy, occurring in up to 25% of hospitalized patients and in 40–60% of those admitted to intensive care units [2]. Type 1 is a rapid and progressive deterioration in renal function that is reflected in the definition and evolves over a matter of weeks (Table 38.4). Type 2 is more indolent and less severe. Type 1 is also associated with more advanced liver disease than type 2 HRS, which is more commonly observed in patients with severe portal hypertension. Both of these types of HRS are independent of intrinsic kidney disease, which is relatively common in patients with cirrhosis. MELD-based organ allocation systems inevitably increase the burden of renal dysfunction in patients receiving liver transplants.

The therapeutic options for HRS include plasma expansion, vasoconstriction and liver transplantation. Terlipressin is the preferred vasoconstrictor in Europe, whilst midodrine or epinephrine is more commonly used in the US. The combination of albumin and terlipressin is considered to be the first-line treatment for type 1 HRS, with improvement in up to 50% of cases [3,4]. The patients who are most likely to respond have earlier disease and exhibit a sustained increase in mean arterial pressure, although the latter does not necessarily result in improved renal function [5]. The value of this therapy should be assessed as a bridge to transplantation rather than the more conventional criterion of direct survival benefit. Transplanting patients with normal or near-normal renal function has significant benefits post transplant in terms of need for renal support, length of stay and survival [2]. There is less evidence to support the use of this regimen in type 2 HRS. Ultimately these patients, together with those with acute tubular necrosis as the basis for renal failure, may proceed to needing renal replacement therapy whilst awaiting liver transplantation.

Table 38.4. Definitions and characteristics of hepatorenal syndromes types 1 and 2

Type 1
Serum creatinine doubling within 2 weeks to exceed 2.5 mg/dL
or
Creatinine clearance reducing by at least half to <20 mL/min
Rapid and progressive in patients with advanced liver failure and in the absence of alternative explanations for renal failure
Type 2
Serum creatinine 1.5–2.5 mg/dL
Increase less rapid than in type 1
Typically liver function better preserved, but with significant portal hypertension

An unintended consequence of MELD-based allocation has been a dramatic increase in the number of patients who have combined liver and kidney transplants. Individual patients with renal failure at the time of liver transplantation benefit from receiving combined liver and renal grafts. However, many patients recover some renal function after a successful liver transplant, including at least 30% of patients who receive combined transplants. Guidelines for selecting patients for the combined approach are being refined but are not yet fully established. Patients with HRS are more likely to recover renal function than those with acute tubular necrosis (ATN). In one study, improvement in renal function was observed at 90 days post-transplant in 88% of HRS patients and 71% of ATN patients [6]. The duration of renal support pretransplant also influences the likelihood of recovery of function. The best outcomes were seen when the duration of dialysis did not exceed 4 weeks [7]. The translation of this criterion to one indicating a combined procedure generated thresholds of between 6 and 12 weeks. A practical implication of this debate is whether a kidney biopsy is required to make the decision in individual cases. Strong prognostic information may be derived from renal histology, but this needs to be balanced with the risk of haemorrhage post biopsy.

Variceal bleeding

Variceal bleeds represent the most acute of the potentially destabilizing events for a patient awaiting liver transplantation. Although the overall survival rate after variceal bleeding has improved dramatically, the risk of death remains significant at around 30% in the type of patient listed for liver transplantation [8]. Primary or secondary prophylaxis with propranalol targeted at maintaining a target pulse rate of around 60 beats per minute should be maintained unless the drug is poorly tolerated. In this situation, low blood pressure is the main limiting factor. There are no data suggesting that the endoscopic surveillance of varices or the use of endoscopic prophylaxis should change after placement on the waiting list for transplantation.

The management of episodes of variceal bleeding follows standard protocols [9]. Endoscopy remains the first-line therapy once the patient has been resuscitated with an appropriate balance of crystalloid, colloids, blood and blood products. Vasoactive drugs such as terlipressin, somatostatin and its analogues can cause spasm of the hepatic artery but this does not prejudice successful transplantation. TIPS is used to salvage patients who fail endoscopic and pharmacological intervention. In this setting, the risk–benefit profile for TIPS is much more favourable than in the context of intractable ascites, and it should be considered in the salvage of patients who are not responding to therapy and/or have bleeding gastric varices. Care should be taken when placing the TIPS not to

extend the shunt into the operative field. Bacterial infection complicates variceal bleeding in up to 66% of patients, which justifies the use of a short course of prophylactic antibiotics [2].

Occasionally, an organ is allocated to a patient at a time when he/she is being managed for active variceal bleeding and a decision needs to be reached on whether it is appropriate to proceed. There are no data to assist with this decision and it will be made on clinical intuition. The portal venous pressure can be reduced early in the transplant process, if necessary by creating a temporary shunt or with extracorporeal veno-venous bypass techniques, and this will help to secure control of the variceal bleeding. Therefore, the bleed itself does not contraindicate transplantation. However, if the bleed has triggered other complications of cirrhosis, as is often the case, it may be better to stabilize the patient before proceeding with surgery. Sepsis, aspiration pneumonia, hemodynamic instability and metabolic abnormalities are among the commoner complications encountered in this situation.

Encephalopathy

Status as a potential liver transplant recipient has limited impact on the approach to the prevention of encephalopathy. Therapies like lactulose and rifaxamin are used in the standard way. However, restriction of dietary protein to reduce the incidence of encephalopathy could conflict with attempts to improve nutritional state and therefore should not be excessive (<60 g/day). The management of an acute episode of encephalopathy is also standard with the three components of intravenous fluids, antibiotics and purgatives. In addition, any precipitating cause should be addressed.

Patients with severe encephalopathy usually require mechanical ventilation to protect the airways and maintain gas exchange. This has relevance to liver transplantation as survival rates are significantly lower in patients who are intubated at the time an organ is allocated. However, there are conceptual reasons to believe this might not apply to the same extent in patients who were intubated primarily to support them through an episode of encephalopathy. These include the relatively short period of intubation usually required and absence of other complications associated with increased mortality. Encephalopathy reverses very rapidly after successful liver transplantation and should not significantly extend the duration of ventilatory support after surgery. In contrast, patients whose encephalopathy is in the context of multiorgan failure at the time of transplantation require extended support with the associated risks.

Nutrition

Body mass index (BMI) has implications for outcomes after liver transplantation and therefore nutrition may be a focus of considerable attention in the pretransplant period. The clearest evidence relates to low BMI (<18 kg/m^2) and such patients need intense nutritional support, accepting that in very advanced liver disease the benefits may be less than anticipated from the number of calories delivered. The general principles of nutritional support apply in this population [11]. Nutritional supplements or nasogastric feeding are regularly used.

The evidence relating to the impact of elevated BMIs is less clear-cut, with some studies finding comparable outcomes in patients with BMIs >35–40 kg/m^2 while others show contradictory data. Irrespective of the evidence, many liver transplant programmes intuitively recommend obese patients to lose weight whilst awaiting transplantation. This patient population is limited in its options with respect to increasing exercise and therefore controlled reduction in calorie intake is the main approach available to them.

Acute decompensation episodes

The complications discussed above often co-exist during an episode of acute deterioration in the patient's clinical condition. Recently, the term acute-on-chronic liver failure has been used to describe this scenario, although acceptance of this term is not universal as some authorities prefer to reserve it for additional insults to the liver unrelated to the cause of the cirrhosis. Infection and variceal bleeding are the commonest triggers of multisystem failure and these in turn precipitate encephalopathy, hemodynamic instability, and renal dysfunction (Figure 38.1). These patients are usually

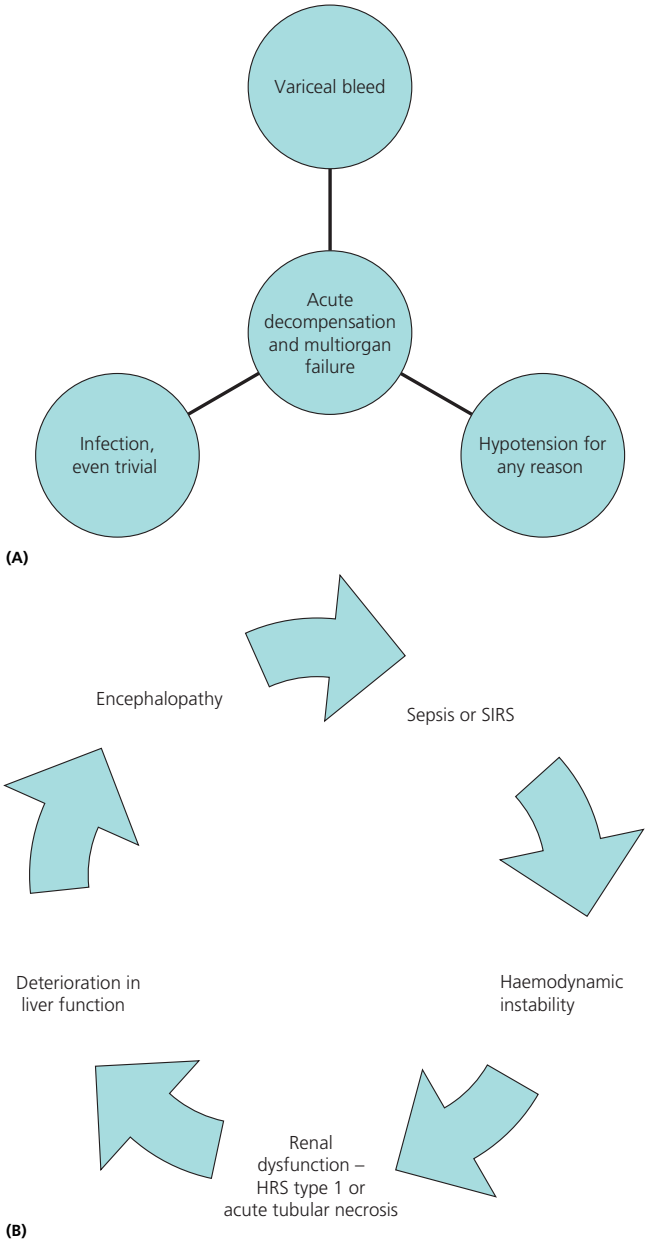

Figure 38.1. (A) Acute decompensation of chronic liver disease has three main triggers and (B) is a vicious circle of interacting complications. SIRS, systemic inflammatory response syndrome; HRS, hepatorenal syndrome.

managed in an intensive care setting and are likely to be intubated.

The decision on whether to proceed to transplantation in the context of an organ offer may be very difficult to make in this setting. The mortality rate is in the order of 40%, with most deaths occurring within a week of admission to an intensive care unit [2]. On the other hand, the 1-year survival rate for liver transplantation in this setting is around 60% or 25–30 percentage points lower than for transplantation of an ambulatory patient with chronic liver disease [1]. A judgement is required on how likely it is that the patient will improve and thereby increase his/her chances of ultimately surviving the transplant. Existing prognostic models do not perform with great precision and ultimately the decision is based on collective clinical judgement. The patients most likely to recover have MELD scores of <26, sequential organ failure assessment (SOFA) scores of <9 and variceal bleeding or encephalopathy as the indication for admission to the intensive care unit [2]. The quality of organ on offer is another dimension of the decision-making process. Patients who are progressively deteriorating may benefit more from the first available organ, while a relatively stable patient's best interests might be served by waiting for a good quality organ.

Antiviral therapy

Depending on geographical location, chronic viral hepatitis is associated with the indication for liver transplantation in between 25% and 50% of cases. Hepatitis C virus (HCV) dominates in the US and Europe, while hepatitis B (HBV) is more prevalent in Asia. HCC is associated with both viral conditions and is present in about one-third of HCV cases. The proportion of patients with HBV who have HCC is higher as antiviral therapy has significantly reduced the need for transplantation for end-stage chronic liver disease. Historically, HBV recurrence was a major problem, but strategies have now been developed to prevent or manage it in most cases. However, recurrence of HCV still is a major problem and it is only recently that the prospect of strategies to combat it have started to take shape.

Hepatitis B

The outcomes after liver transplantation in patients infected with HBV improved dramatically with the introduction of hepatitis immunoglobulin prophylaxis after transplantation. From the outset it was clear that the success of this strategy was dependent on HBV DNA levels in blood being very low at the time of transplantation. The development of a range of antiviral agents effective in controlling HBV replication enabled most patients to be rendered HBV DNA negative at the time of elective liver transplantation. The choice of agents is influenced by the speed with which control of viral replication is required and the drug resistance pattern of the virus. When there is a requirement for a rapid viral response, the use of lamivudine and tenofovir is favoured. Patients maintained on antiviral therapy whilst awaiting transplantation should be screened for emergence of drug resistance at 6-month intervals.

Hepatitis C

As with HBV, there are potential benefits to the patient if viraemia can be eradicated prior to transplantation. HCV infection may not recur in up to 70% of patients who are HCV RNA negative at the time of transplantation. However, this can only be achieved in a minority of HCV-infected liver transplant candidates. Standard therapeutic regimens using pegylated interferon and ribavirin can, realistically, only be considered in patients with well-compensated cirrhosis and thus this option is open only to some patients with HCC. In the remaining patients, an alternative approach has been described called low-dose accelerating dose regimen (LADR), which is a titrated approach with increases in dosing if therapy is well-tolerated [12]. The main concern with antiviral therapy is deterioration in liver function that may not reverse after withdrawal of therapy and can lead to an increase in the urgency with which liver transplantation is required or even death. In theory, the haematological side-effects of antiviral therapy could cause problems in the intra- and post-operative periods, but in reality most of these can be managed effectively.

There is considerable optimism that progress will be made relatively quickly in the treatment of HCV pre transplant. First, the availability of two new agents, telaprevir and boceprovir, has increased the response to interferon-based antiviral therapy and shortened treatment durations. Second, the assessment of patients suitable for antiviral therapy has become more informed on an individual basis by HCV genotype, IL28b genotype and viral kinetics after initiation of therapy. Third, there is an anticipation that drug combinations will emerge that will allow interferon-free suppression of viral replication, mirroring the progress in HBV. There is, however, no current expectation that an immunoglobulin will become available for HCV that would replicate the HBV template for prevention of viral recurrence.

HIV infection

Liver transplantation has been performed in patients infected with human immunodeficiency virus (HIV), but the procedure should still be considered experimental and applied to highly selected patients. Typical protocols will require patients to have undetectable viral loads and options for alternative antiviral drugs if resistance emerges. Consequently, close monitoring of these parameters is required throughout the waiting period. Acquired immune deficiency syndrome (AIDS)-defining illnesses after immune reconstitution generally contraindicate transplantation and should trigger a re-evaluation of transplant candidacy if they develop after placement on the waiting list. The outcomes of liver transplantation in well-selected cases are equivalent to the general population in all aetiologies except hepatitis C, where 3-year graft survival rates are only 53% [13]. Low BMI (<18 kg/m^2) and impaired renal function have been identified as pretransplant correlates of a poor outcome and patients exhibiting these characteristics should not be transplanted.

Liver support devices

Strategies to support the failing liver have a particular relevance to patients awaiting liver transplantation. It is reasonable to broadly conclude that liver support devices have so far failed to improve survival, but a number of studies have supplied evidence that a more realistic expectation from these devices is as a bridge to transplantation. The devices, which are covered in depth in Chapter 47,are categorized as non-biological and biological based on whether or not they contain a cellular component. The cellular components include normal human hepatocytes, cells derived from a human tumour cell line or porcine hepatocytes. Some approaches to liver support should be considered as hybrid as they combine both categories of device. Detoxification is an objective of all artificial devices, and a range of non-biological devices has been

developed to remove water-soluble and/or protein-bound toxins. Protein synthesis is only possible with biological devices that incorporate porcine hepatocytes, human hepatocytes derived from a tumour cell line, or human hepatocytes harvested from human organs deemed unsuitable for liver transplantation.

Albumin-based dialysis is the most widely applied device. Blood or serum coming into contact with albumin allows the transfer of protein-bound toxins. The Molecular Absorbent Recirculating System (MARS) is both the prototype and the most widely used of these devices. An early report showed an impressive improvement in deeply jaundiced patients with renal dysfunction, but in a subsequent systematic review, a 33% reduction in mortality was seen in patients with acute-on-chronic liver failure but no significant benefit in bridging patients to transplantation was identified. Two randomized controlled trials failed to show survival benefit with MARS or Prometheus over standard care [14,15]. Despite having enthusiastic protagonists, albumen-based dialysis has not gained widespread use. Experience with biological systems in patients waitlisted for transplantation is even more limited. A recent trial involving 62 patients did not show either a survival benefit or higher transplant rate with the use of an extracorporeal liver assist device (ELAD) [16].

Hepatocellular carcinoma

The majority of liver transplant programmes apply strict criteria based on tumour bulk (variably defined using number and size of nodules) when listing patients with HCC for liver transplantation, and there is a requirement to remain within these criteria up to the time of transplantation. The original and most widely used criteria are the Milan criteria, which permit a solitary lesion up to 5 cm in diameter or up to three lesions if none exceeds 3 cm in diameter. A number of other criteria are less restrictive by varying margins, including the University of California, San Francisco (UCSF) criteria and rule of 7s. The biological behaviour of HCC varies greatly and is difficult to predict at the outset, as is the response to therapeutic interventions when these are available. The management of these patients on the waiting list can be complex and work intensive with a dynamic approach combining programmed restaging with re-evaluation of therapeutic options.

Patients with advanced liver disease or any evidence of decompensation are unsuitable for locoregional therapy and depend on a favourable biological profile to remain within transplant criteria. Imaging with CT and/or MRI is used at 2–3-month intervals to reassess tumour bulk and confirm the absence of vascular invasion and extrahepatic disease. Alpha-fetoprotein levels, both absolute and relative, are also used to inform this evaluation. On the other hand, patients with well-compensated cirrhosis are likely suitable for locoregional therapy with transarterial chemoembolization (TACE), ablation techniques or a combination of both. It has been suggested that this is considered for patients with tumours of >2 cm in diameter if the anticipated waiting time exceeds 6 months [17,18]. TACE is the most commonly used primary therapy, although there is some evidence that radiofrequency ablation (RFA) may be more effective in generating tumour necrosis [19]. There are no data so far on the use of antiangeogenesis drugs (e.g. sorafenib) prior to transplantation.

The response to locoregional therapy has become an important component of the evaluation of individual patients. A good initial response followed by liver transplantation between 3 and 9 months after listing for transplantation is considered to be the best-case scenario. Up to 25% of patients progress despite locoregional therapy and "fall off" the waiting list. Whilst some consider this to be a failure of therapy, others see it as an important observation of tumour biology and patient selection. Similarly, patients who are outside the listing criteria but down-stage to within the criteria are increasingly being considered favourably for liver transplantation [20,21]. Excellent results have been achieved in patients who are successfully down-staged, with 92% survival rate 4 years after transplantation and an overall intention-to-treat survival rate of 69% at the same time point [21].

Acute liver failure

Patients listed for transplantation either have criteria suggesting a poor prognosis as determined by a range of prognostic models or have been assessed as likely to deteriorate clinically to the point of needing a transplant. The former prognostic models promote specificity over sensitivity and vice versa for assessment. Patients may deteriorate or improve and the need and suitability for liver transplantation must be constantly reviewed. In a number of series, 6–18% of the overall population were removed from the waiting list or died before an organ became available. In this dynamic process, the final decision on transplantation is made when an organ becomes available.

The majority of patients waitlisted for emergency liver transplantation will be in an intensive care environment and be mechanically ventilated for severe encephalopathy. The exception to this may be some patients with subacute liver failure and low-grade encephalopathy. The management of acute liver failure is complex as almost every physiological system is liable to fail [22]. Detailed protocols for the management of these patients are available and will not be reviewed in detail here. There are however a number of aspects of management that may be modified on the basis that the patient is awaiting transplantation. Foremost amongst these is the invasive monitoring of intracranial pressure. This is controversial because of the lack of evidence of a positive impact on survival and the associated risks, particularly intracranial haemorrhage. However, there is a higher level of support for its use when the objective is to bridge the patient safely to transplantation.

Another controversial area is the use of fresh frozen plasma to correct the coagulopathy in the absence of clinical manifestations. The argument against this practice is based on the contention that it is of no obvious clinical benefit and it undermines ongoing assessment of prognosis. In the setting of being waitlisted, the clinical benefit is in preparation for surgery and the prognosis is already considered poor in most cases. Even though the case for using recombinant Factor VII has not been made in general in acute liver failure, its use should be avoided in patients awaiting transplantation because of the possible increased risk for thrombotic events that could have serious consequences after transplantation, e.g. hepatic artery thrombosis.

Untreated sepsis is another complication that can prejudice successful transplantation and the use of prophylactic antimicrobial agents, both antibacterial and antifungal, is justified once patients have been listed for transplantation. There may also be a justification for starting renal replacement therapy sooner in patients on the waiting list to minimize the metabolic disarray at the time of commencing surgery.

The identification of those patients who are too ill to benefit from transplantation, and the deselection of waitlisted patients who deteriorate to the extent that proceeding to transplantation is futile, is

Table 38.5. Correlates of outcomes after liver transplantation for acute liver failure

	Hazard ratio (HR)
United States	
BMI >29 kg/m^2	1.5
Serum creatinine >2 mg/dL	1.4
Age >50 years	1.4
Ventilated	1.4
United Kingdom	
Age >45 years	3.0
On inotropes at point of listing	2.2
"High-risk" donor	2.4
Era effect	1.9

Data from [23] and [24].

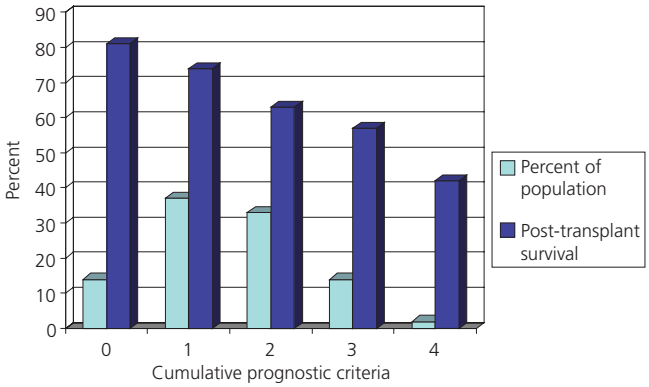

Figure 38.2. Mortality rate increases with the accumulation of poor prognostic indicators. The distribution of patients with these risk factors is shown.

a difficult but important issue to minimize wastage of a valuable resource. Two large studies have identified a number of poor prognostic indicators for liver transplantation but these have limited clinical utility in this context (Table 38.5) [23,24]. Age was identified in both studies as a poor prognostic indicator with respective thresholds of 45 and 50 years. Individually, the other clinical parameters identified are weak correlates, although the US study showed progressive decrease in survival as the prognostic criteria accumulated [23] (Figure 38.2). However, the survival rate with three of four criteria was 57%; only 2% of patients had all four criteria and their survival rate was 42%. The UK study did however highlight the importance of donor quality [24] and this is a component of the decision-making process that may be manipulated to improve outcomes.

Historically, there was a fear of irreversible brain damage after an otherwise successful transplant, but this has receded significantly in recent years. In the US, only 13% of deaths after liver transplantation were considered to be for neurological reasons [23]. However, liver transplantation is contraindicated in patients with fixed dilated pupils or other evidence of brainstem herniation. Intracranial pressure and cerebral perfusion pressure have proven to be unreliable in determining this issue. Accelerating inotrope requirements, uncontrolled sepsis and severe respiratory failure are other imprecise contraindications to transplantation. These contraindications to transplantation are to a degree age sensitive as younger patients are more resilient and more likely to reverse these complications after liver transplantation

Alcohol and smoking screening

Alcohol-related liver disease is the second largest group of patients awaiting liver transplantation. Although there are differences in alcohol policy between programmes, there is a universal expectation that transplant candidates are committed to abstinence from alcohol for life. The time spent awaiting transplantation provides an opportunity to evaluate that commitment. There is great variability in how the commitment is evaluated. A rigorous approach utilizes a written contract allied to random calls for screening of breath, blood or urine for alcohol. Regular interaction with programmes that support abstinence from alcohol may also be a requirement, e.g. Alcoholics Anonymous, although this might not be appropriate for patients with alcohol-related liver disease who are not addicted to alcohol. At the other end of the spectrum, trust-based systems screen for alcohol when a clinical event or laboratory finding arouses suspicion of active alcohol consumption.

As discussed earlier, liver function often improves after patients abstain from alcohol. At the time of listing for liver transplantation it will be assumed that most or all of the capacity for improvement has become evident. However, with lengthening waiting times a small but significant cohort of patients continues to improve for up to 18–24 months after cessation of alcohol consumption. In organ allocation systems like MELD, these patients can remain on the waiting list but with lower levels of priority. However, an active decision to remove these patients from the waiting list is required in allocation systems than couple minimum listing criteria with time spent on the waiting list.

The policy with respect to ongoing cigarette smoking is even less uniform. Patients are likely to be advised to stop smoking when they are placed on the waiting list to improve respiratory performance at the time of transplantation and reduce long-term cardiovascular and malignant risk. The extent to which this policy is monitored is poorly defined.

Summary

The waiting times for liver transplantation are generally increasing, particularly with the use of MELD-based allocation systems, and thus patients awaiting transplantation become quite unwell before an organ is allocated to them, and suffer a mortality of 5–20% between the point of listing and the allocation of an organ. Patients with cirrhosis may deteriorate at a steady rate or may acutely develop life-threatening complications. The most frequently encountered problems in patients with cirrhosis include ascites, variceal bleeding, encephalopathy and renal dysfunction, with each of these potentially triggering a cascade to multiorgan failure. Other problems relate to susceptibility to infection and poor nutrition. Patients with HCC have the additional need for treatment to suppress disease progression beyond criteria for transplantation. The management plans for these conditions may need to be adapted to the needs of patients awaiting liver transplantation. The concept of a "bridge to transplantation" has become established to evaluate some of the more complex or expensive treatments, e.g. liver support devices, as a distinct metric from the more standard survival benefit.

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CHAPTER 39

Waiting List Management for Heart Transplantation

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Introduction

Patients are identified as candidates for cardiac transplantation based on a number of clinical indicators of poor and declining cardiac function and poor prognosis. Failure to respond to standard medical, device-related, and/or surgical therapies may result in the determination that a patient's long-term survival would be improved with cardiac transplantation. Once placed on the transplant waiting list, the potential transplant recipient needs careful follow-up to monitor any changes in their clinical status that might necessitate changes in therapy and perhaps changes in the patient's priority status on the transplant waiting list. This chapter will cover the general management of patients awaiting heart transplantation. It serves as a companion to Chapter 30, which covers indications for heart transplantation, and also will complement the content in Chapters 44, 48, and 49, which detail intensive care unit management of patients with heart failure, ventricular assist devices, and the emergence of the artificial heart, respectively.

Management of patients with advanced heart failure on the cardiac transplant waiting list

Oral agents

Patients placed on the cardiac transplant list should already be receiving optimal or maximally tolerated oral medical therapy. This therapy is directed by guidelines defined by the American College of Cardiology (ACC)/American Heart Association (AHA) and Heart Failure Society of America (HFSA) [1–3]. These therapies include the beta-blockers shown in clinical trials to have survival benefits in heart failure patients [4–7], angiotensin-converting enzyme (ACE) inhibitors, or angiotensin receptor blockers (in patients intolerant to ACE inhibitors), aldosterone antagonists, and especially in African–Americans, the combination of the vasodilators hydralazine and nitrates [8–15]. Inhibitors of the renin–angiotensin–aldosterone system are maintained at target doses as described in the ACC/AHA and HFSA guidelines or as tolerated by the patient as reflected by systemic blood pressure, renal function, serum potassium, and symptoms. Carvedilol, long-acting metoprolol, or bisoprolol are also maintained at guideline-directed target doses or as tolerated by blood pressure. Additional oral medications that are frequently used and should be maintained in patients with advanced heart failure include loop diuretics for symptoms of congestion and in some patients, digitalis glycosides for palliation [16].

Devices

Patients listed for cardiac transplantation are at least New York Heart Association (NYHA) class III in terms of their symptoms of heart failure and will likely have had an automatic implantable-cardioverter defibrillator (ICD) implanted prior to being placed on the transplant list [17–19]. ICDs should be checked at the time of listing and at every outpatient office visit to determine if there have been any arrhythmias requiring antitachycardia pacing or defibrillation as well as the frequency of these arrhythmias. Interrogation of the ICD will also provide information regarding defibrillation thresholds, pacing thresholds, lead function, and battery life. Those patients who remain NYHA class III despite maximal medical therapy who also have QRS duration of >120 ms on their electrocardiograms (ECGs) should have undergone implantation of a biventricular pacemaker along with an ICD to improve exercise tolerance and favorably affect ventricular function and survival [20–22]. These devices should also be interrogated at the time of office visits to determine primarily if patients are being paced and thus undergoing cardiac resynchronization. If patients are spending only a relatively small part of their time with cardiac resynchronization, this could result in a deterioration in exercise tolerance and cardiac function, and could be interpreted erroneously as progression of the patient's heart failure. Efforts should be made to check patients with biventricular pacemakers are truly receiving cardiac resynchronization therapy, including suppression or cardioversion from atrial fibrillation as optimization of biventricular pacemaker function.

Intravenous therapy

Patients who have NYHA class IV symptoms of heart failure despite maximal medical therapy and, if indicated, cardiac resynchronization therapy, should undergo right heart catheterization to assess their cardiac output and index, pulmonary arterial and pulmonary capillary wedge pressure (PCWP), as well as transpulmonary gradient (TPG) and pulmonary vascular resistance (PVR) (see Table 39.1 for formulae to calculate TPG and PVR) [23]. The results of the right heart catheterization are often used to guide subsequent therapy, including further optimization of oral medications if possible, enhanced diuresis if the patients have an elevated PCWP indicative of volume overload, and initiation of inotropic therapy with the intravenous sympathomimetic dobutamine or the phophodiesterase 3 inhibitor milrinone. These may be used for a short period of several days to allow augmentation of oral heart failure medications and diuresis to improve the cardiac output and index,

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Table 39.1. Formulae for calculating pulmonary hemodynamics

Mean PAP = (Systolic PAP + 2 × Diastolic PAP)/3
TPG = Mean PAP − Mean PCWP
PVR = (Mean PAP − Mean PCWP)/CO

where PAP is pulmonary artery pressure; PCWP is pulmonary capillary wedge pressure; TPG is transpulmonary gradient; PVR is pulmonary vascular resistance; and CO is cardiac output.

Normal values
PVR: 1.0–1.5 Wood units (1.0 Wood unit = 80 dyn s/cm^5
TPG: 8–12 mmHg

Source Mehra et al. [23]. Reproduced with permission from Elsevier.

or may be continued indefinitely if the patient proves to be dependent on inotropic therapy to maintain cardiac output. This is usually determined by weaning inotropic therapy and reassessing hemodynamics, including cardiac output with a repeat right heart catheterization. Patients who require indefinite inotropic therapy for maintenance of cardiac output will have an indwelling catheter such as a PICC line or Hickman, placed so that continuous administration of the inotropic therapy can be maintained. These patients can usually wait for transplant at home, but will require interrogation of their ICDs since inotropic therapy is arrhythmogenic and may result in ventricular tachycardia or fibrillation. Continuous use of inotropic therapy is associated with a poor 1-year survival rate and this needs to be addressed in cardiac transplant candidates receiving these agents [24,25]. Home nursing support is usually set up to provide patients with assistance in managing intravenous inotropic therapy at home. Oral heart failure therapy is usually maintained, although doses may be scaled back if inotropic therapy produces hypotension.

Another important issue that needs to be addressed when reviewing the results of right heart catheterization is the presence and type of pulmonary hypertension. Patients with advanced heart failure often have WHO group II pulmonary hypertension [23]. This will already have been assessed during the transplant evaluation and if the TPG or the PVR are elevated (TPG $\geq$15 mmHg; PVR $>$3.0 Wood units; pulmonary artery systolic pressure $\geq$50 mmHg), interventions with intravenous or inhaled pulmonary vasodilators [i.e. nitroprusside, nitroglycerin, nitric oxide, prostacyclin (PGI_2), nesiritide] will have been performed to demonstrate that these hemodynamic parameters can be reduced or reversed while maintaining a systemic systolic arterial blood pressure of $>$85 mmHg. This is important so that post-transplant right ventricular failure, which can be fatal, is prevented. Reversibility of TPG and PVR would have been demonstrated if there is no or minimal intrinsic pulmonary arterial hypertension, and this provides pharmacologic strategies for reducing TPG and PVR at the time of cardiac transplantation. Unfortunately, the prolonged volume overload characteristic of patients with advanced heart failure may result in intrinsic pulmonary arterial damage [23]. Therefore, repeat right heart catheterization is often necessary, especially in patients who previously had elevated TPG and PVR, to make sure that the pulmonary hypertension can still be reversed with vasodilators.

Mechanical circulatory support

Many patients with advanced heart failure who are managed with oral medical and/or inotropic therapy will nonetheless develop progressive hemodynamic deterioration. These patients often present clinically with worsening symptoms of heart failure, including fatigue and abdominal complaints, arrhythmias, hypotension, and evidence of poor perfusion with cool extremities and pulsus paradoxus. Laboratory findings of worsening heart failure, including rising creatinine, blood urea nitrogen, liver function tests, and brain natriuretic peptide, would also provide evidence of hemodynamic deterioration. For these patients, mechanical circulatory support as a bridge to transplant would be required to normalize end-organ function, and improve quality of life and survival to heart transplant, as described in depth in Chapter 48. Ideally, mechanical circulatory support should be initiated before there is significant deterioration in end-organ function. The Food and Drug Administration (FDA)-approved device most commonly used as a bridge to transplant is the HeartMate II continuous flow left ventricular assist device (LVAD), which has replaced earlier pulsatile flow devices as a result of superior 1-year survival, fewer adverse effects, and better durability in randomized clinical trials [26,27]. The HeartWare HVAD has recently also been approved as a bridge to transplant by the FDA while the Jarvik VAD is still under investigation in clinical trials [28]. In the large majority of patients with advanced heart failure requiring mechanical circulatory support, LVAD support alone will be sufficient as a bridge to transplant. However, right ventricular dysfunction after LVAD implantation is a scenario that is not uncommon. Several scoring systems have been developed to predict the likelihood of the development of right ventricular failure prior to LVAD implantation [29,30]. If right ventricular failure develops, this will usually manifest intra- or perioperatively. Strategies to manage this include continued inotropic support and in more serious circumstances, right ventricular support with temporary devices such as the CentriMag. If a patient preoperatively is considered to have a high enough risk of developing right ventricular failure severe enough to compromise the outcome of LVAD support alone, the alternatives are biventricular support with the Thoratec paracorporeal (PVAD) or implantable ventricular assist device (IVAD), both of which allow for discharge to home, or the SynCardia CardioWest artificial heart, which also has an external driver for use at home. The latter device has been shown to provide mechanical circulatory support as a bridge to transplant for critically ill patients and, as opposed to the ventricular assist devices, requires surgical removal of the ventricles [31].

Data from The International Society of Heart and Lung Transplantation (ISHLT) registry demonstrate that the percentage of patients waiting for heart transplant who are supported with mechanical circulatory support has increased between 2000 and 2010 (Figure 39.1). Patients requiring LVAD and right ventricular assist device (RVAD) support had worse outcomes post transplant compared to other groups. Those recipients not requiring mechanical circulatory support had better post-transplant survival than those requiring any form of mechanical circulatory support (Figure 39.2).

Placement of patients with advanced heart failure on the cardiac transplant waiting list

Patients will be placed on the heart transplant waiting list through the local Organ Procurement Organization (OPO) and their priority determined according to the level of medical therapy or mechanical support that they are receiving. This system of prioritization is shown in Table 39.2, and is defined by the United Network of Organ Sharing (UNOS) and the Organ Procurement and Transplant Network (OPTN) of the Health Resources and Service Administration of the U.S. Department of Health and Human Services [32]. Patients with mechanical circulatory support may be listed as status 1A for any 30-day period after implantation,

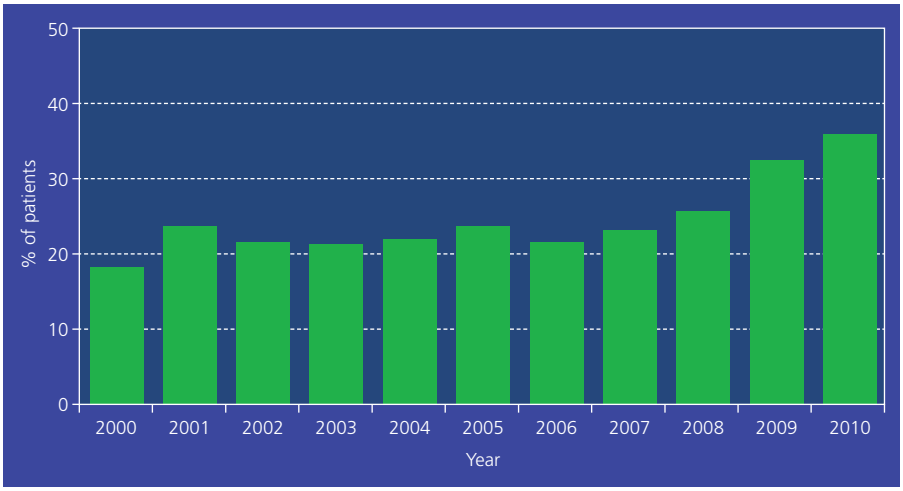

Figure 39.1. Increasing percentage of adult patients on the heart transplant waiting list requiring mechanical circulatory support. Data from the ISHLTr.

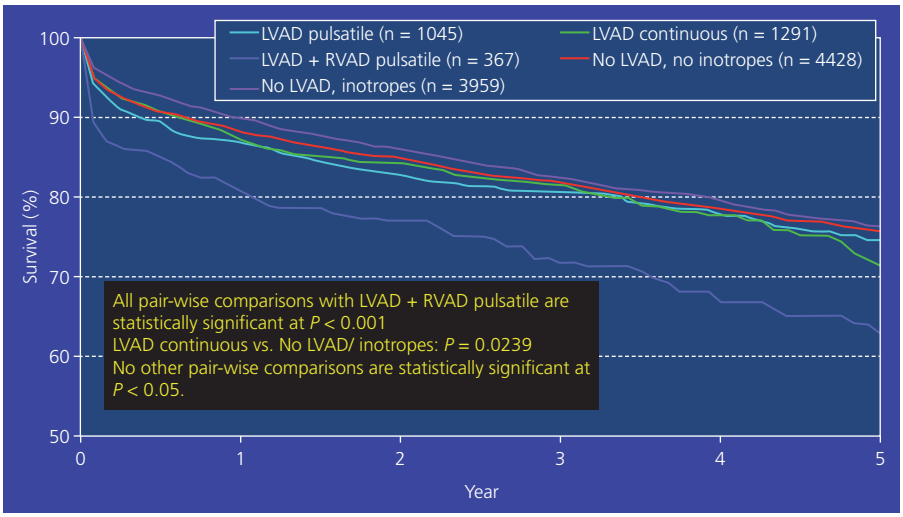

Figure 39.2. Impact of pretransplant mechanical circulatory support and the type of support on post-transplant survival. LVAD, left ventricular assist device; RVAD, right ventricular assist device. Data from the ISHLT registry.

though this is usually done at a time when the patient has recovered from surgery and is ambulatory. Patients with mechanical circulatory support may also be listed as status 1A if they have demonstrable and documented evidence of major device-related complications, such as infection, device mechanical failure, thromboembolism, or potentially lethal ventricular arrhythmias [32]. All other patients with mechanical circulatory support would be listed as status 1B. Additional patients who may qualify as status 1A include those requiring continuous mechanical ventilator support for 14 days with subsequent renewal every 14 days (these patients do have worse outcomes post transplant); and patients receiving continuous infusion of a single high dose of inotropic therapy or multiple inotropes with continuous hemodynamic monitoring of left ventricular filling pressures for 7 days, with the option for a 7-day extension if the above circumstances are unchanged. Finally, patients who do not meet the above criteria may be considered as status 1A if they have compelling urgency and clinical circumstances that can be justified and are approved by the UNOS Regional Review Board [32].

All patients receiving intravenous inotropic support or who have mechanical circulatory support who do not meet the above criterion are listed as status 1B [32]. Patients receiving only oral medications are listed as status 2 and patients on the list who for a variety of circumstances cannot at present be transplanted are listed as status 7 and are "inactivated."

Organ allocation

Donor hearts are allocated according to the policies of the OPTN and administered by UNOS. Donor hearts are preferentially allocated locally within the confines of the local OPO by recipient disease severity. If there is more than one potential recipient at a given status level, priority is given to the recipient with the longest waiting time. If no acceptable donor is identified locally, donor

Table 39.2. Adult cardiac transplant candidate priority status

Status 1A

a i. Patient with mechanical circulatory support with left and/or right ventricular assist device may receive 30 days of Status 1A after device implantation once the patient is felt to be ready clinically for cardiac transplantation (patient may be at home)

a ii. Total artificial heart

a iii. Intra-aortic balloon pump

a iv. Extracorporeal membrane oxygenator

For criteria a ii, a iii, or a iv, patients must be hospitalized at the listing transplant center; initial status 1A is valid for 14 days and must be recertified every 14 days from the initial date of listing by the attending physician caring for the patient

b. Patient with mechanical circulatory support with documented, objective evidence of significant device-related complications, most commonly device mechanical failure, device infection, thromboembolic event or thrombus of the device, or life-threatening arrhythmias. Patients may be at home. Initial status 1A is valid for 14 days and must be recertified every 14 days from the initial date of listing by the attending physician caring for the patient

c. Patient with continuous mechanical ventilation. Initial Status 1A is valid for 14 days and must be recertified every 14 days from the initial date of listing by the attending physician caring for the patient.

d. Patient receiving continuous intravenous infusion of high doses of a single inotrope (dobutamine or milrinone) or multiple intravenous inotropes, as well as continuous invasive hemodynamic monitoring of left ventricular filling pressures. Initial status 1A is valid for 7 days and must be recertified every 7 days from the initial date of listing by the attending physician caring for the patient

Exception – A patient on the transplant waiting list who does not meet the above criteria but who is felt by his/her transplant physician to be of appropriate clinical urgency and who would derive substantial benefit from transplantation. Justification for this request must be submitted by the transplant physician to the Regional Review Board for approval. Listing under this exception is for 14 days. Subsequent extensions of the status 1A-Exception listing must be prospectively approved by the Regional Review Board. Listing as status 1A without approval is referred to the Thoracic Organ Transplantation Committee and the Membership and Professional Standards Committee

The status 1A Justification Form should be submitted via UNet for initial listing or extensions. Once the status 1A time expires, the patient is automatically downgraded to status 1B

Status 1B

aa Left and/or right ventricular assist devices

bb Continuous intravenous infusion of inotropic agents

Exception – A patient on the transplant waiting list who does not meet the above criteria but who is felt by his/her transplant physician to be of appropriate clinical urgency and who would derive substantial benefit from transplantation. Justification for this request must be submitted by the transplant physician to the Regional Review Board for approval. Report of the Regional Review Board decision is referred for review to the Thoracic Organ Transplantation Committee and the Membership and Professional Standards Committee

The status 1B Justification Form should be submitted via UNet for initial listings or extensions

Status 2

Cardiac transplant candidates who do not meet criteria for status 1A or 1B (no inotropes, mechanical circulatory or ventilator support)

Status 7

Patients on the cardiac transplant list who are determined not to be suitable temporarily for transplant. These patients retain their previously accrued transplant waiting time but do not accrue new time and are not offered donor hearts. Patients who are deemed to be unsuitable for transplant indefinitely or permanently should be removed from the list

Source United Network for Organ Sharing/Organ Procurement and Transplant Network of the Health Resources and Service Administration [32]. Available at: http://optn.transplant.hrsa.gov/PoliciesandBylaws2/policies/pdfs/policy_9.pdf

hearts will be allocated preferentially according to geographic distance from the donor hospital. This algorithm follows sequential concentric circles of up to 500 miles from the donor hospital (zone A), 500–1000 miles from the donor hospital (zone B), 1000–1500 miles from the donor hospital (zone C), 1500–2500 miles from the donor hospital (zone D), or >2500 miles from the donor hospital (zone E) [32].

Management of the sensitized patient on the heart transplant waiting list

Patients with advanced heart failure who are waiting for transplants may develop allosensitization from a number of sources, including prior pregnancies, prior transfusions of blood products (including platelets), prior solid organ transplants, or the presence of ventricular assist devices for mechanical circulatory support [33]. Allosensitization presents a challenge in that it puts the recipient at risk for antibody-mediated rejection and graft failure if donor hearts are transplanted into patients with antibody specificities to antigens on the surface of endothelial cells of those hearts. From the ISHLT registry, sensitized patients [panel reactive antibody (PRA) >10%] have a higher 5-year mortality after transplant (Table 39.3). A further challenge is that allosensitization limits, sometimes drastically, the potential donor hearts available for potential recipients as transplant physicians and surgeons seek to eliminate from consideration for transplant those organs against which the recipient is sensitized. The use of the virtual cross-match (see Chapter 89) has helped to optimize donor availability. Nonetheless, it is tempting to try to modulate the alloimmune response to reduce the extent of allosensitization and thereby increase the number of potential donor hearts available for a specific potential recipient on the heart transplant waiting list. This has resulted in the development of a number of desensitization strategies to reduce allosensitization. This topic is considered in depth in Chapter 68. Desensitization protocols often are initiated when the calculated PRA exceeds 10%. These protocols use therapies that are directed against B-cell antibody production and include intravenous immunoglobulin (IVIG), plasmapheresis, mycophenolate mofetil, and rituximab [34]. While IVIG has demonstrated clinical efficacy in clinical studies, no definitive information is available and no clinical trials have been performed in patients awaiting cardiac transplantation [35]. One retrospective study showed that the combination of plasmapheresis, IVIG, and rituximab reduced PRAs from 70.5% to 30.2%, with 5-year survival post transplant and freedom from cardiac allograft vasculopathy similar to those for non-sensitized patients [34]. Often, these therapeutic approaches are ineffective and more recently, the proteasome inhibitor bortezomib has been used in conjunction with plasmapheresis and rituximab in patients who were refractory to other treatments. Two small reports of a total of ten patients suggest that bortezomib may be helpful in reducing PRAs in otherwise refractory patients [36,37]. Unfortunately, there are no prospective controlled trials of desensitization therapy in patients on the heart transplant waiting list, so therapeutic approaches are largely empiric.

Clinical assessment of patients on the heart transplant waiting list

As advanced heart failure is often a dynamic disease, patients on the heart transplant waiting list will need to undergo frequent clinical assessments to determine if their status on the waiting list needs to be changed. For example, patients who are status 2 may require implementation of inotropic therapy or implantation of an LVAD as a result of hemodynamic deterioration. They would then become status 1B. A patient with an LVAD who is status 1B may be upgraded to status 1A as a result of a life-threatening device infection or thrombus. Recommendations for follow-up after listing for cardiac transplantation were included in the ISHLT Listing Criteria Guidelines in 2006 [23]. Table 39.4 summarizes the outpatient visit and procedure schedule for patients who are on the heart transplant

Table 39.3. Elevated pretransplant panel reactive antibody (PRA) (>10%) is a risk factor for increased mortality 5 years post transplant (n = 10 507)

Variable	*n*	Relative risk	*P* value	95% confidence interval
Female recipient with prior pregnancy/male donor vs. male recipient/male donor	780	1.22	0.0286	1.02–1.45
PRA > 10%	766	1.21	0.0066	1.06–1.40
Recipient with infection requiring IV drug therapy within 2 weeks prior to transplant	1161	1.18	0.0082	1.04–1.33
Recipient history of diabetes	2177	1.14	0.0062	1.04–1.26
Diagnosis: coronary artery disease vs. cardiomyopathy	4792	1.14	0.0063	1.04–1.25
Not hospitalized just prior to transplant	5516	0.92	0.0475	0.84–1.00

PRA, panel reactive antigen.
Data from the ISHLT registry.

Table 39.4. Recommended schedule for follow-up of patients once listed for cardiac transplantation

History and physical examination – (including weight, height and calculation of body mass index)	Monthly especially for patients with inotropic or mechanical circulatory support
PRA and flow cytometry	
>10%	Every 1–2 months
VADs	Every 1–2 months
Post transfusion	2 weeks after transfusion then monthly for 6 months
Lab work (CBC, CMP, BNP)	Monthly with office visits
PT/INR	Monthly or as needed if anticoagulated for atrial fibrillation, VADs, or mechanical heart valves
GFR assessment (MDRD method)	Every 3 months
Right heart catheterization with assessment of pulmonary hemodynamics and use of vasodilators as needed	At least every 6 months (and more frequently as needed clinically
Evaluation of VAD	Monthly and more frequently if needed
Interrogation of ICDs, biventricular Pacemakers	Every 4 months and more frequently as needed clinically
Cardiopulmonary stress test	Annually (status 2 patients)
Echocardiogram	Annually; more frequently as needed in patients with VADs
ECG	Annually

PRA, panel reactive antibody; VAD, ventricular assist device; CBC, complete blood count; CMP, complete metabolic panel; BNP, beta-natriuretic peptide; PT/INR, prothrombin time/international normalized ratio; GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; ICD, implantable cardioverter-defibrillator; ECG, electrocardiogram.
Adapted from Mehra et al. [23] with permission from Elsevier.

waiting list. Outpatients, which now includes the majority of ambulatory patients receiving inotropic or mechanical circulatory support, should be seen in the office on a monthly basis for a clinical assessment, which should include history and physical examination, and laboratory assessments of renal and hepatic function and electrolytes. Glomerular filtration rates using the Modification of Diet in Renal Disease (MDRD) formula, should be assessed every 3 months [38,39]. Right heart catheterization should be obtained in all patients on a 6-month basis to determine if there has been an increase in PVR, TPG, and/or systolic pulmonary artery pressure; if these are found to be present, therapy with pulmonary vasodilators as mentioned earlier should be initiated to determine if these markers of pulmonary hypertension are reversible. Additionally, right heart catheterization provides information regarding cardiac output and can trigger more intensive therapy with initiation or enhancement of inotropic therapy or mechanical circulatory support. For patients who have already had elevated TPG, PVR, or pulmonary arterial pressures, these assessments may need to be repeated more frequently and should be performed in patients on the transplant list who present with signs and symptoms of low cardiac output or those who have progressed to NYHA class IV symptoms despite optimal medical therapy. Patients with ICDs or on cardiac resynchronization therapy should have their devices interrogated. Patients with LVADs or other forms of mechanical circulatory support should be seen on a monthly basis and should have their support devices scrutinized. Patients with PRAs of >10% or who have LVADs should have their PRAs checked every 1–2 months [23]. Patients who require blood transfusions should have PRAs measured 2 weeks afterwards and then monthly for 6 months [23]. The transplant center should communicate with the local OPO to update it on unacceptable antigens for specific patients based on the most recent PRA results. As an increasing number of patients are waiting longer than a year for transplant, an echocardiography should be performed annually. Patients with LVADs may require echocardiography more frequently if they develop arrhythmias. Echocardiography is also useful in these patients to assess ventricular emptying from the LVADs. Patients who are status 2 should undergo cardiopulmonary exercise testing to determine if their functional class has improved to the point where they would no longer benefit from cardiac transplantation [23].

Patients who are waiting for transplant in the hospital represent a sicker population who should undergo greater scrutiny. This would potentially include more frequent invasive hemodynamic monitoring and assessment of LVAD function.

Summary

Patients with advanced heart failure who are on the waiting list for a heart transplant should have their heart failure therapies continuously monitored and optimized. Close attention should be paid to clinical deterioration, which might necessitate implementation of more aggressive supportive treatment such as inotropic or mechanical circulatory support. Changes in end-organ function should also be carefully monitored and therapy adjusted to optimize this. Changes in the intensity of therapy should be reflected in changes in the patient's listing status to one that is commensurate with his/her therapy and prognosis prior to transplant. LVAD infection or dysfunction should result in an upgrade in the patient's UNOS status to 1A. PRAs should be assessed longitudinally in those with PRAs elevated by >10% and those at risk for developing increased PRAs, including patients with LVADs or with recent blood transfusions. The role of desensitization in patients on the heart transplant waiting list has not been well established with prospective clinical trials, and may be implemented in patients with very high PRAs who have been unable to receive donor hearts as a result.

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CHAPTER 40

Waiting List Management for Lung Transplantation

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Introduction

Once lung transplant candidates are listed for transplantation, the waiting time to transplant typically varies from weeks to months, a relatively short time reflecting the severity of the conditions impelling candidacy for lung transplantation and the disease severity metrics that drive lung allocation. Thus, an understanding of list management in lung transplantation requires a working understanding of lung allocation and listing requirements as well as the common maladies facing someone with end-stage pulmonary failure. This chapter will review the current scoring system used for allocating lungs, the lung allocation score (LAS). It will address the pragmatic issues that lung transplant centers face with respect to how to educate patients, optimize them for transplant, and ensure that they have the best chance for successful transplantation. Issues including HLA sensitization, physical disability, patient compliance, and recipient size will be discussed. This chapter complements Chapter 31, which covers the specific indications bringing people to the lung transplant list.

Listing for lung transplant

United States programs

The history of the process of listing individuals for lung transplantation and organ allocation through the United Network for Organ Sharing (UNOS) in the US has been recently reviewed [1]. The period from 1984 to 2005 may be viewed as the pre-LAS era, in which organs were allocated regionally to patients accruing the longest waiting times on the transplant list, taking into account specific patient issues of donor–recipient size matching and blood group ABO compatibility. However, waitlist times increased progressively and patients with more rapidly progressive diseases such as idiopathic pulmonary fibrosis (IPF) died prior to transplant at a disproportionate rate. The unacceptably high rate of mortality persisted in lung transplantation in spite of a modification in 1995 to grant newly listed patients with IPF an additional 90 days of accrued waiting time. Concerns with the inequities in the allocation system as well as a desire to conform to the recommendations of the US Task Force on Organ Transplantation ultimately led to the adoption of the LAS in 2005 [2,3], with a goal of balancing the likelihood of surviving while on the waitlist and the probability of surviving at least 1 year following transplant.

In the current LAS era, lung transplant candidates over the age of 12 years are grouped into one of four diagnostic categories (Table 40.1). Various demographic, physiologic, and co-morbid data are entered into a formula [2], and a LAS ranging from 0 to 100 is assigned to the patient, subject to later modification as the patient's condition changes while on the waitlist. A sample LAS calculator is available (http://optn.transplant.hrsa.gov/resources) as a resource to clinicians and patients. The explicit goal of the LAS is to prioritize organ allocation to minimize waitlist mortality and maximize post-transplant survival. Importantly, with the current system, the LAS weights the probability of waitlist mortality twice as high as the odds of post-transplant survival. Organs are still allocated on the basis of recipient height range and ABO compatibility. Donor lungs are first offered locally and the patient with the highest LAS selected for the available organ within each listing region (local, zone A, zone B, etc.). Thus, lungs are first offered to the transplant centers in the local Organ Procurement Organization (OPO). If a suitable recipient does not exist locally, then the lungs are offered by regional zones, with each zone (A, B, etc.) corresponding to concentric geographic circles of 500 nautical mile radius from the donor hospital. Within each list, lungs are first offered to ABO identical patients followed by ABO compatible patients. Since implementation of the LAS system, both waitlist times and waitlist mortality have decreased [1], with a greater number of patients with IPF receiving transplants [4]. Although overall 1-year survival has not changed under the LAS system, there are data that patients with higher LASs (sicker patients) have higher morbidity [5], higher costs [6], and perhaps lower survival over time [7,8].

It has been noted that the LAS system does not adequately measure the likelihood of death on the waitlist for patients with idiopathic pulmonary arterial hypertension (IPAH) [9–11]. For this reason an appeal mechanism was implemented to increase the LAS of selected patients; e.g. an appeal for patients with IPAH increase their LAS to the 90th percentile when their right atrial pressure exceeds 15 mmHg and cardiac index is <1.8 L/min/m^2. Other modifications to the LAS have been made, such as inclusion of P_{CO_2} and bilirubin, and other refinements are likely in the future with additional data. Given that lungs are still first offered in the local OPO list prior to zonal allocation, it is still possible for a recipient to receive a lung transplant in a relatively short period of time, provided there are no other similar patients on the local list. Not

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 40.1. Lung allocation score (LAS) diagnosis groups and major clinical diagnoses

Group A: Obstructive lung disease Including chronic obstructive pulmonary disease (COPD); emphysema; alpha-1 antitrypsin deficiency; lymphangiomyomatosis (LAM); bronchiectasis, including primary ciliary dyskinesia; sarcoidosis with mean pulmonary artery (PA) pressure of ≤30 mmHg
Group B: Pulmonary vascular disease Including primary pulmonary hypertension (PPH); Eisenmenger's syndrome; other specific pulmonary vascular diseases, including pulmonary venous obstructive disease, chronic pulmonary thromboembolic disease
Group C: Cystic fibrosis or immunodeficiency disorders Cystic fibrosis; common variable immunodeficiency; hypogammaglobulinemia
Group D: Restrictive lung disease Idiopathic pulmonary fibrosis (IPF); pulmonary fibrosis (other causes); sarcoidosis with mean PA pressure of >30 mmHg; scleroderma/CREST; bronchoalveolar carcinoma (BAC); bronchiolitis obliterans syndrome (BOS) following lung transplant; primary graft failure following lung transplant; eosinophilic granuloma.

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surprisingly there exists great heterogeneity within the US with respect to median waiting times for transplantation. There have been proposals within the lung transplant community to modify the allocation rules for lungs similar to those for heart, with regional recipients who are very ill taking preference over patients on the local list. At the present time, considerable controversy exists as to how to define such high urgency patients.

Other countries

Whereas the LAS system is unique to the USA, other countries have their own approaches to the issues of equitable distribution of organs. In Europe, for example, seven countries (Austria, Belgium, Croatia, Holland, Luxembourg, Germany, and Slovenia) comprise the Eurotransplant (ET) region. Lung transplant candidates in the ET region are classified as elective, urgent (U), or highly urgent (HU), with HU patients typically being in the intensive care unit and often requiring assisted ventilation [12]. Within these subgroups, time on the waitlist is taken into account. In the paper by Smits et al. [12], a pilot study was employed using LAS and LASplus grouping of patients, and found that LAS was predictive of mortality in their critically ill cohort of patients, indicating that some version of LAS may be implemented in the Eurotransplant region. In England there are six geographic zones that perform lung transplantation. Lung transplant donors are allocated first to the particular regional area in which they reside in, followed by a rotational system where the donor organs are allocated to the zone that is next on the list. There are no standards to rank recipients on the basis of medical urgency, but there are proposals to do so.

There is no comparable UNOS system for organ allocation in Canada, although Canada has a nearly identical lung transplant rate per unit population to the US. An attempt was made to centralize organ allocation including lung transplant starting in 2001, but to date, the majority of organ allocation resides at the provincial level. In 2008 the Canadian Blood Service began to address the mandate to centralize donor allocation within Canada. A national registry for living related kidney donation has been established in 2010 [13]. It is anticipated that eventually a standardized protocol for donor allocation and recipient ranking will be developed. There are five centralized lung transplant centers in Canada, and recently the directors of these centers proposed an algorithm for donor allocation that recommends favoring local allocation followed by regional, then national, similar to the algorithm in the US. With respect to ranking of organ recipients, the current Canadian system ranks patients only as being status 1 or status 2. Status 1 patients are considered stable, while status 2 patients are in need of an emergent transplant. As expected, the median waiting time for status 2 recipients is considerably shorter than for status 1.

Australia utilizes a similar decentralized system for lung transplant allocation. There are four transplant centers within the six Australian states. Donors are allocated first to the local transplant center, and if no suitable donor exists, they are offered to subsequent states on a rotational basis. This allocation system also prioritizes recipients by ABO and size match to the donor. The urgency of the recipient based on illness severity is then used for allocation.

Hence, it is important to note that in both Canada and Australia where a decentralized process of donor–recipient matching is utilized, that a high degree of discretion is required by the transplant program in terms of recipient selection. This discretion is less prominent in the US system where a highly centralized process utilizing the LAS and UNOS is employed.

Patient management while listed

Education

Both before formal listing for lung transplantation and while on the waiting list, it is important that patients and their support person(s) become fully educated about all aspects of the transplant process. Recently, the Centers for Medicare and Medicaid (CMS) in the US have formalized this process with a lengthy education document that the patient must complete at the beginning of the transplant evaluation process. There are eight core aspects to this document:

1. the evaluation process;
2. the surgical procedure;
3. alternative treatments;
4. potential medical or psychosocial risks;
5. national and transplant center-specific outcomes, as taken from the most recent Scientific Registry for Transplant Recipients (SRTR) center-specific report;
6. organ donor risk factors that could affect the success of the graft or health of the patient;
7. the patient's right to refuse transplantation;
8. if the transplant occurs in a non–Medicare-approved center, it could affect the patient's ability to have his/her immunosuppressive medications paid for under Medicare Part B.

It is obviously critical that potential transplant candidates have a realistic understanding of the potential benefits and limitations of lung transplantation and are able to make an informed decision as to whether to proceed. It is important for each patient to understand the process of organ allocation based on ABO blood type, LAS, single versus bilateral transplant, and confounding factors such as presensitization to donor HLA antigens, recipient cytomegalovirus (CMV) and hepatitis B virus (HBV) immune status, among others. Learning about the medications required post transplant, their side-effects, drug interactions, and drugs to be avoided (e.g. non-steroidal anti-inflammatory agents) by both the patient and support person(s) is critical. It is essential to have a plan in place for family or other personal support in the peritransplant period. Understanding the ongoing costs of treatment and medications following transplant and having resources for such costs and/or having a plan in place for dealing with the financial burden is emphasized in the educational process. Underscoring the fact that non-medical factors do impact patient outcome, a recent study reported that the type of insurance

coverage correlates with long-term outcomes after lung transplant [14], with patients having Medicare or Medicaid coverage having lower long-term survival than patients with private insurance. A willingness to partner with their transplant caregivers and to follow instructions for aftercare and medication adherence is also critical to a successful outcome following transplant. Finally, having adequate social support is critical in dealing with the psychological stress and medical issues in the transplant process [15].

Immunization

Prevention of certain infections through routine immunization is accomplished either prior to or while on the lung transplant waitlist. Generally accepted are immunization against *Streptococcus pneumoniae* (one center's retrospective review found only 62% percent of transplant referrals to have prior immunization [16]); HBV if immunity is lacking to allow acceptance of a donor with evidence of prior HBV infection [17]; and annual vaccination against influenza, using a killed rather than a live form of the vaccine. It is important that family members both before and after transplant have annual immunization with the killed vaccine, since immunocompromised transplant recipients may have inadequate antibody responses to the vaccinations themselves [18].

Pulmonary rehabilitation

There are numerous and compelling studies to support the importance of a structured exercise program, preferably through a pulmonary rehabilitation program for patients awaiting lung transplantation [19–26]. Indeed, the distance walked in 6 min, the 6-min walk test (6MWT), has been shown to be strongly predictive of survival while on the lung transplant waitlist [27], especially in IPF [28]. Thus, enrollment in a pulmonary rehabilitation program is prescribed by virtually all lung transplant programs, with some programs even having patients relocate close to their center to attend their own pulmonary rehabilitation program. In some cases, financial constraints and/or lack of third-party payments may require substitution with a home-based exercise program. No matter how it is achieved, patients on the lung transplant waitlist must remain as active as possible and their participation in exercise programs needs to be monitored.

Medical monitoring and treatment while listed for lung transplant

Although the time from listing to transplant under the LAS system has decreased to a median of a few months [29,30], a significant number of patients have more extended waiting times for a variety of reasons. Thus, it is important to have monitoring programs in place to update patients' LAS over time, detect new medical or psychosocial problems, and determine if patients continue to meet the criteria for lung transplantation. Patients and caregivers should understand that the LAS, similar to the Model for End-Stage Liver Disease (MELD) score used to rank liver recipients, is a dynamic score that changes in response to changes in the patient's medical status. Several factors in the LAS that strongly influence the score are the degree of oxygen use at rest and the 6MWT distance. Hence, it is essential that any change in these factors is reported to the transplant center so that the most recent values can be entered into the allocation system. Three factors in the LAS are valid for 6 months; after such time, new testing is required. These are the forced vital capacity, 6MWT distance, and serum creatinine. The infrastructure of a lung transplant program should be competent to make sure these clinical values remain current.

The clinical monitoring and testing for waitlisted patients is relatively intensive. Testing used to assure continued suitability for transplantation may include pulmonary function testing, arterial blood gases, serum creatinine, and bilirubin among other routine labs; sputum cultures in patients with cystic fibrosis (CF) or bronchiectasis; testing for panel reactive antibodies (PRA) against human leukocyte antigens (HLA); chest CT imaging at roughly 6-month intervals in patients at risk for developing malignancy [chronic obstructive pulmonary disease (COPD)/IPF] or fungal infections (CF/sarcoidosis); repeat 6MWT measurements; and repeat transthoracic echocardiogram in patients with pulmonary hypertension and possible repeat right heart catheterization if pressures are rising. Newer data are used to update the LAS as required by UNOS.

During outpatient visits at the transplant center, issues of weight gain or loss can be addressed as both severe malnutrition and obesity are risk factors for adverse outcomes following transplant [31]. Documentation of participation in a pulmonary rehabilitation program can be confirmed, along with general medical compliance and any changes in the patient's psychosocial support and insurance coverage. Random screening for drugs and/or nicotine abuse may be employed in patients considered at risk, with positive findings perhaps being cause for removing such patients from the waitlist.

It is not uncommon for patients to experience anxiety and/or depressive symptoms while waiting for transplant and such issues should be explored and dealt with, either with medication and/or referral to a psychiatrist or psychologist. Depression is associated with increased mortality while awaiting lung transplant [32]. Some transplant programs require relocation of lung transplant candidates to their local area prior to transplantation, which may engender additional hardships on the patient and family, with the disruption leading to further risk of depression. Therefore, it is essential that programs have clear center policies regarding minimal distance from the transplant center and that these policies are communicated early in the pretransplant process.

Adequate control of co-morbid medical problems such as hypertension and diabetes mellitus are assessed during the initial and follow-up visits while on the waitlist. Osteoporosis and vitamin D deficiency are quite common among patients with various lung diseases [33,34], so monitoring with serial bone density scans, along with treatment with calcium, vitamin D, biphosphonates, and/or other agents are employed [35] in waitlisted patients with decreased bone mineral density.

Lung transplant programs also need to establish mechanisms to ensure continued suitability for transplantation of potential recipients on the waitlist. In the US in 2011, roughly 500 patients were removed from the waitlist without having received a transplant. About 300 of these patients were removed for either being too healthy or too sick to undergo transplantation. These data underscore the fact that transplant suitability remains an ongoing question that needs close monitoring. There are no clear guidelines specifically directing how such monitoring should occur, but several lines of broad consensus exist. For example, most believe that sicker patients with a high LAS (>40) should be seen more frequently than more stable patients. Patients with a high LAS, particularly if the patient is deteriorating, should be seen at no longer than a monthly basis. Stable patients with COPD are seen at no longer than 3-month intervals. An additional strategy to ensure suitability for transplantation is to maintain weekly contact with waitlisted patients using methods such as a phone call or email. A second area of broad consensus is that hospitalized

waitlisted patients require a greater level of scrutiny. Most US centers have either an implicit or explicit minimal 6MWT distance that a patient must be able to cover to be considered appropriate for transplantation. A hospitalized patient experiencing an acute deterioration may fall below this threshold and in such cases, a determination may be made to accept donor lungs outside of standard inclusion criteria.

A continuing area of controversy is to what extent lung transplantation should be offered to patients who require either mechanical ventilation and/or extracorporeal membrane oxygenation (ECMO). Registry data from the International Society for Heart & Lung Transplantation (ISHLT) indicate that patients transplanted on mechanical ventilation have a significantly decreased odds of 1-year post-transplant survival [4]. Nevertheless, case series have demonstrated the feasibility of transplantation in very select individuals with results comparable to historical controls [36–38]. A common theme permeating these cases is that the majority of the patients are relatively young and without medical co-morbidities. Hence, if mechanical ventilation is to be used as a bridge to transplantation, one of the guiding principles might be to utilize it for patients in whom the odds of post-transplant survival are relatively high. While there is some uncertainty about transplantation in ventilated patients, the experience with ECMO is even less clear. This may be partly due to the fact that ECMO technology has accelerated rapidly over the past several years. This evolution encompasses both technological advances as well as new clinical decision-making approaches. Regarding the former, the use of self-contained small ECMO circuits, which require much less intensive hands-on technical support and utilize novel bi-lumen cannulas, may ultimately result in a type of ECMO delivery that can practically be tested on large groups of patients for efficacy. Regarding the latter, some clinicians have recommended the use of ECMO as upfront therapy for patients with end-stage lung failure rather than relying on ECMO as a therapy of last resort. The theoretical advantage to upfront ECMO is that it may allow for muscular conditioning and continued oral feeding with less risk of aspiration, thereby optimizing a potential recipient when donor lungs do become available. Several small case series from Europe using ECMO as a bridge to lung transplant have demonstrated the feasibility of this approach; long-term follow-up studies on large groups of patients are still lacking.

Overcoming impediments to lung transplant

There are several factors that may cause certain lung transplant candidates to wait longer than others for a donor lung. In such cases, it may be in the patient's best interest to consider listing at more than one transplant center ("dual listing"). Generally, *dual listing* involves being placed on the lung transplant list of two or more centers, where each center is "local" at a distinct OPO. Such listing effectively allows the recipient to be considered a local recipient on more than one list, thereby increasing the odds of finding a donor. Prior to dual listing, it is essential that the patient and all centers involved agree upon which center will manage the patient's care postoperatively. In most cases short- and long-term management will continue to be the responsibility of the transplanting center as the patient's survival is only counted in the statistics of the transplanting center.

Another common factor limiting individual patients' ability to receive a donor organ in a timely manner is the presence of *anti-HLA antibodies* against prospective donors. This factor limits the acceptable donor pool, thus increasing the time on the waitlist. The traditional approach to preventing acute rejection of a lung allograft by such a sensitized recipient has been to perform a prospective cross-match using recipient serum and donor cells. However, logistical issues and time constraints render such an approach impractical in many cases, thus increasing time on the waitlist. This problem has been addressed by the use of a "virtual cross-match" [39,40]. In essence, pretransplant testing will define the donor-specific class I and class II anti-HLA antibodies. During the donor evaluation process by the OPO, the donor HLA antigens will be defined, allowing the transplant team to determine if the potential recipient has antibodies specific to the donor HLA. The donor lung offer may be accepted or rejected on the basis of these data. There are many caveats to this approach, but the virtual cross-match does offer one way to address the lengthy wait for donors in sensitized patients.

A useful tool to ascertain the chances of finding a suitably matched donor is the CPRA calculator in which unacceptable antigens are entered into a computerized algorithm. A database of over 12 000 previous patient alleles is then run against these unacceptable antigens to determine the odds that the next organ will be free of any unacceptable HLA epitopes. This value can then be used to counsel patients on the chances that they will receive a transplant, as well as to decide in which patients some type of intervention must be undertaken. For example, some programs attempt to remove anti-donor HLA antibodies at the time of transplant with plasmapheresis and intravenous immunoglobulin G (IVIG) [41], or attempt to decrease antibody production in the recipient using various means prior to transplant, a process known as desensitization, which is covered in Chapter 68 [42]. However, none of the approaches to date has been sufficiently studied with long-term follow-up to be considered the accepted approach to managing the sensitized lung transplant patient. Dual listing with another transplant center(s) may also be considered in cases of very high pretransplant anti-HLA antibodies.

Another reason lung transplant candidates may not receive a donor quickly is *small recipient size*. In selected individuals, such as small CF patients, a living donor lobe for lung transplantation may be an option [43], although the clinical experience with this technique is limited to a few centers. Alternatively cadaveric donor lungs may require reduction surgery to fit the small chest of the recipient. Again, in situations where recipient size/geometry issues are predicted to increase waitlist time, dual listing may improve the patient's opportunities for receiving a donor.

Patients may experience clinical worsening while awaiting transplant. If the worsening is due to the development of a superimposed infectious process, e.g. pneumonia, the patient may be temporarily inactivated until the infection has been adequately treated. If clinical worsening results from progression of the underlying disease process, the LAS will be increased and the patient may receive a donor. However, various measures are taken to stabilize and/or improve the status of such patients, including short-term courses of corticosteroids, increased oxygen delivery, and change in oxygen delivery method to either face mask or bi-level positive airway pressure (BIPAP), delivery via a transtracheal catheter or even intubation with mechanical ventilation [44]. As noted above, select patients with profound ventilatory failure have even been supported with ECMO [45–47] until transplant could be accomplished. This and other artificial lung devises are discussed in depth in Chapter 50. Obviously, such critically ill patients have a high mortality [48] while awaiting transplant and higher morbidity and cost immediately after transplant [5,6].

Since the implementation of the LAS system, both waiting time and waitlist mortality have decreased [1], partly as a result of increased organ availability [4]. Nonetheless, there remain insufficient numbers of suitable donor lungs and individuals continue to die while awaiting lung transplant. One promising approach to increasing the number of donor lungs for transplantation is by subjecting lungs of questionable quality to normothermic *ex-vivo lung perfusion* (EVLP; covered in depth in Chapter 26) [49]. After 4 h of EVLP, a high percentage of such lungs was successfully transplanted with excellent short-term results. In the US, studies are underway to gain approval from the Food and Drug Administration (FDA) for implementation of the EVLP device and related materials in clinical lung transplantation practice.

Removal from the lung transplant waitlist

There are several ways in which patients may be removed from the lung transplant waitlist:

1. Patients may be temporarily inactivated due to intercurrent illness, travel, or change in psychosocial circumstances, and then reactivated once these issues are resolved.
2. Patients may be permanently removed from the list because of proven relapse of substance abuse.
3. Patients may be deemed to have progressed to the point of no longer being suitable candidates, e.g. overwhelming infection with septic shock and multiorgan failure syndrome.
4. Rarely, some patients may respond to simultaneous medical therapy and be deemed too well for transplant.
5. The patient may change his/her mind and decide to forego the option of lung transplantation.
6. When suitable organs become available and the patient undergoes the transplant procedure. This is the goal of the entire transplant team and we rejoice with the patient and family whenever this occurs.

Summary

Patients with end-stage pulmonary failure are critically and progressively ill, and require relatively intensive medical monitoring while on the waitlist for a lung transplant. While the recent introduction of lung allocation algorithms has helped to standardize the management of patients awaiting lung transplant, there still is regional heterogeneity in waiting times that relates to local practice patterns and organ supply. The time spent waiting for a suitable organ should be used as far as possible to optimize the cardiopulmonary reserve of the recipient, to immunologically prepare the patient through vaccination and avoidance of sensitizing events, and to educate the patient of the rehabilitation and drug therapy to come.

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CHAPTER 41

Waiting List Management for Pancreas and Islet Transplantation

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Introduction

Numerous pancreas and islet transplant options are available for patients with uremic and non-uremic diabetes. Depending on the center, one or more of these options may be available to patients and all relevant options should be discussed. For uremic patients, simultaneous pancreas–kidney (SPK) transplantation and sequential pancreas after kidney (PAK) transplantation are the principle options unless a center also offers islet transplantation, in which case options including simultaneous islet–kidney (SIK) or more commonly islet after kidney (IAK) transplantation may be offered. For non-uremic patients, the principle options are pancreas transplant alone (PTA) or islet transplant alone (ITA), the latter being restricted to approved clinical trials. For more details on pancreas and islet transplant options see Chapter 32, which covers patient selection and indications for pancreas and islet transplantation. Which one of these various options is ultimately recommended by the transplant center can impact the patient's waiting time. Conversely, the anticipated waiting times can influence which option is chosen. Important factors affecting the decision as to which option to recommend for a particular patient include: anticipated waiting time for a kidney transplant on either the SPK or kidney-alone lists, whether a patient is uremic and currently on dialysis, predialysis or has normal kidney function, allosensitization status, cardiovascular status, pancreas allograft outcomes at a particular center, anticipated pancreas waiting time for different pancreas transplant options, whether an experimental islet transplant program is available, and patient preference. As a consequence of the existence of multiple transplant options for replacing lost beta-cell function in patients with diabetes, patients can be listed on one of several different waiting lists.

The waiting list for patients with diabetes is not a single list but often encompasses multiple distinct lists. For example, at some centers uremic patients with diabetes wait on the same waiting list with other non-diabetic uremic patients, whereas at other centers they wait on a separate SPK waiting list. Patients waiting for a solitary pancreas allograft (PAK and PTA recipients) are often listed together, but on a separate waiting list from those waiting for an SPK transplant. Islet transplant recipients are also usually listed separately on a third waiting list. For uremic diabetic patients, dual listing options (i.e. kidney alone or SPK) are also available. Thus, the decision during the evaluation process to place a particular patient on specific waiting list can be a complex, multifaceted process, yet the management of these patients, *once on the waiting list*, is similar regardless of the waiting list.

This chapter will focus on key health maintenance issues and the frequently changing medical status of patients with diabetes with and without chronic kidney disease (CKD) awaiting transplantation. Among the most important health maintenance issue is cardiovascular disease evaluation and monitoring. This chapter will also outline factors affecting waiting time, and how expected waiting time can influence what type of pancreas transplant is recommended for a specific patient. Finally, the pancreas allocation system in the US will be undergoing changes in the coming years and we will discuss the limitations of the current system and how the new allocation system will hopefully improve allocation efficiency and organ utilization.

Monitoring and maintaining patient's active status on the waiting list

Multitude of medical issues and frequently changing waiting list status

Patients with type 1 diabetes mellitus (T1DM) presenting for transplantation commonly have numerous medical problems, which require close attention and work-up to manage risks appropriately. Common pretransplant medical problems include coronary artery disease, peripheral vascular disease, retinopathy, peripheral and autonomic neuropathy, gastroparesis, hypoglycemia unawareness, or other autoimmune diseases that can result in acute illnesses potentially delaying transplantation. Due to these multiple chronic medical problems and the acute illnesses they may contribute to, actively listed patients frequently need to be placed on hold/inactive status. In fact, it is not uncommon for a patient to be inactive for more than a year. A snapshot look at the United Network for Organ Sharing (UNOS) waiting list for pancreas transplantation indicates that at any one time nearly 50% of all pancreas waitlisted patients are on inactive status [1]. For example, at the end of 2009, approximately 3500 patients were on the waiting list for all types of pancreas transplants, while only 1600 of these were considered active. Within Eurotransplant, 40% of patients on the pancreas transplant waiting list at the end of 2010 were considered not transplantable (i.e. inactive status) [2]. Currently in the US, patients on inactive status (i.e. status 7) can still accrue waiting time points.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

The frequently changing medical condition and waiting list status of diabetic patients awaiting pancreas transplantation poses a significant challenge to transplant centers. One of those challenges is communication. As patients are frequently shifted back and forth between active and inactive status as acute illnesses arise and are resolved, it is essential to maintain an open line of communication with patients and their referring physicians. This communication involves retrieving new records, undergoing repeated evaluations, holding repeated discussions at multidisciplinary candidate selection conferences, and updating the patient's UNOS registration status. As important as communication is between referring/primary care physicians and the transplant center, it is equally important for the transplant center to communicate the patient's status to the patient. A recent survey study of dialysis patients revealed that more than half of patients who were undergoing pretransplantation work-up were unaware of their true listing status [3]. Nearly 89% of these patients who completed the Dialysis Patient Transplant Questionnaire and were unaware of their true listing status mistakenly believed they were listed and 11% were unsure of their current status. Among waitlisted patients, most correctly identified themselves as listed; however, 6% mistakenly thought they were not listed and 12.5% were unsure. Importantly, all of the waitlisted patients who were unaware of their listing status were listed as inactive (i.e. status 7), indicating that they either had developed an intercurrent illness or required additional follow-up testing. Although this single-center study involving predominantly African–American dialysis patients and its findings remain to be validated for other ethnic groups or in the diabetic population, the finding of a substantial lack of listing status awareness emphasizes the need for better communication between the transplant program, patients, and their referring physicians. Thus, initial waiting list status, and all changes subsequently, should be communicated promptly in writing to patients.

The continually changing status of many patients requires a proactive approach to waiting list management, which is labor intensive, yet critical to maintaining patients ready for transplantation. Common problems affecting this diabetic population that often result in a patient being placed on inactive status include a recent cardiac event or need to complete or update cardiac testing, foot infections and/or ulcers, amputations, vascular disease interventions, eye surgeries, pneumonia, and dialysis catheter-related infections, among others. Patients are typically managed by their endocrinologists, nephrologists, or primary care physicians at distant locations and communicating changes in a patient's medical condition to the transplant center is not always seamless. Yet, it is important to avoid the presence of active medical problems at the time of an organ offer. Failing to place a patient on hold (i.e. *inactive* status), delaying treatment of his/her problems, or failing to recognize a worsening medical condition can lead to a patient not being suitable for transplant at the time of an organ offer. If a patient remains active on the waiting list despite ongoing acute medical issues and receives an organ offer, the transplant may need to be canceled, which potentially prolongs the cold ischemia time, or alternatively, a decision is made to proceed with the transplant, placing the patient at unnecessary or unrecognized risk. Thus, despite the challenges of managing a patient remotely, it is in the transplant center's best interest to proactively communicate the need to receive medical condition updates from the patient and his/her referring physicians. Consequently, many centers strive to re-evaluate patients annually and communicate with patients frequently in order to continually assess their medical status and readiness for transplantation. Whereas this re-evaluation process for very large liver or kidney transplant waiting lists may need to be prioritized to focus on only those patients with the longest waiting times, the significantly shorter SPK and solitary pancreas waiting lists at most centers allow most of the waiting list to be addressed. The facts that there are fewer patients on pancreas waiting lists and that SPK recipients are often waiting on the same list as non-diabetic uremic patients further accentuate the need for keeping the patients' medical condition optimized in order to maximize pancreas allocation efficiency and transplant rates.

Factors affecting waiting time

Transplant physicians are often asked by patients and referring physicians: what is the expected waiting time for a pancreas transplant? The answer depends on many factors including transplant center/region and degree of sensitization. Whether the waiting time is anticipated to be short (3–6 months), moderate (6 months to 2 years, or relatively long (>2 years) can influence not only how a patient is clinically managed in the pretransplant period, but may also impact what transplant option is recommended to the patient, so this is not a question to be considered lightly. Thus, knowledge of the key factors affecting waiting times allows transplant clinicians to estimate time to transplant and to formulate local health maintenance and monitoring plans to ensure that suitable candidates are ready for transplantation, thereby minimizing last minute cancellations.

Waiting time varies markedly among regions in the US, particularly for SPK transplants, and cannot always be predicted accurately. A chief determinant of waiting time for SPK recipients is whether these patients are listed with the non-diabetic uremic patients or whether they are listed separately. Those centers where SPK recipients are listed separately generally have shorter waiting times. A UNOS analysis revealed shorter waiting times for separately listed SPK candidates compared to SPK candidates listed on a combined kidney and SPK waiting list (median waiting time: 177 days vs. 645 days, respectively among all SPK candidates, and 144 days vs. 486 days, respectively among non-sensitized dialysis-dependent SPK candidates) [4]. Within a particular center or donor service area, it is easier to predict waiting times, yet these can vary as well and depend on recipient blood type, ethnicity, type of transplant, and degree of allosensitization. These patient-specific factors will be considered below in more detail.

Time on the pancreas transplant waiting list has gradually increased over the last decade in the US [1]. Compared to 1998 when nearly 60% of patients waited <1 year, now only 40% of patients are transplanted within 1 year of listing. Similarly, a decade ago only 1–2% of patients waited >5 years for a transplant, but now approximately 10% wait that long. This gradual increase in waiting time correlates with increasing numbers of inactive patients on the waiting list and an increase in retransplants and sensitized patients who tend to wait longer due to difficulty in finding a suitable match. The longer waiting times correlate with a significant decrease over the last decade in the US in the transplant rates among adult patients waitlisted for a pancreas transplant [1]. For all pancreas transplants taken together, transplant rates were approximately 60 per 100 patient-years in 1998, but have fallen to just below 40 per 100 patient-years in 2009. The fall in transplant rate over this period was greatest for PAK transplants, declining from a rate of approximately 72% in 1998 to only 20% in 2009 [1]. In contrast, the SPK transplant rate has only declined marginally over this period from approximately 60% to 48%.

What is the expected waiting time in the US for a pancreas transplant? The median waiting time for a transplant for *actively listed* candidates from 2005 to 2009 was 7.0 months for PTA, 11.5 months for SPK, and 12.8 months for PAK [1]. Naturally, waiting times for patients registered as inactive at the time of listing are significantly longer than those for active patients. For example, inactive PTA patients have a median waiting time ~40 months, SPK patients ~25 months, and PAK patients >60 months. The longer recorded waiting times for PAK patients may in part be due to the fact that some centers activate uremic T1DM patients on the PAK waiting list at the time of the living donor kidney transplant in case a deceased donor pancreas becomes available at that time, thus artifactually extending their waiting time. Regardless of the type of transplant, the median time to pancreas transplant in the US showed significant geographic variation [1]. Of the three pancreas transplant types, PAK patients have a marginally higher likelihood of still awaiting a transplant after 3 years in the US. For example, at the end of 3 years, 20.0%, 16.1%, and 24.9% of patients were still awaiting PTA, SPK, and PAK transplants, respectively. The increasing waiting times for PAK transplants correlate with the fact that in the US, fewer centers are performing PAK transplants in 2009 compared to 2004. In contrast, over this time period, a steadily increasing number of centers are performing PTA transplants, while the number of centers performing SPK transplants has remained stable.

The median time to pancreas transplant has unfortunately increased in the past decade in the US [1], in part due to the reduced probability that a donor will provide a suitable pancreas graft. The number of pancreata recovered and transplanted per donor has declined steadily since 2002 when it peaked at 0.3 and 0.24, compared to 0.21 and 0.15, respectively, in 2009. The reason for this decline is likely the increasing age, obesity, and diabetes prevalence of the US donor population. In parallel, pancreas discard rates have increased in all age groups since 1998. Despite an increasing percentage of pancreas grafts recovered from donation after circulatory death (DCD) donors across the US and an effort by Organ Procurement Organizations (OPOs) to place the most suitable pancreas grafts, pancreas recovery and transplant rates (i.e. utilization rates) across the US remain highly variable and significantly lower than for kidneys and livers.

Mortality rates and the onset of new medical problems precluding transplant in waitlisted patients affect estimates of whether a patient will receive a transplant. Regardless of the type of pancreas transplant, the overall rate of removal from the list for death or medical reasons tends to be similar [1]. Over 3 years in the US, the rate of removal tends to be up to 20% of patients, again emphasizing the need for frequent monitoring and repeat evaluation. PAK and PTA patients see the highest rates of removal from the list for medical reasons, whereas SPK waiting list patients see the lowest rate of removal for these reasons. On the other hand, SPK patients have the highest mortality on the waiting list of any type of pancreas transplant waitlisted patients, which is a consequence of the combination of uremia and diabetes-related mortality. By 3 years, 3.3% of PTA waitlisted patients had died, as had 9.4% of those listed for SPK and 2.5% of those listed for PAK. This significantly higher mortality in uremic T1DM patients awaiting an SPK transplant coupled with the long SPK waiting times in some US regions is an important consideration when deciding on SPK versus living donor kidney transplant followed by a PAK for this patient population (also see section below).

ABO blood type, sensitization, retransplantation, and ethnicity factors

Waiting time also depends on ABO blood type, degree of HLA sensitization, and retransplant status (covered in detail in Chapter 36) [1]. In the US, patients with blood types O and B wait nearly twice as long as those with blood types A and AB (i.e. median of ~20 months compared to ~10 months in 2009, respectively), a gap which has widened for unknown reasons in the last decade.

Allosensitization of the recipient is a risk factor for a longer waiting time to transplant for all types of pancreas transplants, just as it is for kidney transplants. The proportion of non-sensitized pancreas recipients on the waiting list has decreased over the past few years as the number of retransplant candidates has increased [1]. Additionally, the increasing use of sensitive methods of detecting HLA antibodies, such as Luminex® single bead assays, has probably led to increasing percentages of sensitized patients. Patients who are moderately to highly sensitized [20–79% calculated panel reactive antigen (cPRA)] witness a significantly longer waiting time than less sensitized (<20% cPRA) or non-sensitized patients. For highly sensitized uremic diabetic patients, the clinician should especially consider the several options available to these patients. The best option may be the one that corrects their CKD most expeditiously, thereby reducing excess mortality of this population while on dialysis. One option is SPK transplantation from a deceased donor without waiting list desensitization, which has an expected longer than average waiting time. Another is waiting list desensitization followed by SPK or deceased donor kidney transplantation, although there are logistical challenges with this option. A third is a living donor kidney alone via the National Kidney Registry or local donor exchange programs, if an ABO incompatible or cross-match positive living donor is available. Finally, consideration should be given to dual listing for either a deceased donor SPK *or kidney-alone* transplant, whichever becomes available first and it may also be recommended to consider multiple listing at centers in different donor service areas. An analysis of the UNOS kidney waiting list revealed that multilisted patients experienced 50% reductions in median waiting times and correspondingly higher transplant rates regardless of the demographic, biological, and socioeconomic status of the recipient [5]. Whereas multiple listing at centers with significantly shorter waiting lists has been shown to be beneficial, multiple listing at a second center with similarly long waiting times may not be fruitful [6]. Similar data related to pancreas transplantation are not presently available; however, it is likely these data can be conceptually extrapolated to pancreas transplantation.

There is currently little difference in waiting time when stratified by race in the US, although in the past black/African–American recipients experienced longer waiting times [1].

Transplant options for patients with chronic kidney disease and type 1 diabetes mellitus

For a uremic T1DM patient, there are several pancreas and kidney transplant options [7]. Chief among them is either an SPK transplant or a living donor kidney transplant followed by a deceased donor pancreas allograft. Although these are not the only options, remaining on dialysis or receiving a deceased donor kidney allograft alone are suboptimal outcomes with substantially higher associated mortality in otherwise stable patients with few co-morbidities [8–10]. A sequential PAK transplant involves two

operations and organs from two different donors, each of which requires monitoring for rejection, and generally has worse pancreas allograft survival (3-year pancreas graft survival: PAK 68% vs. SPK 78%) [1]. However, the PAK option has the potential benefit of providing rapid reversal of CKD by virtue of the patient receiving a living donor kidney transplant usually in a very short timeframe. On the other hand, the SPK transplant from the same donor affords the patient simultaneous correction of both kidney disease and diabetes with a single operation and monitoring for rejection focused on one donor, while affording the patient better pancreas graft survival and very similar kidney survival compared to living donor kidney survival [7].

Which of these options should be offered to a patient largely depends on the anticipated waiting time for an SPK transplant in a particular locale. What is the rationale? Given the very high mortality associated with the combination of CKD and diabetes, and the documented strong patient survival benefit of a kidney transplant, such patients cannot afford to wait many years for a kidney transplant due to the expected high waiting list mortality. With long waiting times at some centers in the US and around the world where SPK recipients wait on the same list with the many non-diabetic CKD patients, the primary goal of receiving a successful kidney transplant can best be accomplished with a living donor kidney. Thus, if the waiting time for an SPK transplant is anticipated to be long (perhaps >1–2 years), for either center-specific or patient-specific reasons, then a living donor kidney-alone transplant may be a very beneficial option. In other cases where the SPK waiting time is predicted to be shorter, then the SPK option has advantages over a PAK. The aforementioned recommendation of an living donor kidney followed by a PAK assumes a patient has a living donor kidney option. If not, then an SPK transplant is clearly a better long-term option associated with improved long-term patient survival and better quality of life compared with a deceased donor kidney transplant alone [9].

A few other special considerations that may modulate the relative benefits of each option are worth mentioning. If an HLA-identical living donor kidney is available, then a living donor kidney and sequential PAK option is perhaps a favored choice as HLA-identical kidneys are routinely associated with low rejection rates and significantly longer life span [11]. On the other hand, if the diabetic patient also suffers from impaired awareness of hypoglycemia and frequent hypoglycemic episodes, then there may be a more compelling reason to consider an early pancreas transplant and the simultaneous transplant option. The case of the highly sensitized uremic diabetic patient also merits consideration. Normally, this patient if lacking a living donor for a kidney transplant would be placed on the SPK waiting list because of the added benefits of the dual transplant versus a deceased donor kidney transplant alone. However, as highly sensitized patients can only receive a transplant from a subset of well-matched donors and only a subset of donors (15 of 100 donors on average in the US) provides a suitable pancreas graft, these patients can wait a long time for an SPK transplant. In this circumstance, it may make sense to consider discussing with the patient his/her placement on the kidney-alone waiting list in addition to the SPK list, and the option of accepting a well-matched kidney transplant alone if one becomes available from a donor whose pancreas is not considered transplantable. Finally, for those patients who do not have a suitable living donor matched to themselves, the National Kidney Registry provides an additional avenue to receive a living donor kidney.

Frequency of monitoring

Most centers will re-evaluate recipients on the waiting list annually or every 2 years at a minimum. A survey of transplant programs in the US indicates that 71% of centers attempt follow-up monitoring of all waitlisted kidney transplant candidates by either scheduled appointments or telephone contact [12]. Timely updating of a patient's cardiac, immunological, psychiatric, infectious disease, and malignancy status is essential to avoid non-transplantability at the time of an organ offer or increased perioperative morbidity if such problems are ignored and the transplant proceeds. At the University of Wisconsin, we aim to re-evaluate all patients on the kidney and pancreas transplant waiting lists who have had significant changes in their medical status, significant weight gain, or who have been on the waiting list for more than a year.

Centers typically collect sera monthly for cross-matching and for anti-HLA antibody testing. At the University of Wisconsin, we collect blood samples for Luminex® anti-HLA antibody testing monthly and perform quarterly testing in sensitized patients and semi-annually in non-sensitized patients or after any potentially sensitizing event such as blood product transfusion, pregnancy, or transplantation.

Cardiac screening

The combination of T1DM and CKD places this population at the highest risk for advanced ischemic cardiac disease and unrecognized cardiac events among all patients with CKD. At a minimum, frequent cardiac stress testing is necessary before and after transplantation; however, a strategy relying only on non-invasive stress tests has been challenged [13]. Indeed, diabetic uremic patients exhibit a "perfect storm" of cardiac disease risk and this impacts the need for aggressive cardiac disease screening. The rationale for aggressive cardiovascular disease screening is based on the following observations: (1) a high prevalence of cardiac disease [14,15]; (2) cardiac ischemia and infarction are commonly silent [16]; and (3) a high false-negative rate for stress tests [13,17–19]. For example, Vandenberg et al. performed non-invasive testing (either stress thallium scintigraphy or exercise radionuclide ventriculography) as well as coronary angiography in 47 asymptomatic diabetic transplant candidates. The sensitivity for detecting 75% or greater coronary artery stenosis was 62% for stress thallium scintigraphy and 50% for radionuclide ventriculography [13]. In another prospective study, dobutamine stress echocardiography had a sensitivity of 75% and a specificity of 76% [17]. Selek et al. showed that in diabetic uremic patients being evaluated for transplantation, community performed cardiac stress testing had a sensitivity of 27–47%, whether this was an exercise nuclear stress test, pharmacological stress test, or stress echocardiography [19]. Together these studies and clinical presentation dictate a frequent need for coronary angiography in patients with diabetes for cardiac screening and clearance prior to activating patients on the SPK or kidney-alone waiting list. The benefits of performing coronary angiography as the gold standard cardiac screening test in this high-risk population are further highlighted by the fact that it is not rare to witness SPK candidates dying on the waiting list due to a cardiac event despite a recent previous negative nuclear or treadmill stress test. Also, after SPK transplantation, the most common cause of death is related to a cardiovascular one [9]. Furthermore, SPK recipients exhibit a defined incidence of post-transplant cardiac ischemic events despite negative pretransplant stress tests; as high as 20% in one study [17]. Thus, at the author's

institution the majority of diabetic patients being evaluated for transplantation undergo coronary angiography.

The preference at many major pancreas transplant centers in the work-up of these uremic predialysis SPK candidates is coronary angiography regardless of the stress test result, especially if age >45 years, diabetes duration >25 years, history of tobacco use, or history of cardiac disease prior to waitlisting. It is hoped that such a policy will positively impact the frequency of perioperative cardiac events. This policy is not an issue for those patients already on dialysis at the time of referral. However, in the predialysis patient, a coronary angiogram is associated with some risk of progressive kidney dysfunction and could potentially precipitate need for dialysis initiation, thereby creating a clinical conundrum. Data clearly indicate the benefits of predialysis referral of patients for kidney transplantation and the better associated patient and kidney graft survival [20]. One would expect that this finding can be extrapolated to patients with T1DM and kidney disease as well, given the high mortality rate of patients with diabetes on dialysis. Hence, it seems important to continue to recommend referral of patients for transplantation while they still have early-stage kidney disease. Given the kidney failure risks, centers often selectively perform cardiac catheterization or avoid it altogether in predialysis patients. In this circumstance, patients can either undergo coronary angiography without ventriculogram and/or with renal protection, especially if their creatinine is still relatively low. Alternatively, patients can be added to the SPK waiting list with only a negative stress test result and coronary angiogram deferred until the patient initiates dialysis. Patients can be registered as inactive status on the UNOS waiting list, accrue waiting time, wait until beginning dialysis to undergo their screening coronary angiography, and then be activated for transplantation.

If coronary angiography demonstrates lesions >75%, patients should probably undergo revascularization with angioplasty and stent placement or coronary artery bypass. Manske et al. studied 26 asymptomatic diabetic patients who were found to have >75% coronary stenoses and randomized them to revascularization pretransplant versus medical therapy alone [18]. Patients randomized to medical therapy suffered significantly more post-transplant cardiac events than did patients who underwent interventional therapy.

In summary, the existing data on cardiovascular screening of diabetic uremic candidates for transplantation indicate it is beneficial to take an aggressive stance toward diagnosis and corrective intervention prior to transplantation.

Peripheral vascular screening

When evaluating diabetic patients for pancreas and/or kidney transplantation, it is essential to directly determine the extent and bilaterality of iliac disease as vascular inflow is a key determinant of allograft function. The presence or absence of iliac disease should be the focal point rather than the degree of distal lower extremity disease, which is nearly universally present in this population. Numerous different vascular imaging studies have been used to assess these patients over the years; however, few are better than physical exam. Doppler pulse volume recordings of the lower extremity are notoriously inaccurate due to the non-compressibility of heavily calcified blood vessels, and lower extremity angiograms do not fully assess the region of interest, namely the iliac arteries bilaterally. Plain abdominal X-ray can observe vascular calcifications in the iliac system but is not sensitive enough. The presence of lower limb amputation, particularly if for osteomyelitis, does not necessarily indicate disease involvement of iliac arteries and therefore, does not preclude transplantation. Probably the best indicator of suitable vessels for implantation of one or more allografts is a good femoral pulse exam and/or abdominal/pelvic computed tomography (CT) scan without intravenous contrast. The optimal frequency of vascular screening while on the waitlist has not been determined, yet the interim development of advanced bilateral or severe unilateral disease probably merits re-evaluation of SPK candidacy.

Miscellaneous

Some patients in unique situations may or may not be amenable to receiving organs from Centers for Disease Control and Prevention (CDC) high-risk or hepatitis C-positive donors. Local allocation and transplantation policy should dictate if and how these organs are allocated. Local listing should clearly indicate whether or not a patient is a candidate (willing) to receive a pancreas from a CDC high-risk or hepatitis C-positive donor and this patient choice may change over time while waiting. At the author's institution, the policy is to consider hepatitis C-positive donors only for those SPK candidates who are hepatitis C carriers, the rationale being the same as for allocation of a kidney transplant from a hepatitis C-positive donor to such patients. With regard to allocation of CDC high-risk donors, only those recipients who have consented are eligible and only donors whose nucleic acid testing (NAT) is negative are considered. Importantly, at the time of reassessment of the patient's waitlist status, the patient's wishes regarding CDC high-risk and hepatitis C-positive donors should be re-evaluated.

Waitlist removals

Death, transplantation, and the development of uncorrectable contraindications are reasons for removal from the OPTN waiting list within 24 h of the center being made aware of the event.

Current pancreas graft allocation system in the US

In the US, pancreas allografts are allocated to whole organ pancreas recipients first, followed by islet transplant recipients [21]. Pancreata are first allocated locally within the hospital's donor service area (DSA), then regionally within each of the 11 different regions in the OPTN, and then nationally. Highly sensitized potential recipients who have zero HLA mismatches with the donor are offered the pancreas as a priority over highly sensitized patients who are not a zero mismatch, and over other patients on the list. Within each DSA, sensitized candidates are prioritized ahead of other candidates. Patients are then stratified according to waiting time.

For pancreas and SPK offers from other DSAs, centers may opt to identify center-specific criteria that make an organ unsuitable for transplantation. Pancreata from donors aged <50 years and with a body mass index (BMI) of <30 kg/m^2 are allocated first for pancreas transplant and if rejected by all pancreas recipients, are then offered for islet transplantation or research. If the donor age is >50 years or BMI is >32 kg/m^2, then the pancreas is first offered for islet transplantation. This policy was enacted to facilitate utilization of pancreata generally deemed unsuitable for whole organ transplantation and expedite allocation for islet transplant recipients.

Considering only the allocation of pancreata for whole organ transplant, the current pancreas allocation policy has resulted in a system that is heterogeneous and varies by UNOS region across the

US. In some regions, the "pancreas follows the kidney" and SPK candidates await their turn among the many non-diabetic kidney only recipients on the waiting list, while in other regions the "kidney follows the pancreas" and separate SPK and kidney lists exist. Consequently, centers and regions have developed variances or exceptions, resulting in a patchwork, inconsistent allocation policy for pancreata for whole organ transplantation across the US. In part as a result of this, there remains a great degree of geographic inequity in pancreas utilization, access to transplantation, and transplant waiting time.

Moreover, in the current system, there are no specific diabetes-related qualifying criteria for listing patients for pancreas transplantation, other than "diagnosed with diabetes or has pancreatic exocrine insufficiency," which could adversely lead to more and more patients with type II diabetes and CKD receiving SPK transplants. Finally, many centers maintain at least two separate lists for whole organ pancreas transplant recipients: a SPK waiting list and a solitary pancreas transplant waiting list, which could be simplified into a single list for both groups.

Future US pancreas graft allocation schema

By 2011, the UNOS pancreas transplantation committee had formulated a detailed proposal to develop a more efficient, uniform national pancreas allocation system, which was recently approved by the UNOS Board of Directors and awaits implementation. The goal of the proposed new allocation system is to improve the efficiency of pancreas allocation by disentangling pancreas allocation from kidney allocation, as well as maximizing pancreas graft utilization and correcting geographic inequity in the access and waiting time to pancreas transplantation [4].

The new proposed policy seeks to:

1. Combine pancreas-alone and SPK candidates onto a single waiting list, while allowing candidates who are allocated a pancreas from the combined list but who also require a kidney transplant, to receive a kidney independently of the kidney-alone match run if they meet specific qualifying criteria.
2. Institute objective medical qualifying criteria relating to renal dysfunction and diabetes for SPK candidates to accrue waiting time.
3. Allocate deceased donor pancreata separately from the current kidney allocation system so that pancreas candidates are allocated organs that precede kidney paybacks and pediatric and adult kidney-alone recipients [4].

In an attempt to standardize the eligibility criteria and to prevent an excess number of patients with type 2 diabetes mellitus and uremia from being listed for an SPK transplant, SPK candidates who meet the following medical qualifying criteria will be able to accrue time and receive a transplant [4,22]:

- SPK candidates must qualify for a kidney transplant based on the current qualifying criteria. Thus, they must be on dialysis *or* have a glomerular filtration rate (GFR) of $\leq$20 mL/min *or* creatinine clearance of $\leq$20 mL/min.
- With regard to diabetes eligibility, the SPK candidate must meet one of the following criteria: (1) on insulin *and* with a C-peptide of $\leq$2 ng/mL *or* (2) on insulin *and* with a C-peptide of $>$2 ng/mL *and* BMI of up to the maximal allowable threshold of approximately 28–30 kg/m^2. The BMI threshold will be adjusted dynamically by the OPO contractor.
- Listing criteria for solitary pancreas transplantation will remain the same [4].

By design, the proposed changes create a single list for all pancreas candidates, thereby eliminating some complexities in pancreas allocation and establishing a uniform, national system. In addition, candidates for all types of pancreas transplants have an equal opportunity to receive offers for high quality organs. Importantly, it has been determined through modeling that this system retains high quality kidneys within the kidney allocation system in cases in which the pancreas is used for solitary transplant. Moreover, the new allocation schema where the "kidney follows the pancreas" is consistent with the allocation of kidney allografts with other extrarenal organs. It is anticipated that implementation will occur by the Fall of 2014 and that with implementation, the overall waiting times for SPK transplants will be reduced and overall pancreas graft utilization and pancreas transplant rates will improve.

Islet transplant waiting list considerations

Islet transplantation in the US is offered only within the context of Food and Drug Administration (FDA)- and Institutional Review Board (IRB)-approved clinical trials. As such, waiting list management is largely dictated by the clinical trial design or is established by local policy. One key feature of pancreas allocation for islet transplantation stems from the fact that islets from more than one pancreata are often necessary to achieve insulin independence. As it is widely believed that it is beneficial to have a short intervening period between islet infusions from the two or more islet preparations, recipients who have received at least one islet infusion are typically prioritized for a second infusion and can remain active on the waiting list.

Summary

List management for patients with T1DM is complicated by numerous factors, including significant progressive co-morbidities (particularly cardiovascular), numerous options for beta-cell replacement, and the potential need to be placed on numerous lists. Heterogeneity in allocation policy further complicates the process. Careful consideration of the factors most actively influencing the individual patient is required to navigate the challenges arising prior to a transplant. Engagement is required, particularly in the pursuit of cardiovascular disease and silent myocardial ischemia, to ensure that the patient's health is as good as it can be when a deceased donor organ becomes available.

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CHAPTER 42

Waiting List Management for Intestinal and Multivisceral Transplantation

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Introduction

With increased practicality of intestinal transplantation, alone or combined with other abdominal organs, the number of candidates on the United Network of Organ Sharing (UNOS) waitlist has steadily increased over the last two decades [1,2]. Accordingly, establishment of clinical guidelines for proper management of these complex candidates is essential to reduce waitlist mortality and improve overall outcome with optimal utilization of deceased donor organs.

This chapter will focus on the clinical management of the visceral candidate waitlist with special reference to type of required allograft, current UNOS regulation, and risk factors commonly observed among this unique population.

Type of visceral allograft

The nomenclature of the various visceral allografts has recently been well defined [1,3,4]. Patients with intestinal failure or complex abdominal pathology may require the intestine alone or as part of a composite visceral graft that includes other abdominal visceral and solid organs (Figure 42.1). The three main types are isolated intestine, combined liver–intestine, and multivisceral that includes the stomach with and without the liver. When indicated, a colonic segment and/or a kidney are added to the isolated intestinal or composite visceral allograft.

The indications for visceral transplantation are fully outlined elsewhere [5] and in Chapter 30. In brief, organ failure is the primary basis for replacement. However, inclusion of the pancreas with the combined liver–intestinal graft is commonly required to maintain the allograft vascular integrity and continuity of the gastrointestinal tract. With multivisceral transplantation, both the pancreas and stomach are commonly replaced due to extensive parenchymal and splanchnic vascular disorders.

Current UNOS/OPTN regulations and allocation policy

Listing

After a thorough evaluation, the institutional selection committee determines patient candidacy. After the insurer's financial approval, patients are listed according to the UNOS guidelines. Currently, there is no waitlist designated for patients who need composite visceral allografts; as such each organ is listed separately. With logistics similar to those for other abdominal and thoracic organs, the visceral transplant candidates are listed for the same blood type with preference for small size donors due to contracture of the abdominal domain, particularly in patients with short bowel syndrome.

The UNOS categorical status that the visceral transplant candidates are listed for is based upon the type of required allograft. Patients who are in need of a liver-free allograft are listed status 1 in the presence of imminent life-threatening home parenteral nutrition (HPN)-associated complications, including disappearance of the central venous access and progression of the liver injury. Otherwise, patients are listed as status 2. For composite visceral allografts, priority on the wait list is dictated by the UNOS allocation policy for each organ, specifically the liver.

At the present time, the priority on the UNOS waiting list for liver–intestine or full multivisceral grafts is guided by the Pediatric End-Stage Liver Disease/Model for End-Stage Liver Disease (PELD/MELD) scores. Ironically, the combined liver–intestinal candidates were excluded from the PELD/MELD calculation model because of heavy disease gravity and high patient complexity [6]. The recent evolution of the liver–intestine allocation policy and its impact on current waitlist mortality is discussed below.

The current practice of individual listing of the various visceral and solid organs that constitute a composite visceral graft carries significant pitfalls, including the potential to skew many clinical, biologic, and immunologic metrics, particularly when UNOS or Scientific Registry of Transplant Recipients (SRTR) data are used for clinical research that includes outcome measures. We recently observed that some of our composite visceral allograft recipients were categorized in the SRTR report according to one of the contained organs, overlooking the intestine as the central core of the transplanted allograft. Another potential risk is inclusion of the visceral recipients who received the pancreas en bloc with other organs in any clinical outcome research related to pancreas transplantation, as there will be distinctive immunologic and non-immunologic differences [7,8].

Waiting time

Time spent on the waitlist is the product of many clinical and legislative variables. The most significant of these are type of allograft,

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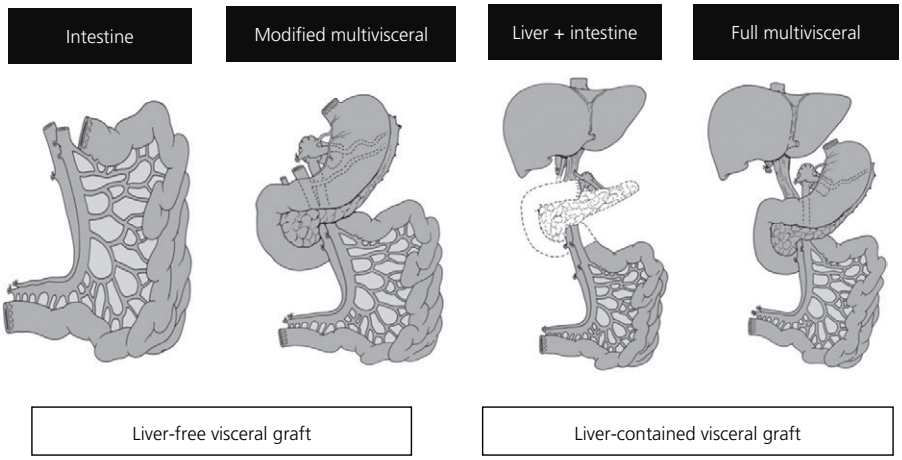

Figure 42.1. Types of visceral allografts: intestine, liver–intestine, and multivisceral. Inclusion of the pancreaticoduodenal complex (unshaded organs) is optional with the liver–intestinal allograft. A multivisceral graft must include the stomach en bloc with the other visceral organs without (modified) or with (full) the liver. The colon, pancreas, and kidney could also be added en bloc to the visceral allograft, with the exception of the liver-free visceral allograft, when the kidney can only be transplanted separately.

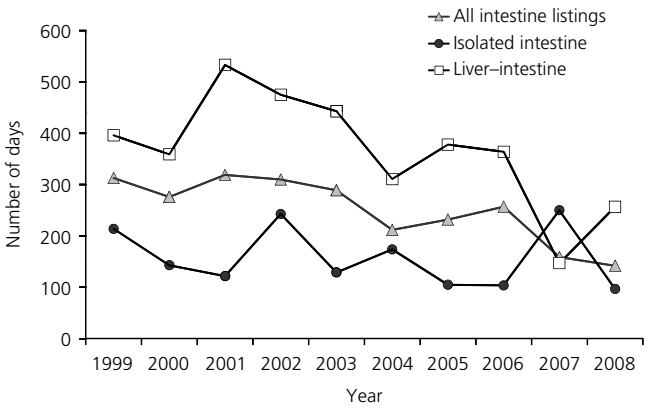

Figure 42.2. Median time to transplant for new intestine United Network of Organ Sharing (UNOS) waitlist registrations, 1999–2008. (Modified from Mazariegos et al. [1], with permission from John Wiley and Sons Ltd.)

status on the waiting list, geographic location of the Organ Procurement Organization (OPOs), and accessibility to regional and national donors. Other determining factors include recipient age, body weight, blood type, and size of abdominal cavity. For patients who are in need of liver-free allografts, the status of cytomegalovirus (CMV) seropositivity and alloantibody presence, particularly donor-specific antibodies (DSAs) when considering transplantation across a positive cross-match, are relevant indices that partly determine the time on the wait list.

In the milieu of the current Organ Procurement and Transplantation Network (OPTN) organ allocation policy, the type of visceral allograft is the major determinant of time from listing to transplantation (Figure 42.2). With the current limited demand for the intestine in isolation, the waiting time for the intestine-only allograft ranges from a few to 250 days [1]. Such a wide range reflects diverse management protocols adopted by different transplant centers, including avoidance of positive cytomegalovirus (CMV) donors for CMV-negative recipients and transplantation of liver-free visceral allografts against a positive lymphocytotoxic cross-match. Other important variables include non-optimal utilization of the intestine by many OPOs, donor geographic location with limits on cold ischemia time (CIT), and stringent donor selection criteria, including young age and hemodynamic stability with no history of significant cardiopulmonary arrest. The pediatric population seems to experience longer waiting times, probably due to body weight and size limitations [1].

The waiting time for patients who are listed for a modified multivisceral transplantation with exclusion of the liver has progressively become protracted over recent years. It has been increasingly difficult to obtain the approval of the liver transplant surgeons nationwide to retain the main celiac trunk with the visceral allograft to preserve integrity of the vascular blood supply to the transplanted stomach [9,10]. The procedure is commonly needed for patients with diffuse gastrointestinal disorders and complex abdominal pathology, particularly those with severe gastric dysmotility, Gardner's syndrome, and prior gastrectomy.

According to the most recent SRTR annual report, the median time to transplant for both the isolated intestine and liver–intestine candidates has been gradually and preferentially reduced (Figure 42.2). Such an achievement reflects the periodic multiple changes that have been made to the UNOS allocation policy combined with the continual advancement of the field. These two important evolutions have been partially beneficial to combined liver–intestine candidates, with no significant impact on the liver-free candidates. Nonetheless, the observed hectic variation in the yearly median time to transplant reflects the orphan nature of the population, with continual changes in the referral pattern, gut rehabilitation activities, and UNOS/OPTN allocation policy (Table 42.1).

Waitlist mortality

Visceral allograft candidates experience high mortality on the UNOS waiting list because of their complexity and disease gravity [11–15]. According to the UNOS database, these patients had a significantly higher unadjusted death rate compared to those listed for other thoracic and abdominal organs (Figure 42.3) [1]. Predictors for urgent referral, death on the waitlist, and early post-transplant mortality are comprehensively discussed elsewhere

Table 42.1. Changes to the Organ Procurement and Transplantation Network (OPTN) policy for liver–intestine candidates

Description	Date implemented in UNet[SM]*
I For candidates awaiting a combined liver–intestine transplant, livers may be allocated using the intestine list unless there is a status 1 liver patient in the region. Different listing status criteria were used over this period	May 1990 to February 2005
II All candidates awaiting a combined liver–intestine transplant who are registered on both waiting lists automatically receive an increase in their MELD/PELD score equivalent to a 10% risk of 3-month mortality	March 2005
III 3.9.3 modified to state that "the liver may be allocated by the local OPO to a local, regional, or national intestine recipient based upon priority for receipt of the intestine using the intestine waiting list unless there is a status 1A or 1B liver candidate locally, regionally, or nationally"	June 2007
IV Pediatric liver–intestine candidates received an additional 23 points to their calculated score. Adults still receive the additional 10%	June 2007
V Pediatric donors (0–11 years) offered preferentially to pediatric liver and liver–intestine patients	June 2007
VI Policy revised to state that livers must be offered sequentially to each potential liver recipient (including all MELD/PELD potential recipients) through national status 1A and 1B offers before being offered to combined liver–intestine potential recipients sequentially according to the intestine match run	N/A
VII Liver offers for 0–10-year-old pediatric donors are extended nationally to 0–11-year-old status 1A pediatric liver and combined liver–intestine candidates before offers are made to local adult status 1A candidates	November 2010
VIII The adult donor algorithm will be modified such that livers are offered to combined liver–intestine candidates *nationally* if there are no regional status 1A/1B candidates, or local candidates with a MELD/PELD score of 29 or higher	November 2011

* UNet[SM], a centralized computer network run by the United Network for Organ Sharing (UNOS) to match organs between deceased donors and potential recipients.
MELD/PELD, Model for End-Stage Liver Disease/Pediatric End-Stage Liver Disease; OPO, Organ Procurement Organization.

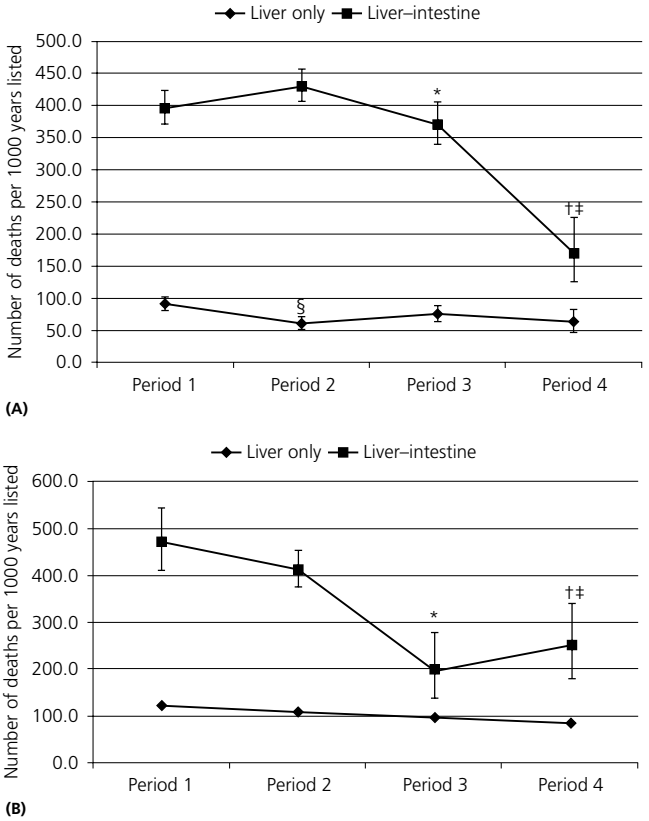

Figure 42.4. (A) The impact of MELD/PELD revisions on the mortality of liver–intestine transplant versus liver-only adult candidates. $^{*}P < 0.05$ compared with period 2; $^{\dagger}P < 0.001$ compared with periods 1 and 2; $^{\ddagger}P < 0.01$ compared with period 3; $^{\S}P < 0.05$ compared to period 1. I bars indicate 95% confidence intervals. (Reproduced from Kaplan et al. [13], with permission from John Wiley and Sons Ltd.) (B) Comparison of mortality rates in pediatric liver–intestine and liver-only candidate groups. $^{*}P < 0.001$ compared with periods 1 and 2; $^{\dagger}P < 0.05$ compared with period 2; $^{\ddagger}P < 0.01$ compared to period 1. I bars indicate 95% confidence intervals. (Reproduced from Kaplan et al. [13]. with permission from John Wiley and Sons Ltd.)

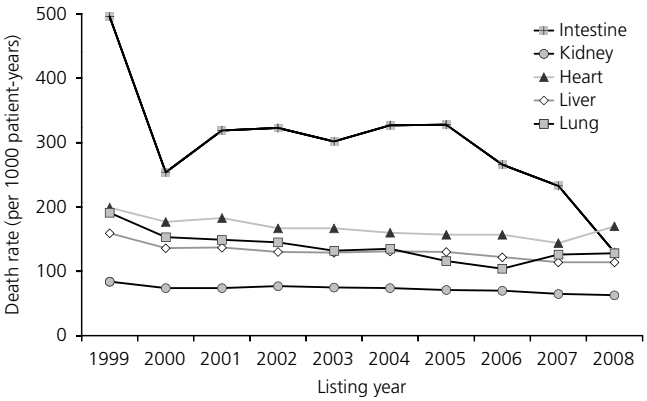

Figure 42.3. Unadjusted death rates per 1000 patient-years at risk for patients on the United Network of Organ Sharing (UNOS) waitlist by organ, 1999–2008. (Modified from Mazariegos et al. [1], with permission from John Wiley and Sons Ltd.)

[16–22]. In brief, the degree of liver damage, hospitalization at time of transplant, cause of intestinal failure, and narcotic dependency are the most significant risk factors for poor outcome.

The series of changes that have recently been introduced to the UNOS/OPTN allocation policy (Table 42.1) have reduced the waitlist mortality of the pediatric liver–intestinal candidates, while the death rate among the adults continues to be clearly higher than that for the isolated liver candidates (Figure 42.4) [13]. Accordingly, the UNOS liver–intestine committee was approached by the primary author of this chapter in June 2009 with an evidence-based recommendation to further amend the current UNOS regulations to increase accessibility of these high-risk intestine–liver candidates to the deceased donor national pool. The aforementioned analysis of the current SRTR data showed that the liver–intestinal patients suffer a three-fold death rate compared to the liver-only waitlist candidates (Figure 42.5). Accordingly, final approval was granted by the UNOS/OPTN board to allow the liver–intestinal candidates with a MELD score of $\geq$29 to have access to the deceased donors nationwide [23].

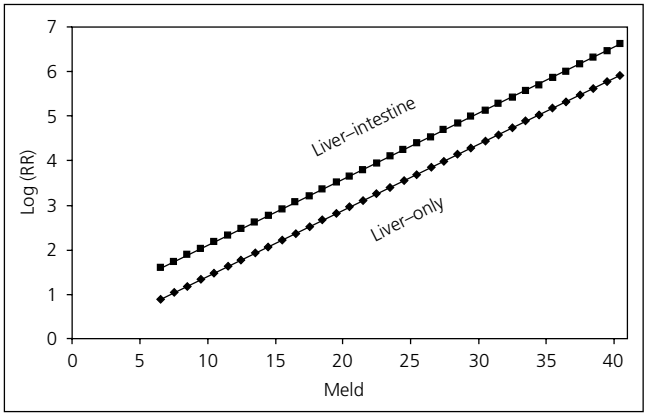

Figure 42.5. Log relative risk (RR) of mortality for liver–intestine compared to liver-only candidates on the Organ Procurement and Transplantation Network (OPTN) waiting list. Data prepared by the Scientific Registry of Transplant Recipients (SRTR) and presented to the United Network of Organ Sharing (UNOS) Liver–Intestine Committee. In November 2011, the UNOS/OPTN board approved the proposal with the modification outlined in Table 42.1.

Efforts toward improving outcomes

With the limited availability of the combined liver–intestinal donors, relentless efforts have been directed toward early referral to eliminate the need for simultaneous hepatic replacement [16–19, 24–27]. Furthermore, the concept of the comprehensive multidisciplinary team approach was introduced utilizing innovative medical and surgical modalities to restore gut functions with possible elimination of the need for transplantation [28].

It is conceivable that reduction of waiting time on the UNOS candidate list for both liver-free and liver-contained allografts could potentially reduce waitlist mortality. For liver-free allografts, technical innovation, utilization of live donors, and judicious expansion of the deceased donor pool has been utilized successfully [9,29–31]. Of great impact was the introduction of simultaneous retrieval of the intestine and pancreas from the same donor and their transplantation to two different recipients [9]. It is our recommendation to use living-related segmental intestinal transplant only for identical twins with no hereditary disorders and in countries where the law does not permit organ donation from brain dead individuals.

For candidates listed for liver-containing allografts, the continual evolution of the UNOS allocation policy has been instrumental in reducing mortality on the UNOS list, particularly among the liver–intestine pediatric candidates. Similar results are anticipated among adults upon implementation of the new algorithm of organ allocation (Table 42.1). Meanwhile, aggressive gut rehabilitation with utilization of an isolated liver allograft in children with short bowel syndrome has been successfully utilized [32]. Reduced and ABO-compatible allografts have also been used for extremely ill candidates [27,33,34]. Combined living-related segmental liver and intestinal transplantation also has been carefully introduced with a cumulative increase in the anticipated potential risks [35].

Regulatory compliance

UNOS/OPTN allocation policies, which are similar to those for other organs, must be followed. These include patient notification of listing and removal, rights for multilisting, and regular candidate status updates. With these regulations, patient care and organ utilization can be better attained.

Clinical management

Patient

Maintenance therapy

Management of intestinal failure, including HPN therapy, is the Achilles' heel of patient care. Of the major life-threatening HPN complications that should be closely monitored are line sepsis, central venous thrombosis, and liver failure. All patients should be monitored for bloodstream infection with weekly surveillance blood cultures. Clinical suspicion of central venous thrombosis should initiate diagnostic imaging with Doppler ultrasound and/or central venographic studies. Liver-free allograft candidates also should be closely monitored for development of significant liver damage that may necessitate simultaneous hepatic replacement. In contrast, candidates who are listed for a liver-contained allograft should be monitored for possible amelioration of the liver damage with elimination of the need for liver replacement. We have observed over the years a few examples of both scenarios with prompt modification of the required allograft.

Follow-up studies

Regular testing is needed weekly for most of the patients, and should include assessments of renal function and a comprehensive metabolic panel to monitor and adjust HPN therapy. Periodic assessment of hepatic enzymes, liver function tests, and measurement of micronutrient serum levels also is required. As indicated in our recent publications, yearly assessment of skeletal health is strongly recommended, including dual energy X-ray absorptiometry (DXA), and hormone and vitamin D serum levels [36,37].

Patients living a great distance from the transplant center should be closely followed by the primary care or referring physician. Weekly contact with the caring physician is mandatory, along with regularly scheduled visits to the designated transplant center.

Gut rehabilitation

Despite being on the transplant waitlist, certain candidates should continue to undergo gut rehabilitation utilizing the best available comprehensive medical and surgical therapy. Candidates on the waitlist with clinical and nutritional data suggestive of significant improvement in gut absorption should undergo gradual weaning of HPN therapy with placement on the inactive waiting list. Meanwhile, autologous surgical reconstruction for patients with preserved liver functions and isolated liver transplantation for those with end-stage liver failure should be judiciously considered [32,38].

Venous access

Management of the indwelling central venous catheter is an essential component of patient care. Sensible central line replacement, preferably by an experienced interventional radiology team, must be adopted to avoid life-threatening technical complications and to preserve a reliable central venous access for volume resuscitation during transplantation. A comprehensive discussion of proper central venous catheter placement and maintenance is beyond the scope of this chapter. However, it is our practice to prohibit the use of a previously placed central venous catheter at the time of transplantation because of the possibility of occult line infection.

Infection

Candidates with fever and/or positive blood culture should be subjected to prompt central line replacement and antimicrobial therapy. Pulmonary aspiration with recurrent pneumonia is not uncommon in patients with ultra-short gut and pseudo-obstruction syndrome, and this risk requires prophylactic measures, diagnostic imaging studies, and active treatment if indicated. Recurrent urinary tract infection is also a common source of infection among candidates with advanced hollow visceral myopathy/neuropathy that can be partly eliminated by sterile self-catheterization and intermittent antibiotic therapy.

In patients with complex abdominal pathology—particularly those with inflammatory bowel disease and irradiation enteritis—careful attention must be paid to the possible development of intra-abdominal abscesses, perforation, and enterocutaneous fistulas. Under such circumstances, patients should be placed on the inactive waiting list and considered for completion enterectomy and control of the abdominal infection, particularly in those with preserved liver functions.

Surveillance protocols

All candidates on the waitlist should undergo annual screening for malignancy, particularly high-risk patients with prior cancer or a strong family history of cancer. The surveillance should include mammogram and cervical smear in females, chest imaging in patients with smoking history, and gastrointestinal endoscopy in patients with neoplastic disorders including Gardner's syndrome. A yearly assessment of cardiopulmonary functions is also recommended for patients with hypercoagulable disorders, including Jak-2 mutations, cardiovascular diseases, or a history of recurrent pulmonary emboli. Development of de-novo malignancy such as melanoma and other skin cancers has been observed in a few of our patients while waiting for visceral transplant. Equally critical is the progression of the primary cardiovascular disease with the development of life-threatening complications that may preclude transplantation.

Allograft

The indications for visceral transplantation with and without simultaneous hepatic replacement are fully described elsewhere [5,27]. It is imperative to note that the pancreas is commonly transplanted en bloc with the liver–intestine or as part of the multivisceral graft due to technical and physiologic considerations [5,7,9]. Inclusion of the stomach as the defining organ of the multivisceral graft should only be utilized for patients with extensive gastrointestinal diseases and those with combined liver failure and portomesenteric/splenic venous thrombosis. The multivisceral graft is also commonly utilized for patients who require retransplantation after a combined liver–intestinal graft with hostile abdomen [27].

For better patient care and optimal utilization of deceased organs, it is imperative to periodically assess the medical necessity of the listed organs. It is not uncommon to add a liver or kidney after the initial listing due to development of liver or renal failure, particularly in patients with a marginal liver who are in need of retransplantation. According to the most recent SRTR/OPTN report, the most common additional organ that intestine candidates were subsequently listed for was the pancreas (20%), followed by the liver (10%) and the kidney (4%). In adults, 58% of candidates who were initially listed for both intestine and liver were subsequently listed for an additional organ, most commonly the pancreas due to the evolution of the technique with en-bloc inclusion of the pancreas [39]. In the pediatric population, 32% of candidates initially listed for intestine-only and 36% of those initially listed for intestine–pancreas were subsequently listed for liver. Furthermore, 27% of pediatric patients initially listed for both intestine and liver were subsequently listed for pancreas [1].

Elimination of one or more of the listed organs should always be considered in candidates who experience significant improvement in gut absorption or functional status of other abdominal organs. With fine adjustment of HPN formula, including the use of omega fatty acids, we have been able to eliminate the need for hepatic replacement and en-bloc pancreas transplant utilizing an isolated intestinal graft. In a few instances, the decision was made during the recipient transplant operation and the reserved organs were utilized for other candidates according to priority on the waitlist.

Donor

With the high susceptibility of the intestine to ischemia reperfusion injury compounded by the complexity of the recipient operation, only optimal donors should be considered for most candidates. The currently adopted donor criteria include young age ($\leq$55 years), low body mass index (BMI; $\leq$30 kg/m^2), hemodynamic stability, minimal pressor support, and no time or short time of cardiopulmonary arrest. Favorable biochemical markers include normal hepatic enzymes, low serum creatinine, normal range of serum lactate levels, and no evidence of disseminated intravascular coagulopathy. Despite organ shortage, particularly with inclusion of the liver, marginal donors should be considered only with great caution [31]. It is also advisable to avoid intestinal allografts with fatty mesentery.

For all visceral candidates, it is our recommendation to use ABO-identical donors. With the current availability of virtual cross-match, it is better to avoid transplanting a liver-free allograft to candidates with multiple unacceptable DSAs [8]. For patients with a contracted abdominal cavity, it is advisable to use small size donors. It is also recommended to use CMV-negative donors for CMV-negative candidates who are in need of liver-free allografts. Equally important is shortening of the cold ischemia time by synchronizing the time of both the donor and recipient operations.

Summary and future considerations

Management of the visceral allograft candidates on the UNOS waitlist is essential for continual improvement of survival outcomes before and after transplantation. Of the utmost importance are early referral, proper management of the waitlist, and further evolution of the current UNOS/OPTN allocation policy. With early referral, it is foreseeable to reduce disease gravity, extent of central venous thrombosis, and simultaneous hepatic replacement. Waitlist mortality can be further reduced by effective prophylactic, pre-emptive, and active measures towards preventing line/systemic sepsis, central venous thrombosis, and hepatic vascular or parenchymal decompensation. With surveillance protocols, malignant, cardiovascular, and other life-threatening diseases can be diagnosed early and treated promptly with better overall outcome. Further refinement of the UNOS/OPTN allocation system will allow better access to the national donor pool, with shortening of waiting time and subsequent improvement in wait list outcome.

For the liver-free allografts, a national policy allowing better HLA match through a new allocation system is essential to reduce time on the waitlist, particularly for those with DSAs and positive virtual cross-match. In addition, future translational research is

Table 42.2. Risk factors for mortality while on the waitlist for visceral transplantation

- Degree of liver damage (histologic and biochemical) [15 – 18,20,22]
- Hospitalization [19]
- Cause of intestinal failure [16,20,22]
- Narcotic dependency [20,22]

required to identify, validate, and ameliorate donor risk factors (Table 42.2) in the hope of increasing organ availability.

For liver-contained allograft candidates, broader access to the UNOS deceased donor national pool is essential to improve outcome at the present time. Similar policies should be adopted worldwide, particularly with the observed increase in the clinical activities of visceral transplantation shown in the recent report of the intestinal transplant registry [40].

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CHAPTER 43

Intensive Care in Hepatic Failure

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Introduction

During the diagnosis, treatment, and management of liver failure in critical care, a rapid distinction needs to be made between acute liver failure (ALF) and acute on chronic liver failure (ACLF). Both types of liver failure can cause multiorgan failure, sepsis, and death. However, their pathophysiology differs, which manifests with distinctive hepatic and extrahepatic organ dysfunction that needs to be anticipated, closely monitored, and treated. In particular, a crucial distinction between ALF and ACLF in the context of hepatic encephalopathy is the potential complication of intracranial hypertension and the risk of herniation in ALF. In the setting of multiorgan failure in advanced ALF or ACLF, an optimization of hepatic and extrahepatic derangements, including cardiopulmonary, renal, and neurologic dysfunction, is essential to the successful transition of the critically ill patient to liver transplantation.

This chapter provides a multiorgan system-based description of the intensive care management of ALF and ACLF. It complements the content in Chapter 38, dealing with the management of patients awaiting liver transplant, and also relates to Chapter 47, which details the use of hepatic assist devices in liver failure.

Acute liver failure

ALF is defined as the onset of hepatic encephalopathy within 8 weeks of the onset of symptoms of hepatic dysfunction in a patient without pre-existing liver disease. A distinct feature of the encephalopathy of ALF is the risk of progression to *intracranial hypertension and cerebral herniation.* The encephalopathy is typically accompanied by a coagulopathy, and the progression of these two parameters is suggestive of worsening hepatic insufficiency, thereby necessitating the need for liver transplantation. The incidence is approximately 2000 cases of ALF a year in the US [1]. In 15% of adult cases, a cause may not be identified [2]. Approximately 45% of patients with supportive care alone will survive, approximately 25% proceed to transplant, and 30% die without transplantation. There are various causes of ALF that portend various prognoses (Table 43.1). The most favorable outcomes are for acetaminophen overdose, hepatitis A, and ischemia, where approximately 60% will survive with supportive therapy. Drug-induced ALF and hepatitis B have a poorer prognosis, with only 25% of patients surviving with supportive therapy alone.

Pathophysiology

The pathophysiology of ALF is massive hepatocellular necrosis. The characteristic clinical manifestations of encephalopathy and coagulopathy are secondary to hyperammonemia from a dysfunctional hepatic urea cycle and the absence of hepatic coagulation factors, respectively. ALF has been delineated into three types depending on the timing of progression from jaundice to encephalopathy: hyperacute (0–7days), in which rapidly fatal cerebral edema may develop, but this has the best overall prognosis; acute (8–28 days); or subacute (5–12 weeks), which has a lower rate of cerebral edema but poorer prognosis [3].

Diagnosis

When a patient presents with possible ALF, a careful and expedited evaluation should be performed to determine the etiology of liver failure (Table 43.1) and to rule out the presence of chronic liver disease that would preclude a diagnosis of ALF. Early in the diagnosis and management of these patients, transplant evaluation should be considered, and any barriers to transplantation such as lack of social support, co-morbidities, and active neoplastic or infectious etiologies should be identified.

A detailed work-up for potential etiologies of ALF (as outlined in Table 43.1) should be performed, and serial assessments of mental status are crucial. Serial measurements of liver enzymes, coagulation parameters including international normalized ratio (INR), arterial pH, and arterial lactate should be followed. Expedited cross-sectional abdominal imaging with computed tomography (CT) or magnetic resonance imaging (MRI) is invaluable in ruling out chronic liver disease, which would manifest as radiographic signs of chronic portal hypertension such as a cirrhotic hepatic contour, splenomegaly, and varices. In addition, the imaging study provides valuable information to the transplant surgeon with regard to assessing vascular patency in preparation for a potential liver transplant. If the patient is insufficiently stable to be transported to the radiology suite for CT or MRI, an abdominal ultrasound with Doppler measurements can provide useful data at the bedside. The utility of liver biopsy in ALF is limited, but may be of use if lymphoma is suspected or if a patient has subacute liver failure of uncertain etiology.

Given the possibility of rapid deterioration in clinical and biochemical status following an initial stable presentation of ALF, early

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Table 43.1. Causes of acute liver failure

Drug-induced (acetaminophen toxicity most common)	
Prescription medications:	Most cases are idiosyncratic and often occur within 6 months of initiating therapy
Antimicrobials	
Anticonvulsants	
Antidepressants	Mechanisms of injury include hepatocellular necrosis, cholestasis, mixed pattern, steatosis, autoimmune, hypersensitivity, phospholipidosis, mitochondrial toxicity, sinusoidal obstruction syndrome
Lipid-lowering agents	
Antihypertensive agents	
Glucose-lowering agents	
Chemotherapeutic agents	
HIV medications	
Over-the-counter analgesics	If hypersensitivity suspected, steroids may have a role
Recreational drugs	
Viral (12% of cases in US; hepatitis A and B are most common)	Hepatitis A
	Hepatitis B (may treat with antiviral)
	Hepatitis D (super- or co-infection)
	Hepatitis E (20% mortality if pregnant)
	Hepatitis C (rare)
	Herpes simplex (treat with acyclovir)
	Varicella zoster (treat with acyclovir)
	Cytomegalovirus (treat with antiviral)
	Epstein–Barr virus
Toxins	*Amanita phalloides* (mushrooms)
	Organic solvents
	Herbal medicines
	Bacterial toxins (e.g. *Bacillus cereus*)
Wilson's disease	May have findings of chronic disease
	May have hemolytic anemia and renal failure
	May have low–normal alkaline phosphatase
	May have low uric acid
	Nearly 100% mortality without transplant
Pregnancy related (often treated with delivery of fetus)	Acute fatty liver of pregnancy
	HELLP syndrome
Autoimmune hepatitis	Treat with steroids
	May have findings of chronic disease
Malignancy	Lymphoma
	Diffuse metastatic disease
Hepatic ischemia	Shock
	Cocaine
	Acute arterial thrombosis/injury
Heat stroke	Clinical history important
Acute Budd–Chiari syndrome	Hypercoagulable states
Post extensive hepatic resection	Based on percentage volume removed as well as state of remaining hepatic parenchyma
Indeterminate (15–20% of cases)	Up to 20% of these cases may be due to undiagnosed acetaminophen toxicity
	Liver biopsy may or may not be helpful

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transfer of patients to a liver transplant center is recommended. A detailed communication between the transferring and receiving facility should address the existing neurologic, cardiopulmonary, hepatic, hematologic, and renal status. Prior to transfer, it may be necessary to electively intubate a patient for airway protection, as advancing encephalopathy can occur unexpectedly in transit. Since patients with advanced ALF present with multiorgan system failure, management should follow an organ system-based, goal-directed approach.

Management

Cerebral edema and intracranial hypertension

A crucial feature of the management of ALF involves the assessment and therapy of *cerebral edema and intracranial hypertension*. In contrast to ACLF, the acuity of hyperammonemia in ALF carries the risk of causing cerebral edema and intracranial pressure (ICP) elevation due to astrocyte swelling. Of ALF patients, 20–30% will develop intracranial hypertension [4]. Serial neurologic assessments are essential to detect the onset of progressively worsening intracranial hypertension, which will manifest as worsening encephalopathy. The West Haven criteria for grading hepatic encephalopathy (Table 34.2) are useful in assessing and documenting serial neurologic exams, and in decision-making regarding elective intubation and the placement of ICP monitors. Advanced grades of encephalopathy (grades III and IV) are associated with cerebral edema and require emergent intubation for airway protection, and ICP monitoring and management to aggressively lower ICPs. It must be emphasized that brain imaging with CT or MRI is not sensitive enough to assess for cerebral edema and elevated ICP associated with ALF and therefore, should not be used to rule out intracranial hypertension.

Intracranial monitoring and management

ICP monitoring practices vary across institutions. There are no randomized trials studying the efficacy of ICP monitoring in ALF. However, the utility of ICP monitors is supported in the general neurointensive literature, extrapolation of these concepts to the context of ALF is being applied in certain centers in Europe and the US, though widespread use has been limited by the risk of intracranial bleeding in the setting of coagulopathy. In the presence of advanced encephalopathy requiring intubation and mechanical ventilation, the presence of sedation limits the utility of the neurologic exam and therefore, a more accurate and objective measure of ICP using a monitoring device would enable determination of intracranial hypertension and guide decisions regarding therapy, prognosis, and candidacy for liver transplantation. The intraparenchymal monitor appears to offer the optimal balance between accuracy and safety, and is the most widely used ICP monitor in ALF.

Intraparenchymal monitoring can be placed rapidly and safely at the bedside by trained specialists. The coagulopathy of ALF can be corrected rapidly with recombinant Factor VIIa at a dose of 40 μg/kg, thereby minimizing the risk of procedure-related bleeding. Elevated ICP is defined as an ICP of >25 mmHg for over 10 min. Monitoring the ICP also allows calculation of cerebral perfusion pressure (CPP), which is the mean arterial pressure (MAP) minus ICP (CPP = MAP – ICP). Therapeutic targets for the ICP and CPP are <25 mmHg and >60 mmHg, respectively. The optimization of CPP, especially in the setting of an elevated ICP, involves the pharmacologic induction of a therapeutic hypertension (elevated MAP) with a vasoactive agent such as norepinephrine. With regard to the management of elevated ICP, Figure 43.1 outlines an algorithm for pharmacologic therapy, which includes mannitol, hypertonic saline, hypothermia, and barbiturate coma [5].

Prior to the initiation of pharmacotherapy, patients with grade III or IV encephalopathy should be intubated for airway protection. Endotracheal or intravenous lidocaine prior to intubation may be helpful in reducing increases in ICP. Any seizure activity should be treated with dilantin; prophylactic dilantin is not currently recommended. In the intubated patient, patient–ventilator synchrony with adequate sedation and analgesia should be maintained to prevent surges in ICP due to tracheal irritation.

Pharmacotherapy for elevated intracranial pressure

The use of mannitol is efficacious in the initial correction of elevated ICP in ALF, with repeated dosing limited by serum osmolality and renal function (since the drug is renally cleared). Mannitol increases blood osmolarity, which creates an osmotic gradient that

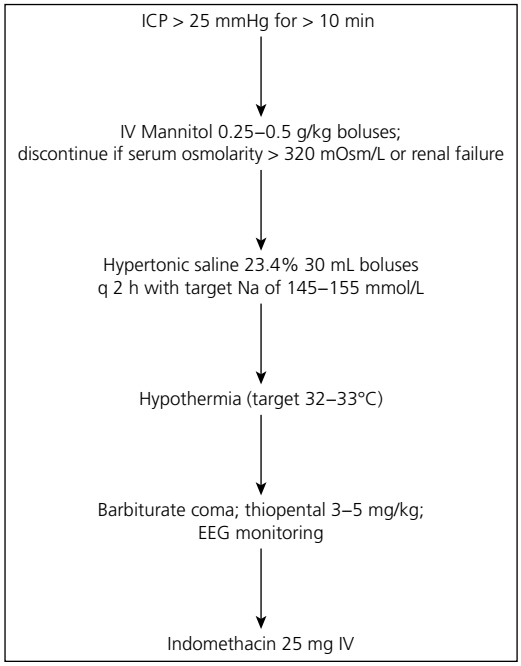

Figure 43.1. Algorithm for the management of intracranial hypertension in acute liver failure. EEG, electroencephalography. (Reproduced from Ford et al. [19], with permission from Elsevier. Copyright ©2010, Elsevier.)

draws fluid from the brain to the blood. Its ability to lower cerebral edema is associated with increased survival [6]. Bolus doses of 0.5–1 g/kg may be given to achieve correction of elevated ICP as long as the serum osmolarity does not exceed 320 mOsm/L and the patient is not in renal failure. Hypernatremia and hyperosmolarity resulting in pulmonary edema and hypervolemia can ensue; therefore, frequent vigilant monitoring of osmolarity and chemistries are essential.

Following initial intervention with mannitol, hypertonic saline is used repeatedly to decrease and maintain ICPs by increasing blood osmolarity. This intervention has been shown to successfully decrease ICPs in ALF [7]. Intravenous 23% hypertonic saline in 30-mL aliquots can be given as often as every 2 h to target a serum sodium level between 145 and 155 mmol/L. In addition, moderate hypothermia, with a goal core body temperature of 32–34 °C, can also effectively reduce ICP, especially in the setting of ICP elevations refractory to the above pharmacologic interventions [8]. Furthermore, in the authors' experience, the combination of hypertonic saline and hypothermia is effective in most cases of elevated ICP in ALF. In cases of refractory intracranial hypertension despite the above therapies, the induction of barbiturate coma can be used. Continuous electroencephalogram (EEG) monitoring is needed to titrate the barbiturate to achieve burst suppression on the EEG tracing. This treatment has the potential side-effects of myocardial suppression and hypotension, which limit its use [9]. A form of salvage therapy is intravenous indomethacin, which is a cerebral vasoconstrictor and should be used only as the last line of therapy due to its transient effects on lowering ICP [10]. It should be noted that the above interventions to optimize ICP and CPP are typically implemented for a finite time period in order to stabilize the neurologic status while the patient awaits definitive therapy with liver transplantation. In the event that an ICP monitor is placed pretransplant, it should be kept in for 24 to 48 hours post-transplant to confirm the presence of normal intracranial pressures.

Hematologic derangements

Coagulopathy as manifested by an elevation in the prothrombin time/INR) is a characteristic feature of ALF. Since hepatically synthesized factors (e.g. Factors V and VII) and the INR are the most sensitive biochemical indicators of hepatic synthetic function and recovery, the INR should be used as a *measure of potential hepatic recovery*, and *should not* be corrected with blood products in the absence of bleeding. Also, in addition to the loss of procoagulant factors, the synthesis of hepatic anticoagulant proteins, such as protein C and S, is decreased; hence, an elevated INR does not translate to an increased risk of bleeding. If the INR needs to be corrected for invasive procedures or bleeding, activated recombinant Factor VIIa can temporarily correct the INR. A minimum dose of 40 μg/kg is recommended to achieve transient reversal of coagulopathy, with a therapeutic window of approximately 2 h to perform invasive procedures. The transient effect of recombinant Factor VIIa is ideally suited to the context of ALF, since the temporary correction of coagulopathy to facilitate invasive procedures safely is followed by a return of the intrinsic INR to its uncorrected value; this can be used as a biochemical marker to trend hepatic function.

Thrombocytopenia is often present in ALF. Despite the low platelet count, patients do not typically exhibit signs of bleeding. Since ALF does not manifest the derangements of chronic portal hypertension, the risk of variceal bleeding is minimal. If the patient does not show active signs of bleeding, routine platelet transfusion is not recommended unless the count is $<10\,000/mm^3$. If a procedure is planned, a platelet count of $>50\,000/\ mm^3$ is commonly recommended.

With regard to the correction of hematologic derangements, the risk of transfusion-related acute lung injury (TRALI) should be recognized. The risk is greatest with fresh frozen plasma (FFP), but has been associated with platelet and packed red blood cell (pRBC) transfusions as well. The characteristic of TRALI is the onset of acute lung injury within 6 h of product administration; therapy is supportive.

Cardiopulmonary derangements

Cardiovascular

As the course of ALF progresses, the systemic inflammatory response induces the physiology of a hyperdynamic circulation and a low systemic vascular resistance. Given a compromised hepatic reticuloendothelial system, the patient with ALF is an immunocompromised host and is at risk for infectious complications that can worsen existing hypotension. Vasoactive agent support includes agents typical for septic shock, including norepinephrine and low-dose vasopressin. In the setting of cardiac dysfunction, ionotropic support with dobutamine or milrinone may be considered. As mentioned previously, there is a role for vasoactive agents such as norepinephrine in the setting of an elevated ICP in order to induce an iatrogenic hypertension to optimize CPP.

Pulmonary

The presence of advanced encephalopathy necessitates elective intubation for airway protection, and adequate short-acting analgesia (e.g. fentanyl) and sedation (e.g. propofol, dexmedetomidine) to ensure patient–ventilator synchrony. There are no evidence-based guidelines regarding the optimal mode of mechanical ventilation;

a volume-controlled or pressure-controlled mode can be used. With respect to pulmonary derangements, patients with ALF are at risk for acute lung injury due to infectious and non-infectious causes. TRALI is a non-infectious form of acute lung injury that the patient with ALF may be at particular risk for, given the need for correction of coagulopathy and thrombocytopenia with blood products. With respect to prophylactic measures, the routine intensive care unit (ICU) practice of head of bed elevation at 30º serves to decrease the risk of ventilator-associated pneumonia, and assists in decreasing ICP as well. With regard to ventilator settings, maneuvers to decrease intrathoracic pressures will facilitate venous drainage from the CNS circulation, thereby favorably influencing ICP values; therefore, tidal volumes in the range of 6–8 mL/kg of predicted body weight, and the lowest positive end-expiratory pressure (PEEP) to maintain adequate oxygenation should be utilized.

Renal failure

Acute kidney injury (AKI) is commonly seen in ALF and is associated with a poor prognosis [11]. The work-up for AKI should address pre-, intra-, and post-renal etiologies. It must be noted that AKI in the setting of ALF is unlikely to be hepatorenal syndrome (HRS), since the physiology of HRS requires the pathophysiologic derangements of chronic portal hypertension. AKI in the setting of ALF often necessitates renal replacement therapy, especially for the indication of a rapidly worsening anion-gap metabolic acidosis due to hyperlactatemia. If renal replacement therapy is initiated, the *continuous mode* of dialysis therapy with a bicarbonate bath should be used, even in hemodynamically stable patients, since intermittent hemodialysis can induce significant fluid shifts and cause dramatic rises in ICP [12]. In addition, high-volume hemofiltration has been shown to reduce serum lactate, base deficits, and vasopressor requirements [13].

N-Acetylcysteine therapy

For treatment of hepatic dysfunction in acetaminophen- or non-acetaminophen-induced liver failure, N-acetylcysteine (NAC) can be used, especially if given within the first 12 h of acetaminophen ingestion. NAC increases glutathione production, which is an antioxidant that ameliorates hepatoxicity. It is useful for up to 48–72 h from ingestion [14]. For non-acetaminophen liver failure, studies have shown that NAC improves end-organ oxygen delivery and hemodynamic stability [15]; therefore, all patients with ALF should receive NAC independent of etiology. NAC may be given orally or intravenously, and it is well tolerated with minimal side-effects. The oral loading dose is 140 mg/kg, followed by 70 mg/kg every 4 h for 17 doses. The intravenous dose is 150 mg/kg (in 5% dextrose solution) followed by 50 mg/kg over 4 h, followed by 100 mg/kg over 16 h [16]. NAC therapy can be discontinued when there is clinical improvement in hepatic dysfunction as evidenced by an improvement in encephalopathy and coagulopathy (INR <1.5).

In summary, the management of ALF involves addressing derangements in multiple organ systems, with specific attention to the management of hepatic encephalopathy and coagulopathy. In multiple prognostic models, these two factors have demonstrated utility for predicting spontaneous hepatic recovery or the need for liver transplantation.

Acute on chronic liver failure

The pathophysiology of ACLF involves the splanchnic and systemic derangements induced by chronic portal hypertension. Multiple organ systems can be involved that can lead to life-threatening derangements such as variceal bleeding, shock, abnormalities in gas exchange, and HRS-induced AKI. Early recognition of these disease states and anticipatory critical care support are lifesaving interventions prior to definitive therapy with transplantation.

Portal hypertension

Portal hypertension in cirrhosis is a consequence of the combined effects of intrahepatic resistance to portal flow and increased portal inflow. The resistance to portal flow consists of both fixed and functional components. The fixed component occurs from sinusoidal fibrosis and compression by regenerative nodules. The functional component is secondary to vasoconstriction, resulting from both decreased intrahepatic nitric oxide and increased intrahepatic vasoconstriction. The paradoxical decreased intrahepatic nitric oxide and overproduction of extrahepatic nitric oxide produces splanchnic vasodilation and increased portal inflow. Combined, the effects of the intrahepatic resistance to flow and increased portal inflow result in portal hypertension [17]. In addition, the pathologic splanchnic vasodilation results in a shunting of the cardiac output to the splanchnic circulation, and an associated decrease in effective systemic arterial blood volume perfusing other organs. These hemodynamic derangements in the splanchnic and systemic circulation form the basis for current management strategies in decompensated cirrhosis prior to liver transplantation. A central theme in the management of splanchnic and systemic derangements in ACLF is the reversal of splanchnic vasodilation with splanchnic vasoconstrictors (e.g. octreotide, terlipressin).

Hepatic encephalopathy

Hepatic encephalopathy is a serious complication of portal hypertension occurring in ACLF. Its neuropsychiatric clinical presentation ranges widely from mild cognitive impairment to frank coma. The pathophysiology is accepted to be a result of impaired hepatic clearance of toxins from the gastrointestinal tract, particularly ammonia.

While the debate continues over which toxins mediate the development of hepatic encephalopathy, elevated ammonia levels have long been implicated to play a role in its pathogenesis. Specifically, ammonia's effect on brain astrocytes is suspected in the development of hepatic encephalopathy. The astrocytes in chronic liver disease take on an Alzheimer's type morphology known as Alzheimer type II astrocytosis. In chronic liver disease, excess serum ammonia levels alter astrocytic neuronal proteins, leading to abnormal glutamate trafficking. This alteration in glutamate is thought to be partially responsible for the abnormal neurotransmission seen in hepatic encephalopathy. Other studies have suggested the involvement of seratoninergic and GABA receptors, manganese, as well as catecholamine pathways in the pathogenesis of hepatic encephalopathy [18].

Patients may present with symptoms ranging from subtle changes in the sleep–wake cycle, to lethargy, to worse levels of consciousness, including somnolence and coma. The West Haven criteria grades hepatic encephalopathy from grade I to grade IV based on varying levels of consciousness, intellectual function, and behavior (Table 43.2). Neurologic abnormalities on physical exam may be seen in more advanced presentations and include asterixis, hyperactive deep tendon reflexes, and hemiplegia.

Initial management of hepatic encephalopathy involves determining the grade of encephalopathy with prompt ICU transfer and elective intubation for airway protection in grades III and IV. The

Table 43.2 West Haven criteria for semi-quantitative grading of mental state

Grade 1	Trivial lack of awareness
	Euphoria or anxiety
	Shortened attention span
	Impaired performance of addition
Grade 2	Lethargy or apathy
	Minimal disorientation for time or place
	Subtle personality change
	Inappropriate behavior
	Impaired performance of subtraction
Grade 3	Somnolence to semi-stupor, but responsive to verbal stimuli
	Confusion
	Gross disorientation
Grade 4	Coma (unresponsive to verbal or noxious stimuli)

requirement for sedation during mechanical ventilation is typically minimal. Potential sedative agents include propofol and dexmedetomidine; benzodiazepines should be avoided given their risk of exacerbating hepatic encephalopathy. Imaging of the brain should also be considered to rule out other etiologies of altered mental status, including cerebrovascular accident (CVA), intracranial bleed, or masses. The *precipitating factor* of hepatic encephalopathy must be identified and treated. These include gastrointestinal bleeding, infection, electrolyte disturbances, dehydration, recent placement of a transjugular intrahepatic portosystemic shunt (TIPS), constipation, medication non-compliance, sedatives, or progressive hepatic dysfunction. Supportive care with intravenous fluid hydration, correction of electrolyte disturbances, and aspiration, and fall precautions should be instituted.

In contrast to ALF, the risk of developing cerebral edema and intracranial hypertension in the setting of hepatic encephalopathy in ACLF is negligible; therefore, ICP monitoring and osmotherapy are not indicated. A putative rationale for the lack of cerebral edema in ACLF is that the chronicity of hepatic dysfunction provides time for extrahepatic tissues such as muscle to up-regulate ammonia fixing mechanisms; therefore, in the setting of an acute insult that results in a hyperammonemic state, the central nervous system circulation is not subject to elevated ammonia levels that would lead to astrocyte edema (as seen in ALF).

Non-absorbable disaccharides such as lactulose are the main pharmacologic agents to aid in the clearance of ammonia in the treatment of hepatic encephalopathy. These drugs work by decreasing ammonia production in the gastrointestinal tract and increasing fecal nitrogen excretion. Specifically, when oral lactulose reaches the cecum, it is metabolized by enteric bacteria, causing a drop in the pH. This leads to a shift in bacteria favoring uptake of ammonia, leaving fewer for mucosal absorption. If the patient is unable to take oral lactulose, then a nasogastric tube must be placed for luminal administration or lactulose enemas. The dosage should be titrated to approximately three bowel movements per day. Antibiotics, including flagyl, rifaximin, and vancomycin, have also been studied and shown to be effective in the treatment of hepatic encephalopathy. These work primarily by eliminating urease-producing bacteria flora. While these agents can reduce blood ammonia levels and improve mentation, it is important to note that the degree of encephalopathy has not been shown to correlate with specific ammonia levels. Other treatment methods, including zinc administration and protein restriction, are also used, but lack strong clinical supporting evidence. In addition to the above agents targeted at elimination of ammonia, the treatment of the inciting factor is essential; in particular, the identification and treatment of a potential infectious etiology is critical.

Hemodynamic derangements

Due to the systemic vasodilatation from cirrhosis, which leads to low systemic vascular resistance, cardiac output is increased to facilitate end-organ perfusion. The hemodynamic pathophysiology mimics the features of septic hypotension, with characteristic features of a low MAP, a wide pulse pressure, and tachycardia. In the setting of worsening hepatic function or an active infection, this septic physiology can be exacerbated, leading to rapid progression to severe shock. With regard to vasoactive agent support, norepinephrine is typically the first agent of choice, since it acts on α and $\beta 1$ receptors, causing increased vascular tone and preserving cardiac output. Low-dose vasopressin can be added for distributive shock refractory to initial norepinephrine therapy. Dopamine should be used judiciously since it causes splanchnic vasodilatation, which can worsen portal hypertension. Volume resuscitation should be monitored closely since it can exacerbate ascites, which in turn can induce an abdominal compartment syndrome and subsequent hypovolemic hypotension due to inferior vena cava (IVC) compression and decrease in cardiac preload [19].

Pulmonary complications

Hepatopulmonary syndrome

Hepatopulmonary syndrome (HPS) is characterized by hypoxemia, and is defined as an increase in the alveolar-to-arterial oxygen gradient (A–a) and pulmonary vasodilatation in the setting of liver disease [20]. The frequency of HPS has been reported to range from 4% to 29%, with approximately 20% of patients with HPS awaiting transplant [21]. HPS is caused by pulmonary vasodilatation or pulmonary arteriovenous malformations. Proposed experimental models implicate pulmonary endothelin B receptor stimulation, which increases nitric oxide and in turn causes pathologic pulmonary vasodilatation. The dilated pulmonary vasculature induces hypoxemia due to a diffusion-impeded transfer of oxygen from the alveolus to the hemoglobin molecule in the capillary.

Clinical manifestations are tachypnea, hypoxemia, clubbing, cyanosis, platypnea (shortness of breath while upright), and orthodeoxia (hypoxemia while upright). The rationale for an exacerbation of the hypoxemia in the upright position is a basilar preponderance of the pathologic pulmonary vasodilation; therefore, in the upright position, since more blood flow is diverted to the lung bases, the hypoxemia associated with HPS is exacerbated. Patients with HPS typically have normal chest imaging studies, and the presence of hypoxemia in the absence of a radiographic shunt lesion should raise the possibility of HPS.

Diagnosis is based on a PaO_2 of <70 mmHg on room air with an increased A–a gradient of >20 mmHg [22], and diagnostic evidence of intrapulmonary shunting either by echocardiogram with a bubble study or a perfusion scan. The former evaluates for a right-to-left shunt with respect to the right and left heart. Agitated saline bubbles are introduced into the right heart via a peripheral vein. The appearance of delayed bubbles in the left heart after three to six cardiac cycles indicates an intrapulmonary shunt, with the bubbles having traversed the entire pathologically dilated pulmonary circulation; this finding of delayed bubbles needs to be contrasted with an "early bubbles" result, which would occur in the setting of an intracardiac shunt. Apart from echocardiographic data, a scintigraphic perfusion scan can be used to diagnosis intrapulmonary shunting. In a normal patient, technetium-99-labeled macroaggregated albumin is large enough to be trapped in the pulmonary vascular bed, but in patients with dilated pulmonary

vasculature, it can pass through the lungs and appear in the kidneys and brain.

Treatment is supportive with oxygen therapy. For patients who have an arteriovenous malformation on thoracic imaging, embolization may be curative. Patients with HPS who have a PaO_2 of <60 mmHg on room air are eligible for Model for End-stage Liver Disease (MELD) exception points, and transplantation can be curative, with a complete resolution of the hypoxemia. Patients with severe HPS, defined as a room air PaO_2 of <50 mmHg, have been reported to be at increased risk for perioperative mortality [23], although more recent evidence suggests that patients with this severity of HPS can be transplanted without increased mortality and therefore, it should not be a contraindication to transplantation [24].

Portopulmonary hypertension

Another distinct pulmonary entity seen only in cirrhotic patients is portopulmonary hypertension (PPH), which is a form of pulmonary arterial hypertension. It is estimated that 2–10% of cirrhotic patients are at risk of developing pulmonary hypertension, and in those referred to transplantation, 16% have pulmonary hypertension [22]. The diagnostic criteria of PPH include a mean pulmonary arterial pressure (mPAP) of ≥25 mmHg, peripheral vascular resistance (PVR) of ≥120 dynes·s/cm^5, and pulmonary capillary wedge pressure (PCWP) of ≤15 mmHg [22].

The pathogenesis is linked to portal hypertension, although the mechanism is not clearly elucidated. Impaired hepatic clearance of putative endotoxins and cytokines leads to the passage of these factors into the pulmonary circulation, which triggers pulmonary arterial endothelial proliferation. Proliferation of the pulmonary arterial endothelial cells and smooth muscle cells results in pulmonary vasoconstriction and pulmonary artery medial hypertrophy, causing obliteration of the vessel lumen and formation of plexiform lesions, which are the hallmark features of pulmonary hypertension [25]. Severe cases of PPH can lead to cor pulmonale and right heart failure. Patients with moderate-to-severe PPH, defined as a mPAP of ≥35 mmHg and a PVR of ≥250 dynes·s/cm^5, have a >50% mortality during liver transplantation due to the development of acute right heart failure following reperfusion of the hepatic graft; therefore, a mPAP of ≥35 mmHg in the setting of a PVR of ≥250 dynes·s/cm^5 is a contraindication to liver transplantation [23].

Typical symptoms include dyspnea on exertion, palpitations, syncope, and chest pain, with possible progression to symptoms of right heart failure. An echocardiogram is a useful non-invasive screening diagnostic tool for pulmonary hypertension. An estimated pulmonary arterial pressure of >50 mmHg on echocardiography is an indication to obtain more objective data with right heart catheterization. With respect to therapy, intravenous epoprostenol has been studied in the setting of PPH [22], with the demonstration of favorable effects on decreasing the mPAP to levels that are compatible with liver transplantation. Other agents currently being evaluated for the treatment of PPH include sildenafil, a phosphodiesterase-5 inhibitor, and endothelin receptor antagonists (e.g. bosentan). In contrast to HPS, PPH is less reversible following transplantation; most patients with pretransplant PPH require continued pharmacologic therapy in the short term, with potential weaning off therapy over time.

Hepatic hydrothorax

Hepatic hydrothorax is ascitic fluid accumulation in the pleural space, which causes dyspnea and potential respiratory failure. Fluid accumulation in the abdomen migrates through small diaphragmatic defects, and can rapidly accumulate in the pleural space given the relatively negative intrathoracic pressure compared to the peritoneal space. Most commonly, the effusion is right sided (seen in up to 66% of patients with hydrothorax). The mainstay of therapy is diuretic therapy. In the setting of acute dyspnea, thoracentesis can be performed. A large-bore chest tube is not recommended given the risk of inducing hypovolemia and hypovolemic shock. Pleurodesis is not a viable option for recurrent hepatic hydrothorax, since persistent accumulation of fluid prevents optimal apposition of the visceral and parietal pleura. In the setting of a recurrent hepatic hydrothorax process that is refractory to maximal diuretic therapy and thoracentesis, a TIPS may be curative.

The pleural fluid is most commonly transudative. A pleural fluid analysis that demonstrates exudative characteristics should raise the possibility of a para-pneumonic effusion. A hepatic hydrothorax can become infected and develop spontaneous bacterial pleuritis (defined as a transudate with an absolute polymorphonuclear cell count of >250/mm^3 or a positive fluid culture) and should be treated like spontaneous bacterial peritonitis [26].

Respiratory failure

Cirrhotic patients in the ICU often require mechanical ventilation due to an altered mental status, acute lung injury, sepsis, or multiorgan failure. Cirrhotic patients are at risk of developing acute lung injury/acute respiratory distress syndrome [27] due to pulmonary or extrapulmonary insults. Although there are no evidence-based guidelines on the optimal ventilator strategy for cirrhotic patients with acute lung injury or acute respiratory failure [acute respiratory distress syndrome (ARDS)], it is commonly recommended to extrapolate from the Acute Respiratory Distress Syndrome Network study and use a low tidal volume of 6 mL/kg predicted body weight [28]. In addition, ascites, pleural effusions, and chest wall edema commonly seen in cirrhotic patients can further compromise lung mechanics, thereby increasing the risk of barotrauma and volutrauma during mechanical ventilation. In the setting of prolonged mechanical ventilation for airway protection due to persistent hepatic encephalopathy, tracheostomy may have to be considered using a percutaneous or surgical technique.

Variceal bleeding

Gastroesophageal variceal bleeding is a life-threatening complication in cirrhotic patients. Portal hypertension causes increased vasodilatation of the splanchnic circulation, resulting in increased portal inflow. This increased flow, coupled with intrahepatic resistance, causes the formation of portosystemic variceal collaterals in gastroesophageal venous plexus. The development of varices is directly related to the hepatic sinusoidal resistance as measured by the hepatic venous pressure gradient (HVPG), and develops at pressures of around 10–12 mmHg [29]. These varices are at risk for rupture and can cause massive gastrointestinal hemorrhage.

Initial management includes the placement of two large-bore intravenous (18 G and higher) or large-bore central venous access catheters, and fluid and blood resuscitation. Careful hemodynamic monitoring is needed because overly aggressive fluid resuscitation can lead to increased portal pressure and worsening hemorrhage. In the setting of hematemesis or shock, elective intubation is indicated, which also facilitates the performance of endoscopy. It is critical that the patient's anemia and coagulopathy is aggressively managed, with a combination of pRBCs, platelets, and FFP to reverse the hematologic derangements. In addition, prophylactic

antibiotics should be administered to decrease the rate of bacterial infection from the variceal bleeding; either a quinolone or cephalosporin should be initiated and continued for 7 days. The initiation of antibiotics in this setting has been associated with a decrease in the rates of rebleeding and mortality [30].

The prompt initiation of pharmacologic therapy with a splanchnic vasoconstrictor such as octreotide (somatostatin analog) or terlipressin (vasopressin analog) is recommended. In addition, emergent endoscopy with therapeutic intent should be performed. In the setting of esophageal variceal bleeding, endoscopic band ligation can be successful in achieving hemostasis. In the setting of isolated gastric variceal bleeding or esophageal variceal bleeding that is refractory to endoscopic hemostasis, TIPS therapy by interventional radiology can be lifesaving. While awaiting TIPS therapy after a failed endoscopic approach, balloon tamponade therapy with gastric and esophageal balloon insufflation can provide a successful temporizing measure to stabilize active variceal bleeding.

Renal dysfunction and hepatorenal syndrome

Renal failure is a common complication of ACLF. The etiology of renal dysfunction can be pre-, intra-, or post-renal. Standard work-up to identify the cause of kidney injury includes an analysis of the urine sediment and indices, and imaging with ultrasound. The causes of AKI in ACLF include, shock, nephrotoxic drugs, and intrinsic renal disease. HRS is a unique prerenal etiology seen in cirrhotic patients and carries an increased mortality. HRS occurs due to splanchnic vasodilatation in advanced portal hypertension, which results in a reduction in the effective arterial blood volume. This arterial hypotension causes renal hypoperfusion, a compensatory renal vasoconstriction, and a consequent reduced glomerular filtration rate (GFR) [31].

HRS is classified into two types. Type 1 HRS is rapidly progressive AKI characterized by a doubling of the serum creatinine to >2.5 mg/dL or a 50% reduction of the initial 24-h creatinine clearance to a level <20 mL/min in <2 weeks. Type II HRS is defined as renal insufficiency (serum creatinine of 1.5–2.5 mg/dL) without rapid progression, and is typically associated with refractory ascites. HRS is a diagnosis of exclusion, and needs to be distinguished from prerenal azotemia by the lack of a renal response to a fluid challenge with crystalloid or colloid [32]. Combination therapy targeting splanchnic and systemic vasoconstriction with the use of octreotide (somatostatin analog) and midodrine (α-adrenergic agonist), respectively, is a potential treatment option for HRS. Terlipressin is a potent splanchnic vasoconstrictor, and has been shown to improve renal function in HRS [33–35]. It is currently in phase III clinical trials in the USA, and not yet FDA approved for use. In the setting of AKI due to HRS that is refractory to pharmacologic therapy, renal replacement therapy may have to be initiated. HRS and especially prolonged renal replacement therapy beyond 6 weeks should trigger a consideration for combined liver and kidney transplantation [36].

Ascites and spontaneous bacterial peritonitis

Ascites

Ascites is caused by portal hypertension and the derangement of the renin–angiotensin–aldosterone system, which leads to the pathologic renal absorption of sodium and water. New-onset or worsening ascites should trigger an evaluation for acute mesenteric venous thrombosis, and in particular portal vein and hepatic vein thrombosis. In the setting of preserved renal function, combination diuretic therapy with spironolactone and furosemide can be optimized to decrease ascites. If ACLF is complicated by AKI, pharmacologic therapy with diuretics is contraindicated, and large volume paracentesis (LVP) is indicated. If >5 L of fluid is removed during the paracentesis, albumin at a dose of 5 g/L of ascites removed should be administered to help prevent post-paracentesis circulatory dysfunction, which can lead to hypotension and renal impairment. If recurrent LVP is required for the management of persistent ascites, definitive therapy with a TIPS may be considered.

It must be noted that in the critical care setting, tense ascites can induce intra-abdominal hypertension, and the associated physiology of abdominal compartment syndrome (ACS). ACS is characterized by hypotension due to decreased venous return secondary to IVC compression, oliguria due to renal vascular compression, elevated peak and plateau pressures on the ventilator due to decreased chest wall compliance, and potentially, mesenteric ischemia due to compromise of splanchnic blood flow. Bladder pressure measurements using the Foley catheter can provide some objective evidence for intra-abdominal pressures, with a value of >20 mmHg being pathologic. Therapeutic interventions for ACS include large-volume paracentesis and gastrointestinal luminal decompression with nasogastric and rectal tubes.

Spontaneous bacterial peritonitis

Patients with persistent ascites can develop bacterial spontaneous peritonitis (SBP). It has been reported that up to 12% of cirrhotic patients requiring inpatient management for ACLF have SBP; therefore, cirrhotic patients admitted to the hospital should undergo evaluation for SBP with paracentesis [32]. The diagnosis of SBP is made if the ascitic fluid absolute neutrophil count is >250 cells/mm^3 or if the ascitic fluid culture is positive for an organism. Treatment is with a third-generation cephalosporin (e.g. cefotaxime 2 g/every 8 h). With regard to duration of antibiotic therapy, a randomized controlled study found that treatment for 5 days was just as effective as treatment for 10 days [37]. Of note, in addition to bacterial SBP, there is a rising incidence of fungal SBP and therefore, a lack of response to empiric antibacterial therapy should raise the possibility of a fungal infection.

In addition to antibacterial therapy, co-administration of albumin at a dose of 1.5 g/kg within 6 h of detection of SBP and 1 g/kg on day 3 following diagnosis, has been shown to decrease the incidence of renal impairment and death compared to antibiotic treatment alone [38].

Sepsis

Patients with cirrhosis are at increased risk of developing sepsis, severe sepsis, and septic shock. It has been reported that cirrhotic patients with septic shock have an inhospital mortality of up to 70% [39]. Although the mechanisms for increased risk of bacterial and fungal infection in cirrhotic patients are not clear, there has been evidence that cytokine deficiency and macrophage dysregulation play a role. The most common infection is SBP followed by urinary tract infection, pneumonia, bacteremia, and cellulitis. Cultures are positive in about 50–70% of cases and are normally due to Gram-negative bacilli, especially *Escherichia coli*. Gram-positive cocci are seen in 30–35% of cases. Given the increased use of empiric antibacterial therapy, the incidence of nosocomial fungal infections appears to be increasing. Due to the baseline low MAP and systemic vascular resistance, infectious triggers can precipitate rapid hemodynamic deterioration and florid septic shock. Furthermore, cirrhotic patients with septic shock have a higher incidence of adrenal

insufficiency (51–68%), which may be due to chronically decreased adrenal perfusion [40].

Treatment for sepsis and septic shock in cirrhosis is similar to the established management strategies for septic shock in general intensive care; these interventions include early goal-directed therapy aimed at ensuring adequate end-organ perfusion with volume resuscitation and vasoactive agent support, empiric broad-spectrum antimicrobial therapy, and a lung protective ventilatory strategy for acute lung injury.

Summary

The critical care management of patients with liver failure requires a detailed multiorgan, system-based approach to critical illness. The differing pathophysiology of ALF and ACLF requires specific management strategies to address hepatic and extrahepatic derangements. Clinical decision-making involves a team-based approach that incorporates the input of the transplant hepatologist, transplant surgeon, and intensivist, with a goal to stabilize the patient prior to liver transplantation.

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CHAPTER 44

Intensive Care in Cardiac Failure

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Introduction

Cardiovascular disease increasingly complicates the care of transplant patients regardless of the organ system considered. Many indications for non-cardiac transplantation, such as hypertensive nephrosclerosis or insulin-dependent diabetes, represent serious risk factors for concomitant ischemic heart disease, and the generally aging recipient population adds cardiovascular risk as a general concern in most transplant settings. Brain death also markedly impacts cardiac function and thus, the issue of cardiac support impacts the procurement of essentially all organs. The hemodynamic alterations of advanced heart failure frequently compromise the function of vital organs, and result in the need for critical care interventions. In this chapter, we review the approach to a critically ill heart failure patient in need of vasoactive therapies, as well as the role of intra-aortic balloon counter-pulsation and mechanical ventilation in this clinical setting. The chapter complements Chapter 39, which covers waitlist management of critically ill patients awaiting heart transplantation, and provides companion information for Chapter 48, which covers the use of ventricular assist devices. The pathophysiology and approaches to management of disturbances of other organ systems that result from compromised cardiac function, such as pulmonary hypertension, the cardiorenal syndrome, and liver failure, are also discussed. As such, the information contained herein is applicable to most areas of transplantation that deal with critically ill patients and advanced cardiovascular co-morbidities.

Intravenous vasoactive drugs

Patients with advanced heart failure may develop hemodynamic instability and present critically ill with hypotension, respiratory distress, or progressive end-organ dysfunction. In these clinical scenarios, vasoactive agents are often required to improve hemodynamics and preserve end-organ function.

The selection and dosing of vasoactive agents should be based on careful assessment of the patient's hemodynamics. The main parameters of interest are systemic blood pressure, volume status, cardiac output, and systemic vascular resistance. Physical examination can provide a good estimate of these parameters, and objective data can be obtained through right heart catheterization (using a pulmonary artery, Swan-Ganz, catheter). The benefit of pulmonary artery catheter placement in critically ill patients has been questioned. In fact, randomized studies have shown that the routine use of pulmonary artery catheters does not provide a mortality benefit or reduce hospitalizations [1–3]. However, there are scenarios where the use of a pulmonary artery catheter would be reasonable. Typically, right heart catheterization should be considered in a critically ill heart failure patient, especially in the presence of progressive end-organ dysfunction, in whom physical examination does not provide an unequivocal indication of hemodynamic status, or in whom therapy instituted based on clinical assessment does not lead to the expected results [4–7]. Hemodynamic measurements obtained through right heart catheterization are then used to select the appropriate vasoactive agents.

Classes

Inotropic agents

Marked reduction of cardiac output is often the reason for severe symptoms and end-organ dysfunction in patients presenting with heart failure exacerbation. Dobutamine and milrinone are the two most common inotropic agents used to improve cardiac output in heart failure. Both act by increasing cyclic AMP (cAMP) in myocardial and vascular smooth muscle cells (Figure 44.1) [8–11]. In myocardial cells, this leads to a Ca^{2+}-mediated increase in contractility and enhanced chronotropic response. In vascular smooth muscle, cAMP leads to enhanced Ca^{2+} uptake by the sarcoplasmic reticulum and vasodilation.

Dobutamine, a synthetic catecholamine, is a strong agonist of $beta_1$ and $beta_2$ receptors. Binding to the $beta_1$ receptors in the myocardium leads to an increase in cAMP synthesis and a strong inotropic, as well as weaker chronotropic, effect [12]. The effect of binding to $alpha_1$ and $beta_2$ receptors on the vascular smooth muscle cells is dependent on the dose used—at lower doses ($\leq$5 μg/kg/min) the net effect is mild vasodilation, at moderate doses (up to 15 μg/kg/min) peripheral resistance is unchanged, and at higher doses (>15 μg/kg/min) vasoconstriction predominates [13].

Milrinone increases cAMP by inhibiting phosphodiesterase 3 (PDE 3), an enzyme that breaks down cAMP into AMP in the myocardium and vascular smooth muscle (Figure 44.1) [14]. In the myocardium, milrinone produces a potent inotropic effect and improves diastolic relaxation (lusitropy) [15–17]. Vasodilation in

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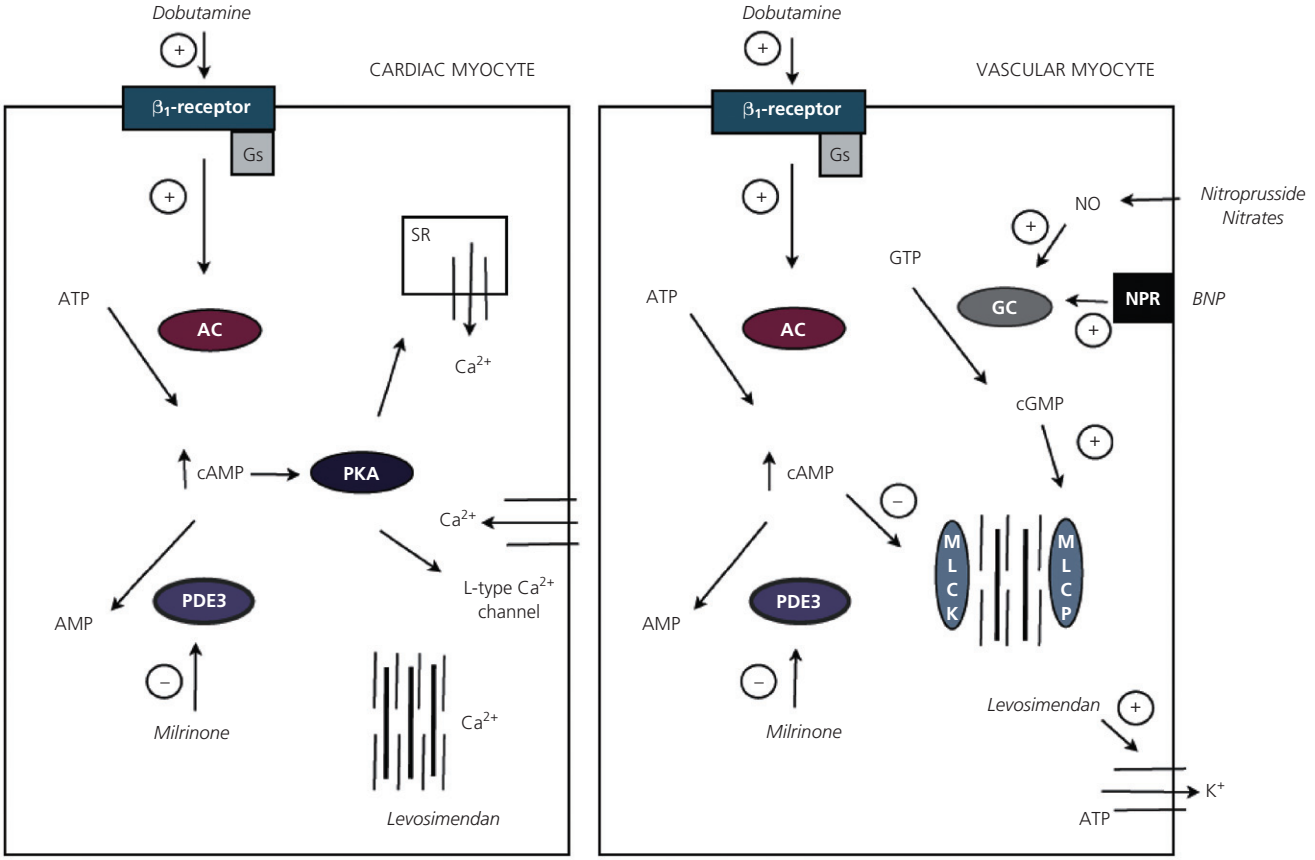

Figure 44.1. (A) Beta-agonists such as dobutamine activate the adenylcyclase (AC) system via G proteins (Gs), leading to an increase in cyclic AMP (cAMP) concentration. Milrinone increases cAMP by inhibiting phosphodiesterase 3 (PDE3) and the breakdown of cAMP into adenosine monophosphate (AMP). In cardiac myocytes, cAMP activates protein kinase A (PKA), leading to phosphorylation of L-type calcium channels and increased Ca^{2+} entry and greater release of Ca^{2+} from the sarcoplasmic reticulum (SR). Ca^{2+}-mediated activation of the actin–myosin–troponin system results in increased contractility. Levosimendan increases contractility by increasing the sensitivity of the myofilaments to Ca^{2+}. (B) In vascular smooth muscle, cAMP inhibits myosin light chain kinase (MLCK) and leads to relaxation. Nitric oxide (NO) and the natriuretic peptide receptor (NPR) act through guanylylcyclase (GC) to increase cyclic GMP (cGMP), activating myosin light chain phosphatase (MLCP) and causing relaxation. Levosimendan activates ATP-dependent K^+ channels, inducing vasorelaxation.

the systemic and pulmonary vasculature is more pronounced than with dobutamine [18,19]. Milrinone is cleared renally, so its dose may need to be adjusted in patients with renal dysfunction.

Direct comparisons of dobutamine and milrinone have shown similar clinical outcomes, including similar hemodynamic efficacy and arrhythmogenic potential [20]. There are situations, however, where one agent may be preferred over the other. Dobutamine leads to less systemic vasodilation and may be preferred in a hypotensive patient, particularly when the systolic blood pressure is $\leq$85 mmHg. Due to blunted beta-adrenergic receptor responses, or due to treatment with beta-blockers, some heart failure patients show diminished efficacy with dobutamine therapy [21]. As milrinone acts through non–beta-adrenergic pathways, these factors do not blunt the response to milrinone. In addition, due to more potent pulmonary vasodilation and greater right ventricular (RV) afterload reduction, milrinone may be preferred in patients with pulmonary arterial hypertension and/or RV dysfunction, as discussed later in this chapter. No mortality differences have been found in the chronic use of dobutamine versus milrinone. While acute administration of these inotropic agents leads to predictable improvement of hemodynamics, chronic administration of both dobutamine and milrinone is associated with increased myocardial oxygen consumption and possibly increased mortality [22–27].

Calcium-sensitizing agents

Calcium sensitizers represent a newer class of inotropes, with levosimendan being the most widely studied agent. In the myocardium, levosimendan binds to troponin C, sensitizing the myofilaments to calcium, which results in increased contractility. In vascular smooth muscle, levosimendan opens ATP-dependent potassium channels, resulting in vasodilation and decrease in preload and afterload [28,29]. Studies have shown improved symptoms in patients with acute decompensated heart failure treated with levosimendan, but survival was no different compared to patients treated with continuous dobutamine [30,31].

Other sympathomimetic agents

Dopamine is a norepinephrine precursor with cardiac and vascular effects that differ according to the dose [32,33]. At low doses (1–3 μg/kg/min), stimulation of dopaminergic receptors in the kidney and splanchnic arteries promotes vasodilation and increases blood flow through these vascular beds. At intermediate doses

(3–10 μg/kg/min), dopamine binds to $beta_1$ receptors, resulting in increased cardiac contractility and chronotropy and a mild increase in peripheral resistance. At higher doses (10–20 μg/kg/min), $alpha_1$-adrenergic stimulation in the peripheral vasculature predominates, resulting in peripheral vasoconstriction and an increase in blood pressure.

Norepinephrine is a potent $alpha_1$ receptor agonist with weaker beta-receptor binding. Norepinephrine administration results in intense vasoconstriction with less pronounced inotropic and chronotropic effects. It may be used temporarily in the setting of significant hypotension; however, prolonged use may be associated with direct myocardial toxicity and arrhythmias.

Epinephrine acts as a strong agonist of cardiac and vascular alpha and beta receptors. At low doses the beta-adrenergic effects predominate, while at higher doses alpha-adrenergic stimulation is greater and results in pronounced arterial and venous vasoconstriction. Epinephrine is often used in the setting of low cardiac output following heart transplantation. Its use in heart failure patients is more limited, as it is associated with a marked increase in myocardial oxygen consumption, an increase in afterload, and increased arrhythmias; it may be used temporarily when severe hypotension is present.

Isoprotenerol is a non-selective beta-receptor agonist with minimal effect on alpha-receptors. It has potent inotropic and chronotropic effects, and is often used immediately following heart transplantation where maintenance of a higher heart rate is desirable [34]. The use of isoproterenol in advanced heart failure patients outside of this setting is limited.

Arginine vasopressin

Vasopressin is an agonist of vasopressin V1 receptors in the vascular smooth muscle. Vasopressin administration results in peripheral vasoconstriction and increases the response of vascular smooth muscle to catecholamines [35]. The use of vasopressin can be considered in patients with heart failure and hypotension believed to be due to marked peripheral vasodilation. Unlike many other vasopressor agents, vasopressin does not have significant arrhythmogenic effects.

Intravenous vasodilators

Chronic heart failure results in activation of the sympathetic nervous system and peripheral vasoconstriction. This increase in peripheral vascular resistance and afterload limits cardiac output and leads to progression of cardiac dysfunction. As long as systemic blood pressure is not too low, intravenous vasodilators may favorably alter hemodynamics.

Sodium nitroprusside is a potent arterial and venous dilator in both the systemic and the pulmonary vasculature. The vasodilation results from reduction of nitroprusside by intracellular glutathione, a reaction that produces nitric oxide (NO). In patients with reduced cardiac output but without hypotension, nitroprusside might be preferable to inotropes as it does not increase myocardial oxygen uptake and is not arrhythmogenic [36,37]. However, the use of nitroprusside may be limited by hypotension. Other adverse effects are related to its metabolites. Cyanide, a product of nitroprusside metabolism, can accumulate in patients with hepatic dysfunction, but cyanide toxicity has also been described in patients with normal liver function. Manifestations of cyanide toxicity include lactic acidosis, nausea, restlessness, and methemoglobinemia. Thiocyanate (a metabolite of cyanide) toxicity is seen in patients with renal dysfunction, and presents as weakness, tremor, confusion, nausea, hyperreflexia, and rarely coma [38,39].

Nitrates are NO donors and their primary effect is venodilation, although arterial dilation can be seen at higher doses. Patients with decompensated heart failure refractory to diuretic therapy, particularly those with disproportionate right-sided failure, may benefit from intravenous nitroglycerin administration. Its use is limited primarily by a rapid onset of tolerance [40,41].

Nesiritide is a recombinant form of human B-type natriuretic peptide (BNP) and mimics the effect of endogenous BNP. It produces arterial and venous dilation and lowers pulmonary capillary wedge and right atrial pressures. Studies of nesiritide in acute decompensated heart failure have shown improvements in symptoms, but no mortality benefit [42–44]. In heart transplant candidates, nesiritide has occasionally been used to bridge critically ill patients unresponsive to other therapies to transplant [45]. The main adverse effect of nesiritide is hypotension.

Clinical application

The use of vasoactive agents in patients with heart failure has been contentious. Clinical trials examining the benefit of inotropic agents in advanced heart failure patients presenting in cardiogenic shock are lacking. Clinical trials evaluating inotropic therapy in less sick patients with advanced heart failure not awaiting heart transplantation have shown increased morbidity and mortality with both short-term and chronic use [24,46,47]. The mechanisms for these undesirable effects are believed to be an increase in intracellular calcium concentration and calcium overload of the sarcoplasmic reticulum, leading to arrhythmias and maladaptive remodeling [48,49]. Clinical guidelines recommend against routine use of these agents in patients hospitalized with acute decompensated heart failure, but note that chronic use is appropriate in inotrope-dependent patients awaiting heart transplantation, or as palliative therapy for severely symptomatic patients as part of end-of-life care [50].

Vasoactive agents are often initiated in heart transplant candidates who present in acute heart failure exacerbation. The specific agents are selected based on the patient's hemodynamics and the attributes of the individual agents reviewed above. Patients with marked systemic hypotension require the use of agents without vasodilatory effect, such as dopamine, epinephrine and norepinephrine. Studies comparing outcomes of the different agents with vasopressor properties in heart failure patients are limited [51]. In patients with low cardiac output without marked hypotension, milrinone or dobutamine are the most frequently used inotropes. Compared to dobutamine, milrinone is associated with less drug tolerance and more vasodilation of pulmonary vasculature; however, in the absence of compelling comparative clinical trials, the two drugs are believed to provide similar results [52]. When used in combination, the beta-agonist effect of dobutamine and the PDE 3 inhibition of milrinone may produce benefit in patients with an inadequate response to either agent alone.

Once hemodynamics are improved, the goal is to transition the patient to oral therapy. In a subset of patients, however, withdrawal of the intravenous vasoactive support will again lead to hemodynamic instability, worsening heart failure, and end-organ dysfunction [48]. In the absence of other reversible factors, these patients are deemed inotrope dependent, and may require continuous inotropic support as a bridge to transplant. This clinical scenario is frequent; between 2002 and 2010, 45% of the 27 387 patients registered in the Scientific Registry of the International Society for Heart

& Lung Transplantation (ISHLT) were on inotropic support at the time of transplant [53]. Patients bridged with inotropes while awaiting transplant require close follow-up. Given the arrhythmogenic properties of inotropes, an implantable cardiac defibrillator should be placed before discharge to outpatient care [54–56]. As inotrope dependency represents a truly advanced stage of heart failure and is associated with higher risk of mortality on the transplant waitlist [57], some organ allocation systems afford these patients a higher urgency status. In the US, for example, patients on a continuous infusion of a single high-dose intravenous inotrope (dobutamine $\geq$7.5 μg/kg/min, milrinone $\geq$0.5 μg/kg/min, dopamine $\geq$7.5 μg/kg/min, or epinephrine $\geq$0.02 μg/kg/min) or two or more intravenous inotropes, and with continuous hemodynamic monitoring, are eligible for the highest urgency 1A status. Patients receiving a continuous infusion of intravenous inotropes, including those maintained on a single agent and as outpatients, will qualify for the next urgency status 1B [58].

Inotrope-dependent patients whose waiting time for transplant is expected to be prolonged, as well as patients with progressive hemodynamic deterioration on inotropic therapy, should be considered for mechanical assist support as a bridge to transplant, either using a ventricular assist device or a total artificial heart. These topics are discussed in more detail in Chapters 48 and 49, respectively.

Intra-aortic balloon counter-pulsation

Intra-aortic balloon pump (IABP) counter-pulsation is often considered in patients with heart failure refractory to medical therapy. The concept of intra-aortic counter-pulsation was first developed by Moulopoulos and colleagues in the early 1960s [59], and applied clinically by Kantrowitz in 1968 [60]. Current applications for the IABP include support in cardiogenic shock, use in high-risk coronary interventions, treatment of intractable angina, and support during weaning from cardiopulmonary bypass [61,62]. The IABP remains the most widely used form of mechanical circulatory support.

Hemodynamic effects

The IABP consists of an inflatable balloon placed percutaneously, usually through the femoral artery, into the descending aorta. An external console controls inflation and deflation of the balloon at precise time intervals during the cardiac cycle: the balloon is inflated at the onset of ventricular diastole and deflated just before the onset of ventricular systole. Balloon inflation in diastole displaces blood from the descending aorta, resulting in increased diastolic pressure and coronary perfusion, as well as enhanced blood flow to other organs during diastole. In systole, balloon deflation creates a vacuum effect, reducing afterload and improving forward blood flow. Many believe it is this reduction in afterload that is most beneficial to a failing left ventricle (LV) [63,64]. Scheidt et al. reported that in a patient with cardiogenic shock, the average result of IABP counter-pulsation was a 20% decrease in systolic pressure, a 30% increase in diastolic pressure, a 20% increase in cardiac output, and a 20% decrease in mean pulmonary capillary wedge pressure (PCWP) [65]. For optimized hemodynamic results, inflation and deflation of the IABP must be synchronous to a patient's cardiac cycle. Technological advancements have allowed for IABP control systems to automatically optimize balloon inflation and deflation timing.

Clinical application

The IABP has been successfully used to bridge approximately 7% of patients to transplant over the past two decades [53]. Patients bridged to transplant with the IABP do not have an increase in post-transplant mortality [53,66].

The IABP has been used most frequently in patients who present with cardiogenic shock due to acute ST-segment elevation myocardial infarction (STEMI). As opposed to inotropes and vasopressors, an IABP does not increase oxygen consumption in the ischemic myocardium. The efficacy of the IABP has been undergoing some renewed scrutiny. A meta-analysis of nine studies of STEMI and cardiogenic shock found that the IABP was associated with a reduction in 30-day mortality in patients treated with thrombolysis, but no significant benefit in the overall cohort (n = 10 529) [67]. Observational data from the National Registry of Myocardial Infarction 2 did not show a mortality reduction with IABP use associated with primary percutaneous coronary intervention (PCI), although a benefit was found in hospitals with a higher volume of IABP use [68]. Most recently, a multicenter randomized trial assigned 600 patients with cardiogenic shock due to acute myocardial infarction, and plan for revascularization, to IABP vs. no IABP. This study has not shown a difference in 30-day mortality in the two treatment groups [69]. Despite the lack of unequivocal clinical efficacy data, the predictable favorable hemodynamic response to IABP counter-pulsation in this clinical scenario is such that STEMI clinical guidelines recommend use of an IABP in cardiogenic shock that is not quickly reversed with pharmacologic therapy [70]. The IABP can then be used as a bridge to revascularization, other mechanical circulatory support, or heart transplant.

The IABP also has a role in patients with non-ischemic cardiomyopathy who present with decreased cardiac output and end-organ dysfunction. While IABP will favorably influence the oxygen supply-to-demand ratio in the myocardium, its ability to improve cardiac output is limited by overall myocardial reserve and contractility [71]. The benefits might be less in patients without coronary artery disease and myocardial ischemia. In a series of patients supported with IABP counter-pulsation, non-survivors were more likely to have chronic heart failure, a LV ejection fraction of $<$30%, New York Heart Association (NYHA) functional class IV heart failure, and acute myocardial infarction [72]. Therefore, in cardiomyopathy patients with profound shock, end-organ dysfunction, or significant arrhythmias, an IABP may be helpful, but plans for more definitive support with other mechanical assist devices should also be made.

IABP counter-pulsation, in combination with vasoactive drugs, has also been used to decrease elevated pulmonary artery pressures in patients awaiting heart transplantation. Newer therapies that include the use of phosphodiesterase 5 (PDE 5) inhibitors and profound unloading with LV assist device (LVAD) implantation, which will be discussed later in this chapter, make this approach less practical.

Contraindications to the IABP use include significant peripheral vascular disease and greater than moderate aortic insufficiency. Potential complications include vascular events such as limb ischemia, bleeding, and vessel laceration, as well as infection and stroke. A major limitation of the IABP is that the patient is immobile, which makes this therapy attractive only for a relatively short duration of time. While IABP placement through the left axillary artery allows the patient to be ambulatory, this method is not widely used [73]. As more permanent ventricular assist devices are now available, it appears reasonable to limit the use of the IABP to

approximately 1 week in patients who have alternative mechanical assist options.

Mechanical ventilation in a transplant candidate

Patients with advanced heart failure may present with respiratory insufficiency requiring mechanical ventilation. The goals of mechanical ventilation include improved gas exchange, decreased work of breathing, and decreased myocardial demand. The interactions between the cardiac and pulmonary systems in the setting of mechanical ventilation are complex [74,75]. Here we briefly review important basic principles relevant to the heart failure patient.

Hemodynamic effects

In normal spontaneous breathing, alveolar and pleural pressures are lowered with inspiration, augmenting venous return of blood to the heart [76]. The application of positive pressure ventilation increases the intrathoracic pressures relative to the extrathoracic vascular beds and alters the pressure gradients for both systemic venous return and LV outflow [77]. The result is a decrease in systemic venous return of blood and a reduction in LV afterload. The net effect is an overall decrease in intrathoracic blood volume and decreased myocardial work [75]. These hemodynamic changes are theoretically beneficial to patients with cardiogenic pulmonary edema. Further benefits of mechanical ventilation occur through improved arterial oxygenation and reduced respiratory muscle oxygen demand [78].

The effect of mechanical ventilation on RV function is less well described and has been best studied in patients with acute respiratory distress syndrome (ARDS). Initiation of positive pressure ventilation is accompanied by a drop in RV preload. During mechanical ventilation, high plateau pressures and high positive end-expiratory pressure (PEEP) may result in increased RV afterload [79,80]. These effects should be considered in patients with RV dysfunction in need of mechanical ventilation.

Clinical application

A common indication for mechanical ventilation in a patient with acute decompensation of heart failure is cardiogenic pulmonary edema. In this setting, the increase in left atrial pressure causes transudation of fluid into the interstitium and the alveoli, leading to increased lung stiffness and ventilation–perfusion mismatch causing hypoxemia, CO_2 retention, and an increased work of breathing. In addition to diuretics and vasodilators, initial treatment may include non-invasive positive pressure ventilation (NIPPV), which has been shown to improve oxygenation and reduce the need for invasive ventilation in patients with cardiogenic pulmonary edema [81–85]. When these treatments fail, however, or if a patient has hemodynamic instability or respiratory muscle fatigue, endotracheal intubation and invasive mechanical ventilation may be necessary. Other indications for mechanical ventilation in a patient with advanced heart failure include mechanical complications of myocardial infarction, refractory arrhythmias, pulmonary infection, and multisystem organ failure. Benefits of mechanical ventilation in these settings include maximal oxygen delivery to the tissues, correction of acid–base disturbances, and a decrease in myocardial demand.

Regardless of the specific indication, the need for mechanical ventilator support at the time of heart transplant remains a strong risk factor for increased mortality, and only a small number of patients are considered for heart transplantation while requiring mechanical ventilation. Of the 10 271 patients in the ISHLT Registry who underwent heart transplant between 2004 and 2009, <3% were supported with mechanical ventilation at the time of transplant. Mechanical ventilation was associated with a 60% increase in 1-year mortality, a risk that persisted after adjustment for other clinical co-variates in a multivariate analysis [53]. Therefore, activation of mechanically ventilated patients for transplantation should be discouraged. If the primary reason for mechanical ventilation is hemodynamic instability due to exacerbation or progression of heart failure, then considerations for inotropic support or mechanical circulatory support should be made to allow for stabilization, extubation, and functional recovery of the patient prior to transplant whenever possible. If the respiratory failure is not caused by hemodynamic instability, outcomes with either transplant or permanent mechanical circulatory support are likely to be poor.

Lungs in end-stage heart failure

Pulmonary hypertension due to LV dysfunction has been referred to as pulmonary hypertension with left heart disease, secondary, pulmonary venous, or postcapillary pulmonary hypertension [86]. Pulmonary hypertension is present in as many as 60% of patients with severe LV systolic dysfunction and an even greater proportion of patients with diastolic dysfunction [87], and is associated with increased morbidity and mortality [88,89]. In one study, mortality of patients with heart failure and moderate pulmonary hypertension was three-fold that of patients without pulmonary hypertension [90].

Up to 50% of patients referred for cardiac transplant evaluation have elevated pulmonary vascular resistance [91,92]. A transpulmonary gradient (TPG) of >16 mmHg carries an increased risk of RV failure and early mortality after transplant [91–93]. Patients with a pulmonary vascular resistance (PVR) of >2.5 Wood units (WU) and/or TPG of >15 mmHg despite vasodilator therapy have a three-fold higher risk of death early post transplant and also have compromised late post-transplant outcomes. Patients who demonstrate significant reversibility of pulmonary hypertension (PVR <2.5 WU) without the development of systemic hypotension have a post-transplant outcome similar to that for those with a PVR of <2.5 WU at baseline [92].

Pathophysiology of cardiopulmonary disease

The pathogenesis of pulmonary hypertension due to left heart disease is complex (Figure 44.2) [94]. Elevated left heart pressures due to systolic [88,90], diastolic [95], or valvular heart diseases [96] can result in a passive increase in pulmonary venous pressure and elevated mean pulmonary artery pressure. Two hemodynamic profiles have been described in patients with postcapillary pulmonary hypertension [97]. The first pattern, passive pulmonary hypertension, involves elevation in mean pulmonary artery pressure as a reflection of elevated PCWP with a minimal increase in TPG. This postcapillary pulmonary hypertension likely represents a compensatory response necessary to maintain forward blood flow against increased downstream pressure [97]. Reduction of PCWP in patients with passive, postcapillary pulmonary hypertension results in a decrease in mean pulmonary artery pressure [98]. The second pattern of pulmonary hypertension in left heart disease involves reactive pulmonary arterial vasoconstriction, superimposed on passively elevated pulmonary artery pressure. The exact mechanisms by which pulmonary venous congestion leads to a reactive increase in PVR are not known. The endothelium plays a central role in vascular

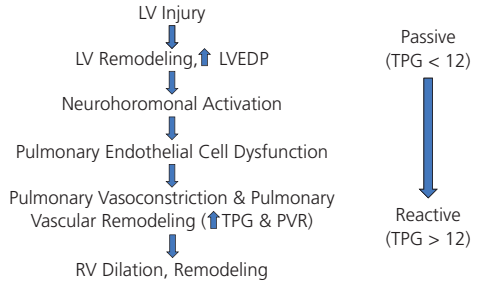

Figure 44.2. Pathobiology of pulmonary hypertension due to left heart disease. Chronic heart failure leads to neurohormonal dysregulation, an imbalance between the vasodilatory effects of nitric oxide (NO) and the vasoconstrictive effects of increased endothelin-1 (ET1). Left ventricular (LV) dysfunction, either systolic or diastolic, results in increased left ventricular end-diastolic pressure (LVEDP), which is passively transmitted to the pulmonary vascular bed, leading to elevated pulmonary artery pressures. Transpulmonary gradient (TPG) is initially low and pulmonary artery pressures are elevated proportionately to LV pressure elevation. Endothelial dysfunction may lead to reactive pulmonary arteriolar vasoconstriction and pulmonary vascular remodeling, where pulmonary artery pressure rises disproportionately to the degree of elevation of LV pressure (TPG increases). Pulmonary hypertension will lead to compensatory remodeling of the right ventricle (RV) and eventual RV dysfunction.

tone through the regulation of nitric oxide and endothelin pathways [94]. Nitric oxide-dependent vasodilation is impaired in patients with heart failure [99], while endothelin release is up-regulated [94]. Abnormalities in pulmonary vascular reactivity in heart failure are believed to be, at least in part, due to endothelial dysfunction and the imbalance of these pathways [94]. In patients with reactive pulmonary hypertension, TPG and PVR remain high even after LV filling pressure is reduced. Reactive pulmonary hypertension over time results in remodeling of the pulmonary vascular bed [100]. Thickening of both the alveolar and capillary basal laminae [101,102] reduces capillary filtration and microvascular permeability. Vascular remodeling likely serves initially to decrease the development of pulmonary edema; however, similar to the neurohormonal cascade in heart failure, these compensatory changes become maladaptive and lead to reduced lung compliance, increased PVR, reduced gas diffusion, and inefficient ventilation [101,102]. Acute reversibility of reactive pulmonary hypertension can be tested with vasodilator therapy. The lack of reduction of pulmonary hypertension, TPG, and PVR with acute hemodynamic tests has been termed "fixed pulmonary hypertension;" however lack of acute response does not necessarily indicate a permanent change. Some patients who do not show decrease in pulmonary pressures with acute testing will show gradual improvement of pulmonary hemodynamics over weeks or months when appropriate medical therapy is introduced [97]. More recently, the profound unloading that a LVAD exerts on the LV has been shown to predictably reduce pulmonary hypertension in most patients where the abnormal hemodynamics are due to left heart disease [103,104].

In a study of patients hospitalized with acute decompensated heart failure, 51% had passive and 25% had reactive pulmonary hypertension [105]. Compared to passive pulmonary hypertension, reactive pulmonary hypertension was more strongly correlated with death (adjusted hazard ratio 4.8 for reactive and 2.8 for passive pulmonary hypertension, compared to normal pulmonary pressures; $P = 0.0001$) [105].

The pulmonary circulation is the major determinant of RV afterload, and mortality due to cardiopulmonary disease is strongly related to RV dysfunction [106–108]. RV failure leads to a reduction in cardiac output and backwards congestion with resultant organ failure (see Kidney and Liver sections). The thin-walled RV can accommodate large increases in venous return and still pump blood through the typically low-resistance pulmonary circuit [109]. Increased RV afterload leads to distension of the RV, increased oxygen consumption, and reduced contractility [110]. Due to ventricular interdependence, RV distension also leads to paradoxical intraventricular septal motion with transmission of elevated right-sided filling pressures to the LV, potentially impairing LV filling [111–114]. Furthermore, increased wall tension from volume overload and myocardial distension may increase fiber length beyond a point where the Frank–Starling mechanism is active, and ventricular contraction may fail [115]. Pulmonary artery pressure further depends on RV function. A normal RV can generate peak systolic pressures up to 40–50 mmHg, but with gradual hypertrophy, much higher pressures can be generated [94]. When the RV begins to fail, the mean pulmonary artery pressure may fall despite a markedly elevated PVR [113].

Diagnosis of pulmonary hypertension

Echocardiography may be used as a screening test to assess right-sided pressures, as the tricuspid jet can be used to estimate RV systolic pressure. Echocardiographic findings suggestive of LV diastolic abnormalities include left atrial enlargement, LV hypertrophy, an abnormal mitral inflow profile, and abnormal mitral annular tissue Doppler signals [116,117]. However, invasive hemodynamic evaluation with right heart catheterization remains the objective diagnostic standard for pulmonary hypertension [87,116]. Right heart catheterization measures right- and left-sided filling pressures, pulmonary arterial pressure, PCWP, and estimated cardiac output. With this information, TPG and PVR can be calculated. In the presence of pulmonary hypertension, its pre- or post-capillary etiology can be determined. Vasoreactivity testing with pulmonary vasodilators is recommended to assess for reversibility of pulmonary hypertension in patients undergoing cardiac transplant evaluation. Vasodilators, including sodium nitroprusside, inhaled nitric oxide, or prostanoids, are typically administered to patients who have a systolic pulmonary artery pressure of >50 mmHg, a TPG of >15 mmHg, or PVR of >3 WU, with the goal of achieving a systolic pulmonary artery pressure of <40 mmHg, a TPG of <12 mmHg, and a PVR of <3 WU, without a drop in cardiac output and while maintaining a systolic blood pressure of >85 mmHg [118].

Management of pulmonary hypertension

Management should be directed at the underlying cause of disease and optimization of LV filling pressures [116]. The overall goal should be to improve physiologic coupling between the RV and pulmonary artery to improve pulmonary blood flow. A number of pharmacologic (diuretics, nitrates, hydralazine, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, nesiritide, milrinone) and mechanical (valve surgery, LVAD implantation) interventions may lower mean pulmonary artery pressures via a reduction in left-sided filling pressures. Additionally, a number of therapies target improvement in RV function. Specific therapeutic approaches are described below.

Vasodilators

Both non-selective and pulmonary vasodilators can reduce PVR and augment RV output via reduction in RV afterload. Inhaled

nitric oxide and prostanoids are pulmonary vasodilators with acute, favorable hemodynamic effects [119–121]. Nitric oxide needs to be continuously delivered into a ventilator circuit and rebound pulmonary hypertension may occur upon weaning [122]. Trials of selective pulmonary vasodilators in pulmonary hypertension with left heart failure have not yielded favorable effects on clinical outcomes. Epoprostenol resulted in higher mortality compared to standard therapy in a large, randomized controlled trial [123]. The endothelin antagonist bosentan failed to demonstrate beneficial effects in patients with chronic heart failure, although patients with postcapillary pulmonary hypertension were not specifically selected [124]. Pulmonary selective vasodilators may increase right-sided cardiac output to a poorly compliant LV and precipitate acute pulmonary edema [119,125–127].

Sodium nitroprusside reduces both pulmonary and systemic vascular resistance, leading to a reduction in LV afterload, which may offset any increase in LV filling due to improved RV function. Sodium nitroprusside has been used for acute hemodynamic testing of pulmonary hypertension reversibility, as well as for short-term treatment in acute decompensated heart failure [128]. The recombinant brain natriuretic peptide nesiritide also reduces pulmonary artery pressures and PVR in patients with postcapillary pulmonary hypertension [43,129]; however, benefit beyond improvement of hemodynamics has not been confirmed.

Sildenafil, a PDE 5 inhibitor, is a partially selective pulmonary vasodilator [130–132]. Some clinical investigations suggest that sildenafil may improve hemodynamics, exercise capacity, and quality of life in patients with chronic heart failure due to reduced LV function [133–135]. It has also been demonstrated to improve measures of diastolic function [136]. Sildenafil may also exert a milrinone-like effect through PDE 3 inhibition, augmenting RV function [137]. However, in a randomized trial, compared to placebo sildenafil did not improve exercise capactiy in patients with diastolic heart failure [138]. The efficacy of sildenafil in the management of acute postoperative RV dysfunction has been reported [139,140]. While routine use of PDE5 inhibitors for the management of postcapillary pulmonary hypertension cannot be recommended without more robust data, they are frequently used to reduce pulmonary pressure in heart transplant candidates [140–142].

Management of right ventricular dysfunction

There is lack of effective therapies for the management of RV dysfunction due to pulmonary hypertension. Management strategies include optimization of RV preload and systolic function, and reduction in RV afterload [115]. Attempts to also correct any reversible causes of elevated PVR such as hypoxia should be made. Sleep apnea is prevalent in heart failure patients and its treatment may reduce pulmonary hypertension and improve RV function. RV contractility can be optimized with inotropic agents, including dobutamine and milrinone, which are discussed earlier in this chapter.

Aggressive volume loading for RV failure may adversely affect LV filling by inducing septal shift and reducing LV volume [143–145]. Unmonitored fluid challenges are not advised in the setting of RV dysfunction [115]. Invasive hemodynamic monitoring may be employed to target an initial central venous pressure of 10 mmHg.

Transplant and mechanical support

In patients with end-stage heart failure and pulmonary hypertension that is not reversible with medical treatment, LVAD therapy may be considered [146]. This commonly results in a decrease in PVR over time due to normalization of LV filling pressures [103,104]. In cases of severe RV failure, a RV assist device (RVAD) may be necessary in conjunction with a LVAD to bridge patients to cardiac transplantation. Intra-aortic balloon counter-pulsation and extracorporeal membrane oxygenation have been used as rescue therapies in biventricular failure while awaiting more definitive therapy [147,148]. Protective lung strategies should be employed in patients requiring mechanical ventilation to reduce plateau pressures and avoid further strain on RV function [149]. Carefully selected patients with truly fixed pulmonary hypertension despite medical and device therapy may be candidates for combined heart–lung transplantation [150].

Kidneys in end-stage heart failure

The cardiorenal axis is important for regulation of fluid and electrolyte balance, blood pressure, and systemic perfusion. Acute or chronic dysfunction in either the heart or the kidneys can alter the function of the other organ, which is commonly referred to as the cardiorenal syndrome [151]. In patients with acute heart failure, the cardiorenal syndrome may result in a scenario where therapy aimed at relieving congestion results in further worsening of kidney function. The challenge of successful heart failure management lies in addressing fluid overload while avoiding further impairment in glomerular filtration rate (GFR).

Avoiding progressive renal dysfunction is critical, as reduced GFR in this patient population is a stronger predictor of mortality than reduced LV ejection fraction [152], and this risk is independent of the severity of heart failure symptoms [153–159]. In a systematic review of >80 000 heart failure patients, the mortality rate at 1 year was 24% in those with a normal GFR compared to 38% in patients with mild and 51% in patients with moderate-to-severe reductions in GFR [160]. Increase in serum creatinine during treatment for heart failure is also associated with worse outcomes, including longer length of hospital stay, more frequent readmissions, and increase in both inhospital and long-term mortality [157–159,161–167].

In patients with heart failure and elevated serum creatinine, it is important to distinguish between the cardiorenal syndrome, a result of hemodynamic derangements associated with heart failure, and underlying intrinsic chronic kidney disease. This distinction may be difficult given that in many heart failure patients both conditions can be contributing to abnormal renal function [168]. Findings suggestive of intrinsic kidney disease include significant proteinuria (>1 g/day), an active urine sediment (hematuria, pyuria, or cellular casts), and/or structural abnormalities on kidney imaging.

Pathophysiology of the cardiorenal syndrome

The cardiorenal syndrome is a complex vasomotor nephropathy with an array of factors contributing to the development of worsening kidney function [161,162,169,170]. Known pathophysiological mechanisms include neurohormonal activation, reduced renal perfusion, increased renal venous pressure, RV dysfunction, and systemic inflammation.

Neurohormonal activation

The decreased stroke volume and cardiac output seen in heart failure result in decreased effective arterial blood volume and arterial under-filling, producing decreased triggering of mechanoreceptors in the LV, aortic arch, carotid sinus, and baroreceptor-like

juxtaglomerular apparatus. This in turn results in compensatory activation of a number of neurohormonal pathways—increased sympathetic outflow, activation of the renin–angiotensin–aldosterone system, and the release of arginine vasopressin and endothelin-1, all aimed at restoring organ perfusion via systemic vasoconstriction and salt and water retention [171–173]. Over time, these processes become maladaptive and overwhelm the counter-regulatory vasodilatory and natriuretic pathways of natriuretic peptides, nitric oxide, prostaglandins, and bradykinin [162,169,174]. The effects of these mechanisms in the kidney are attenuated due to down-regulation of natriuretic peptide receptors, renal vasoconstriction, and reduced delivery of sodium to the distal nephron [172,175], which cause sodium and water retention despite elevated filling pressures in the heart [171,176,177].

The use of loop diuretics in heart failure produces increased sodium delivery to the distal tubule, which results in increased adenosine secretion [178]. Elevated serum adenosine levels stimulate afferent arteriolar constriction and proximal tubular sodium reabsorption [163,179]. These actions further reduce GFR, promote sodium and water retention, and may contribute to diminished diuretic responsiveness.

Thus, neurohormonal activation in heart failure results in vasoconstriction, increased afterload, reduced cardiac output, and reduction of renal blood flow.

Reduced renal blood flow

Worsening kidney function during heart failure exacerbation is often attributed to reduced cardiac output and renal hypoperfusion. This is certainly the case in patients who are hypotensive or in cardiogenic shock; however, the correlation between cardiac output and kidney function is not linear [164,165], as renal autoregulatory mechanisms maintain renal blood flow until the cardiac index falls below 1.5 L/min/m^2 [166]. As the majority of patients hospitalized for heart failure with kidney dysfunction are neither hypotensive nor in shock [153,180,181], it appears that factors other than low cardiac output likely play a greater role in the pathogenesis of the cardiorenal syndrome.

Venous congestion

Most patients admitted for acute heart failure exacerbation have signs and symptoms of systemic venous congestion [153]. However, even in the absence of peripheral edema, patients may have an increase in blood volume and elevated intracardiac filling pressures [167].

Ascites, visceral edema, and engorgement of the splanchnic circulation lead to increased intra-abdominal pressure, extrarenal compression, and a decline in kidney function [181]. As renal perfusion pressure is determined by the difference between the mean arterial pressure and renal venous pressure [182], progressive venous congestion may impede forward blood flow through the kidney [183,184], reduce urine generation, and decrease sodium excretion [185]. This explains why systemic venous congestion has been shown to be independently associated with decline in GFR [168,186,187] and why inadequate reduction of systemic venous pressures during treatment of heart failure exacerbation correlates more closely with decline in kidney function than reduction in cardiac output [168]. In the ESCAPE Trial [1] right atrial pressure was the only hemodynamic variable associated with baseline renal function [164].

Because the renal capsule is not distensible, interstitial edema will further increase interstitial and renal venous pressures (Figure 44.3) [182]. Increases in renal venous pressure also stimulate local

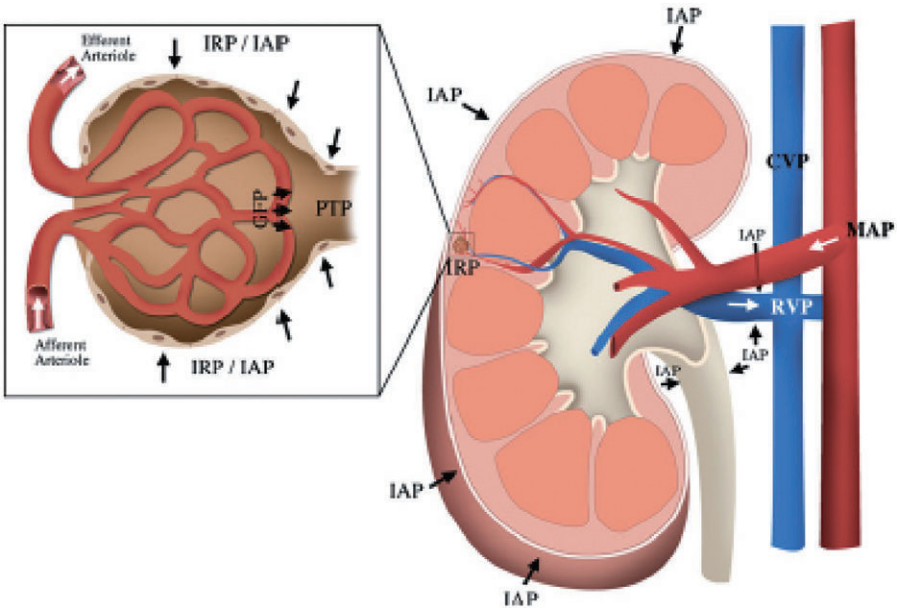

Figure 44.3. Hemodynamic determinants of glomerular filtration rate (GFR). In addition to the number and quality of nephrons, GFR is dependent on multiple hemodynamic factors. The mechanical force driving fluid from the glomerular capillaries to the capsular space is the (renal) filtration gradient (FG), which is equal to: glomerular filtration pressure (GFP) – proximal tubule pressure (PTP). PTP is dependent on the interstitial renal pressure (IRP) and intra-abdominal pressure (IAP), both of which are increased by venous congestion. GFP is dependent on renal blood flow (renal perfusion pressure; mean arterial pressure [MAP] – renal venous pressure [RVP]/renal vascular resistance). RVP is closely related to central venous pressure (CVP) and thus, is increased when venous congestion is present. GFP is further regulated by the complex interplay between afferent and efferent arteriolar vasoconstriction and vasodilatation. (Reproduced from Dupont et al. [182], with kind permission from Springer Science+Business Media.)

sympathetic nerve activity, causing intrarenal arterial vasoconstriction and a further decline in GFR [188–190].

Right ventricular function

Patients treated for acute heart failure exacerbation who have echocardiographic evidence of RV dysfunction typically have greater impairment in renal function compared to patients with normal RV function [191]. Dysfunction of the RV contributes to elevation of systemic venous pressures, which can affect renal function, as described above. In addition, due to pericardial constraint, RV dilatation impairs LV filling, and limits stroke volume and cardiac output [192]. Through both of these mechanisms, RV dysfunction likely plays an important role in progression of the cardiorenal syndrome.

Inflammation

Heart failure is known to result in endothelial activation [193–195]. In response to changes in circumferential stress, endothelial cells produce inflammatory mediators such as endothelin-1, interleukin-6, and tumor necrosis factor-alpha [182,196–199]. Oxidative stress further reduces nitric oxide bioavailability [182], which impairs endothelial nitric oxide-mediated regulation of venous tone, and may contribute to the cardiorenal syndrome [197].

Factors that may affect the response to therapy

Plasma refill rate

Plasma refill refers to fluid shift from the extra- to intra-vascular compartment in response to reduced blood volume, e.g. from diuresis or fluid removal by ultrafiltration. Plasma refill rate is dependent on oncotic and hydrostatic pressure gradients between the extra- and intra-vascular compartments [200]. If plasma refill rate is exceeded, worsening of renal function and hypotension may result. Therefore, a rise in serum creatinine with diuresis may not indicate sufficient decongestion; rather, it may be a result of excessive fluid removal from the intravascular space not matched by plasma refill [201].

Diuretic resistance

Diuretic resistance is defined as a diminished pharmacologic response to a given diuretic dose [202]. A number of mechanisms contribute to diuretic resistance. Often, drug bioavailability is reduced due to reduced gut perfusion and visceral edema [203]. Advanced heart failure may also lead to protein wasting and hypoalbuminemia [204], decreasing intravascular oncotic pressure, increasing the volume of distribution of loop diuretics, and impairing their delivery to the proximal tubule. Reduced renal blood flow may also impair tubular drug delivery [205]. Kidney dysfunction results in the accumulation of organic acids that inhibit tubular secretion of loop diuretics [185]. The pharmacodynamic effect of diuretics may be impaired even in the presence of adequate drug absorption and delivery. Impaired renal perfusion reduces sodium filtration, limiting the diuretic response. Chronic blockade of the proximal $Na^+/K^+/Cl^-$-transporter by loop diuretics also leads to increased sodium delivery to the distal tubule and eventual hypertrophy of the distal tubular cells [185], and a "braking effect," where increased sodium delivery causes a rebound increase in distal sodium reabsorption [206]. The effects of neurohormonal activation also counteract the effects of the diuretics in the kidney; conversely, decreases in norepinephrine levels in response to heart failure therapy have been shown to improve diuretic responsiveness [207].

Management of the cardiorenal syndrome

In the absence of specific therapies directed at increasing the GFR, the goal of therapy in heart failure with the cardiorenal syndrome is relief of systemic congestion and restoration of end-organ perfusion. Favorable hemodynamic changes may re-establish an effective renal arterial venous pressure gradient and improve the GFR [168]. Fluid removal with diuresis, ultrafiltration, or paracentesis may lower intra-abdominal pressure and improve kidney function [208].

Modification of sodium and fluid intake

While not tested in randomized trials, salt restriction is essential in avoiding worsening congestion [209] and it is recommended that sodium intake be limited to 2 g/day. A total fluid intake of <2 L/day is also frequently recommended, especially for patients with hyponatremia (<130 mEq/L) or those who have become diuretic resistant [209].

Diuretics

Diuretics remain the mainstay of managing congestion despite a lack of data regarding their safety and efficacy [209]. Patients with heart failure frequently have an unpredictable dose–response curve to loop diuretics, and these agents require careful titration until the desired response is achieved (Figure 44.4) [210]. Excessive diuresis can lead to hypovolemia, hypotension, reduced cardiac output, and neurohormonal activation, resulting in further decline in kidney function, and higher doses of diuretics have been associated with increased mortality [211–213]. In patients with diuretic resistance,

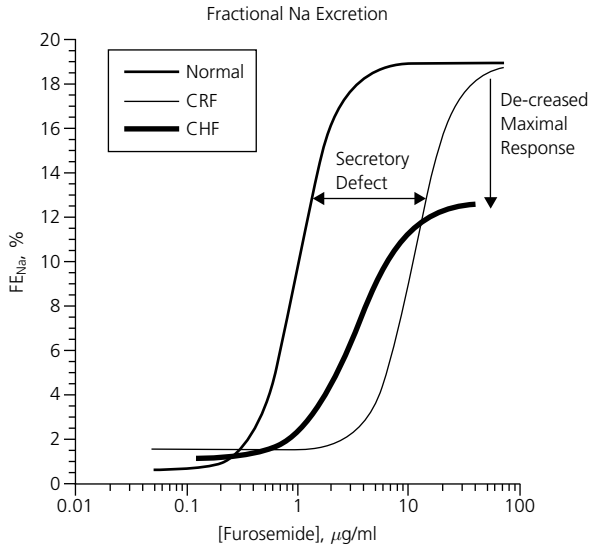

Figure 44.4. Dose–response curves for loop diuretics. Fractional Na^+ excretion (FE_{Na^+}) as a function of loop diuretic concentration. Compared with healthy controls, patients with chronic renal failure (CRF) show a rightward shift in the curve, owing to impaired diuretic secretion. The maximal response is preserved when expressed as FE_{Na^+}, but not when expressed as absolute Na^+ excretion. Patients with chronic heart failure (CHF) demonstrate a rightward and downward shift, even when the response is expressed as FE_{Na^+}, and thus are relatively diuretic resistant. (Reproduced from Ellison [210], with permission from S. Karger AG, Basel.)

diuretic responsiveness may be restored by increasing the diuretic dose. Continuous diuretic infusion or intermittent bolus dosing appear to result in similar volume loss at an equivalent daily dose [214]. Addition of thiazide diuretics can also decrease diuretic resistance. Thiazide diuretics act on the distal tubule and function synergistically with the more proximally-acting loop diuretics. Combined use of thiazide and loop diuretics overcomes the tubular hypertrophy and rapid sodium reabsorption that occur in response to chronically increased distal sodium delivery from long-term loop diuretic use [215].

All diuretics can alter serum electrolyte concentrations, with the potential for cardiac rhythm and neuromuscular disturbances. Careful electrolyte monitoring and replacement is warranted with all diuretic therapy, especially when loop and thiazide diuretics are used in combination. Potassium supplementation and potassium-sparing diuretics act to counter-balance potassium loss. The goal should be to maintain a potassium level at >4.0 mEq/L and a magnesium level of at least 1.5–2.0 mEq/L.

Ultrafiltration

Ultrafiltration has been proposed as a treatment option in patients with heart failure and volume overload with suboptimal response to diuretics. Although diuretics achieve increased sodium excretion, the urine is hypotonic relative to the plasma, with the average urinary sodium concentration following administration of a loop diuretic being 60 mEq/L, compared 140 mEq/L in the plasma [210]. Ultrafiltration has the theoretical advantage of removing greater amounts of salt by isotonic fluid removal. By controlling the rate of fluid removal, ultrafiltration also has the potential advantage of being able to tailor fluid removal to the estimated plasma refill rate. Initial experience with veno-venous ultrafiltration in heart failure suggests that this therapy is well tolerated, and unlikely to cause adverse hemodynamic effects [216,217]. In comparison with intravenous diuresis, ultrafiltration was associated with greater weight loss and fluid removal, with similar changes in serum creatinine [216,218]. If fluid removal does not exceed the plasma refill rate of approximately 12–14 mL/min, further activation of the renin–angiotensin–aldosterone system is avoided [200].

Whether ultrafiltration could aid in resolution of the cardiorenal syndrome remains to be seen. Some studies have shown that the weight loss achieved with ultrafiltration resulted in improved diuretic responsiveness and decongestion was better maintained compared to in patients who were treated with loop diuretics [207,218–220]. Excessive ultrafiltration may result in increased neurohormonal activation, decreased renal perfusion, and deterioration of renal function [183]. In a randomized trial of patients hospitalized with acute heart failure, persistent congestion and worsened renal function, while weight loss at 96 hours was similar, compared to a stepped pharmacologic-therapy algorithm, ultrafiltration was associated with greater rates of worsening renal function and increased adverse events [221]. Currently, ultrafiltration is typically reserved for patients who have failed to achieve a negative fluid balance with aggressive diuresis [209,222,223]. Patients with severe kidney dysfunction may require hemofiltration or hemodialysis rather than veno-venous ultrafiltration. Ultrafiltration should also be avoided when venous access cannot be obtained, in the presence of hypercoagulable states, or when shock or hypotension requiring vasoactive therapy is present [183].

Vasoactive therapies

Data regarding the role of vasoactive therapies in the management of the cardiorenal syndrome are limited. Many commonly used vasoactive drugs, including nitroglycerin, nitroprusside, nesiritide, and dobutamine, have shown variable results as far as improvement of kidney function, despite predictable improvement in cardiac output [224–227]. Many of the agents reduce preload and afterload through combined arterial and venous dilation [228]. By increasing renal blood flow and reducing renal venous pressures, vasodilators may improve kidney function. However, depending on a particular agent's relative effects on the renal vasculature (afferent versus efferent glomerular arteriolar tone) and the particular hemodynamic circumstances of the individual patient, the net effect on the renal blood flow and GFR may not be easily predictable. Systemic hypotension could offset any favorable effects of vasodilators on venous pressure and cardiac output [229]. Among patients with acute heart failure and renal dysfunction, compared to placebo, low-dose nesiritide (0.005 mcg/kg/min) did not enhance decongestion or improve renal function when added to diuretic therapy [230]. Although routine use of a pulmonary artery catheter has not been shown to improve outcomes, its use prior to implementing vasodilator therapy in patients in whom volume status and cardiac filling pressures are uncertain based on clinical assessment is warranted [209].

The utility of low-dose dopamine in preventing worsening of renal function in patients with heart failure and cardiorenal syndrome is uncertain as clinical investigations have shown inconsistent results [231,232,233]. In one study, a combination of low-dose dopamine (5 μg/kg/min) and a low-dose furosemide infusion (5 mg/h) has been shown to be as effective as a high-dose furosemide infusion (20 mg/h) at relieving congestion, with less kidney dysfunction and improved potassium homeostasis [234]. However, in a separate study of patients with acute heart failure and renal dysfunction, compared to placebo, low-dose dopamine (2 mcg/kg/min) did not enhance decongestion or improve renal function when added to diuretic therapy [230]. Additional well-powered investigations are needed and are currently in progress.

Use of intravenous inotropes in patients with acute heart failure may improve symptoms and kidney function by augmenting cardiac output and reducing cardiac filling pressures. Evidence from a number of clinical investigations raises concerns of increased mortality with inotropic treatment [27,235]. While milrinone leads to minor improvements in kidney function, this benefit does not translate to decreased rates of death or readmission [236]. As a result, routine use of intravenous inotropic agents in patients with chronic heart failure is generally not recommended. In heart transplant candidates with kidney dysfunction, intravenous inotropes can be used to stabilize hemodynamics and bridge these patients to implantation of mechanical circulatory support or transplantation.

Transplant and mechanical support

In patients with advanced heart failure and irreversible kidney disease, combined heart–kidney transplantation can be considered. According to data from the United Network for Organ Sharing (UNOS), between 1995 and 2005, 1.4% (n = 264) of heart transplant recipients in the US received a simultaneous kidney transplant [237]. While there are no professional society-endorsed guidelines to inform patient selection for combined heart–kidney transplantation, patients with an estimated GFR (eGFR) of <33 mL/min and no expected improvement in renal function may be good candidates for this therapy and gain a survival benefit compared to heart transplant alone [237]. Expected survival for heart–kidney transplant recipients is similar to that for patients who receive an isolated heart transplant [238]. In contrast, survival following heart transplantation alone in dialysis-dependent candidates is subopti-

mal and end-stage renal disease is therefore considered to be a contraindication to heart transplant, unless a kidney transplant is also planned [238].

In patients with advanced heart failure and cardiorenal syndrome, mechanical circulatory support often results in improvement of kidney function [239,240]. Other reports indicate that improvement in renal function following LVAD implantation may largely be transient [241]. However, LVAD insertion should be avoided in patients whose kidney function is unlikely to recover despite improved hemodynamics, with the exception perhaps in patients who are candidates for combined heart–kidney transplantation.

Liver in end-stage heart failure

There are two clinical syndromes that comprise the "cardiohepatic" syndrome. The first, ischemic hepatitis or "shock liver," is associated with acute heart failure and cardiogenic shock. The second, congestive hepatopathy, is more closely associated with chronic heart failure. However, there is significant clinical overlap between these syndromes. There is considerable interpatient variability such that some patients with mild heart failure have liver function test (LFT) abnormalities, while others with severe hemodynamic derangements do not have evidence of liver dysfunction [242]. What underlies this heterogeneity is unclear. When heart failure patients present with LFT abnormalities, one should also investigate the possibility of concomitant, intrinsic liver disease. Infectious, autoimmune, and biliary processes should always be excluded when evaluating LFT abnormalities in heart failure patients.

Ischemic hepatitis

Ischemic hepatitis or "shock liver" is characterized by a marked and reversible elevation in serum aminotransferase levels to at least 20 times the upper limit of normal in the absence of other causes [243,244]. The most common causes of ischemic hepatitis are cardiogenic—cardiogenic shock resulting from acute myocardial infarction, acute exacerbation of chronic heart failure, dysrhythmia, cardiac tamponade, etc. [243]. Less commonly, ischemic hepatitis may be caused by hypovolemia. The incidence of acute ischemic hepatitis in patients with cardiogenic shock is estimated to be around 20% [245].

Pathophysiology

The pathophysiology of ischemic hepatitis is not fully understood. The liver is highly vascular with a dual blood supply. Approximately 80% of hepatic perfusion is derived from the portal vein and the remaining 20% from the hepatic artery [244,246]. With hypotension, compensatory neuroendocrine and sympathetic nervous system responses redistribute cardiac output, with priority given to perfusion of the heart and brain [244]. Splanchnic vasoconstriction decreases portal vein flow; however, the liver is able to maintain normal oxygen uptake by increasing oxygen extraction [246]. This compensatory mechanism makes the liver relatively resistant to ischemic injury and may account for the relatively low incidence of liver injury in other forms of shock [247,248]. Additionally, ischemic injury is typically self-limited and reversible, unless the liver had been previously damaged [246]. Ischemic hepatitis presents with centrilobular necrosis [243,249], as the centrilobular areas are the most sensitive to oxygen deprivation [244]. The most commonly proposed mechanism is a sudden impairment in hepatic perfusion from hypotension and reduced cardiac output leading to hepatocyte hypoxia [243], with hepatic venous congestion also playing an important role [243]. Indeed, patients with low cardiac output and elevated central venous pressure are more likely to develop ischemic hepatitis than patients with low cardiac output alone [245], and patients with severe shock from trauma seldom develop LFT abnormalities [243]. It is therefore believed that passive congestion may predispose hepatocytes to hypoxic injury, as central venous congestion results in portal hypertension, which may alter hepatic blood flow [243]. The already abnormal hepatic blood flow may be further reduced in the setting of hypotension. Alternatively, hepatocytes chronically exposed to increased pressure may be more susceptible to injury. In addition, hepatocytes in the livers of patients with chronic heart failure may be subject to chronic recurrent injury from unrecognized episodes of underperfusion and hypoxia [243]. Thus, ischemic hepatitis likely requires derangements in both forward and backward blood flow [242].

Management

Supportive therapies targeting the underlying hemodynamic derangements should be employed to improve hepatic perfusion and relieve hepatic congestion. These therapies, which include pharmacologic (vasopressors, inotropes, vasodilators, diuretics) and mechanical support (IABP, ventricular assist devices, ultrafiltration), are discussed in greater detail earlier in this chapter.

Chronic congestive hepatopathy

Congestive hepatopathy is characterized by heart failure, abnormal LFTs, and the exclusion of alternative etiologies of liver dysfunction [250]. Congestion from right heart failure, restrictive cardiomyopathy, or constrictive pericarditis may all lead to congestive hepatopathy. Right heart failure is common in patients with LFT abnormalities [249] and hepatomegaly is commonly found on physical examination in patients with right heart failure [251]. LFT abnormalities due to heart failure most commonly demonstrate a cholestatic rather than a hepatic profile [250,252]. Of the 2679 patients randomized in the Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity program (CHARM), 13% had elevations in total bilirubin levels, 14% had elevations in alkaline phosphatase, and 18% had low serum albumin [252]. Direct bilirubin was elevated in 4% of patients, and only 3% and 4% of patients had elevations in alanine aminotransferase and aspartate aminotransferase levels, respectively [250,251]. Total bilirubin is typically higher in patients with volume overload [252]. The severity of tricuspid regurgitation is also associated with LFT derangements and patients with moderate-to-severe tricuspid regurgitation often have a significant increase in cholestatic LFTs [250]. Total serum bilirubin level is a strong predictor of death in patients with heart failure [204,252]. When elevated preoperatively, total bilirubin is also a marker of reduced survival following LVAD surgery [253,254].

Pathophysiology

Backward congestion of hepatic venules and pulsatile injury from tricuspid regurgitation may cause liver injury in patients with chronic heart failure [250] and result in hepatocyte dysfunction and elevation in both direct and indirect bilirubin [255]. Histopathologic studies describe hyperemia and congestion of the central zone of the hepatic lobule, sometimes described as "nutmeg liver" [250,255]. Congestive liver fibrosis may ensue, ranging from mild deposition of sinusoidal collagen to more severe fibrosis [246]. These changes are thought to result from a combination of increased venous pressure, hypoxia, and/or hepatocellular necrosis [256]. Congestive liver fibrosis can progress to cirrhosis. Interestingly,

synthetic function may be preserved in cardiac cirrhosis and patients can present without any perturbations in LFTs. The diagnosis of cardiac cirrhosis is suggested by right heart failure with hepatomegaly and ascites refractory to diuretic treatment [246]. The ascitic fluid protein content of cardiac cirrhosis is usually high (>2.5 g/dL), with a serum–ascites albumin gradient of >1 g/dL, as is common with disorders characterized by portal hypertension [246,257].

Management

The treatment of congestive hepatopathy is similar to the management of chronic heart failure. Congestion is managed with vasodilator therapy, diuretics, and salt restriction. Paracentesis can be performed for refractory ascites; however, because synthetic function is preserved in cardiac cirrhosis, albumin replacement is not necessary [246].

Patients with ascites who are undergoing cardiac transplant evaluation should have hepatic imaging with ultrasound or computed tomography, and if cirrhosis is suggested, a liver biopsy should be done. If cirrhosis is present, combination heart–liver transplant may be considered in eligible patients. Heart transplant alone is not recommended in the setting of cirrhosis. There are no professional society-endorsed guidelines for combined heart–liver transplantation, which is an uncommon procedure, with only 51 recorded in the registry of the ISHLT between 2005 and 2010 [53]. Reported 1- and 3-year survival rates following combined heart–liver transplant (80% and 70%, respectively) approach those for heart transplant alone [258]. Mechanical support may be an option for patients ineligible for cardiac transplantation due to cirrhosis; liver function abnormalities frequently improve following implantation of a LVAD [239]. However, elevated LFTs have been associated with the need for RV support after LVAD implant [254].

Summary

Cardiac failure plays a role in most aspects of transplantation, either as a direct indication for heart replacement or as a consequence of co-morbid conditions. Similarly, cardiac failure can present with numerous adverse effects on non-cardiac end organs, and cardiac failure remains in the differential diagnosis of hepatic, pulmonary, and renal system failure. As such, cardiac support and intensive care remain important topics of which all transplant professionals should have cognizance. Numerous cardioactive drugs are available to support the failing heart, and sophisticated mechanical devices ranging from IABP counter-pulsation to totally artificial hearts exist to bridge patients across episodes of heart failure. The ubiquity of heart failure across the spectrum of transplantation and the ever-increasing complexity of its management make robust cardiology and cardiothoracic surgery programs important parts of even non-cardiac transplant programs.

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CHAPTER 45

Intensive Care in Pulmonary Failure

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Introduction

Respiratory failure may be acute, chronic, or a combination of the two, and has the potential to proceed to end-organ failure necessitating lung transplantation. As the outcome of a transplant is intimately dependent on the condition of the recipient at the time of the procedure, attention to the proper care of critically ill patients in respiratory failure is necessary. Indeed, respiratory failure is also part of the spectrum of disease seen in critically ill patients requiring other transplants, particularly heart and liver. This chapter will review the physiology and pathophysiology of acute and chronic respiratory failure. Pharmacologic and non-pharmacologic means of optimizing lung function will be discussed. Mechanical ventilation will be reviewed briefly with special consideration given to ventilator strategies specifically designed to avoid further injury to the lung or other end organs. In addition, a disease-specific review will cover the most common diseases of the lung that benefit from solid organ transplantation.

Respiratory physiology and pathophysiology

The adequacy of oxygen and carbon dioxide exchange is determined by inspired gas transit into alveolar units, ventilation (V), and blood flow into alveolar capillaries [perfusion (Q)]. The ideal situation in the lung occurs when the ratio of ventilation to perfusion equals 1 ($V/Q = 1$) [1]. Perfusion without adequate ventilation is known as shunt ($V/Q < 1$). Ventilation without adequate perfusion ($V/Q > 1$) is known as dead space [1]. Anatomic dead space (V_{Danat}) is the volume of gas in the large, non-exchanging airways, i.e. trachea, bronchi, and conducting airways [2]. A portion of tidal volume (V_T) makes its way to alveoli that are not perfused with blood ($Q = 0$), which is known as alveolar dead space (V_{Dalv}) [2]. In most individuals, this volume is minimal, but in the diseased lung V_{Dalv} can rise rapidly [2]. The anatomic and alveolar dead space together are known as the physiologic dead space ($V_{Dphysiol}$) [2]. The physiologic dead space is what is relevant to the patient. The dead space relationships are summarized by the following equation:

$$V_{Dphysiol} = V_{Danat} + V_{Dalv}$$

In the normal person, the sum of anatomic and alveolar dead space represents approximately 20–30% of the tidal volume [1]. When the $V_{Dphysiol}$ reaches 100%, the V/Q ratio approaches infinity ($V/Q = \infty$). Disease processes that decrease the available surface area of lung parenchyma increase dead space, e.g. emphysema. Processes that limit or alter pulmonary blood flow also can increase dead space, as in pulmonary embolism or during hemorrhagic shock. An increase in dead space can result in both hypercarbia and hypoxia.

Complete equilibration between the alveolar and pulmonary capillaries occurs in each perfused alveolus. This means that the arterial gas tension should be equal to the alveolar gas tension. Making these assumptions allows for calculation of dead space. The equation for physiologic dead space is known as the Bohr equation [2]:

$$V_{Dphysiol}/V_T = PaCO_2 - PECO_2/PaCO_2$$

It is important to note in this equation that $PECO_2$ is the mean exhaled PCO_2 and is not the same as end-tidal CO_2 on capnography [1]. $PaCO_2$ is the tension of CO_2 in arterial blood.

Perfusion without ventilation is known as shunt ($V/Q < 1$). Much like dead space, shunt can be divided into true shunt (total absence of gas exchange) and venous admixture (partial gas exchange between alveoli and pulmonary capillary blood). In the true shunt, the V/Q ratio equals zero ($V/Q = 0$). Disease processes that obstruct gas exchange regions or cause excessive blood flow through pulmonary capillaries increase shunt fraction. Examples include acute respiratory distress syndrome (ARDS), pneumonia, hepatopulmonary syndrome, and pulmonary edema. Any alveolar filling process that is accompanied by incomplete hypoxic pulmonary vasoconstriction will also result in some degree of shunt. Shunt results in hypoxemia with either an increase in $PaCO_2$, or no significant change in $PaCO_2$. The higher the shunt fraction, the less responsive the patient will be to oxygen therapy. In fact, at approximately 50% shunt fraction, PaO_2 will be essentially independent of changes in inspired oxygen concentration (FiO_2) [3]. Figure 45.1 shows examples of V/Q abnormalities from shunt (Figure 45.1b) to dead space (Figure 45.1c). Ideal V/Q matching (Figure 45.1a) is 1:1.

Shunt fraction can be calculated using the following equation, where CcO_2 is the oxygen content in the end-pulmonary capillary blood, CaO_2 is the oxygen content in the arterial blood sample,

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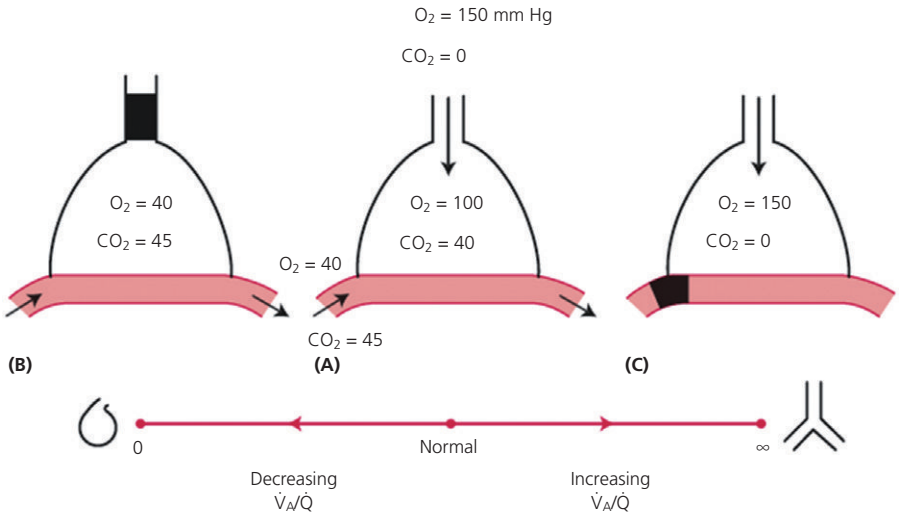

Figure 45.1. (A) Ideal *V*/*Q* matching. (B) Pure shunt. (C) Pure dead space where V/Q = infinity. (Adapted From West JB. Respiratory Physiology: The Essentials, 9th ed. 2011. Reproduced by permission of Wolter Kluwer Health.)

and Cvo_2 is the oxygen content of the pulmonary mixed venous blood:

$$Qs/Qt = (Cco_2 - Cao_2)/(Cco_2 - Cvo_2)$$

This is a somewhat cumbersome appearing equation. The denominator (Cco_2 – Cvo_2) represents total pulmonary blood flow and the numerator represents non-gas exchanging blood flow. In other words, shunt is a comparison between the systemic (Cao_2), the mixed venous (Cvo_2), and the pulmonary capillary blood oxygen content (Cco_2). Although interesting, the clinical applicability of this equation is limited due to the inability to sample post-pulmonary capillary blood. In practice, the more popular Pao_2/Fio_2 ratio can be used to estimate shunt fraction in disease states like ARDS [4]. A Pao_2/Fio_2 ratio of <200 is suggestive of a shunt fraction of >20% (*Q*s/*Q*t) [4].

In clinical practice, *V*/*Q* abnormalities can also be estimated by calculating the gradient between alveolar partial pressures (A) and arterial oxygen tension (a), known as the A–a gradient. The alveolar gas equation is as follows:

$$PAo_2 = PIo_2 - (Paco_2/RQ)$$

where PAo_2 is the partial pressure of oxygen in the alveolus, PIo_2 is the partial pressure of oxygen in inspired gas, $Paco_2$ is the carbon dioxide tension in arterial blood gas, and RQ is the respiratory quotient that defines the relative rate of gas exchange. At sea level and breathing room air, the normal A–a gradient would be 10 mmHg. Age increases the A–a Po_2 gradient because as the human lung ages, small airways lose their architectural strength, resulting in collapse at progressively higher lung volumes, thus causing shunt during normal tidal breathing. There is significant increase in *V*/*Q* heterogeneity due to this increase in closing volume with aging [5]. In addition, the A–a Po_2 gradient increases as the Fio_2 increases. This is thought to be due to the attenuation of hypoxic pulmonary vasoconstriction in local areas of blood that are poorly ventilated. In this way, an increase in alveolar oxygen partial pressure may worsen *V*/*Q* matching. This can increase shunt fraction and widen the A–a gradient [1].

Pharmacology of the lung

Respiratory failure is treated by correcting underlying pathology and by optimizing pulmonary function and mechanics. The goal in doing so is to allow the patient to resume his/her normal quality of life upon discharge. The following provides a focused review of the mechanisms of action of both intravenous and inhaled therapies used to treat the patient in respiratory failure.

Vasodilators: oxygen, nitric oxide, and epoprostenol

Oxygen therapy is the first line of therapy for respiratory failure. As noted above though, in certain pathologies, such as a high percentage of shunt, oxygen therapy does little to increase the arterial partial pressure of oxygen in blood. It is also important to remember that partial pressure of oxygen in arterial blood does little to increase oxygen content in the blood as long as the patient has a normal amount of functional hemoglobin. This is demonstrated by the following equation:

$$Cao_2 = (Hgb \times 1.36 \times Sao_2) + (0.0031 \times Pao_2)$$

where Cao_2 is the oxygen content, Hgb is the hemoglobin concentration in g/dL, and Sao_2 is the percentage saturation of the hemoglobin in arterial blood.

Oxygen decreases hypoxic pulmonary vasoconstriction (HPV) in the lungs, which, in turn, decreases pulmonary vascular resistance. This effect is beneficial in diseases such as pulmonary arterial hypertension, but may result in an increased A–a gradient in other cases by promoting more *V*/*Q* mismatch. Attenuation of hypoxic pulmonary vasoconstriction resulting in worsening *V*/*Q* mismatch during the use of oxygen therapy is especially evident in patients with chronic obstructive pulmonary disease (COPD) [6]. The premise of providing supplemental oxygen is to avoid end-organ damage by providing adequate oxygen delivery. However, even in patients with severe pulmonary insufficiency at rest (arterial Pao_2 in the 20s–30s of mmHg), blood levels of lactate were not elevated [7]. Organs and tissues at rest have extremely low oxygen tension, so the preoccupation with oxygen delivery in the setting of pulmonary insufficiency may be overemphasized in clinical medicine [1].

Oxygen therapy may not be benign either. Oxygen toxicity may occur, probably caused by the generation of peroxides and other reactive oxygen species that cause inflammatory damage to cells [8]. Neonates especially have been shown to be at risk of complications such as retinopathy of prematurity [9]. For these reasons, oxygen therapy should be minimized when possible. Reducing Fio_2 to <60% seems to be a reasonable goal.

Nitric oxide (NO) is produced in the body and in the lung itself by a host of cells, including vascular endothelial cells, epithelial cells, smooth muscle cells, and inflammatory cells among others [10]. In the vascular endothelium, NO acts to increase levels of cyclic guanosine monophosphate (cGMP), which in turn causes relaxation by means of decreasing calcium availability in vascular smooth muscle [10]. Administration of inhaled NO (iNO) is thought to be advantageous because it is preferentially delivered to ventilated alveoli, thus limiting systemic side-effects. This in turn causes more of the cardiac output to preferentially flow towards the adequately ventilated portion of lung. Most patients who are given iNO demonstrate improved *V*/*Q* matching and increase in oxygenation; however, the therapy has not been shown to reduce mortality in ARDS [11]. NO is especially appealing because it is rapidly metabolized in the blood stream and its vasodilatory effects are localized to the lung [10]. Common clinical uses for iNO include management of pulmonary artery hypertension and right ventricular failure [10].

Epoprostenol occurs in the body naturally as a product of arachidonic acid metabolism. Its effect on the vascular endothelium is to increase intracellular levels of cyclic adenosine monophosphate (cAMP), which then decrease levels of available calcium, causing vessel relaxation [10]. It can be given in both intravenous and inhaled forms. Delivery of the intravenous form requires a special pump. The inhaled form has gained popularity recently as a treatment for refractory hypoxemia by improving *V*/*Q* matching, largely due to the cost advantage over iNO. There have been no randomized controlled trials of epoprostenol in ARDS [12].

Bronchodilators: beta$_2$-agonists and anticholinergics

Beta$_2$-agonists

Beta$_2$-agonists can be given intravenously (epinephrine, isoproterenol), but when treating bronchospasm and elevated airway resistance, aerosolized preparations are the most efficacious. In addition, inhaled administration minimizes unwanted side-effects in distant organs. Beta$_2$-agonists act on G-protein receptors and increase levels of cAMP, thus decreasing the availability of intracellular calcium and causing relaxation of airway smooth muscle [13]. Short-acting agents (albuterol, levalbuterol) are used for acute bronchospasm, whereas long-acting agents, such as fometerol and salmeterol, are used for long-term management in patients with asthma or COPD. Long-acting agents have been proven effective in decreasing exacerbations and symptoms in patients with asthma, but there are safety concerns in some patients with a trend towards increased mortality [14]. As such, many clinicians discontinue long-acting bronchodilators in the acute setting in favor of short-acting beta-agonists. The side-effect profiles of beta-agonists are often problematic. This is especially true of the tachycardia they often cause. One particular drug, levalbuterol, has been marketed as an alternative to albuterol to avoid tachycardia and tachyarrhythmia. However, this has not been shown in clinical studies in patients with asthma [15].

Anticholinergics

Intravenous anticholinergic agents (glycopyrrolate and atropine) are rarely used for bronchodilation. Inhaled anticholinergic agents work on the muscarinic receptors (M_1, M_2, and M_3) of the bronchial airway smooth muscle. The inhaled anticholinergic agents ipratropium and tiotropium are quaternary ammonium compounds. Ipratropium is a non-selective muscarinic antagonist, blocking all three M receptors. Tiotropium also antagonizes all three muscarinic receptors in the lung, but the half-life is longer on M_1 and M_3 receptors, which may result in more bronchodilation (M_2 antagonism causes bronchoconstriction) [16]. Because inhaled anticholinergics are quaternary compounds, they are charged and lack the ability to cross the blood–brain barrier, so anticholinergic syndrome is unlikely [16]. Ipratropium is used as second-line therapy in acute asthma exacerbations, but tiotropium is used only in chronic COPD treatment [16].

Anti-inflammatory treatments: steroids and leukotriene inhibitors

Steroids

Steroid therapy for lung failure is a heavily studied and oft debated topic. Lung tissue may be damaged by the inflammatory cascade set in motion by many diseases, infectious and non-infectious alike. Steroids decrease the body's inflammatory response and spare the tissues from further damage. Systemic steroid therapy has proven beneficial in COPD and asthma exacerbations [17]. "Doubling dosing" of inhaled steroids in an asthma exacerbation, however, has not been shown to be beneficial in preventing rescue dosing with systemic steroids in asthma exacerbation [18]. Corticosteroids have also been extensively studied in ARDS in adults, but they have not been shown to have a significant mortality benefit [19].

Leukotriene inhibitors

Leukotrienes are by-products of arachidonic acid metabolism during the inflammatory cascade. As such, they play a significant role in inflammatory conditions of the lung, most notably asthma. Leukotrienes are potent bronchoconstrictors. Antagonism of this action has proven to be a beneficial adjunct in the treatment of exercise- and aspirin-induced asthma. In addition to their role in bronchoconstriction, leukotrienes play an important role in the modulation of pulmonary capillary permeability, mucus production, and neuronal input [20]. Despite their benefit in the chronic treatment of inflammation-mediated pulmonary disease, leukotriene inhibitors have very little role in the acute setting. Rather, their main utility is as an adjunct in the chronic treatment of atopic asthma.

Phosphodiesterase inhibitors

Phosphodiesterases (PDEs) are enzymes that catalyze cyclic nucleotide hydrolysis. There are 11 identified isoforms, each with varying effects on target tissues. Generally speaking, PDEs modulate smooth muscle constriction. Variable expression of the different isoforms of PDEs in different tissues has allowed for targeted drug development in the hope of achieving specific ends [21]. Theophylline is one of the oldest PDE inhibitors still in use for its bronchodilator effects, despite its weak inhibition of PDE [21]. PDE 3 inhibition results in positive ionotropy, but it also has effects on pulmonary and systemic vascular smooth muscle, resulting in vasodilation [21]. For example, milrinone, a PDE 3 inhibitor, is used in the treatment of end-stage pulmonary hypertension with

secondary right heart failure for its inotropic and vasodilatory effects, particularly in the pulmonary vascular bed. The PDE 5 inhibitor sildenafil has been safely and successfully used in the treatment of end-stage pulmonary hypertension [22]. PDE 5 inhibitors can be added in the intensive care unit (ICU) setting as de-novo therapy or to support weaning from inhaled pulmonary vasodilators, like epoprostenol or iNO.

Mucociliary drugs

N-acetylcysteine, guaifenesin, and iodinated glycerol represent a class of drugs that over the years has been used to alter mucus quality or production to allow improved clearance of pulmonary secretions [23]. Interestingly, there are little to no data supporting their use in the critically ill. Illustrative of this point, N-acetylcysteine has well-known antioxidant properties, but in a series of studies, no statistically significant effect on mortality has been shown [24].

Mechanical ventilation for respiratory failure

Command of mechanical ventilator management is important in properly supporting patients with respiratory failure until their underlying condition can be reversed or improved. Mechanical ventilation is not a therapy for the lungs. Rather, mechanical ventilation provides support for the pulmonary system by providing ventilation and oxygenation when the patient is incapable of doing so to a sufficient extent to allow survival. It is important to bear in mind that mechanical ventilation exposes the patient to ventilator-mediated injury, however, and prompt liberation from mechanical ventilation is paramount.

A detailed discussion of the inner workings of the modern mechanical ventilator is beyond the scope of this chapter. Suffice it to say that mastery of ventilator terminology is necessary for the modern clinician to successfully manage a patient in respiratory failure. Modern ventilators are best understood in terms of *trigger variables*, *limit variables*, and *cycle variables*. A *trigger variable* is the input the ventilator microprocessor uses to register patient initiation of a breath, and it is determined by the mode selected by the operator. The specific trigger variable values are often preset, but may be altered if necessary. For example, a patient may trigger a supported breath from the ventilator by achieving a flow rate (in L/min) or a negative pressure deflection (in cmH_2O). A *limit variable* is what the ventilator tries to achieve during the breath, much as a driver tries to achieve the speed limit for maximum efficiency during a trip. The limit variable is generally an inspiratory pressure or a flow rate. The positive pressure breath is ended by the *cycle variable*. The cycle variable can either be a time (in seconds), a volume (in mL), or a flow rate (in L/min).

The most commonly used ventilator modes are *assist-control mode*. In this mode, the clinician sets the respiratory rate. If the patient is apneic, the ventilator will supply positive pressure breaths to achieve that set rate. If the patient initiates a breath, the ventilator will assist the patient by giving the patient a breath at the set tidal volume. Both the control breath and the assisted breath will abide by the limit variable and cycle variable set by the clinician. The clinician selects whether to give a set volume during each breath or a set pressure during each breath. Hence, the full name of the ventilation mode will be assist control-volume (ACV), or assist control-pressure (ACP). ACV is volume limited and time cycled. ACP is pressure limited and time cycled.

Synchronized intermittent mandatory ventilation (SIMV) is a modification of assist control. Like assist-control ventilation, SIMV may be volume or pressure controlled. In this mode, the clinician sets a respiratory rate. If the patient's respiratory drive is blunted and he/she is breathing at a rate less than the set rate, his/her respiratory rate will be the same as the set respiratory rate. If the patient breathes faster than the set respiratory rate, then the ventilator will assist the patient with a predetermined pressure support breath. Pressure support breaths are pressure limited and flow cycled. This support is designed to allow a patient to use more of his/her respiratory muscles to support ventilation. It has been postulated that SIMV with pressure support could be used for patients with prolonged respiratory failure who are difficult to wean from mechanical ventilation. However, review of the existing literature does not show any clear benefit in weaning [25]. Pressure support may be used as a stand-alone mode of ventilation. In this setting, the patient determines the respiratory rate and minute ventilation by triggering support. An important safety feature is a back-up mode of ventilation that provides ventilation in case the patient becomes hypopneic or apneic.

Pressure-regulated volume control (PRVC) is a mode of ventilation that is pressure limited and time cycled. However, the clinician sets a tidal volume goal. The microprocessor evaluates whether the tidal volume was reached, and then changes the pressure limit to attain the set goal. This mode is representative of a movement toward "smart ventilators," though it remains imperative that clinicians understand ventilator modes. As an example of the dangers of PRVC, imagine that a spontaneous pneumothorax occurs with some degree of tension. In this case, the PRVC-programmed ventilator will continue to increase the delivered pressure to achieve the tidal volume set by the clinician. This feedback loop will continue until dangerously high pressures are used to deliver volumes, thus worsening the pneumothorax and eventually resulting in fulminant cardiopulmonary collapse. These "smart ventilator" modes have alarm values to mitigate these situations, but clinicians must be keenly aware of the ways in which the ever-changing limit variable may result in changes in respiratory compliance without immediate changes in tidal volume.

Airway pressure release ventilation (APRV) is a novel ventilator strategy aimed at maintaining a relatively high mean airway pressure (MawP), thus maximizing oxygenation. It is a time triggered, pressure limited, and time cycled type of ventilation [26]. In this mode, a high pressure (P_{high}) and a time high (t_{high}) are set, as well as a low pressure (P_{low}) and time low (t_{low}). A normal starting point for settings could be 6–10 cycles/min with the following settings: $P_{high} = 30$ mmHg, $t_{high} = 5.2$ s, $P_{low} = 0$ mmHg, and $t_{low} = 0.8$ s. In the interest of comparison with conventional ventilator modes, most alveolar recruitment occurs as a result of distension of alveoli with positive end-expiratory pressure (PEEP) at low lung volumes. By contrast, in APRV, the t_{low} is the more important determinant of recruitment. A short t_{low} allows for incomplete expiration and the development of intrinsic PEEP. The clinician thus uses the intrinsic PEEP to recruit lung near to its closing pressure. What makes APRV particularly attractive is the avoidance of alveolar atelectatic shearing trauma. This mode is advantageous because it supports spontaneous ventilation throughout the respiratory cycle with no closing of valves, i.e. the patient can take many small breaths during P_{high} or P_{low}. Because of this, it is thought that patients are more comfortable as they can breathe at any time during the cycle without resistance and without setting off alarms. Spontaneous breathing

improves *V*/*Q* matching and hemodynamics as well. APRV can improve oxygenation in hypoxemic patients because of the prolonged time spent at higher inflation, thus increasing the MawP versus in conventional modes of ventilation. APRV has been shown to increase lung aeration in patients with ARDS more than pressure support ventilation [27]. Importantly, this mode of ventilation can be dangerous in patients with obstructive lung disease requiring a prolonged expiratory phase. Another potential disadvantage of this mode is that the clinician cannot control volumes achieved while at P_{high}. This has the theoretic potential to result in hyperinflation of the lung, resulting in volutrauma [26]. Overall, this mode of ventilation is compatible with the open lung model, which assumes that, by recruitment and maintenance of alveoli, more efficient oxygenation and ventilation will translate into gains for the patient. However, no studies have shown outcome benefits from this mode of ventilation [26].

High-frequency oscillatory ventilation (HPOV) is a mode used in patients with non-compliant lungs in ARDS. A piston oscillator moves a diaphragm, much like a speaker vibrates on a stereo. The rates are 3–10 Hz, which is equal to 180–600 breaths/min [28]. Fresh gas inflow, known as bias flow, is at 40–60 L/min to achieve an FiO_2 of 0.8–1 [28]. An expiratory valve allows for gas escape and for the mean airway pressure to be set [29]. One advantage of HFOV is the ability to separate oxygenation and ventilation [28]. Parameters that affect oxygenation are FiO_2, mean airway pressure, and bias flow [28]. Parameters that affect ventilation are frequency and amplitude (power) [28]. More rigorous study is needed, but there may be a 30-day mortality benefit from using HFOV versus conventional mechanical ventilation in patients with acute lung injury/ARDS [30].

As already mentioned, mechanical ventilation is not therapeutic for the lungs during respiratory failure. With that in mind, the medical team should be constantly assessing the patient's ability to be liberated from mechanical ventilation. It is well documented that, without good vigilance, patients have the tendency to remain mechanically ventilated for longer than necessary. Daily cessation of sedating drugs has been shown to shorten the amount of time patients are mechanically ventilated [31]. The Awakening and Breathing Controlled Trial paired daily sedation interruption with a spontaneous breathing trial and showed a decrease in ICU length of stay and hospital length of stay [32]. Incorporating sedation interruptions and spontaneous breathing trials into protocols will limit the time spent on mechanical ventilation and the complications of mechanical ventilation.

Acute respiratory distress syndrome

ARDS was first described in a case series in 1967. Since then, ARDS has been the focus of many clinical investigations. From an initial mortality of above 60%, the rate has decreased to about 30% [33]. The characteristics of the syndrome were codified in a consensus definition in 1994 that included: (1) acute onset; (2) PaO_2/FiO_2 <200; (3) bilateral infiltrates on chest radiograph; and (4) pulmonary capillary wedge pressure (PCWP) of <18 mmHg [34]. Because of the overall decline in the use of pulmonary artery catheters, a lack of evidence of a cardiac cause of pulmonary edema is an acceptable substitute for a PCWP of >18 mmHg. ARDS is generally caused by a disruption of the alveolar–capillary membrane.

Three phases of pathology have been described (though not all patients traverse through all three): the exudative, proliferative, and fibrotic phases [35]. The exudative phase involves disruption of the alveolar–capillary membrane, which then allows a significant quantity of protein-rich fluid and neutrophils to flood the alveoli. The proliferative phase refers to hyaline membrane reorganization. This phase is followed by the fibrotic phase, classically occurring around the 2-week mark in the disease process, and resulting in scarring that causes a fibrotic appearance of the lung. Starting in the first phase, diffuse alveolar damage means that gas exchange does not take place normally because of the development of a large functional shunt, resulting in hypoxemia. As discussed earlier, when the shunt becomes large, the patient may no longer be responsive to oxygen therapy. In addition, a vast increase in dead space ventilation early in the course of ARDS is associated with higher mortality [36].

Causes of ARDS include direct insults to the lungs, like aspiration, near drowning, pneumonia, toxic inhalation, or pulmonary contusion [34]. Causes may also be indirect or remote from the lungs, such as non-thoracic trauma, sepsis, blood product transfusion, and cardiopulmonary bypass [34]. Once the alveolar–capillary membrane has been disrupted, the inflammatory cascade results in further damage to the lungs.

Numerous pharmacologic interventions have been tried with limited success. For example, surfactant therapy should theoretically provide benefit because the action of surfactant is lost during the flooding of the alveoli during the exudative phase. However, no mortality benefit has been shown for broadly defined groups of ARDS patients. That said, meta-analysis has shown potential benefit for patients who develop ARDS from aspiration or pneumonia [37]. iNO improves oxygenation, but no decline in mortality has emerged as a result [38]. Corticosteroids have not been shown to change mortality, but they may reduce the number of ventilator days [39].

A landmark study published in 2000 by the Acute Respiratory Syndrome Network (ARDSNet) compared patients with ARDS ventilated with "conventional" tidal volumes (12 mL/kg predicted body weight) versus low tidal volumes (6 mL/kg predicted body weight). The study was stopped on ethical grounds after enrollment of around 800 patients because the low volume ventilation group had a mortality reduction from 39% to 31% [40]. This study built upon many prior studies in animals and humans that pointed toward decreasing lung injury when smaller tidal volumes were used.

In ARDS, the damage to the lungs when viewed on chest computed tomography is fairly heterogeneous, with areas of good lung interspersed with areas of damaged lung [41]. As referenced above, the capillary leak of fluid in the alveolus causes regional changes in compliance. When a positive pressure breath is administered to a patient, the volume preferentially goes to the healthier, more compliant regions of the lungs. This redistribution causes more stretch and shear force on the previously healthy lung parenchyma, causing more inflammation and a worsening of lung injury. A lower tidal volume ventilation strategy is likely protective because it mitigates ventilator-induced injury to the healthy regions of the lung.

The lung in ARDS is under constant assault at the hands of inflammatory mediators and it is at significant risk for ventilator-associated lung injury (VALI). There are several types of injury that can happen during mechanical ventilation: barotrauma (high volume trauma), atelectatic trauma (low volume trauma), and biotrauma (distant trauma to organs other than the lung) [41]. *Barotrauma* is caused by overstretching of the alveoli at high tidal volumes, resulting in damage to the alveolar–capillary membrane.

The bedside strategy for minimizing barotrauma includes use of lung protective ventilation with smaller tidal volumes and maintenance of plateau pressures (a surrogate for alveolar pressures) at or below 35 cmH_2O [41]. It is important to keep in mind that peak pressures are not reflective of pressure transmitted to alveoli, but rather are a function of resistance between non-exchanging airways and the ventilator circuit itself [41]. *Atelectrauma* occurs when the closing capacity is greater than the functional residual capacity. With each positive pressure breath, the smaller exchanging airways and alveoli collapse and then reopen, resulting in sheer stress and injury as those units open and close [41]. Application of PEEP stents open lung units that would otherwise close. This has been shown to be beneficial in animal models. However, large randomized controlled trials comparing higher and lower PEEP strategies utilizing low tidal volume ventilation showed no difference in mortality or other clinical endpoints in the comparison groups [42]. Therefore, it seems that minimization of tidal volume is more important in the prevention of VALI [42]. Utilization of a PEEP sufficient to allow adequate oxygenation can be recommended, though the specific strategy should be left to the clinician's discretion. *Biotrauma* is the third type of injury that can be associated with mechanical ventilation in patients with ARDS. Mechanical ventilation can induce the release of some inflammatory cytokines from the lungs and these travel to other distant organs, and are possibly a cause of multiorgan system failure [41]. Additionally, inflammatory mediators released by other organs may result in damage to the lungs. The ARDSNet trial found lower levels of serum interleukin (IL)-6 in the low tidal volume ventilation group [40]. Low tidal ventilation may minimize IL-6–mediated injury.

In summary, ARDS is a clinical syndrome that most likely represents the final common pathway of several different pathologies. Multiple pharmacologic and mechanical ventilatory interventions have been studied with a few generalizable successes. Few patients with ARDS die from irreversible lung injury; rather, mortality is associated with other causes, most notably sepsis [43]. The one "treatment" that we do have for ARDS is treatment of the underlying cause, with supportive care until the patient can recover and the use of lower tidal volume lung protective ventilation in the interest of mitigating VALI.

Diseases of chronic respiratory failure

As mentioned above, most patients with ARDS die from causes other than irreversible lung injury. However, diseases of chronic respiratory failure are often progressive, marked by steady deterioration with intermittent acute exacerbations brought on by infection or inflammation. Most are relatively manageable until the respiratory failure becomes such a threat to life that lung transplantation offers the only hope of prolonged survival with reasonable quality of life.

Lung transplantation is reserved for high mortality conditions that are progressive and will not recur in the donor lungs. A worldwide survey of lung transplantation reveals that the greatest percentage of lung transplants were performed for the following indications: COPD (31%), idiopathic pulmonary fibrosis (21%), cystic fibrosis (16%), and alpha-1 antitrypsin deficiency (7%) [44]. We will briefly review the pathology and management of the diseases for which the majority of lung transplantation is done. Additional discussion of these conditions can be found in Chapter 31.

Chronic obstructive pulmonary disease

The disease burden of COPD, both in the US and worldwide, is great [45]. By 2020, COPD will be the fifth leading cause of death worldwide [46]. The term COPD encompasses both chronic bronchitis and emphysema. Both diseases have an obstructive pattern of airflow on spirometry. Chronic bronchitis is characterized by small airways disease that causes obstruction during the expiratory phase. Emphysema has a similar pattern, but is further complicated by the loss of elastic tissue in the lung parenchyma, resulting in further functional impairment of the expiratory phase.

The diagnosis of COPD can be suspected from clinical history and confirmed with the spirometry values. In practice, manifestations of both emphysema and chronic bronchitis frequently are seen in the same patient. In addition, asthma may be present in some patients, complicating the diagnosis. Pulmonary function testing before and after the administration of $beta_2$-agonists can help to define the amount of reversibility of bronchoconstriction. COPD can been divided into mild, moderate, severe, and very severe based on the forced expiratory volume in 1 s (FEV_1)/forced ventilatory capacity (FVC) ratio and symptoms. Each level of severity is accompanied by an additional therapy. Mild COPD, defined as an FEV_1/FVC of <0.70 and an FEV_1 of $>80\%$ predicted, is managed by reducing risk factors, providing appropriate vaccinations, and using short-acting bronchodilators when necessary. As mild progresses to moderate disease, defined as an FEV_1/FVC of <0.70 and an FEV_1 of between 50% and 80% predicted, one or more long-acting bronchodilators is added, and pulmonary rehabilitation should be considered. Further progression from moderate to severe disease, defined as an FEV_1/FVC of <0.70 and an FEV_1 of between 30% and 50%, inhaled glucocorticoids may be added for exacerbations. Finally, as severe disease progresses to very severe disease, defined as an FEV_1/FVC of <0.70 and an FEV_1 of $<30\%$ predicted or FEV_1 of $<0\%$ complicated by chronic respiratory failure, then consideration should be given to chronic oxygen therapy or surgical treatment options [47].

Because of the combination of obstruction to expiration and loss of elastic tissue, multiple complications occur in COPD, including pneumothorax, cor pulmonale, giant bullae, and pneumonia [48].

The pathogenesis of COPD involves multiple proposed mechanisms of damage to the lungs. Most often, an environmental pathogen, such as cigarette smoke, induces inflammatory changes in the lung. In large airways there can be an increase in the production of mucus. This results in goblet cell hyperplasia, a characteristic of chronic bronchitis [49]. Despite the increase in the production of mucus and goblet cell hyperplasia, the airway obstruction of COPD is not large airway obstruction, but rather smaller airway obstruction, especially of airways of <2 mm in diameter [49]. The exact cascade of factors is not completely understood, but neutrophilic infiltration into bronchioles and lung units clearly occurs [50]. Hypertrophy of smooth muscle may also contribute to resistance to airflow. There is also destruction of the architecture of the lung, leading to deterioration in elastic recoil and increased dead space. The relative contributions of airflow deficit from emphysema and from airway obstruction are still unknown, and likely vary among patients. In addition to the elastic changes in the lung, there is loss of pulmonary capillary blood flow due to parenchymal remodeling, further contributing to a decrease in the diffusion capacity of carbon monoxide [51].

Treatment now is directed toward smoking cessation, bronchodilator therapy, and specific pulmonary rehabilitation therapy. In severe cases, inhaled corticosteroids are added. Threats to life

occur when patients have "COPD exacerbations," defined as acute worsening of symptomatology. The first step in the care of the COPD patient is the prevention of COPD exacerbations. Inhaled corticosteroids have been shown to reduce exacerbations by 20% [47]. Tiotropium has been shown to improve the COPD patient's quality of life and reduce COPD exacerbations by 24% over ipratropium [52]. A combination of salmeterol and fluticasone has been shown to reduce COPD exacerbations by 25%. Bronchodilator therapy with short-acting and long-acting beta$_2$-agonists can be used to improve FEV_1 by about 100–150 mL [48]. Empiric treatment with antibiotics with the onset of COPD exacerbation has been shown to decrease treatment failure by 46% and inhospital mortality by 78% [53]. A short course of 14 days or fewer of systemic steroids has also been shown to reduce treatment failure by 46% [53]. Oxygen therapy should be directed to keep saturations to >88–90% [49]. For hypoxemic patients with COPD, long-term oxygen therapy improves survival and has better results when administered for 24 h rather than for part of the day [54].

Further discussion is warranted with respect to ventilatory support in patients with COPD exacerbations. During COPD exacerbations, total lung volumes expand due to restriction of airflow in the small airways [55]. This hyperinflation strains respiratory muscles and increases the work of breathing. Furthermore, the inability to exhale completely during the respiratory cycle leads to a respiratory acidosis, which further impairs ventilation [55]. A review of randomized controlled trials has shown that use of non-invasive positive pressure ventilation (NIPPV) for support of the patient with a COPD exacerbation reduced mortality by 48% compared to the usual medical care [55]. In addition, the rate of endotracheal intubation was reduced by 59% [55]. The profound benefit of NIPPV is unique to this group of patients and to patients with acute congestive heart failure. In most other groups, the benefit of NIPPV could not be shown, and at times caused harm by delaying the inevitable placement of the endotracheal tube. The striking reduction in mortality in COPD and congestive heart failure (CHF) may be due to the avoidance of endotracheal intubation and its associated risks to the patient, which include decreased secretion clearance, increased requirements for sedation and analgesia, and ventilator-associated pneumonia.

Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutation of the cystic fibrosis transmembrane conductance regulator (CFTR). The disease has variations in its severity, but it is primarily characterized by chronic infection, airway obstruction, deficiency of the exocrine pancreas, and gastrointestinal tract dysfunction [56]. It is most common in descendants of northern Europeans, with between 2% and 5% of Caucasians being carriers for the CF gene mutation [56]. In CF, the lung epithelia are dysfunctional. CFTR protein is important in chloride transport and defects in the protein result in a depletion of airway surface water [57]. The remaining mucus is thicker and more adherent to the cells, which causes airway obstruction and provides a nidus for infection [56]. The mucus is more difficult for the mucociliary elevator to clear. Because of chronically obstructed airways, the patient becomes colonized with potentially pathogenic bacteria.

Treatment of the patient with a CF exacerbation focuses on: (1) antibiotic therapy to reduce bacterial burden; (2) airway clearance; and (3) maintenance of proper nutrition [58]. The most common organisms cultured include *Staphylococcus aureus* and *Pseudomonas aeruginosa* [56]. Patients who present to the hospital with respiratory insufficiency secondary to CF exacerbation should have special CF sputum cultures sent to determine species and sensitivities of each bacterial culture. Because patients receive courses of antibiotics with each exacerbation, multidrug-resistant Gram-negative cultures, such as *Burkholderia cepacia*, are found in approximately a third of CF patients [59]. Initial antibiotic selection is based on the patient's prior cultures and involves two classes of antipseudomonals [58]. Consulting infectious disease specialists can help to guide therapy. Suppressive antimicrobial therapy with inhaled tobramycin as an outpatient has become prevalent and has been shown to decrease rates of CF exacerbation [58]. Macrolide (mainly azithromycin) therapy has been shown to improve lung function, particularly in those patients with pseudomonal infections [60]. Since it does not have activity against *Pseudomonas*, it is thought that modulation of inflammation and immunity may be the mechanism by which azithromycin improves lung function [60].

Airway clearance is achieved by physical drainage assistance and inhaled expectorants. Postural drainage, percussion, and vibration are associated with an increase in sputum expectoration [61]. When postural drainage, precussion, and vibration are used in combination with exercise, a measurable increase in FEV_1 can be achieved [61]. Therapeutic apparatus include vibrating beds and special vests. In terms of expectorant therapy, recombinant human deoxyribonuclease I (rhDNase I) is a biologically manufactured copy of the human enzyme. Extracellular DNA contributes to the viscous nature of mucus in patients with CF. When used twice daily by inhalation, rhDNase reduced CF exacerbations by 37% versus placebo [62]. There is less convincing evidence that nebulized hypertonic saline increases mucus clearance in CF. The purported mechanism for the benefit of hypertonic saline is that the inhalation of salt increases osmotic flow of water, resulting in rehydration of mucus with the resulting reduced viscosity allowing for expectoration [63].

Maintaining nutritional status should be a cornerstone of CF treatment, as poor nutrition and low body mass index (BMI) have been linked to poor outcomes [58]. No specific diet is recommended, but high calorie foods are encouraged, along with appropriate pancreatic enzyme supplementation [58]. Low BMI can even be a barrier to lung transplantation.

Supportive care of the pulmonary system in CF is much the same as in other etiologies of respiratory failure. Oxygen therapy likely has benefit by reducing pulmonary hypertension and cor pulmonale, but studies in the CF population are lacking, and much of the data are extrapolated from the COPD population [58]. Short-term non-invasive ventilation for hypercarbia has been described, but it is unclear whether this is beneficial or harmful in this population [64]. Support of other failing organ systems is necessary because of the expression of CFTR in other tissues, especially the gastrointestinal system. Pancreatic insufficiency is managed by replacement of digestive enzymes. Hyperglycemia generally presents in the second or third decades of life [56]. Patients often have renal insufficiency due to multiple exposures to aminoglycosides. Hepatic cirrhosis may occur as well, in concert with all of its associated complications [56].

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) generally has an insidious onset with non-productive cough and exertional dyspnea. It is

characterized as a restrictive lung disease due to the loss of lung compliance associated with fibrosis. Diagnosis of IPF is generally accomplished with a surgical biopsy of the lung [65]. IPF is fatal unless remission is achieved. At diagnosis the 5-year survival rate is 20–30% [66]. Mortality from progression of disease is 39%, 24% from cardiovascular causes (especially cor pulmonale), 10% bronchogenic carcinoma, and 7% pulmonary embolism [67].

Corticosteroids are the mainstay of treatment, but there is no evidence to support their use as sole therapy [68]. The diagnosis of IPF should prompt an immediate referral of the patient to a center with treatment protocols and consideration for lung transplantation. The lung allocation score was implemented in 2005 with the aim of reducing the number of patients who die on the lung transplant waitlist. It has resulted in a significant increase in the number of patients with IPF who receive transplants [69]. At this time, treatment involves experimental protocols of anti-inflammatory drugs and support. There are currently multiple trials ongoing testing antifibrotic and anti-inflammatory drugs.

It is important to note that pulmonary hypertension is prevalent in this population, and should be investigated with right heart catheterization and treated as appropriate [70]. Intubation should be avoided in patients with advanced IPF where possible unless an immediate treatment can be initiated [71]. In a series of patients with IPF intubated and ventilated for acute respiratory failure, only one survived by having a lung transplant 6 h after mechanical ventilation [71].

Pulmonary arterial hypertension

The definition of pulmonary artery hypertension is a mean pulmonary artery pressure of $\geq$25 mmHg and a pulmonary artery occlusion pressure of $<$15 mmHg during right heart catheterization [72]. The World Health Organization divided pulmonary artery hypertension into groups (Table 45.1) [73]. Idiopathic pulmonary artery hypertension (IPAH) is attributable to group 1. There is much still unknown about this disease process, but current evidence points to the growth and proliferation of cells in all three layers of the blood vessel [73]. Dyspnea is the most common presenting symptom and, though echocardiographic findings are helpful, diagnosis should be made with the aid of a right heart catheterization [73]. Treatment consists of supportive therapy, light physical exercise as tolerated, anticoagulation with warfarin, diuretic therapy, and pulmonary vasodilator therapy [73]. After classification of the patient's functional status, a graded approach to pulmonary vasodilation therapy is recommended [72]. By following these general guidelines, pulmonary hypertensive patients are surviving longer with a better quality of life with vasodilator therapy, making them less likely need a lung transplant in the future.

Table 45.1. World Health Organization categorization of pulmonary artery hypertension

Group 1	Pulmonary arterial hypertension
Group 1′	Pulmonary veno-occlusive disease
Group 2	Pulmonary hypertension from left heart disease
Group 3	Pulmonary hypertension from chronic lung disease or hypoxia
Group 4	Chronic thromboembolic pulmonary disease
Group 5	Pulmonary hypertension with unclear multifactorial mechanisms

Extracorporeal life support

Patients may have such respiratory failure that mechanical ventilation is no longer able to provide sufficient oxygenation to their end organs. In this case, additional support may allow the lungs time to recover or provide a bridge to lung transplantation [74]. Most pulmonary support is given by extracorporeal membrane oxygenation (ECMO) (see Chapter 50). There are two main types of ECMO: veno-venous (V-V) and veno-arterial (V-A). V-V ECMO drains deoxygenated blood from a central venous cannula, oxygenates it, and then returns it into the central venous circulation for delivery to the pulmonary circulation and beyond. This serves as a pulmonary support device in patients who have no evidence of right heart failure associated with their respiratory failure. In contrast, V-A ECMO removes blood from a central vein and returns oxygenated blood under pressure into the arterial system. V-A ECMO serves as cardiopulmonary support and is similar to cardiopulmonary bypass used during heart surgery. Any mortality benefit conveyed by ECMO likely depends upon patient selection, the underlying disease process, and the expertise at the ECMO center. There have been a few recent randomized controlled trials evaluating ECMO that demonstrate benefit. Prior studies failed to find a survival benefit. A recent, well-publicized study found that patients with severe ARDS who were referred to an ECMO center had a survival benefit when compared to a cohort who remained on mechanical ventilation in a non-ECMO center [75]. It is unclear whether the intervention of ECMO was integral in the survival benefit or if transfer to a center of excellence was more important.

Summary

The lung is an integral organ necessary for the maintenance of homeostasis by providing oxygenation, ventilation, and hormonal input. A healthy pulmonary system has a large amount of intrinsic reserve, as evidenced by the body's ability to tolerate single lung ventilation. However, because of their direct exposure to the environment, the lungs are subject to severe injury by aspiration, inhalation, and aerosolized exposure to infectious agents. The advent of mechanical ventilation has allowed for maintenance of tissue oxygenation and ventilation when a patient is no longer capable of doing so to a sufficient extent to allow survival. At times, the lungs may be so damaged that ECMO must be employed to support the body until sufficient recovery or transplantation can be accomplished.

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CHAPTER 46

Dialysis

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Introduction

The current state of kidney transplantation is inextricably entangled with the practice of dialysis. Unlike other life-sustaining areas of organ transplantation, where reliable, long-term surrogates for end-organ failure are either absent (e.g. liver and lung) or only recently gaining a reliable foothold (heart and intestine), kidney transplantation has emerged in parallel with the emergence and refinement of techniques for dialysis. Indeed, it is reasonable to say that the bulk of kidney transplantation exists because of the ability to mitigate the immediate consequences of renal failure and in so doing allow the medical team to attend to the logistics of transplantation. Similarly, the fiscal management of renal failure has been greatly influenced by policies related to the coverage of dialysis (as discussed in detail in Chapters 140 and 143). As such, an understanding of essentially all aspects of kidney transplantation requires some understanding of the current practice of dialysis, and the techniques of achieving and maintaining dialysis access are an accepted part of the repertoire of all clinicians with specialty certification in transplantation.

The chapter will provide the reader with a general understanding of dialysis as it relates to transplantation. It is divided into three sections: a review of epidemiological data regarding the impact of dialysis exposure and dialysis modality on kidney transplant outcomes; an overview of dialysis and related extracorporeal therapies; and a detailed discussion of dialysis management considerations in renal and extrarenal transplant recipients. The chapter can be viewed as a companion to Chapters 28 and 37, which cover patient selection and indications for transplantation, and the management of patients awaiting transplantation, respectively.

Epidemiology

Impact of dialysis on kidney transplant outcomes

Dialysis provides limited clearance of metabolic waste products. Chronic uremia and hypervolemia together with other metabolic abnormalities (e.g. anemia, abnormalities of calcium and phosphate metabolism) cause accelerated progression of pathological conditions, including atherosclerotic disease, vascular calcification, and left ventricular hypertrophy, which lead to excess morbidity and mortality in dialysis patients. Advances in dialysis and medical therapies have improved dialysis survival; however, the annual death rate among waitlisted transplant candidates remains in the range of 3–5% per year [1]. Patient tolerance of uremia is variable, and diabetic and elderly patients have the highest risk of mortality on dialysis.

A longer duration of pretransplant dialysis treatment is associated with decreased allograft survival, presumably because the progression of co-morbid disease during dialysis is not reversed after transplantation [2]. This is in part due to the fact that the patients with a functioning allograft have decreased kidney function, and continue to be at risk for chronic kidney disease-related complications [3]. Complete avoidance of dialysis with pre-emptive transplantation is associated with the best post-transplant outcomes [4]. To what extent the survival advantage of pre-emptive transplantation is due to differences between pre-emptive and non–pre-emptive recipients, rather than a detrimental effect of even relatively short exposures to uremia, cannot be discerned from available observational studies.

The timing of pre-emptive transplantation remains a subjective science. In a study of adult pre-emptive transplant recipients in the US between 1994 and 2000, there was no difference in post-transplant outcomes between the patients who had an estimated glomerular filtration rate (GFR_ of less or greater than 15 mL/min at the time of transplantation [5]. In clinical practice, pre-emptive transplantation may be considered at any time when the GFR is $<$20 mL/min, with avoidance of uremic signs and symptoms being the overriding consideration. The rate of kidney function decline as well as signs and symptoms of uremia should be closely followed in pre-emptive transplant candidates. A short course of dialysis to correct uremia may be necessary in pre-emptive candidates who develop symptoms or biochemical abnormalities that cannot be managed medically (Table 46.1).

Impact of dialysis modality on kidney transplant outcomes

The ability to determine an association between dialysis modality and post-transplant outcomes is limited by the fact that the existing single-center studies have inadequate power to detect a difference in transplant outcomes, and registry analyses are prone to residual confounding. Peritoneal dialysis (PD) is self-administered and

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 46.1. Indications for acute dialysis

- Refractory hyperkalemia
- Refractory acidosis
- Refractory pulmonary edema
- Uremic pericarditis or pericardial effusion
- Uremic encephalopathy

preferred by patients who require flexibility in their dialysis treatment schedule and/or the ability to travel. Accordingly, PD patients may be more active, have a higher functional status, and have a lower burden of co-morbid disease than hemodialysis (HD) treated patients, and such differences are likely inadequately accounted for in registry analyses. Consistent with this view is the finding that PD patients are more likely to receive a transplant than HD patients [6].

In an analysis of United States Renal Data System (USRDS) data, there was no difference in long-term allograft survival or death between PD and HD patients despite a small but significant increased risk of death-censored allograft failure among PD patients [6]. The long-term increased risk of death-censored graft loss was driven by a higher risk of graft failure during the first 3 months after transplantation. This high early risk of death-censored graft loss in PD patients was observed despite a lower rate of delayed graft function (presumably due to higher levels of residual native kidney function in PD patients). The reason for increased early allograft failures in PD treated patients could not be determined. However, in a subset of patients with available information regarding the cause of allograft failure, a higher rate of graft thrombosis was noted [6,7]. No differences in acute rejection were observed. An increased risk of allograft thrombosis in PD patients has been noted in other studies and in theory could be related to an acquired thrombophilic state from loss of anticoagulant proteins across the peritoneal membrane [7]. A small retrospective single-center study found that institution of a prophylactic aspirin protocol reduced the observed incidence of renal vein thrombosis [8]. However, larger and controlled studies are necessary to recommend any specific intervention in PD patients.

Existing studies do not suggest a difference in acute rejection between PD and HD patients. In the setting of delayed graft function, previous studies have suggested a higher clearance of mycophenolic acid (MPA) in PD patients [9]; however, more recent analyses have failed to show any difference in clearance between HD and PD patients, and no adjustment of MPA dosing appears to be necessary to maintain therapeutic efficacy in dialysis patients. However, MPA glucuronide (MPAG) does accumulate in dialysis patients and may lead to increased gastrointestinal intolerance [9,10].

A higher rate of bacterial intra-abdominal, bloodstream, surgical wound, or catheter site infections in PD patients is inconsistently reported in the literature, and it is unclear whether there is a higher risk in PD compared to HD patients [11,12].

No studies have examined whether the dialysis prescription (i.e. increased clearance of metabolic waste products with nocturnal hemodialysis, daily hemodialysis, or hemodiafiltration) mitigates the impact of uremia on post-transplant outcomes.

Dialysis and related extracorporeal therapies

Indications for dialysis

Acute dialysis is indicated to correct the potentially life-threatening complications of uremia (Table 46.1) that cannot be medically managed. The indications for acute dialysis are the same as for chronic dialysis patients, patients with advanced chronic kidney disease not yet initiated on chronic dialysis, and patients with acute kidney injury, including delayed graft function.

Hemodialysis

Procedure

During HD blood is circulated through an extracorporeal circuit containing a dialysis membrane where solute and water are removed, and subsequently returned to the patient [13]. Blood and dialysis fluid (dialysate) are pumped in a counter-flow direction on either side of the dialysis membrane. Solute removal is achieved through diffusion of molecules across the dialysis membrane, while fluid removal is achieved through application of hydrostatic pressure across the membrane. In conventional HD, diffusion is the main form of solute clearance, while in hemodiafiltration, solute clearance is also achieved by convection.

Circuit

Components of the dialysis circuit include a vascular access to extract blood from the patient, dialysis tubing to connect the patient to the extracorporeal circuit, the dialysis machine, dialysate, and the dialysis membrane (Figure 46.1). Modern dialysis machines allow for tight control of fluid removal, and the ability to provide isolated fluid removal without diffusive clearance (isolated ultrafiltration). Anticoagulation is usually required to prevent clotting in the extracorporeal circuit, commonly in the form of heparin.

Prescription

The dialysis prescription specifies the duration and frequency of each treatment, the blood and dialysate flow rates, the type of dialysis membrane, and the composition of the dialysate. The frequency and duration of treatments can be increased to enhance clearance of metabolic waste products and fluid, with a number of demonstrable clinical benefits in chronic HD patients [14–16]. In the acute setting, the frequency and duration of dialysis treatment is dependent on the indication for treatment. Table 46.2 shows a typical dialysis prescription along with common variations in the prescription.

Vascular access

The principal types of vascular access in chronic HD patients are native arteriovenous fistulas (AVF), arteriovenous grafts (AVG), and central venous catheters (CVC) that are typically tunneled under the skin prior to entering the central circulation (tunneled cuffed catheter).

AVFs are created through direct anastomosis between an artery and a vein, while AVGs use synthetic material to connect an artery and vein (Figure 46.2). Both AVFs and AVGs require time for maturation (4 –8 weeks) before use. During dialysis, needles are inserted directly into the AVF or AVG for blood removal and return. CVCs are double-lumen catheters that are usually inserted in the internal jugular, subclavian, or femoral veins (Figure 46.2). CVCs can be used immediately after placement. Between treatments, CVCs are locked with anticoagulants.

Complications

Due to fluid and solute removal

Dialysis disequilibrium syndrome is the occurrence of neurological signs and symptoms due to cerebral edema [17]. The exact cause is unclear, but is related to rapid removal of fluid and solutes. Cardiac

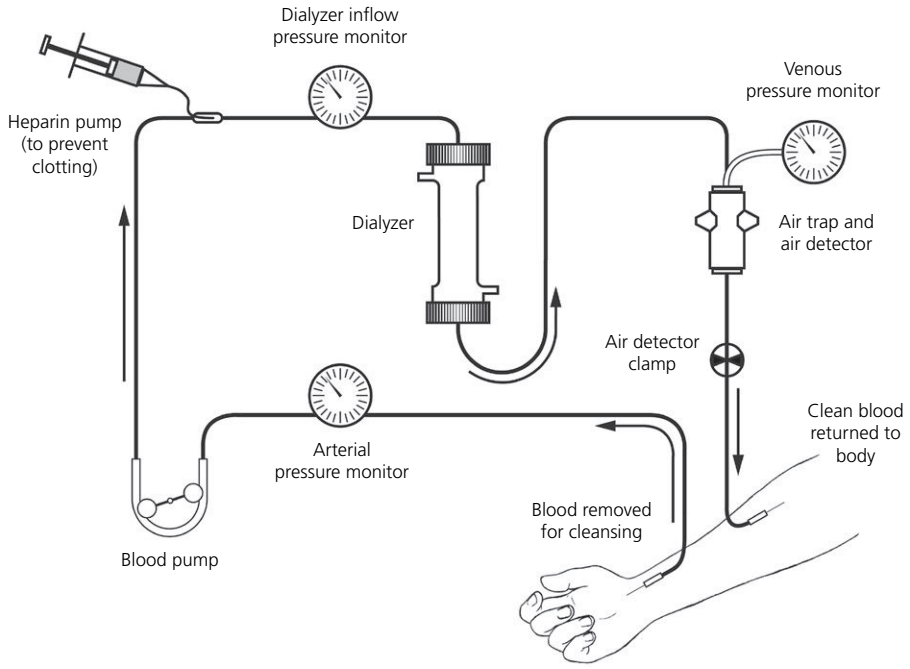

Figure 46.1. Hemodialysis circuit. Components of the dialysis circuit include a vascular access to extract blood from the patient, dialysis tubing to connect the patient to the extracorporeal circuit, the dialysis machine (not shown), dialysate (not shown), and the dialysis membrane (dialyzer). Blood and dialysis fluid (dialysate, not shown) are pumped in a counter-flow direction on either side of the dialysis membrane. Solute removal is achieved through diffusion of molecules across the dialysis membrane, while fluid removal is achieved through application of hydrostatic pressure across the membrane. The circuit is commonly anticoagulated with heparin to prevent clotting. (Reproduced from http://www.catalog.niddk.nih.gov/imagelibrary.)

Table 46.2. Hemodialysis prescription

	Typical prescription	Potential variations
Dialysis membrane	Synthetic hollow fiber High flux Surface area 1.8 m^2	Cellulo-synthetic hollow fiber Low flux Surface area 1.8–2.5 m^2
Dialysate:		
Sodium (mEq/L)	138–140	130–155
Potassium (mEq/L)	2	0–4
Bicarbonate (mEq/L)	35	28–40
Calcium (mmol/L)	1.25	1.25–1.75
Magnesium (mmol/L)	0.75	0.5–1.0
Glucose and chloride	Standard	
Dialysate flow rate (mL/min)	500	300–800
Blood flow rate (mL/min)	350	200–400
Anticoagulation	Heparin 500-U load/500 U/h maintenance infusion	Heparin 500–1500-U load/500–1500 U/h maintenance Other alternatives: no heparin, regional citrate, regional heparin with protamine reversal, citrasate dialysate, danaparoid
Duration of run (h)	4	2—8
Frequency of runs (number/week)	3	3–7
Fluid removal (L/treatment)	2 L per treatment	0–5L per treatment
Dialysate temperature (°C)	36.5	35–37

arrhythmias due to rapid changes in serum potassium and calcium concentrations, seizures due rapid alterations in serum sodium and calcium concentrations, and hypotension due to rapid or excessive fluid removal are other common complications [18].

Due to exposure of blood to the extracorporeal circuit

Despite technologic advances, the removal of blood from the central circulation together with the contact of blood with components of the dialysis circuit can lead to a variety of problems, including bleeding, clotting, thrombocytopenia, infection, and air embolism. Cytokine release and complement activation occur when blood comes into contact with the dialysis membrane. Cytokine release is decreased with modern dialysis membranes made from more biocompatible synthetic materials, compared to that with the earlier cellulose-based membranes; however, dialyzer reactions still occur. The severity of dialyzer reactions ranges from mild allergic reactions to anaphylaxis [19].

Dialysate-related complications

HD patients are exposed to vast quantities of water, which is mixed with dialysate concentrate to create dialysate. Water must be filtered, softened, temperature and pH adjusted, and purified by reverse osmosis, and organic solutes must be absorbed prior to entering the dialysis circuit. Patients may be exposed to trace mineral contaminants, chemical contaminants, and bacterial endotoxins leading to infection or hemolysis [20].

Vascular access-related complications

The major complications with AVF/AVGs are infection, thrombosis, and steal syndrome. Infections can present with local signs of

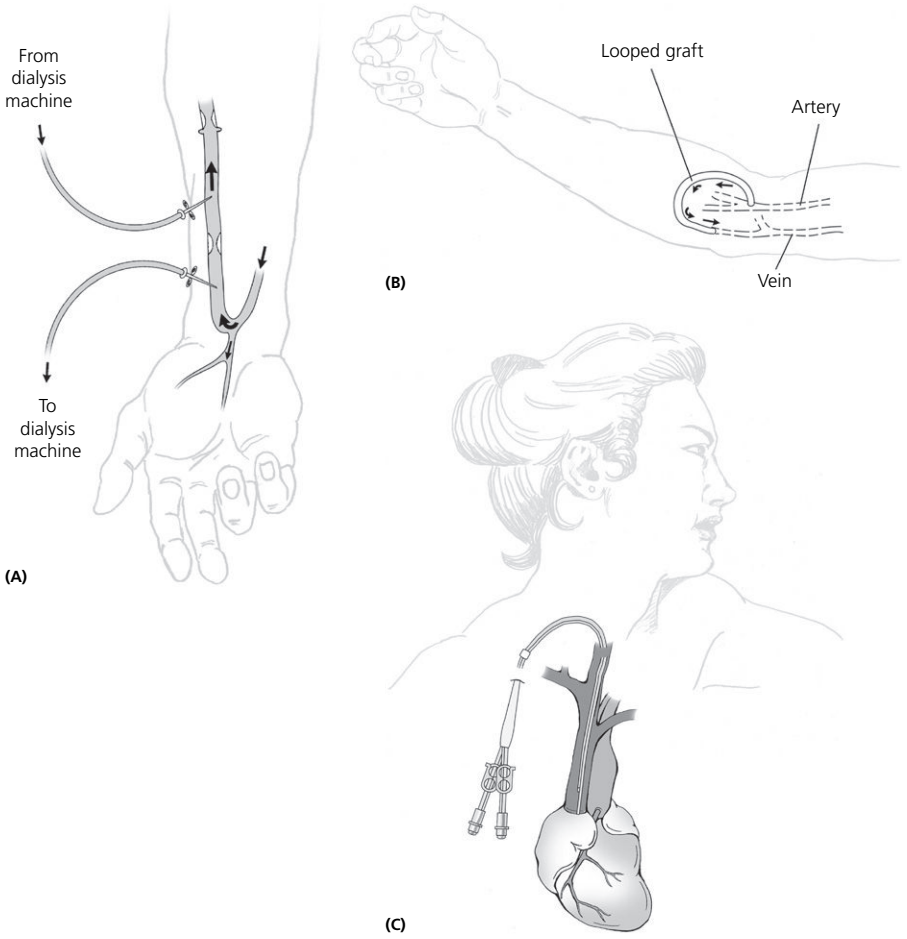

Figure 46.2. Types of vascular access used in hemodialysis. The principal types of vascular access in chronic hemodialysis patients are (A) native arteriovenous fistulas (AVF), (B) arteriovenous grafts (AVG), and (C) central venous catheters (CVC) that are typically tunneled under the skin prior to entering the central circulation (tunneled cuffed catheter). (Reproduced from http://www.catalog.niddk.nih.gov/imagelibrary.)

inflammation, septicemia, or peripheral seeding (endocarditis, osteomyelitis). Management includes investigation (cultures of blood and local drainage), antibiotic therapy, and occasionally surgical intervention [21]. Thrombosis can result from stenosis or poor flow due to local or systemic hypotension. Blood pressure measurements, phlebotomy, and insertion of peripheral intravenous lines should be avoided in the extremity where an AVG or AVF is present. Avoidance of systemic hypotension and local compression are additional important considerations [22]. Diversion of arterial blood flow through an AVF/AVG may lead to ischemia in the distal extremity (steal syndrome). Evidence of ischemia or pain in the extremity, particularly during dialysis, is suggestive of steal syndrome. Signs and symptoms suggestive of steal syndrome should be assessed prior to surgery to inform the need for pre-emptive surgical correction or interventions to protect the extremity during the transplant surgery [23].

CVCs have a higher rate of infection than AVF/AVGs. Infections can be localized or systemic. Investigation and treatment of suspected infections are similar to those for AVF/AVG infections. The decision to remove the catheter is based on patient hemodynamic instability, microbiologic culture results, extent of response to treatment, and whether co-existent tunnel or exit site infection are present [24]. Catheter dysfunction manifests as the inability to reach appropriate blood flow rates during dialysis and a decrease in clearance of metabolic waste products due to high rates of recirculation within the catheter. The main causes of catheter dysfunction are improper positioning, kinking, and intraluminal fibrin sheaths. A variety of interventions including repositioning of the patient or catheter, intraluminal thrombolytics, and even catheter replacement may be required [25].

Development of central vein stenosis can occur with all types of vascular access [26]. This usually manifests as swelling of the upper extremity; other presentations include pleural effusions, development of collateral veins across the trunk, difficult cannulation, and superior vena cava syndrome.

Peritoneal dialysis

Procedure

An indwelling PD catheter is used to instill and remove dialysate from the peritoneal cavity. The patient's own peritoneal membrane acts as the dialysis membrane and allows for diffusion and convection of molecules and fluid into the dialysate, which is drained after a specific dwell period. PD solutions contain osmotic molecules, which promote movement of fluids out of the peritoneal vasculature across the patient's peritoneal membrane into the dialysate. Through repeated fill–dwell–drain exchanges, PD achieves gradual and continuous solute and fluid removal [27].

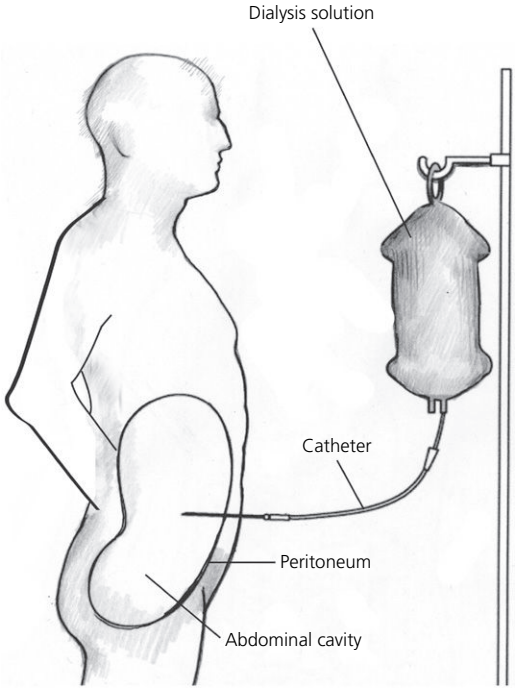

Figure 46.3. Peritoneal dialysis (PD) procedure. The PD set-up includes the catheter, dialysis solution, connection, and transfer sets. An indwelling peritoneal dialysis catheter is used to instill and remove dialysate from the peritoneal cavity. The patient's own peritoneal membrane acts as the dialysis membrane and allows for diffusion and convection of molecules and fluid into the dialysate, which is drained after a specific dwell period. (Reproduced from http://www.catalog.niddk.nih.gov/imagelibrary.)

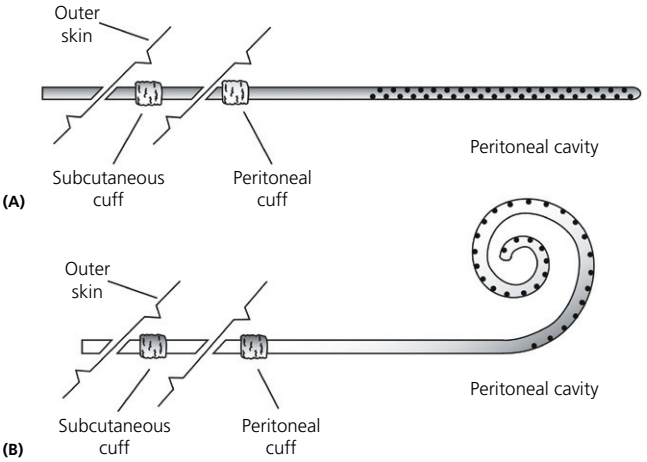

Figure 46.4. Two-cuff Tenckhoff peritoneal dialysis catheter, the most common catheter in use. The peritoneal dialysis catheter consists of a distal intraperitoneal portion, a mid-portion that is implanted into the abdominal wall, and a subcutaneous track. (Reproduced from http://www.catalog.niddk.nih.gov/imagelibrary.)

Table 46.3. Peritoneal dialysis (PD) prescription

	Typical prescription	Range of variations
Frequency of exchanges	3 per day	3–5 per day (CAPD) 3–5 per night (CCPD)
Fill volumes per exchange (L)	2	1.0–2.5
PD solution	1.5% dextrose based	0.5%, 1.5%, 2.5%, or 4.25% dextrose based Variations: bicarbonate based, amino acid based
Long dwell	Overnight 7.5% icodextrin based	Overnight dry abdomen (no fluid) Long day dwell if CCPD
Anticoagulation	None	Heparin injection into PD solution bags
Standard electrolyte composition	Sodium 130–137 mEq/L Potassium 0–2 mEq/L Calcium 1.25–1.75 mmol/L Magnesium 0.5–1.5 mmol/L Lactate 35–40 mEq/L	

CAPD, continuous ambulatory peritoneal dialysis; CCPD, continuous cycler peritoneal dialysis.

Patients are trained to perform PD daily at home, either manually [continuous ambulatory peritoneal dialysis (CAPD)], or using a cycler machine [automated peritoneal dialysis (APD)]. During times when no exchanges are being performed, the peritoneal cavity can be left empty ("dry abdomen") or filled with solution ("wet abdomen"). Patients on CAPD typically perform three to four exchanges during the day using dextrose-based solutions, followed by an overnight dwell. Patients on APD typically perform several exchanges during the night, followed by a day dwell. The choice of initial therapy is usually based on patient preference and suitability. Systemic anticoagulation is usually not required.

Apparatus

The PD set-up includes the catheter, dialysate solution, and connection and transfer sets (Figure 46.3). Dialysate solutions are supplied in prepackaged bags of sterile fluid with fixed concentrations of osmotic agents, electrolytes, and buffers. The type and concentration of the osmotic agent and the duration of the dwell determines the amount of fluid and solute removed during an exchange. Higher osmotic strength solutions remove more fluid and solute. The most common osmotic agents used are dextrose and icodextrin, a high molecular weight glucose polymer. The PD catheter consists of a distal intra-abdominal portion, a mid-portion that is implanted into the abdominal wall, and a subcutaneous track [28]. Variations of the catheter include the nature of the distal end of the intra-abdominal portion (straight vs. coiled), the number of cuffs used for implantation into the abdominal wall (one vs. two), and the design of the subcutaneous track (straight vs. permanently bent). The most common catheter in use is the two-cuff Tenckhoff catheter (Figure 46.4).

Prescription

Components of a typical PD prescription and variations are summarized in Table 46.3. The dialysis prescription requires frequent re-evaluation because the transport characteristics of the peritoneal membrane and the amount of residual native kidney function vary over time, leading to alterations in the amount of fluid and solute removal [29]. Peritonitis can also cause acute changes in membrane characteristics, requiring alteration in the PD prescription.

Complications

Infectious complications

PD catheter-related infections can be in form of an exit site infection, tunnel infection, or peritonitis. Exit and tunnel track infec-

Table 46.4. Diagnosis of peritonitis

Presumptive PD peritonitis	Confirmed PD peritonitis	Suspected secondary peritonitis
At least one of: Abdominal pain Peritoneal signs Fever Cloudy dialysis fluid Serum leukocytosis *Plus:* Dialysate WBC > 100 cells/mL with neutrophils >50 cells/mL	Positive dialysate Gram stain or culture	Positive dialysate culture for multiple pathogens Fecal material in dialysate High dialysate amylase High dialysate lipase

PD, peritoneal dialysis; WBC, white blood cell.

tions present with local signs of inflammation and/or drainage. Peritonitis usually results from contamination of the peritoneal cavity during instillation or drainage of dialysate. Suggestive features and diagnostic criteria for peritonitis are shown in Table 46.4. Distinction from peritonitis due to intra-abdominal pathology should particularly be considered in the perioperative setting and usually requires abdominal imaging studies. Isolated and uncomplicated exit site infections can be eradicated with careful exit site care and antimicrobial (topical and oral) therapy. If the exit site infection progresses or concurs with peritonitis or a tunnel infection, the PD catheter must be removed [30]. Peritonitis is usually treated with intraperitoneal antimicrobial therapy, but catheter removal is necessary for fungal, mycobacterial, polymicrobial, refractory, or recurrent peritonitis.

Mechanical complications

The presence of an intraperitoneal catheter with a subcutaneous track and cutaneous exit site may weaken the abdominal wall, resulting in hernia formation. Pericatheter leaks manifest as subcutaneous swelling, reduced outflow volumes, or fluid accumulation around the catheter site. In some patients, abdominal or genital wall edema can develop and may require surgical intervention and/or cessation of PD [31]. The presence of a unilateral pleural effusion may indicate communication between the peritoneal and pleural space. Outflow failure is a common problem related to constipation or catheter-related problems, including malposition, kinking, intraluminal occlusion by thrombus, or compression by the omentum. The intra-abdominal portion of the catheter may rarely cause contusion or laceration to intra-abdominal organs, resulting in a bloody dialysate. Other causes of hemoperitoneum include cyst rupture in polycystic kidney disease patients, ovulation, endometriosis, or retrograde menstruation.

Peritoneal dialysis solution-related complications

Traditional PD solutions are lactate buffered, slightly acidic in pH, and contain dextrose which when metabolized leads to the production of glucose degradation products. All these factors render PD solutions somewhat bio-incompatible which may contribute to loss of peritoneal membrane function over time. Dextrose-based solutions can lead to systemic absorption of glucose with resultant metabolic abnormalities, including caloric loading, hyperglycemia, and dyslipidemia [27].

Continuous renal replacement therapies

The main types of continuous renal replacement therapies (CRRT) are continuous veno-venous hemodialysis (CVVHD), continuous veno-venous hemofiltration (CVVH), and continuous veno-venous hemodiafiltration (CVVHDF). The addition of hemofiltration allows convective clearance of larger molecules that are not removed by diffusion during HD alone.

Table 46.5. Comparison of typical continuous renal replacement therapy (CRRT) [continuous veno-venous hemodiafiltration (CVVHDF)] and conventional intermittent hemodialysis (HD) prescriptions

	CRRT	Intermittent HD
Prescribed daily duration (h)	24 (continuous)	4
Blood flow rate (mL/min)	100–200	250–350
Dialysate flow rate (mL/min)	15–45	300–800
Replacement fluid flow rate (mL/min)	15–45	N/A
Hourly ultrafiltration rate (mL/h)	50–150	100–1500
Dialysis membrane surface area	Usually smaller ($0.9–1.2\,m^2$)	Usually bigger ($1.8–2.5\,m^2$)
Anticoagulation options	**1** Regional citrate **2** Heparin **3** None	**1** Heparin **2** Citrasate dialysate **3** Danaparoid **4** Normal saline flushes
Composition of dialysate	Fixed (several available solutions)	Modifiable
Composition of replacement fluid	Fixed (several available solutions)	N/A

Compared to conventional HD, CRRT operates at lower blood and dialysate flow rates using smaller HD membranes, thereby making it a less efficient form of dialysis [32]. The inefficiency of the dialysis procedure is compensated for by prescribed continuous treatment over a 24-h period so that total daily solute and fluid removal can approximate that achieved with intermittent dialysis.

Technique

While the basic set-up of a CRRT circuit is similar to a conventional HD set-up, some noteworthy distinctions exist (Table 46.5). Most CRRT machines use prepackaged bags of sterile dialysate and replacement fluid. The electrolyte and buffer content within these bags is fixed and cannot be altered as can be done during conventional HD. Resultant derangements in patient electrolyte or acid–base balance therefore require systemic correction. The continuous nature of therapy usually precludes use of a pre-existing AVF/AVG and a dialysis catheter is preferred. Regional or systemic anticoagulation is required. Regional citrate anticoagulation is preferred in most critically ill patients.

Advantages, disadvantages, and indications

CRRT is better tolerated in hemodynamically unstable patients than conventional dialysis. CRRT provides slow but continuous removal of solutes and fluid, which can be adjusted in real time in response to changes in clinical and fluid status. In most patients, this facilitates administration of blood products, total parenteral nutrition, and medications by avoiding volume overload and electrolyte abnormalities.

The delivered dialysis dose is often much less than the prescribed dose in CRRT. Transient CRRT interruptions may be necessary for a variety of reasons, and it is difficult to compensate for this lost time with this slow modality. Despite advances in anticoagulation, the half-life of a CRRT dialysis filter is limited and filter replacement is typically required every 48–72 h [33]. The avoidance of systemic heparin administration with regional citrate anticoagulation requires frequent monitoring to avoid hypocalcemia, hypernatremia, and alkalosis. Therefore, CRRT is an expensive and labor-intensive dialysis modality [34].

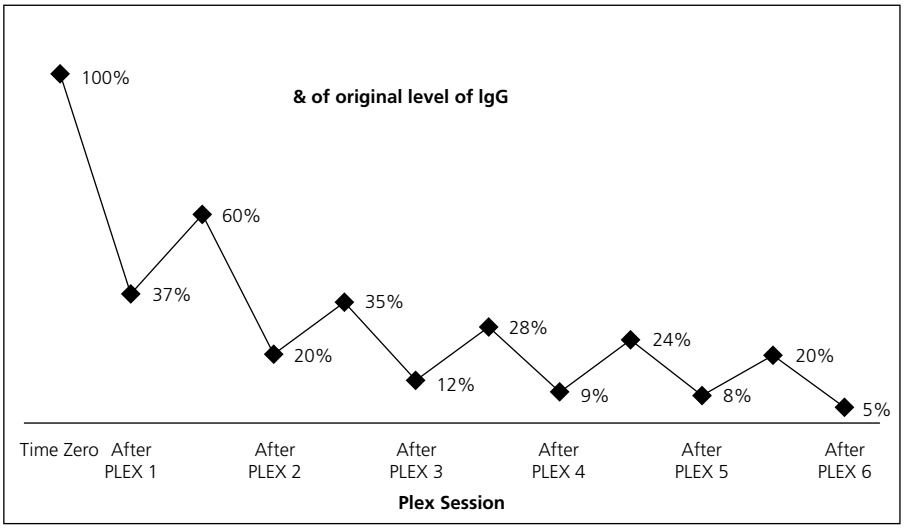

Figure 46.5. Serum levels of immunoglobulin G (IgG) after consecutive therapeutic plasma exchange (TPE; PLEX) treatments. The exchange of one plasma volume will typically lower plasma IgG levels by 65%. The removal of macromolecules with plasma exchange is limited by redistribution from the extravascular space after each treatment (rebound). Typically three to five treatments are required before a sustained reduction in antibody levels is achieved.

Despite its advantages, CRRT does not improve survival or renal recovery in critically ill patients [35]. Nonetheless, it is often the most feasible method to deliver dialysis in hemodynamically unstable patients. CRRT should not be used in clinical settings requiring rapid fluid or solute removal, including in patients with toxic ingestions and life-threatening hyperkalemia.

Therapeutic plasma exchange

Therapeutic plasma exchange (TPE) is an extracorporeal treatment designed to remove large molecular weight substances (e.g. antibodies, para-proteins) from the plasma or to replace deficient plasma factors.

Technique

Separation of plasma from blood can be performed by centrifugation or by membrane filtration [36]. Centrifugation is usually performed using a specialized centrifugal device. Advantages of centrifugation include the ability to remove and return blood from the peripheral venous circulation using two large-bore intravenous catheters and the ability to selectively remove cells from blood (cytapheresis). Disadvantages of this low-efficiency technique include longer treatment times, an increased frequency of thrombocytopenia, and complications related to use of citrate for anticoagulation. In contrast, membrane filtration uses a highly permeable membrane and a standard dialysis machine operating in ultrafiltration mode. Membrane filtration requires placement of a double lumen CVC to allow adequate blood flow rates. Although anticoagulation can be performed with citrate, heparin is commonly used.

When plasma is removed, the intravascular volume must be repleted to prevent hypotension. The choice of replacement fluid depends on the indication for TPE [37]. Fresh frozen plasma (FFP) replaces the normal plasma proteins and avoids depletion of coagulation factors or immunoglobulins. Disadvantages of FFP include increased risk of blood-borne disease transmission, transfusion-related lung injury, citrate-induced hypocalcemia, and urticaria, and rarely anaphylactoid reactions. FFP is only recommended when replacement of plasma factors is required, as in cases of HUS/TTP or bleeding risk. Albumin is favored as replacement fluid in most other conditions because it carries no risk of blood-borne disease transmission and minimizes the risk of allergic reactions. Saline in combination with albumin may be used when hyperviscosity or cost is a consideration. The rapid removal and infusion of protein-containing solutions during plasma exchange primarily involve the intravascular compartment; therefore, even small mismatches between removed and infused volumes can have a large impact on blood pressure. Accordingly, the amount of saline infused, which is distributed both intra- and extra-vascularly, should be limited to 20–40% of the total replacement volume.

The exchange of one plasma volume will typically lower plasma immunoglobulin G (IgG) levels by 65%, while an exchange of 1.5 $\times$ plasma volume lowers plasma levels by 75% [38]. The plasma volume that is exchanged is dependent on the patient's body weight and hematocrit (hct), and can be estimated using a simplified equation:

$$\text{Plasma volume} = 0.07 \times \text{weight (kg)} \times (1 - \text{hct})$$

The number of therapeutic exchanges is dictated by the indication for plasma exchange, patient tolerance, and clinical response. The removal of macromolecules with plasma exchange is limited by redistribution from the extravascular space after each treatment (rebound). Typically, three to five treatments are required before a sustained reduction in antibody levels is achieved, as shown in Figure 46.5. Table 46.6 shows a typical plasma exchange prescription along with common variations.

Complications

Reported rates of minimal reactions, including minor allergic symptoms (e.g. pruritus, hives), are between 5% and 10%. Rates of moderate reactions such as hypotension or cardiac arrhythmias (due to hypokalemia or hypocalcemia) are around 5%, while rates of severe anaphylactoid reactions are $<3\%$, with procedure-related mortality rates being very low ($<0.05\%$) [39].

Table 46.6. Therapeutic plasma exchange prescription

	Typical prescription	Alternatives
Mode	Centrifugal or membrane filtration	
Exchange volume	1 × plasma volume	1.0–2.0 × plasma volume
Replacement fluid	Albumin plus crystalloid (normal saline or Ringer's lactate)	Fresh frozen plasma
Plasma removal rate (mL/min)	60	30–100
Anticoagulation	Citrate (with prophylactic calcium infusion)	Heparin
Treatment regimen	Individualized based on indication and response to therapy	

Table 46.7. Pretransplant assessment of dialysis patients

Dialysis history	Date of first chronic dialysis treatment Timing and reasons for change in modalities
Dialysis access	Hemodialysis vascular access Peritoneal dialysis catheter
Dialysis adequacy	Clinical assessment of adequate dialysis Measurements of adequate dialysis
Residual renal function	Estimation of residual renal function Preservation of residual renal function
Management of chronic medications	Before transplantation After transplantation

Complications during TPE are more common when FFP is used as the replacement fluid, and include anaphylactoid reactions, severe bronchospasm, and transfusion-related lung injury. Allergic reactions may be more frequent in patients prescribed angiotensin-converting enzyme (ACE) inhibitors. Citrate contained within FFP or used for anticoagulation binds to calcium and reduces the ionized but not total calcium concentration, leading to a variety of symptoms including paresthesias, muscle cramps, nausea, vomiting, prolongation of QT and arrhythmia, hypotension, and tetany. Prophylaxis with intravenous calcium may be used (e.g. 10 mL of 10% calcium chloride infused over 30 min, beginning 15 min after initiation of plasma exchange). Citrate may also cause alkalemia, especially in patients with concomitant renal failure. Volume-related complications include hypotension and pulmonary edema due to a mismatch between fluid removal and replacement. Hypotension and dyspnea may also be due to complement activation owing to contact between the blood and filter in the extracorporeal circuit, or sensitivity to ethylene oxide that is used to sterilize the filter. Arrhythmias due to hypokalemia are observed with albumin replacement. Potassium is often added to albumin to minimize the risk of hypokalemia. Removal of clotting factors may predispose to bleeding. In patients at risk of bleeding, FFP may be indicated as a replacement fluid. Removal of immunoglobulins and complement can lead to an increased risk of infection, which may be aggravated by concomitant use of immunosuppressant agents. Plasma exchange removes drugs very efficiently; therefore, medications are typically administered following plasma exchange and increased monitoring of critical dose medications may be necessary.

Dialysis management considerations in renal and extrarenal transplant recipients

Kidney transplant candidates

Pretransplant evaluation

The initial pretransplant evaluation of kidney transplant candidates may occur anywhere from a few days to years before the date of transplantation. Table 46.7 shows the dialysis-related issues most relevant for transplantation.

Dialysis history

Knowledge of the date of first chronic dialysis treatment is essential to project the anticipated date of transplantation, and to devise an appropriate waitlist management plan. Although the timing of transplantation cannot be precisely determined for deceased donor candidates, knowledge of ABO blood group and panel reactive antibody (PRA) can provide a reasonable estimate of waiting time. Based on anticipated waiting time and co-morbid disease history, a waitlist management strategy, including need for longitudinal investigations and repeat medical or surgical evaluation, should be determined. Waitlist management is not covered in this chapter and the reader is referred to published literature on this topic [40–42].

Dialysis access

The site, type, and health of the current HD and PD access should be determined.

HD patients with multiple or prolonged use of CVCs are prone to the development of scarring and central vein stenosis, which may complicate establishment of central venous access at the time of transplantation. A history of frequent AVF/AVG thrombosis should be ascertained and may require further evaluation. It is unclear how patients with a history of recurrent vascular access thrombosis should be managed at the time of transplantation. Most commonly, the cause of thrombosis can be ascribed to vascular access stenosis, and no specific management prior to transplantation is necessary. In some cases, however, a thrombophilic work-up may be necessary to exclude a systemic clotting disorder, which may lead to allograft thrombosis. In cases where the only indication for anticoagulation is maintenance of vascular access and no thrombophilic disorder is identified, anticoagulants should not be required during or after transplantation.

In PD patients, details of previous PD catheter-related complications and reasons for previous PD catheter loss should be established. Removal of previous PD catheters due to serious peritoneal infection together with ongoing symptoms suggestive of intermittent abdominal obstruction or hemoperitoneum may suggest the presence of encapsulating peritoneal sclerosis, an infrequent condition that can complicate transplant surgery or lead to intermittent bowel obstruction after surgery [43].

Dialysis adequacy

An assessment of the dialysis adequacy consists of clinical assessment of signs or symptoms of uremia, supplemented by mathematical formulas to estimate the clearance of waste products during dialysis (Table 46.8). Various methods to estimate the clearance of metabolic waste products with either PD or HD are available but their description is beyond the scope of this chapter [44,45]. Inadequate dialysis can suggest problems with the dialysis access or dialysis membrane (in the case of PD patients) or non-adherence with the dialysis prescription. Modification of the dialysis prescription may be necessary to optimize patient management prior to transplantation.

Residual kidney function

Knowledge of residual native kidney function and urine output is necessary to assess immediate allograft function after transplantation. Because of the non-linear relationship between serum creatinine and GFR, a small change in GFR will cause a large change in serum creatinine in patients with advanced kidney disease. Residual kidney function should not be monitored by serum creatinine alone. A 24-h urine collection for urea and creatinine clearance can provide a more accurate assessment of residual kidney function. However, the usefulness of this test is limited because it is inconvenient for patients and prone to collection error. Further, tubular secretion of creatinine may result in overestimation of kidney function by this method.

A variety of serum creatinine-based equations have been developed to estimate kidney function, as detailed in Table 46.9 [46]. The Cockcroft–Gault equation predicts creatinine clearance based on age, weight, height, and plasma creatinine. The requirement for weight and height somewhat limit the use of this equation in clinical practice. Equations derived from the Modification of Diet in Renal Disease (MDRD) study do not require height and weight measurements and are now the most commonly used in clinical practice. The original six-variable MDRD equation required measurement of serum albumin and urea, and was surpassed by the simpler four-variable equation. The MDRD equation has not been validated in morbidly obese patients and in ethnic minority groups other than African–Americans. To overcome biased GFR estimates related to the method of serum creatinine measurement in the MDRD study, a unified effort to standardize creatinine measurements to the reference isotope-dilution mass spectrometry (IDMS) method was encouraged in laboratories around the world. With this, a new factor of "175" (as opposed to "186") was subsequently recommended in the MDRD equation for creatinine assays that are IDMS aligned. MDRD equations perform poorly in patients with near-normal kidney function, and in these situations the more recently developed Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is preferred [47]. Either the Cockcroft–Gault equation or the MDRD equation may be used for monitoring kidney function in patients with advanced chronic kidney disease, including pre-emptive transplant candidates.

Residual kidney function is an important predictor of survival in dialysis patients [48]. Every effort should be made to preserve residual kidney function, especially in pre-emptive and PD patients. This includes avoidance of potentially nephrotoxic medications, hypotension, and radiocontrast agents. Expert consultation with the nephrologist should be sought when these insults are unavoidable as prophylactic measures to mitigate the nephrotoxicity of these insults may exist.

Medication management

The majority of medications prescribed to chronic dialysis patients are discontinued after surgery. However, there are certain chronic medications that will require dose adjustment after transplantation because of drug interactions with immunosuppressant medications or because of the establishment of kidney allograft function

Table 46.8. Assessment of dialysis adequacy

	Hemodialysis patients	Peritoneal dialysis patients
Clinical signs of symptoms suggestive of inadequate dialysis	Refractory hypertension Refractory congestive heart failure Progressive left ventricular hypertrophy Refractory hyperphosphatemia Refractory acidemia Confusion, altered cognition Nausea, vomiting Pruritus Unexplained fatigue	
Mathematical indicators of inadequate dialysis	Kt/V < 1.2 (target in clinical practice > 1.4) URR < 65% (target in clinical practice > 70%)	Weekly Kt/V < 1.7 Daily ultrafiltration volume achieved by PD <750 mL (or <250 mL if no residual renal function)

Kt/V, dialysis dose, where K is the dialyzer clearance (mL/min), t is the duration of dialysis treatment (min), and V is the volume of distribution of urea (mL)
URR, urea reduction ratio

Table 46.9. Equations used to estimate native kidney function

Equation	Formula	Variables
Creatinine clearance (mL/min) *Requires adjustment for body surface area*	$GFR = [U_{Cr} \times V]/S_{Cr}$	U_{Cr} = urine creatinine concentration (μmol/L) S_{Cr} = serum creatinine concentration (μmol/L) V = 24-h urine volume (L)
Cockcroft–Gault [creatinine clearance, (mL/min)] *Requires adjustment for body surface area*	[(140 – Age) × W (kg)/Cr × 72] × 0.85 (if female)	W = lean body weight (kg) Cr = serum creatinine concentration (mg/dL)
Original six-variable MDRD [GFR (mL/min/1.73 m^2)]	$170 \times S_{Cr}^{-0.999} \times Age^{0.176} \times BUN^{-0.170} \times Alb^{+0.318} \times 0.762$ (if female) x 1.18 (if black)	S_{Cr} = serum creatinine concentration (mg/dL) Age in years BUN = blood urea nitrogen concentration (mg/dL) Alb = serum albumin concentration (g/dL)
Modified four-variable MDRD [GFR (mL/min/1.73 m^2)]	$186.3 \times S_{Cr}^{-1.154} \times Age^{-0.203}$ x 0.742 (if female) x 1.21 (if black)	S_{Cr} = serum creatinine concentration (mg/dL) Age in years
Modified four-variable MDRD IDMS [GFR (mL/min/1.73 m^2)]	$175 \times S_{Cr}^{-1.154} \times Age^{-0.203} \times 0.742$ (if female) x 1.21 (if black)	S_{Cr} = serum creatinine concentration (mg/dL) Age in years
CKD-EPI [GFR (mL/min/1.73 m^2)]	$141 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa, 1)^{-1.209} \times 0.993^{(age)}$ x 1.018 (if female) x 1.159 (if black)	S_{Cr} = serum creatinine concentration (mg/dL) κ = 0.7 for females and 0.9 for males α = –0.329 for females and –0.411 for males min = minimum of Scr/κ or 1 max = maximum of Scr/κ or 1 Age in years

GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease, IDMS, isotope-dilution mass spectrometry; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

Table 46.10. Peritransplant medication management

Medications that are usually stopped	Continued medications where dose adjustment may be necessary
Phosphate binders (e.g. calcium carbonate, sevalemer, lanthanum)	Insulin and oral hypoglycemic agents
Coumadin (if sole indication for vascular maintenance of vascular access)	Anticonvulsant medications
Platelet $P2Y_{12}$ receptor blockers* (clopidogrel, ticlodipine, prasugrel)	Aspirin
Vitamin D analogs (e.g. 1-α calcitriol)	Antiarrhythmic medications
Diuretics	Antidepressant medications
ACE inhibitors	Beta-blockers
Statins	Clonidine

*Depends on indication. Bare metal coronary stents require dual antiplatelet therapy with aspirin and $P2Y_{12}$ blocker for a minimum of 1 month and preferably 6 months. Drug-eluting stents usually require a minimum of 6 months and preferably 12 months of dual antiplatelet therapy. Consultation with a cardiologist is recommended.
ACE, angiotensin-converting enzyme.

(Table 46.10). A discussion of all potential medication adjustments is beyond the scope of this chapter, and consultation with the hospital pharmacist is advised to ensure patient safety.

Perioperative management

Dialysis in the immediate pretransplant setting

It is easy to overlook dialysis-related management issues at the time of transplantation. The absolute indications for dialysis immediately preceding transplantation are shown in Table 46.1. In the absence of absolute indications, dialysis is administered based on the timing of the last dialysis treatment, clinical evaluation of the patient, or when delayed graft function is anticipated based on donor characteristics or cold ischemic time.

Modifications to the pretransplant prescription

Heparin is typically eliminated from the HD prescription to minimize the risk of operative bleeding. Measurement of the serum potassium immediately prior to HD and adjustment of the potassium concentration in the dialysate minimize the risk of intraoperative hyper- or hypo-kalemia. Depending on the timing of surgery, the duration of HD may be lengthened to achieve target goal weight in hypervolemic patients. Concerns regarding activation of inflammatory mediators with pretransplant HD treatment, particularly with bio-incompatible membranes, remain theoretical. The widespread use of biocompatible membranes has diminished the relevance of this issue and biocompatible membranes are preferred for pretransplant dialysis treatments if possible.

PD can be continued until the time of surgery. Should acute correction of volume overload or hyperkalemia be necessary, rapid PD exchanges can be performed every 30–90 min if necessary, either manually or using a cycler machine [49]. Icodextrin should not be used during rapid PD exchanges. The peritoneal cavity should be emptied completely prior to surgery.

Patients on prolonged or more frequent dialysis regimens, including nocturnal dialysis, may have lower perioperative blood pressures, although rates of delayed graft function have not been shown to be higher in these patients [50,51]. Some centers routinely switch nocturnal dialysis patients to a conventional HD schedule for a period of 1 week prior to living donor transplantation to avoid the risk of post-transplant hypotension (personal communication, Chris Chan, Toronto General Hospital).

Dialysis access management

Preventative measures to protect AVF/AVGs should be prioritized in the perioperative period (see above) [52], and CVC catheters should be locked with anticoagulant preparation as per local dialysis unit practice prior to surgery. The PD catheter can be either removed or left in situ at the time of surgery for later removal. It is advantageous to maintain the PD catheter as this allows for the provision of dialysis after transplantation if required, while the major disadvantages of doing so are the risk of catheter-related infection, the need for catheter care after transplantation, and the need for a further procedure to remove the catheter [53]. This decision to maintain or remove the PD catheter should consider the likelihood of post-transplant dialysis and risk of PD catheter-related infection. The risk of peritonitis may be informed by history of peritonitis and clinical exam of the PD exit site prior to surgery [54].

Postoperative management

Determination of the need and timing of post-transplant dialysis

The absolute indications for dialysis after transplantation are shown in Table 46.1. In the unstable post-transplant patient, the overriding priority is patient safety, and the need for urgent dialysis may supersede the performance of diagnostic imaging tests to establish the cause of allograft dysfunction.

The presence of severe postoperative hyperkalemia is a medical emergency that requires temporizing medical management while preparations are made for urgent dialysis. This includes administration of intravenous bicarbonate, calcium, insulin, and glucose. Rectal administration of sodium polystyrene sulfonate (Kayexalate) may cause colonic dilatation or even perforation and is best avoided in the immediate postoperative period.

Oliguric or anuric patients are often volume expanded as a result of repeated volume challenges in the intraoperative or postoperative setting. Tolerance of hypervolemia is influenced by preexisting cardiopulmonary status, including history of congestive heart failure, valvular heart disease, chronic pulmonary disease, and sleep apnea, as well as body habitus (obesity). In such patients it may be advisable to limit volume resuscitation in the absence of hemodynamic instability. In situations where urine output is tenuous, it may be prudent to avoid extubation until urine output is established or until after dialysis is completed. Urgent volume removal may be achieved by conventional HD or isolated ultrafiltration.

The immediate postoperative physical evaluation should include confirmation of a functioning dialysis access. Fistula patency may be compromised and dialysis catheters may be displaced during surgery. Clinical exam of the fistula and radiographic confirmation of the location of a new or pre-existing central venous dialysis catheter is required prior to initiation of HD. Rarely, it may be necessary to access the dialysis catheter for infusion of critical medications, and the location of any indwelling dialysis catheter should be confirmed prior to use for non-dialysis indications. In the case of PD catheters, direct discussion with the transplant surgeon is necessary to confirm the integrity of the peritoneal membrane prior to performing PD.

Knowledge of pretransplant residual kidney function and urine output, as well as the timing of dialysis prior to transplantation is needed for the evaluation of allograft function in the oliguric patient. The anesthetic record should be reviewed to determine the

volume and type of intravenous solutions or blood products administered, the occurrence of hypotension or arrhythmia, amount of blood loss, and amount of intraoperative urine output. Review of the surgical record and discussion with the transplant surgeon is essential to understand any donor-related or surgical issues that may impact decision-making regarding the need or timing of dialysis in the immediate postoperative period.

The decision to proceed with dialysis may be informed by knowledge of allograft characteristics and intraoperative events. Specifically, transplantation from an expanded criteria deceased donor, prolonged cold or warm ischemic times, and a complicated vascular anastomosis may provide important prognostic information. Knowledge of the presence or absence of delayed graft function in the mate kidney may also be helpful.

Dialysis management of delayed graft function

In the absence of absolute indications, the decision to proceed with dialysis is often subjective and variable between transplant programs. Therefore, defining delayed graft function (DGF) by the use of dialysis in the first post-transplant week is problematic and contributes to variability in both the incidence and associated consequences of DGF in the literature. Further, because DGF has been associated with decreased allograft survival in some studies, defining DGF by the requirement for dialysis leads to the perception that dialysis itself is harmful to the allograft.

Experimental and clinical observations suggest that DGF may be prolonged by the exposure of blood to bio-incompatible dialysis membranes. Three small randomized trials in the late 1990s failed to show a difference in DGF between patients treated with biocompatible and bio-incompatible membranes [55–57]. Although none of these studies had sufficient power to definitively answer the question, the widespread use of biocompatible membranes has obviated the need for further studies.

Intradialytic hypotension may lead to hypoperfusion of the allograft, prolongation of DGF, or even graft thrombosis. In HD patients, more frequent and longer dialysis sessions as well as a cool temperature dialysis can prevent development of intradialytic hypotension [58]. Alternatively, isolated ultrafiltration can be performed to achieve isolated fluid removal, which is generally better tolerated. In patients with a functional PD catheter and no surgical contraindications, PD may be continued in the setting of DGF as this modality is generally associated with a lower risk of hypotension compared to HD. In hemodynamically unstable patients, CRRT is preferred and is recommended in patients requiring inotropic support.

Careful assessment of volume status and limiting or avoiding the use of medications known to aggravate hypotension are important management considerations. In particular, it may be beneficial to administer lymphocyte-depleting antibodies and HD on alternate days in patients with established DGF.

Post-transplant management

Hemodialysis catheters

In successful transplant recipients who do not require ongoing dialysis, arrangements are made for removal of the catheter within the first few weeks after transplantation to avoid the risk of catheter-related infection. While the catheter is in situ, the health of its tunnel and exit site should be regularly assessed and the catheter should be flushed and locked weekly with an anticoagulant until removal.

Hemodialysis, arteriovenous fistulas, and grafts

Spontaneous AVF/AVG closure occurs in the minority of patients [59]. There is no consensus on routine surgical closure of functioning AVF/AVGs in patients with established allograft function [60]. Advantages of maintaining the fistula include access for future dialysis and avoidance of surgery. Fistula maintenance may be favored in patients who have exhausted vascular access options in the past. Disadvantages include a potentially deleterious effect on cardiac function [61]. Cardiac failure has been reported in dialysis patients with pre-existing cardiac disease after fistula creation [62].

Studies examining the impact of surgical fistula closure in transplant recipients have produced conflicting results. Two small prospective observational studies have shown improved echocardiographic cardiac dimensions, but it is unknown whether these changes will result in improved patient outcomes and routine surgical closure of AVFs cannot be recommended [63,64]. Potential indications for surgical closure of AVFs include worsening cardiac hemodynamics, fistula-related complications (e.g. infection or steal syndrome), and safety considerations in patients involved in high-risk activities [60].

Peritoneal dialysis catheters

PD catheters should be flushed and inspected weekly for signs of infection until removal. Timely removal is indicated to minimize the risk of exit site infection or peritonitis [65].

Heart transplant candidates

Chronic dialysis patients awaiting transplantation

A complex interplay of factors, including decreased cardiac output, deranged neurohormonal pathways leading to salt and water retention, and medication effects make it difficult to remove fluid in dialysis-dependent patients awaiting heart transplantation [66]. Conventional HD is particularly poorly tolerated due to the rapid shifts in fluid and solute. An increase in the frequency and/or duration of dialysis treatments may be required to manage these patients. PD may be the ideal dialysis modality because fluid and solute removal is achieved gradually and is generally better tolerated [67].

Patients with decompensated heart failure not requiring dialysis

In heart failure patients without end-stage kidney disease but with diuretic resistance or acutely decompensated heart failure, peripheral veno-venous ultrafiltration (UF) and slow continuous UF can facilitate fluid removal. In a non-blinded study, 200 patients with acute decompensated heart failure were randomized to treatment with UF or diuretics. There was no difference in the primary endpoint of 48-h weight loss and improved dyspnea. However, there was a greater reduction of mean weight and net fluid loss, as well as lower rates of rehospitalization within 90 days in the UF group as compared to the diuretic group. There was no difference in kidney function and less hypokalemia in the UF group. Proposed mechanisms for the sustained improvements in the UF group include removal of isotonic fluid with UF compared to hypotonic fluid removal with diuretics [68]. UF may provide an important alternative to high doses of diuretics for decompensated heart failure, especially in patients with some degree of kidney dysfunction or diuretic resistance. However, further studies are needed before UF can be routinely employed as a management strategy for advanced heart failure patients.

Critically ill patients in need of acute dialysis

In the setting of cardiogenic shock, CRRT is the preferred dialysis modality. When CRRT is not available, the conventional HD circuit and prescription can be modified to simulate the technical characteristics of CRRT. Performance of frequent (ideally daily) and long (>8 h) dialysis sessions with low blood and dialysate flow rates can approximate the operating characteristics of CRRT. This technique [slow, low efficiency dialysis (SLED)] has been shown to be as well tolerated as CRRT in hemodynamically unstable patients [69].

Patients with mechanical assist devices

Dialysis-specific considerations in patients requiring cardiac support by intra-aortic balloon pump or ventricular assist devices include knowledge of the intravascular location of the device, site of percutaneous access, and type of anticoagulation [70]. A potentially dangerous interaction between a continuous veno-venous hemodialysis (CVVHD) system and an intra-aortic balloon pump (IABP) counter-pulsation device has been reported [71]. Electrical interference created by the roller pump action of the CVVHD system was identified by the balloon pump as cardiac in origin, and it responded by inflating and deflating. Interference between different electrical support systems may occur, and systems should be tested for compatibility before their combined use.

Patients on extracorporeal membrane oxygenation

Patients in refractory cardiogenic shock with hemodynamic collapse may require complete cardiopulmonary bypass in the form of veno-arterial extracorporeal membrane oxygenation (VA-ECMO) [72]. During conventional VA-ECMO, a large volume of blood is extracted from the femoral vein, circulated by a mechanical pump through an oxygenator and heat exchanger where hemoglobin is saturated with oxygen and carbon dioxide is removed, and subsequently returned into the femoral artery. Initial blood flow rates through the ECMO circuit are between 4 and 5 L/min. The ECMO circuit requires continuous systemic anticoagulation and is associated with significant risk of both hemolysis and bleeding.

ECMO patients in cardiogenic shock frequently develop acute kidney injury requiring renal replacement therapy. This may be delivered using one of three methods [73–75]. The first method is to establish a separate vascular dialysis access and run the CRRT circuit independently from the ECMO circuit. This may be challenging in the face of limited venous access and systemic anticoagulation during ECMO. The second method is through the in-line inclusion of a HD membrane after the membrane oxygenator. A limitation of this method is that it does not allow dialysis parameters to be controlled separately from ECMO parameters. The third method is to siphon off a portion of blood from the ECMO circuit, process it through a CRRT circuit, and subsequently return it back into the ECMO circuit to undergo membrane oxygenation. Local center and physician experience should dictate which method is used.

Intraoperative dialysis

Hemodialysis can be performed intraoperatively by connecting a HD circuit in series with a cardiopulmonary bypass pump (CBP). This allows for control of electrolytes (especially potassium) and fluids during surgery and may avoid the need for urgent HD immediately after surgery [76].

Dialysis after cardiac transplantation

Patients requiring dialysis after cardiac transplantation can be treated with either CRRT or conventional HD. Special attention should be paid to anticoagulation of the dialysis circuit and volume assessment, which can be difficult due to the presence of right ventricular strain or failure. Patients established on PD may continue with this modality after transplantation as long as volume status and ventilation parameters can be managed. Rapid PD exchanges (every 30–90 min) using low fill volumes may be required to manage volume overload.

Liver transplant candidates

Patients with liver disease who develop kidney dysfunction are at high risk of death [77]. As discussed in Chapter 43, their management is challenging and often requires support for concomitantly failing kidneys. Dialysis should only be offered to liver failure patients who have acute kidney injury that is expected to recover, and liver transplant candidates or patients undergoing evaluation for liver transplantation [78]. Dialysis is technically difficult due to systemic vasodilation and shunting leading to systemic hypotension, reduced plasma oncotic pressure due to malnutrition and decreased hepatic function, and coagulopathy and thrombocytopenia leading to increased risk of bleeding [79]. Reduced hepatic clearance of vasodilatory molecules leads to decreased effective circulating volume and increased salt and water retention. The resultant volume overload with ascites and peripheral edema is difficult to remove during HD in the face of systemic hypotension.

The decision to treat patients with intermittent dialysis or CRRT is based on hemodynamic stability and tolerability. Theoretic advantages of CRRT include more gradual correction of hyponatremia, and less fluctuation in intracranial pressure [78]. Regional citrate anticoagulation of the CRRT circuit should be avoided as it can lead to citrate toxicity in patients with impaired liver function [33]. Alternatives include high-flow CRRT without anticoagulation and regional heparin–protamine anticoagulation. CRRT is favored in patients with fulminant hepatic failure because in contrast to conventional HD it does not increase intracranial pressure [80].

While achieving reasonable small solute and uremic toxin removal, conventional HD modalities do not provide adequate clearance of larger and protein-bound toxins that accumulate in hepatic failure. In patients with severely impaired hepatic function, there may be a benefit to maximizing clearance of larger molecules that accumulate due to impaired hepatic clearance, and modalities that utilize hemofiltration may be of value, but these require further study [81,82].

PD may be the most feasible dialysis modality in some liver failure patients. Advantages over HD include no need for anticoagulation, more gradual and better tolerated fluid removal, continuous solute clearance, drainage of ascitic fluid, and caloric loading with dextrose-based PD solutions. Disadvantages include potential protein losses into the dialysate [83]. Historic concerns regarding higher rates of peritonitis and patient drop-out due to impaired manual dexterity to perform exchanges remain unproven.

A number of new techniques have been developed to provide support for patients with combined liver and kidney failure. These include membrane- and adsorbent-based systems to provide dialysis and detoxification (e.g. hemoperfusion, hemodiabsorption, plasmapheresis, and selective plasma filtration therapy), as well as

cell-based therapies to replicate the metabolic functions of the liver. None of these approaches has been rigorously tested and should be considered experimental. The MARS (molecular adsorbent recirculation system) removes albumin-bound toxins by albumin dialysis and CRRT [84]. Albumin dialysate is regenerated using a combination of charcoal adsorption and an anion exchange resin. MARS has been studied in a small number of patients with hepatorenal syndrome and was associated with improved survival and hemodynamics compared to hemodiafiltration [85]. Other studies have failed to show any benefit in patients with hepatorenal syndrome that is resistant to vasoconstrictor therapy [86]. A similar but less complex system, single pass albumin dialysis (SPAD) uses a conventional HD circuit, but infuses albumin as the dialysate. Albumin is passed once through the dialysis circuit and then discarded. Preliminary comparisons of SPAD to MARS suggest similar efficacy [87].

During liver transplantation, the inferior vena cava and portal vein are cross-clamped during hepatectomy, which reduces venous return and cardiac output by 40–50%. Following completion of the portal anastomosis, portal unclamping results in large amounts of desaturated blood, potassium, and lactic acid entering the main circulation, which induces hypotension, hyperkalemia, metabolic acidosis, and coagulopathy (the post-reperfusion syndrome) [88,89]. Commonly, large amounts of blood and plasma products may be required intraoperatively. In an effort to mitigate the impact of these factors, intraoperative dialysis (administered as CRRT) has been performed in patients undergoing liver transplantation, with successful intraoperative fluid, acid–base balance, and electrolyte management. This may apply in particular to patients with severe renal dysfunction or who require renal replacement preoperatively. There is considerable experience with intraoperative CRRT in selected centers; however, studies are needed to clarify the indications for intraoperative CRRT [89,90].

Summary

Dialysis provides limited but reliable and sustainable clearance of metabolic waste products, and in doing so controls the uremia, acute electrolyte disturbances, and volume overload associated with renal failure. Numerous forms and methods of dialysis exist. These have been adapted to various settings of renal failure, from the temporary support of a critically ill patient awaiting renal recovery, to the long-term support of a patient awaiting a kidney transplant or in whom transplantation is not an option, to a destination therapy. Given that renal failure often accompanies liver and heart failure, the use of dialysis is a critical supportive modality influencing not only kidney transplantation, but also heart and liver transplantation. The practice of dialysis, and the myriad methods of access required for dialysis, remains a critical part of any transplant clinician's armamentarium.

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CHAPTER 47

The Artificial Liver, in vivo Tissue Engineering, and Organ Printing

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Introduction

Liver transplantation has evolved since 1963 from an experimental procedure to the standard treatment for the life-threatening syndrome of liver failure. Success in liver transplantation is increasingly limited because of donor organ scarcity. To address the growing disparity between the numbers of suitable donor organs and patients waiting for transplantation, efforts have been made to optimize the allocation of organs, find alternatives to deceased donor liver donation, and develop extracorporeal methods to support or replace the failing organ. Clinicians and basic scientists earnestly look for alternative procedures and technical substitutes for conventional transplantation. To expand the donor pool, the procurement of marginal organs and donation after circulatory death have become more widely accepted. However, organ and patient survival is reduced with these alternatives. Liver support devices are used to improve outcome by providing an environment for regeneration of the patient's organ ("bridging to regeneration") or to support the patient until liver transplantation can be performed ("bridging to transplantation"). Liver cell transplantation (LCT) was developed as a therapeutic alternative to solid liver transplantation in the management of liver-based metabolic disorders and may be useful for the treatment of acute or chronic liver failure. Bioprinting, or the layer-by-layer additive biofabrication of tissue and organoid constructs, and the decellularization of donor organs to provide an acellular, natural three-dimensional biologic scaffold are emerging technologies that promise to transform tissue engineering. This chapter will summarize the latest developments in extracorporeal liver support, LCT, and highly innovative bioengineered alternatives to conventional orthotopic liver transplantation (OLT). It complements Chapters 38 and 43, which discuss the care of patients awaiting liver transplantation and the intensive care of patients in liver failure, respectively.

Extracorporeal liver support

Liver support devices are intended to improve the clinical course of the patient in liver failure by providing an environment for regeneration of the patient's organ ("bridging to regeneration") or to support the patient until liver transplantation can be performed ("bridging to transplantation"). Therefore, a liver support device should provide the three main functions of the liver: detoxification, synthesis, and regulation.

The most critical issue of the clinical syndrome in liver failure is understood to be the accumulation of toxins not cleared by the failing liver. Based on this hypothesis, the removal of lipophilic, albumin-bound substances, such as bilirubin, bile acids, metabolites of aromatic amino acids, medium-chain fatty acids, and cytokines, should be beneficial to the clinical course of a patient in liver failure. This theory has led to the development of artificial filtration and adsorption devices—so-called *artificial liver support* devices. The complex tasks of regulation and synthesis remain to be addressed by the use of liver cells—made available in *bioartificial liver support systems*.

Artificial liver support

Artificial liver support systems intend to support or replace the detoxification functions of the liver. Plasma exchange, widely performed in the 1980s, has recently re-emerged as a treatment option. A large multicenter trial by Larsen et al. demonstrated a significant improvement in transplant-free survival when patients with (hyper) acute liver failure (ALF) were treated with three sessions of high-volume (10 L) plasma exchange [1]. Conventional hemodialysis (HD) and continuous veno-venous hemodiafiltration (CVVHDF) have been shown to be effective in the removal of water-soluble toxins. To clear the blood of albumin-bound, hydrophobic substances, additional adsorber or acceptor substances are necessary to enhance mass exchange. Most artificial liver support devices are based on membrane separation associated with sorbents, including charcoal and anion or cation exchangers. Because albumin is the predominant carrier of toxins in the patient's blood, it may also serve as an appropriate acceptor substance. Based on this idea, albumin has been added to the dialysate of modified HD systems. Stange and Mitzner from Rostock, Germany introduced a detoxification system based on albumin dialysis, the *molecular adsorbent recirculation system* (MARS; Gambro, Stockholm, Sweden) [2]. An albumin solution, which is separated from the patient's blood by a high-flux HD filter, is circulated in a closed circuit (Figure 47.1A). The albumin acts as the acceptor substance for albumin-bound toxins and is partly regenerated by passing an anion exchanger and a charcoal adsorber in a closed circuit. *Single-pass albumin dialysis* (SPAD) is a simple method of albumin dialysis using standard renal replacement therapy machines without an additional perfusion pump system, in which the patient's blood flows through a circuit with a high-flux hollow-fiber hemodiafilter. The other side of this

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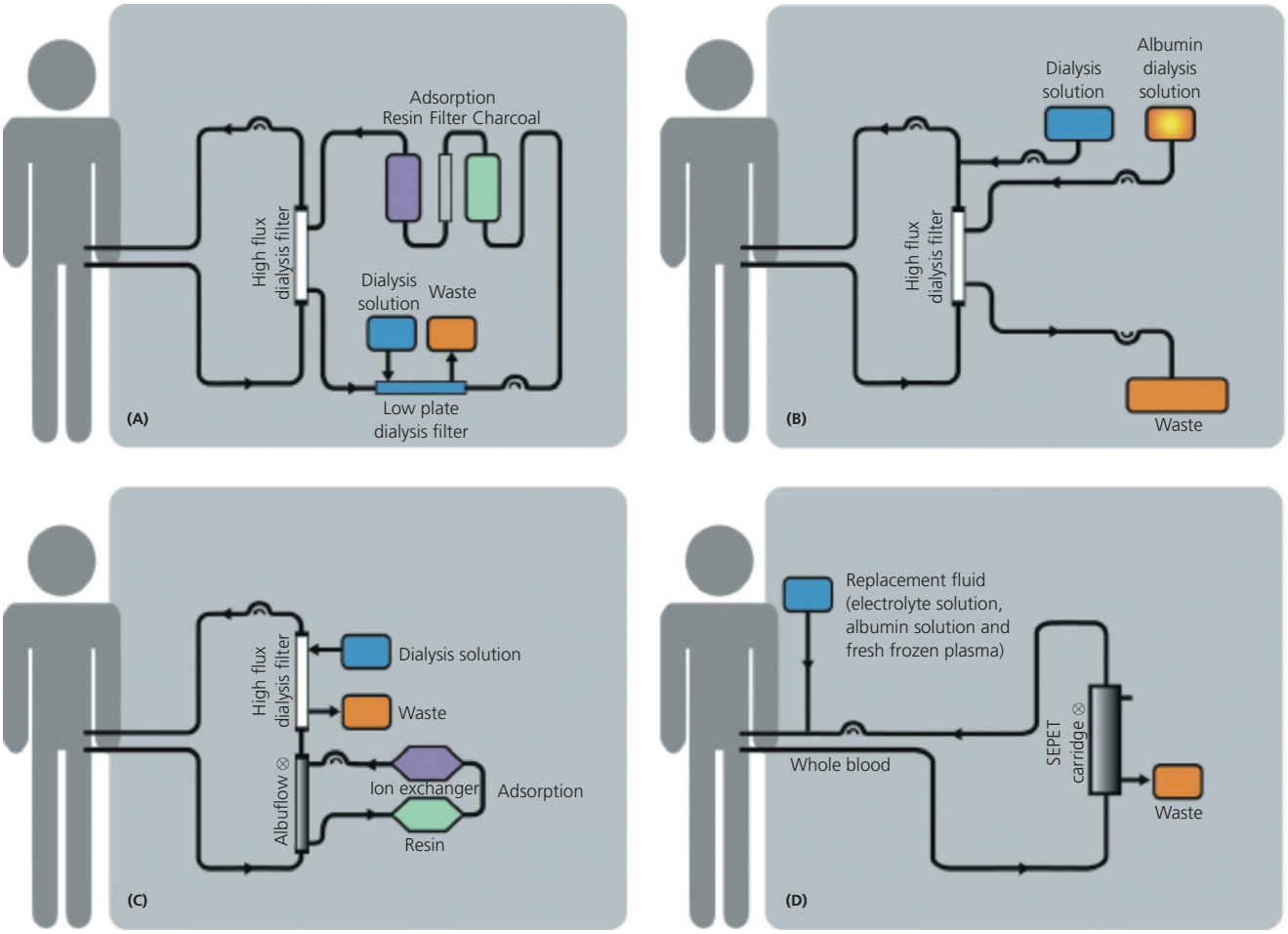

Figure 47.1. Artificial liver support systems. (a) The molecular adsorbent recirculation system (MARS); (b) single pass albumin dialysis (SPAD); fractionated plasma separation and adsorption (FPSA, Prometheus), and (d) the selective plasma filtration therapy (SEPET). (Reproduced from Jörres A, Ronco C, Kellum JA. (2009) Management of acute kidney problems: Extracorporeal liver support, with kind permission of Springer Science + Business Media.)

membrane is cleaned with an albumin solution in counter-directional flow, which is discarded after passing through the filter (Figure 47.1B) [3].

The use of membranes with larger pores, separating a certain fraction of plasma proteins, including the toxin-loaded albumin from the patient's blood, is a second approach. *Fractionated plasma separation and adsorption* (FPSA; Prometheus, Fresenius Medical Care, Bad Homburg, Germany) allows the patient's own albumin to be regenerated via passage through two adsorption matrices in a secondary circuit (Figure 47.1C). In contrast to SPAD and MARS, an albumin-permeable polysulfone membrane is used to filter the patient's albumin fraction into the secondary circuit, where direct purification from albumin-bound toxins by different adsorbers takes place. Afterward, conventional high-flux dialysis is performed inside the primary circuit [4].

Selective plasma-exchange therapy (SEPET; Arbios Systems, Allendale, NJ, USA), developed by Rozga et al., attempts to combine the advantages of SPAD with the advantages of Prometheus (Figure 47.1D). As in the Prometheus system, the fractionated plasma passes through an albumin-permeable, size-selective membrane. Substances with a molecular weight (MW) of <100 kDa are removed, whereas larger molecules (MW >100 kDa), including immunoglobulins, complement system proteins, most blood-clotting factors, and stimulators of hepatic regenerative response, are largely retained in the blood circulation. However, as in the SPAD system, there is no regeneration. Instead, the albumin fraction containing the patient's toxins is discarded and replaced by an electrolyte solution, albumin solution, and fresh frozen plasma [5].

The *BioLogic-DT* system developed by Ash et al. combines hemodiabsorption with push–pull, sorbent-based pheresis that is based on a cellulosic plate dialyzer with a suspension of powdered charcoal and cation exchangers as dialysate. The system was redesigned and renamed the *liver dialysis device* (HemoCleanse, Lafayette, IN, USA), but is no longer marketed [6].

Bioartificial liver support

Artificial liver support devices only support the failing detoxification function of the diseased liver. As the liver does more than detoxify, the clinical syndrome of the failing liver will most likely also be determined by the failing regulatory (e.g. acid–base status, amino acids) and, even more importantly, synthetic functions (e.g. albumin, glucose, lipids, coagulation factors, even unknown substances). These tasks have to be addressed with the use of liver cells.

Extracorporeal liver perfusion, initially performed in the 1960s, requires complex logistics because the organ has to be freshly explanted prior to the treatment session and then connected to the patient's blood circulation under sterile conditions. To address these problems, several different liver support bioreactors were developed, which enabled the cultivation of isolated liver cells in a more suitable mode for integration into clinical perfusion systems and for prolonged cell culture times.

The *HepatAssist* system developed by Demetriou et al. is based on 5–7 $\times$ 10^9 cryopreserved porcine hepatocytes within the intercapillary space of a device resembling a modified dialysis cartridge. The patient's plasma ultrafiltrate is passed through the cartridges via an activated charcoal adsorber and an oxygenator [7].

The *extracorporeal liver assist device* (ELAD), developed by Sussman et al., uses approximately 200 g of cells of a cell line originating from human hepatoblastoma (C3A, derived from HepG2) in a similar setting. The cells are separated from the patient's plasma by hollow-fiber membranes, and an integrated charcoal adsorber and a membrane oxygenator support detoxification and maintain the oxygen supply of the cells [8].

A bioreactor introduced by Gerlach et al. in the 1980s was later integrated into the *modular extracorporeal liver support* (MELS) system. The bioreactor consists of two hydrophilic polyethersulfone membrane bundles and a hydrophobic multilayer hollow-fiber bundle for oxygenation. The bioreactor is charged with primary porcine [9] or human liver cells [10]. MELS combines this bioreactor with CVVHDF and SPAD for the removal of water-soluble and albumin-bound toxins, respectively.

The *Academisch Medisch Centrum bioartificial liver* (AMC-BAL), developed by Chamuleau et al., differs from other clinically applied systems in one major aspect: the patient's plasma is in direct contact with the cells rather than separated from the extracorporally applied liver cells by a membrane. Within a cylindrical housing, a nonwoven polyester matrix offers a large surface area to which the inoculated primary porcine cells can attach and form aggregates between the fibers [11].

Evaluation

The design of clinical studies with respect to liver failure is difficult. ALF and acute on chronic liver failure (AoCLF) are very heterogeneous diseases with respect to the capacity for parenchymal regeneration and prognosis. In ALF, liver support treatment will usually be terminated within 24–48 h of the patient being listed for high urgency liver transplantation due to organ availability. Additionally, sensible clinical endpoints are lacking [12].

There is a lack of appropriate randomized controlled and adequately powered studies because most clinical studies are nonblinded and uncontrolled. There are significantly more clinical data available for artificial liver support systems than for bioartificial concepts.

A Cochrane systematic review of extracorporeal liver support systems for clinical use in ALF and AoCLF, which reviewed articles published before September 2002, concluded that these systems had no significant effect on mortality, but there appeared to be a survival advantage in AoCLF [13]. More recently, Stutchfield et al. published a meta-analysis with more stringent criteria for selection of randomized controlled trials (RCTs) concerning the effect of extracorporeal liver support on survival in both ALF and AoCLF. A systematic review of the wider effects of extracorporeal liver support on clinical and biochemical parameters was also undertaken [14]. Stutchfield et al. included 74 clinical trials (17 RCTs, five case–controlled studies, and 52 cohort studies). Eight RCTs investigating the effects of extracorporeal liver support in ALF (three studies, 198 participants) and AoCLF (five studies, 157 participants) were suitable for inclusion. In six studies, artificial liver support devices (five for MARS, one for Biologic DT) were used; two studies evaluated bioartificial systems (ELAD and HepatAssist). None of the included trials reported a significant difference in adverse events between the group treated with extracorporeal liver support devices and the group treated with standard medical therapy. No specific biocompatibility issues were raised by any of the trials. Interestingly, overall extracorporeal liver support therapy significantly improved survival in ALF (risk ratio 0.70; $P = 0.05$). No significant survival benefit was demonstrated in AoCLF (risk ratio 0.87; $P = 0.37$).

The results of the HELIOS trial, a randomized controlled European multicenter trial of Prometheus/FPSA therapy in a total of 145 patients with AoCLF, were not included in the above meta-analysis. In the HELIOS trial, there was no statistically significant difference in the overall survival between the study groups. However, a significant survival benefit was observed with FPSA therapy in two predefined subgroups, namely patients with hepatorenal syndrome type I and those with a Model for End-stage Liver Disease (MELD) score of >30 [4].

Compared with the large clinical effects of liver grafting after transplantation, these results appear limited and ask a number of questions. Are the concepts being evaluated inadequately? Are the concepts right but their scale too small and performances too low? Most studies have evaluated artificial liver support systems—are biological components mandatory? Cell-based, bioartificial liver support systems address all of the complex tasks of detoxification, regulation, and synthesis, but have some biological and physical limitations in common: most of these systems use hollow-fiber membranes, which limit the mass exchange between the patient's blood and extracorporeal liver cells. For technical reasons, the perfusion of bioreactors is limited to 100–300 mL/min of plasma, which is rather low compared with the blood flow rate of the natural human liver in situ of approximately 1500 mL/min. Therefore, the maximum clearance rate for any substance in the bioreactor, which involves the plasma volume passing through the bioreactor, must be expected to be much lower than in the human liver. Another characteristic of current bioreactors is the absence of functional biliary excretion into a dedicated compartment. Liver cells in three-dimensional culture conditions can form functional bile canaliculi, but it is unknown to what extent biliary compounds still accumulate intracellularly and whether these will shorten the vitality of the cells [15]. However, this concern can be addressed by the addition of certain detoxification components, such as SPAD in the MELS concept or the charcoal adsorber in the ELAD concept.

Finally, liver cell mass and source appear to be limited. Usually, bioreactors in liver support systems are charged with 100–200 g (some bioreactors even contain 600 g) of hepatic parenchymal cells, which seems to be insufficient. In living donor liver transplantation (LD-LTx), a graft size of 40% or less of the recipient's ideal liver mass (approx. 1500 g) is likely to be insufficient to meet the recipient's metabolic needs, and the recipient is at risk of suffering graft failure [16]. An even higher number of cells must be provided when they are applied within an extracorporeal circuit—especially with respect to the limitations of plasma flow and mass exchange [17].

The dominant limitation of all bioartificial support systems is still the cell source. Widely available cells, unfortunately, are associated with the metabolic incompatibility and risk of xenozoonosis

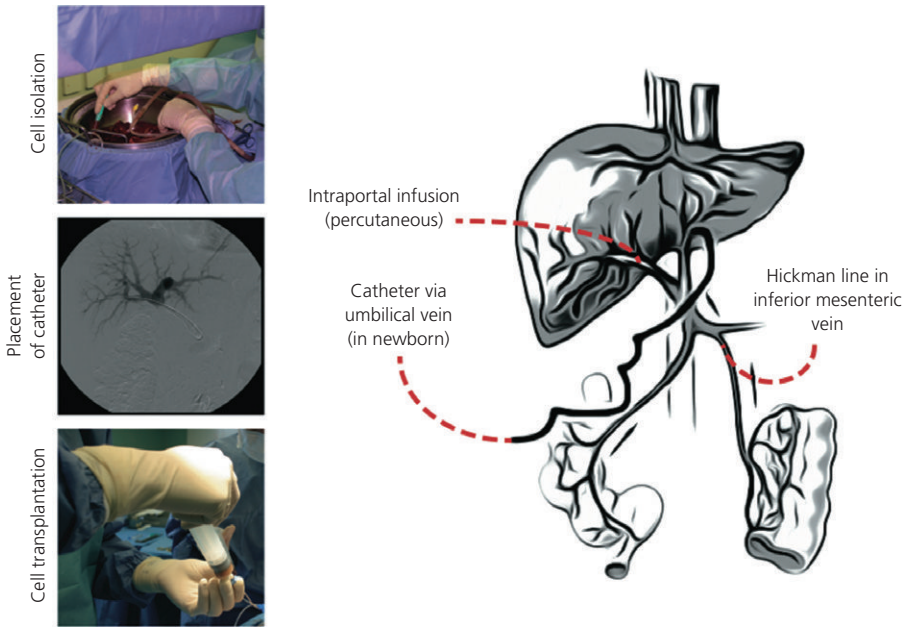

Figure 47.2. Liver cell transplantation. Hepatocyte transplantation is based on the administration of isolated liver cells in suspension. Liver cells are isolated from donor livers using enzymatic perfusion technique. Hepatocytes are infused into the liver of the recipient through the portal circulation. Vascular access to the portal system is performed by percutaneous or transjugular hepatic puncture, umbilical vein catheterization, or insertion of a Hickman line in the inferior mesenteric vein.

(porcine cells) or with the risk of metastases and a lower liver-specific metabolic performance (tumor cell lines). The use of primary human liver cells is increasingly favored for clinical application. Discarded donor organs might serve as a source for the isolation of primary human hepatocytes. However, in addition to their limited availability, primary human liver cells originating from these organs are impaired with respect to their metabolism and regenerative capacity because of histological alteration, preservation, and isolation processes. Until we can establish a reliable, safe, highly metabolically active and easily expandable human cell source, a successful breakthrough in bioartificial liver support systems appears to be unlikely.

Liver cell transplantation

LCT was developed as a therapeutic alternative for solid liver transplantation in the management of liver-based metabolic disorders and acute or chronic liver failure [18]. LCT is based on the administration of isolated liver cells in suspension (Figure 47.2). Liver cells are isolated from donor livers or liver lobes rejected or unused for whole organ transplantation [19]. Cell isolation is performed using collagenase perfusion techniques, followed by centrifugation for cell purification. Hepatocytes are transplanted immediately following isolation or after cryopreservation and thawing. Isolated hepatocytes are infused into the liver of the recipient through the portal circulation or transplanted to an ectopic implantation site, e.g. the spleen or peritoneum. Intraportal infusion is currently the main cell delivery route, with the liver serving as a biological matrix for the transplanted hepatocytes. Vascular access to the portal system is gained by percutaneous or transjugular hepatic puncture, inferior mesenteric vein catheterization, or, in infants, revascularization of the umbilical vein. Hepatocytes that accumulate in the portal periphery, which causes transient local hypertension and ischemia-reperfusion injury, adhere to the activated endothelium, translocate through the sinusoidal fenestration, and engraft in the liver plates. Under the appropriate conditions, e.g. in the presence of a regenerative stimulus or survival advantage, donor hepatocytes proliferate and replace the recipient liver. The infusion of up to 10^8 cells/kg of body weight can be safely performed under continuous monitoring of the portal pressure and lead to a replacement of 5% of the recipient liver cell mass [20].

From a conceptual point of view, cell transplantation offers various advantages compared with whole organ transplantation:

1. Liver cells can be cryopreserved and stored in cell banks, which enables timely and scheduled applications.
2. Cell infusions are less invasive than whole-organ transplantation, offering the chance to treat critically ill or very young patients who are not suitable for whole organ transplantation.
3. The native liver is left in place, allowing its potential recovery and meaning that graft failure need not be life threatening.
4. Cell infusions can be dosed according to the individual needs of the recipient, and the cells can be genetically modified or labeled with a contrast agent prior to their transplantation.

Based on preclinical studies in animal models of ALF or metabolic disorders, which have demonstrated improved survival or improvements in biochemical abnormalities, clinical LCT has been developed over the last 10 years, and current reviews list over 80 case reports of clinical human hepatocyte transplantation from approximately 13 centers [21]. This section will discuss the current status of clinical LCT for the treatment of metabolic liver disorders, ALF, and chronic liver diseases, and highlight the conceptual and practical limitations.

Treatment of metabolic liver diseases

Among all indications, LCT is currently most effective in the treatment of inborn metabolic liver disorders. In cases where a specific

gene is missing, donor hepatocytes expressing the gene are transplanted to replace the missing function without the need to replace the whole organ. The transplanted cells integrate into the normal host liver and improve specific functions of the failing liver, without the need to replace all hepatic functions. Approximately 35 children and adults who received LCT for liver-based metabolic liver disease have been reported in the literature, with the majority of studies performed within the last few years [21–24]. In all cases, LCT was performed through intraportal infusion, by single or repeated infusion, using both fresh and cryopreserved cells. As a first attempt to treat metabolic liver disorders with hepatocytes, Grossmann et al. described LCT for the treatment of familial hypercholersterolemia in 1994 [25]. They isolated autologous hepatocytes from liver tissue resected from five adult patients, transduced the cells in vitro with an LDL receptor expressing retroviral vector, and infused these intraportally 3 days after resection, taking advantage of the regeneration stimulus after partial hepatectomy. The authors observed an approximately 20% reduction in LDL cholesterol levels, but transgene expression declined early after transplantation. Today, patients with urea cycle defects are the most common group of patients to undergo LCT [21]. Among them, 10 children (aged 1 day to 5 years) with ornithine transcarbamylase (OTC) deficiency showed decreased ammonia levels after LCT and could be successfully bridged to OLT in most cases. Two patients with citrullinemia and one patient with arginine succinylase deficiency were metabolically stabilized. Eight cases of Crigler–Najjar syndrome type 1 treated with hepatocytes have been reported. The patients showed a 30–60% decrease in bilirubin levels after the intraportal infusion of 5% of their calculated liver mass. However, OLT was necessary after a few months in most of these cases. Other conditions under which individual patients have been successfully treated with LCT are glycogen storage disease, Refsum syndrome, and Factor VII deficiency.

In conclusion, the published cases indicate that LCT is safe and feasible for at least the temporal treatment of metabolic liver disorders. Major medical complications associated with LCT for the treatment of metabolic disorders have not been described in the literature. However, the correction of metabolic deficiency has been temporary in most cases, indicating declining activity of the transplanted cells due to insufficient engraftment [26] or graft rejection [27]. Moreover, the lack of controlled trials makes the interpretation of these findings difficult.

Treatment of acute liver failure

Based on small animal studies with D-galactosamine poisoning, in which splenic, portal, and peritoneal hepatocyte transplantation was shown to reverse the disease, LCT has been investigated in humans with ALF as a bridge to recovery of the native liver or to OLT [28,29]. Fisher et al. reviewed 37 patients with ALF who were allotransplanted with human hepatocytes [30]. Among them, 10 children were treated with intraportal hepatocyte infusion. Three children were successfully bridged to OLT and two children recovered without OLT. Of the 27 adults transplanted with hepatocytes, four could be bridged to OLT and five showed full recovery without organ replacement. Of the 13 adult patients who received cells into the spleen, three were successfully bridged to OLT and one recovered fully. Cell infusion-associated complications, such as hepatocyte lung emboli, splenic and mesenteric vein thrombosis, or bleeding due to interventional hepatic access, were observed in the critically ill ALF patients [31,32]. LCT in patients with ALF resulted in the reduction of ammonia and bilirubin levels and improvements in hepatic encephalophathy in some patients, but the reported morbidity and mortality were relatively high; this may be because an insufficient mass of cells to rescue the patients was transplanted by intraportal or intrasplenic infusions. Because both the treatment protocols (e.g. number of cells or implantation site) and the patients transplanted with hepatocytes vary among the published reports and because RCTs are not available, the efficiency of LCT for the treatment of ALF cannot be truly assessed.

Treatment of chronic liver disease

In chronic liver disease, LCT has been performed to replace the function of the diseased liver by hepatocyte transplantation to ectopic implantation sites or to repopulate the diseased liver with transplanted cells. Published data are available on 20 patients with chronic liver disease and transplanted hepatocytes [21]. Small animal studies have shown that the deranged liver architecture prevents successful hepatocyte engraftment and intrahepatic shunts caused a systemic distribution of intraportal transplanted cells; thus the spleen was the implantation site in the majority of the published clinical cases [33].

Mito et al. were the first to treat cirrhotic patients with hepatocytes [34]. They resected the left lateral liver segment of cirrhotic hepatitis C patients and isolated and retransplanted the autologous hepatocytes into the spleen of the patients. One patient fully recovered and hepatocytes could be detected by radioisotope imaging 11 months after LCT; however, no clinical benefit of splenic hepatocyte injection could be observed in the other nine patients. In a further 10 cases, allogeneic hepatocytes isolated from non-cirrhotic liver donors were used for transplantation.

While some clinical improvements have been observed, LCT appears to be least successful in those patients with chronic liver disease, which may be due to the low number of cells infused in the majority of the published cases. Cirrhotic liver disease patients may benefit from alternative extrahepatic sites for liver cell implantation, but if the native cirrhotic liver is left in place, the management of co-existing portal hypertension and the risk of development of hepatocellular carcinoma in the native liver will not be resolved [35].

Conceptual and practical limitations

Although the safety and feasibility of LCT has been well demonstrated over the last decade, especially for the treatment of metabolic liver disorders, there are still many questions and barriers regarding the successful treatment of liver disease by hepatocyte transplantation.

Cell source for hepatocyte transplantation

Along with the critical shortage of donor organs for whole organ transplantation, donor organs for hepatocyte isolation are scarce. To provide a sufficient cell supply for LCT, alternative sources, such as marginal organs or livers from non-heart beating donors, must be established for clinical hepatocyte isolation [36]. Another valuable alternative for hepatocyte support could arise from stem cells or induced pluripotent cells [37,38]. Despite the encouraging results regarding the generation of hepatocyte-like cells from such precursor cells, there are still safety concerns, particularly concerning the oncogenic potential of stem cells.

Engraftment and survival of transplanted cells

Another critical aspect in LCT is the insufficient engraftment and long-term survival of transplanted hepatocytes. It is likely that

many cells do not survive long enough to enter the liver structure, and animal studies have shown that up to 80% of the transplanted cell mass is lost within 24–48 h after cell infusion [39]. In-vitro studies have indicated that, as in islet transplantation, isolated hepatocytes induce an immediate blood-mediated inflammatory reaction, which could be responsible for significant cell loss in vivo [40]. Also, due to the innate immune system, hepatocytes face reactions mediated by the adaptive immune system and non-specific mechanisms, such as apoptosis or specific cell-mediated or humoral rejection, even when immunosuppression is used after OLT [41]. Hepatocyte engraftment is also hampered by the need for the cells to translocate through the sinusoidal fenestration to integrate into the liver parenchyma. Histopathological analyses in animal models and explanted livers of humans transplanted with liver cells commonly reveal cells entrapped in distant portal branches [26]. It is likely that these cells are not metabolically active in the long term. Two strategies are currently being discussed to enhance hepatocyte engraftment in clinical LCT: liver irradiation and partial embolization of the liver. Large animal studies have showed that hepatic irradiation could arrest native hepatocyte proliferation, disrupt the sinusoidal endothelium, and inhibit the phagocytic function of Kupffer cells [42]. Partially reversible embolization of the portal vein could stimulate a regenerative response in the liver, which could also be of benefit to the transplanted cells [43]. It is possible that a combination of reversal portal embolization and hepatic irradiation may produce the best effect.

Tracking of transplanted liver cells

Unfortunately, little is known about the processes during and following LCT in humans because investigations of donor liver cell distribution and engraftment in humans are limited to the examination of tissue samples. A clinically applicable method for the visual monitoring of transplanted cells is currently not available. Cell labeling with radioisotopes was used in a patient to demonstrate hepatocyte biodistribution after transplantation [44]. However, radioisotope imaging is limited by the short half-life of the radioisotope and the lack of anatomical resolution. Magnetic resonance imaging (MRI) is currently the main clinically relevant technology for real-time and non-invasive monitoring of cell transplantation. MRI offers high spatial resolution in parenchymal organs, and dynamic imaging is possible. Cells must be labeled with intracellular contrast agents to be detectable by MRI. In-vitro studies have demonstrated the feasibility of labeling human hepatocytes with MRI contrast agents, and animal models have demonstrated the feasibility of tracking transplanted liver cells with MRI under clinical conditions [45,46]. However, further work is necessary to translate this approach to clinical use in LCT.

Implantation sites for liver cells

Ectopic implantation sites for hepatocytes are necessary, especially for the treatment of chronic liver disease [21]. Clinical studies have focused on the spleen, but the long-term survival of hepatocytes has not been conclusively demonstrated, and the hepatization of the spleen remains a mystery. The required mass of cells necessary for support during ALF cannot be safely transplanted into the spleen. Moreover, the exposure of hepatocytes to the immunological environment of the spleen is undesirable. The encapsulation of hepatocytes is a promising approach to immunoprotect them and enable ectopic implantation at the same time. Mei et al. showed that hepatocytes encapsulated in alginate beads could be implanted into the peritoneal cavity in a sufficient mass (50% of the native liver weight) to rescue animals with fulminant liver failure [47]. Further work is necessary to identify and evaluate clinically workable extrahepatic sites or tissue engineering-based constructs enabling in-vivo extrahepatic liver cell support [35].

Bioengineered solutions

Given the scarcity of donor organs and the unresolved issues of extrahepatic liver support and LCT, other solutions are necessary. Bioengineering approaches, which combine in-vitro and in-vivo tissue engineering, could reach the ultimate goal of total bioartificial liver grafting. This section will focus on two of the currently most exciting concepts regarding the bioengineered liver: organ printing and organ recellularization.

Organ printing: biofabrication of human organ constructs

Organ printing is a technology designed for the complete de-novo biofabrication of three-dimensional vascularized functional human organs. Organ printing is based on the layer-by-layer robotic biofabrication of functional three-dimensional tissue [48]. Cells of different origin are placed next to each other in a physiological manner to mimic the cellular distribution in the intact template organ. Robotic bioprinters, which are based on the concept of conventional inkjet printers, and bioreactors are the essential components of an organ biofabrication line (Figure 47.3A) [49].

Compared with other tissue engineering-based approaches designed for the liver, such as the use of scaffolds for ectopic hepatocyte seeding or the transplantation of liver cells, organ printing has certain competitive advantages: it allows for a precise simultaneous three-dimensional positioning of several cell types, ideally as found in nature; it enables the creation of tissue with a high level of cell density; it can solve the problem of vascularization in thick tissue constructs; and it can be automated and offers a pathway for the scalable production of engineered organs [50].

The fabrication of vessels for blood supply and bile removal is usually the limiting issue in tissue engineering-based approaches for the construction of functional liver tissue. Organ printing is capable of addressing these issues, e.g. using the technique of layer-by-layer deposition [51]. As described by Visconti et al., a sheet of biocompatible hydrogel is printed and tissue spheroids are embedded into the hydrogel [52]. Tissue spheroids are prepared from cell suspensions and can be homogeneous or heterogeneous, containing a single cell type or a mixture of several cell types (e.g. hepatocytes combined with endothelial cells or cholangiocytes). The deposition of layers of hydrogel and tissue spheroids is pursued according to the predefined blueprint of the desired three-dimensional structure, e.g. a tubular construct. The fusion of the tissue spheroids and the removal of the hydrogel leads to the generation of a hollow tube after a few days. The living cell-based construct that results from the printing and postprinting fusion process is then placed in an incubator, where it attains its final three-dimensional structure and appropriate biomechanical properties. Proof-of-concept studies have already been performed to show that thick tissue sheets and straight and branched tubes can be "built" [53].

Organ printing is a highly innovative and ambitious technology. To date, only a few successful applications have been published. For example, bone grafts have been generated using a bioprinter-based approach. Fedorovich et al. applied organ printing technology

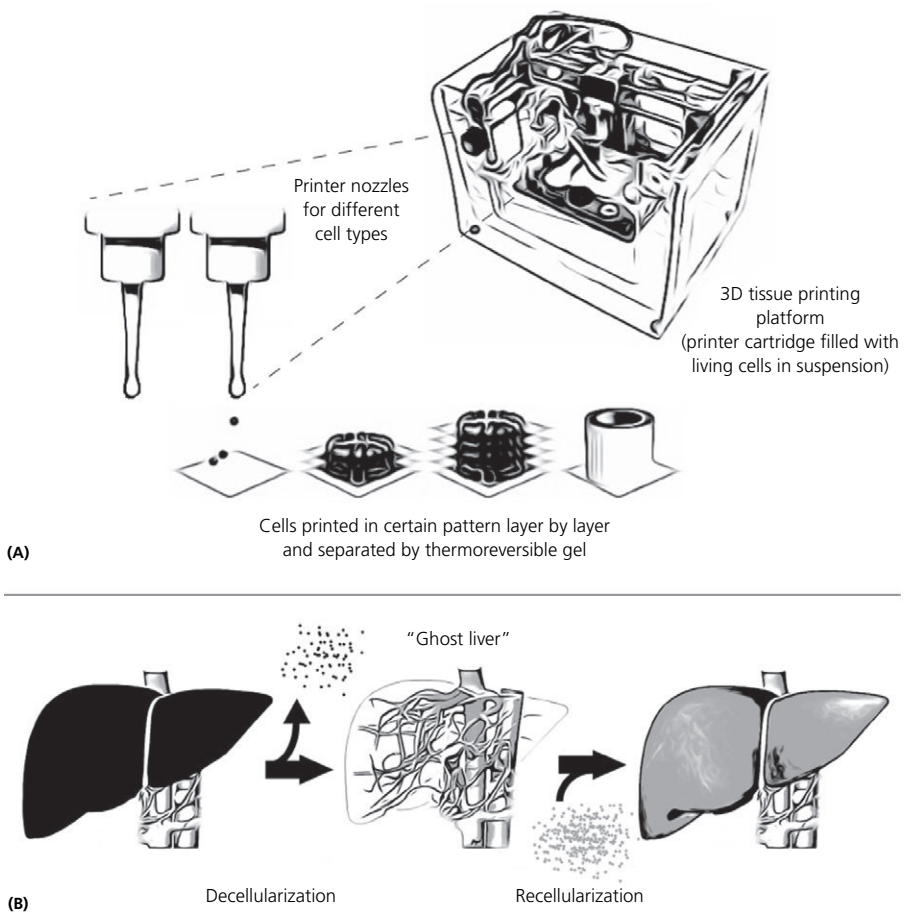

Figure 47.3. Bioengineered solutions for the liver. (a) Organ printing is based on three-dimensional printing of cells or spheroids. Cells are printed in a certain pattern layer by layer and separated by thermoreversible gel to generate tubular structures. A living cell-based construct results from cell printing and postprinting fusion of the cells. (Adapted from Mironov et al. [51].). (b) Decellularization and recellularization of the liver. Livers are recellularized by perfusion with a detergent that lyses cells and solubilizes cytoplasmic components. The decellularized "ghost liver" can then be sequentially recellularized with hepatocytes and endothelial cells or progenitor cells to generate functional liver tissue.

to fabricate porous constructs containing endothelial progenitors and multipotent stromal cells [54]. They showed that these grafts retained heterogeneous cell organization after subcutaneous implantation in immunodeficient mice. Cell differentiation led to tissue formation at the site of the deposited progenitor cells. While perfused blood vessels were formed in the endothelial progenitor cell-laden part of the constructs, bone formation was taking place in the multipotent stromal cell-laden part of the printed grafts. In another example of the successful application of bioprinting technology, Xu et al. showed that automated cell printing systems could be used to print a three-dimensional co-culture model of cancer cells and normal fibroblasts. Bioprinting allowed for high spatial control over the printing of co-cultured cells, which is helpful for investigating specific cell–cell interactions [55]. Moreover, bioprinting was applied for embryoid body formation, providing an effective tool to generate controllable uniform-sized embryonic bodies [56].

Studies on organ printing for the liver have not yet been published. However, it is very likely that this technology will be used to fabricate blood vessels and bile ducts to place hepatocytes and non-parenchymal liver cells in the spaces between these structures. Based on the promising results reported for other cells and tissues and the ongoing development of this young technology, it is conceivable that organ printing may ultimately be successful in the biofabrication of human liver constructs suitable for surgical transplantation.

Recellularization of decellularized organs

Recellularization is another young concept that has rapidly evolved in recent years. This concept is based on the idea of removing all cellular and antigen-presenting components from a donor liver and reseeding this *ghost organ* with cells from a different origin (Figure 47.3B). Decellularization ideally preserves the macrovascular skeleton of the entire organ, including the functional aspects of the native microvasculature, the extracellular matrix, and the three-dimensional architecture, which makes decellularized organs in a biomatrix ideally suited for reseeding with cells [57]. Because organs from non-heart beating donors (and foreseeably organs of xenogeneic origin) can be used for decellularization, this concept is promising in overcoming the issues of organ scarcity [58]. Recellularization could be performed with the patient′s own cells, either using stem cells or induced pluripotent stem cell technology, which might prevent the need for life-long immunosuppression following transplantation.

Following the successful demonstration of decellularized matrices in the tissue engineering of other tissues, including the bladder, arteries, and the trachea, a few groups have started to develop techniques for the de- and re-cellularization of the liver. Uygun et al. were the first to report on the fabrication of a transplantable recellularized liver [59]. They performed whole organ decellularization of rat livers by perfusing them with SDS, an anionic detergent that lyses cells and solubilizes cytoplasmic components. After complete decellularization, the authors restored the parenchyma of the liver with repeated hepatocyte infusions via the portal vein and additionally perfused the liver with microvascular endothelial cells as the non-parenchymal component. As a proof of principle, they connected the bioengineered liver graft to the renal artery and renal vein of a nephrectomized rat and perfused it for 8 h in vivo. Histological analysis of the perfused organ showed that hepatocytes had engrafted around the larger vessels and repopulated the surrounding parenchyma, suggesting that the cells migrated beyond the matrix barrier to reach the decellularized sinusoidal spaces. The endothelial cells were capable of lining the vasculature encircled by hepatocytes. Following implantation, the hepatocytes retained both normal morphology and hepatic function.

Based on these encouraging results, further studies were performed to optimize the decellularization and recellularization procedures for the liver. Baptista et al. showed that livers from mice, rats, and adult pigs could be successfully recellularized with human endothelial cells and human fetal liver cells [60]. Soto-Gutierrez et al. developed a minimally disruptive whole organ decellularization protocol for the liver [61]. Using this protocol, they were able to preserve the native microvascular network, the bile drainage system, and up to 50% of the growth factor content of the liver. Bao et al. modified the decellularized graft with heparin depositions to avoid intravascular thrombosis [62]. Using this internal surface anticoagulant modification, they were able to increase the number of reseeded cells by up to 10% of whole liver equivalents. They implanted the recellularized graft in series with the residual native liver, perfused it with portal vein blood, and demonstrated improved liver function and the prolonged survival of 90% of hepatectomized rats over a period of 72 h. Finally, Barakat et al. engineered a humanized porcine liver by transplanting human fetal hepatocytes co-cultured with fetal stellate cells into the decellularized matrix of a porcine liver [63].

The fascinating results of these pilot studies demonstrate the feasibility of creating recellularized liver grafts. However, tremendous efforts are still needed before recellularized liver grafts are suitable for clinical use. The decellularization protocols need to be further optimized and standardized to enable the efficient removal of all cellular components while conserving the microarchitecture and extracellular matrix of the liver. The number of cells that can be reseeded must be increased, and the reseeded cells need to be stimulated to repopulate the decellularized matrix. Moreover, further investigation is needed to determine whether hepatocytes in recellularized grafts retain their physiological properties and are capable of building functional tissue. Finally, animal studies need to be performed to show that a recellularized liver performs similarly to a native liver graft. Despite these challenges, it is already evident that this technology, if ultimately successful, will have a broad impact on the clinical setting of liver transplantation and has the potential to overcome the organ scarcity facing liver transplantation.

Summary

Liver transplantation remains limited by organ supply and an incomplete ability to prognosticate a patient's ability to recover from ALF. Liver support devices have been used in various forms for several decades, and conceptually remain exceedingly attractive as a means of bridging patients to recovery or to transplantation. However, their efficacy remains to be proven and considerable optimization is needed before these devices reach their conceptual potential. Suitable artificial processes capable of replacing the myriad processes of the liver and a suitable source of hepatocyte biomass for bioartificial hybrid devices remain the limiting factors. LCT has been developed as a therapeutic alternative for solid liver transplantation, particularly for the treatment of liver-based metabolic disorders, and may be useful for the treatment of acute or chronic liver failure. Given the clear need for alternatives to donor transplantation and the shortfalls of other existing technology, numerous novel approaches are being tested, including bioprinting and the use of decellularized organs that are subsequently recellularized to provide a new source of hepatocyte mass.

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CHAPTER 48

Ventricular Assist Devices

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Introduction

The use of ventricular assist devices (VADs) to treat patients with advanced cardiac failure and as a bridge to cardiac transplantation has become an accepted and routine practice in clinical cardiovascular medicine. Their prevalence and general incorporation into the care of patients being considered for heart transplantation now necessitates their consideration in tandem with heart transplantation, and indeed, VAD services are frequently combined with transplant services as part of a multidisciplinary care team for patients with cardiac failure. This chapter will cover the origins, development, and modern use of VADs, and serves as a companion chapter to Chapters 30 and 39, which consider the preoperative assessment and waitlist management of patients awaiting heart transplantation, respectively.

History

Research into temporary mechanical support of the circulation was initiated by Gibbon, who was the first to successfully use the cardiopulmonary bypass machine in a clinical setting [1,2]. In addition, Dodrill demonstrated a successful mitral valve repair utilizing a left heart bypass. This technique, however, was envisioned as an approach specific to mitral valve surgery, not as a means for prolonged mechanical left ventricular (LV) support [3]. Observations of these first patients to receive cardiopulmonary support demonstrated that sometimes prolonged support after surgery could allow the heart to recuperate over time. Once function had been restored, these patients could be successfully weaned off the heart–lung machine. This finding was an important impetus to the development of mechanical circulatory support. However, oxygenators in use at the time caused blood damage, which limited the success of cardiopulmonary bypass for ventricular recovery. Therefore, cardiac recovery by use of VADs in this era could be achieved only if the patient's own lungs could be used as an oxygenator and only the circulation required support.

In 1961, Dennis et al. [4] employed an external roller pump to directly assist a failed LV. Following extensive research and detailed anatomic dissections, they were able to place a cannula transseptally into the left atrium via the right jugular vein. Blood was then returned by the roller pump via the common femoral artery. The trans-septal inflow cannula was placed blindly, and this, remarkably, was successful in supporting seven of eight patients with heart failure following extensive myocardial infarction [4]. (This approach, first applied by Spencer and Dennis, formed the basis of the now widely used Tandem Heart.) Likewise, in 1965 Spencer et al. also successfully used roller pumps for temporary circulatory support, allowing ventricular recovery [5].

DeBakey and Spencer simultaneously reported the successful recovery of a patient after prolonged support with partial cardiopulmonary bypass [5,6]. This work evolved in an era of very high mortality following open heart surgery. The initial goal of this research, therefore, was to develop a pump that would temporarily support the heart until sufficient myocardial function had returned to allow pump removal. In 1963, the Baylor group led by Dr. DeBakey reported the first implantation of an intracorporeal, pulsatile left ventricular assist device (LVAD) [7]. Circulation was well maintained and the myocardium showed improvement through the duration of support; however, the patient failed to recover from a neurologic injury that occurred during this procedure. In August 1966, Dr. DeBakey implanted another LVAD, now placed extracorporeally, in a 37-year-old woman who could not be weaned from bypass after a double valve replacement [6]. After 10 days, the pump was successfully removed, and she survived for 6 years with good cardiac function, only to eventually die tragically in an automobile accident.

Work on short-term LVADs continued through the 1960s, but it was soon overshadowed by the beginning of cardiac transplantation: the first successful heart transplant was performed by Barnard in 1967 [8]. However, in spite of the short-term surgical and medical success of heart transplants, the disappointing long-term outcomes led to a general abatement of this program and stimulated a renewed interest in both short- and long-term mechanical circulatory assist devices. In 1972, under the auspices of the National Institutes of Health (NIH), a committee of senior academic clinicians met in Washington and recommended further development of an implantable, long-term LVAD. As transplantation was not considered an option at that time, these devices were to be developed with long-term cardiac support as a goal ("destination therapy"). However, the initial implantable LVADs were once again employed only as a short-term bridge to recovery for patients who could not be weaned from cardiopulmonary bypass following open heart surgery. At the time, the Texas Heart Institute had the largest clinical experience

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Table 48.1. Two-staged cardiac replacement

Patient	Diagnosis	Year	Procedure	Duration
47-year-old man	CAOD, LVA	1969	TAH	64 h
		1969	Allograft	32 h
21-year-old man	SBE, MR, AR, stone heart	1978	LVAD	5 days
		1978	allograft	14 days
36-year-old man	CAOD	1981	TAH	54 h
		1981	allograft	7 days

CAOD, coronary artery occlusive disease; LVA, left ventricular aneurysm; SBE, subacute bacterial endocarditis; MR, mitral regurgitation; AR, aortic regurgitation; TAH, total artificial heart; LVAD, left ventricular assist device.

with intra-abdominal LVADs under the guidance of Dr. John Norman [9]. Although the use of these LVADs did not result in meaningful long-term survival, these devices were successful at providing short-term circulation support, allowing the first bridge to transplant with a LVAD to be performed in 1978 [10].

In parallel with the above efforts to develop the temporary intra-abdominal LVAD, efforts were also underway to develop a long-term, totally implantable LVAD. This research was also supported by the National Heart, Lung, and Blood Institute (NHLBI). Requests for proposals (RFPs) were issued in 1976 and 1978 for the development of the latter with the ultimate goal of returning a terminal heart failure patient to a New York Heart Association (NYHA) class I cardiac functional status. As noted, these were only LV pumps; it was hoped that the right ventricle (RV) could be supported by long-term LV unloading and temporary pharmacologic assistance.

As stated earlier, the emergence of cardiac transplantation was an important stimulus to the clinical application of implantable LVADs. Before the introduction of cyclopsorine, three LVADs were implanted for use as a bridge to transplant (Table 48.1). Unfortunately, although the mechanical support as a bridge to transplantation was effective, these patients all eventually died of overwhelming sepsis secondary to infections resulting from the profound pan-immune suppressive drugs employed at that time to prevent transplant rejection. With the advent of cyclosporine, the use of these pumps as a bridge to transplant could once again be explored. The survival of a patient who underwent cardiac transplantation while suffering from active ongoing staphylococcus, streptococcus, and candida sepsis at our hospital in 1984 showed that even severely infected patients could successfully undergo cardiac transplantation utilizing the new immune suppression drug [11]. Therefore, it was believed that patients receiving LVAD support could also potentially undergo successful cardiac transplantation with cyclosporine immunosuppression, thereby making LVADs a viable option as a bridge to transplant. Shortly after this case was reported, Portner and Oyer described the use of a Novacor LVAD as a bridge to transplant (Figure 48.1) [12]. In addition, the Thermo Cardiosystems, Inc. (TCI) LVAD, also developed with NHLBI support, was first implanted in 1986 as a bridge to transplant [13].

Although bridge to transplant was not the original use envisioned for this technology, the introduction of cyclosporine, which allowed successful transplantation following the use of mechanical assist devices, renewed interest in long-term, implantable circulatory support [14]. As a result, the use of these implantable pump technologies as a bridge to transplant began to see an increase through the 1980s, and the 1990s saw a period of exponential growth in this field [14].

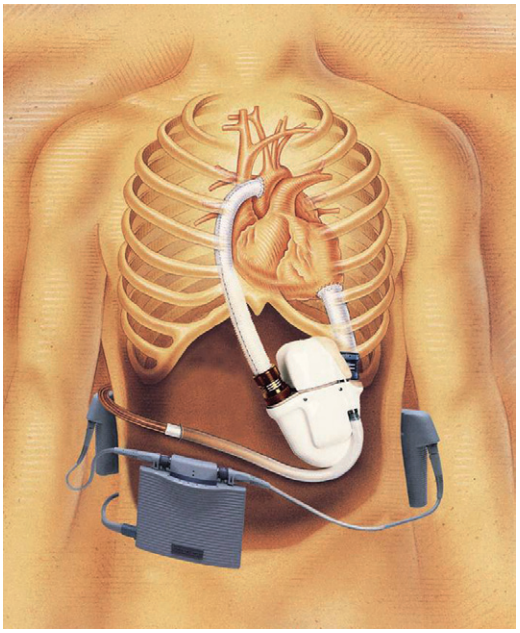

Figure 48.1. Novacor electronically powered implantable left ventricular assist device. Oyer and Portner used it as the first successful bridge to transplant in 1984.

As donor organ waiting times began to increase, the duration of LVAD support also began to increase. Transplant candidates who received the initially available tethered pneumatically powered LVADs were rehabilitated from a NYHA class IV status to a NYHA class I or II status, but unfortunately, they were forced to remain hospitalized until after transplantation. A significant advancement in LVAD technology occurred in 1991, when the first patient received an untethered, wearable, implanted, electric-powered TCI LVAD (Figure 48.2). This patient was eventually discharged from the hospital in 1993, becoming the first such patient to be discharged with an implantable LVAD [15]. In addition to providing patients with the freedom to be discharged from the hospital, these portable devices were also shown to improve end-organ function and exercise tolerance, normalize hemodynamics, and improve quality of life [16–19].

At the time, the two dominant pulsatile implantable pumps were the Novacor and the TCI [19]. The first pump to receive US Food and Drug Administration (FDA) approval was the pneumatic HeartMate (formerly known as the TCI LVAD) in 1994. The electrical versions of both the TCI and the Novacor implantable pumps received FDA approval in 1996.

REMATCH and destination therapy: the end of the beginning

Since the original RFPs for the development of these technologies were initiated in the 1970s as an alternative to transplantation, it was important for the NHLBI to assess these technologies with that goal in mind. Accordingly, under the auspices of the NHLBI, the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure Trial (REMATCH) was initiated. This study analyzed the use of long-term LVADs in patients who were not candidates for heart transplantation and compared their outcomes to those in a randomized cohort of medically treated patients. This study demonstrated that implanting an LVAD in

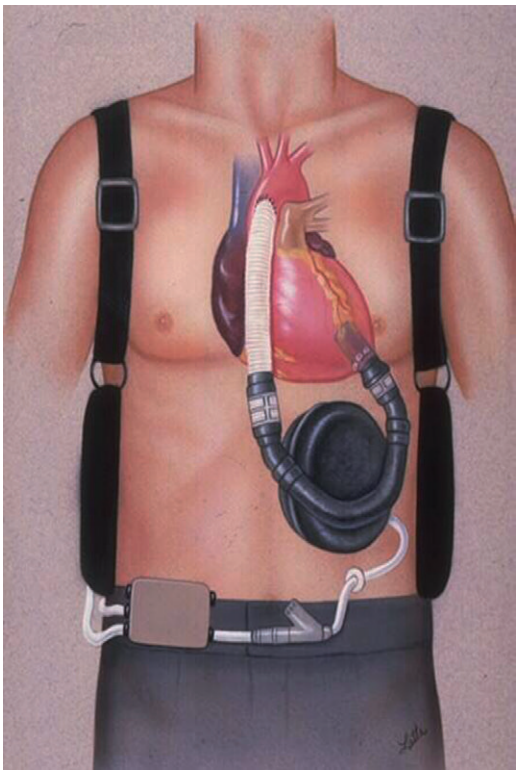

Figure 48.2. TCI HeartMate left ventricular assist device (LVAD), which was the first untethered LVAD, allowing patients to be discharged from the hospital.

patients with NYHA class IV symptoms was an acceptable alternative therapy and that the use of LVADs in these patients resulted in clinically meaningful survival benefit and an improved quality of life [20].

These implantable, pulsatile devices played an important role as life-saving technologies when applied to select patients facing imminent death from heart failure. They performed effectively as a bridge to transplant in these patients. The main limitations of these implanted, pulsatile pumps were size and durability. Because of the large size of these pumps, they could only fit comfortably in normal sized adults. In addition, as waiting times for transplantation increased, the greatest limitation for these pumps became durability. A goal of 2 years of functionality was an ambitious one, considering that the pumps required a flexing, non-lubricated membrane to be activated >100 000 times every 24 h or a minimum of 38 million times in a year. Accomplishing the goal of 2 years was a remarkable achievement. However, successful use beyond that time interval was limited, and multiple exchanges were frequently required even to obtain that degree of durability. This durability barrier, thus, prevented the application of pulsatile pumps for meaningful long-term cardiac support. As a result, the main use of this technology remained only as a bridge to transplant.

Rotary blood pump systems

Since the first use of the heart–lung machine in the 1950s, there has been an interest in the use of continuous flow pumps. Saxton et al. [21] introduced the concept of using continuous flow pumps for long-term support as early as 1960. However, at that time this technology was only used clinically for temporary support in an immobilized patient. Rotary blood pump technology as a long-term cardiac support was not pursued at the time because of engineering limitations and the perceived physiologic barriers to this approach.

The Hemopump, introduced by Dr. Richard Wampler in 1986, showed that implantable continuous flow technology could feasibly support a failed heart. To explore the potential for this device, the senior author (OHF) and Dr. Wampler conducted in-vivo research using an implanted Archimedes screw pump rotating at over 25 000 rpm to directly unload the failed ventricle. These studies showed that this pump worked efficiently, could pump up to 4 L against physiologic pressures, and that the rapid rotation of this pump did not cause hemolysis. The first successful clinical application of the Hemopump occurred in 1988. At the same time, our lab was also working with Robert Jarvik to develop a long-term, implantable continuous flow Archimedes' screw-type pump utilizing blood-immersed bearings. Before the above studies were conducted, not only was rapid rotation considered too hemolytic for intravascular application, but the common wisdom was that non-lubricated, blood-washed bearings could not be implanted in the vascular system long term. These experiments were essential to the clinical application of rotary blood pumps for both temporary and long-term cardiac support.

Rotary blood pumps have several advantages over the earlier volume-displacement pumps. They are smaller than their precursors and therefore applicable to small adults and even children. This allows easier surgical implantation and permits a more acceptable anatomic placement. Importantly, they do not require valves, eliminating a large expense because the flow is unidirectional. They usually contain only one moving part, the impeller or rotor, which increases mechanical longevity; and they have smaller drivelines. The percutaneous driveline required in pulsatile pumps as a vent for the negative pressure created by their pulsatility is not necessary in continuous flow pumps, allowing for transcutaneous power delivery and eliminating external contamination completely. Also, rotary pumps have much lower power requirements than the earlier volume displacement pumps to deliver the same level of support [22].

In the late 1990s and early 2000s, the use of clinical implants with rotary blood pumps began in Europe and the US. The initial pumps were axial flow, Archimedes screw pumps utilizing blood-immersed bearings. The MicroMed, Jarvik, and HeartMate II pumps were introduced clinically in 1998, 1999, and 2001, respectively. All of these pumps are similar in operation, the main differences between them being the length or presence (Jarvik) of the inlet cannulas, the anatomic location of the pump relative to the heart, and the type of blood-immersed bearings used. In June 2000, the Jarvik pump was the first of these long-term pumps to be applied as destination therapy, rather than as a bridge to transplantation. This patient was supported by the pump for 7.5 years. At the time of his death, the pump remained in good working order with minimal bearing wear (Figure 48.3).

Continuous flow pumps in current clinical use

Axial flow pumps

HeartMate II

The HeartMate II (Figure 48.4; Thoratec Corporation, Pleasanton, CA, USA) is an implantable axial flow-type LVAD that is basically a long-term application of the Hemopump principle (Figure 48.5).

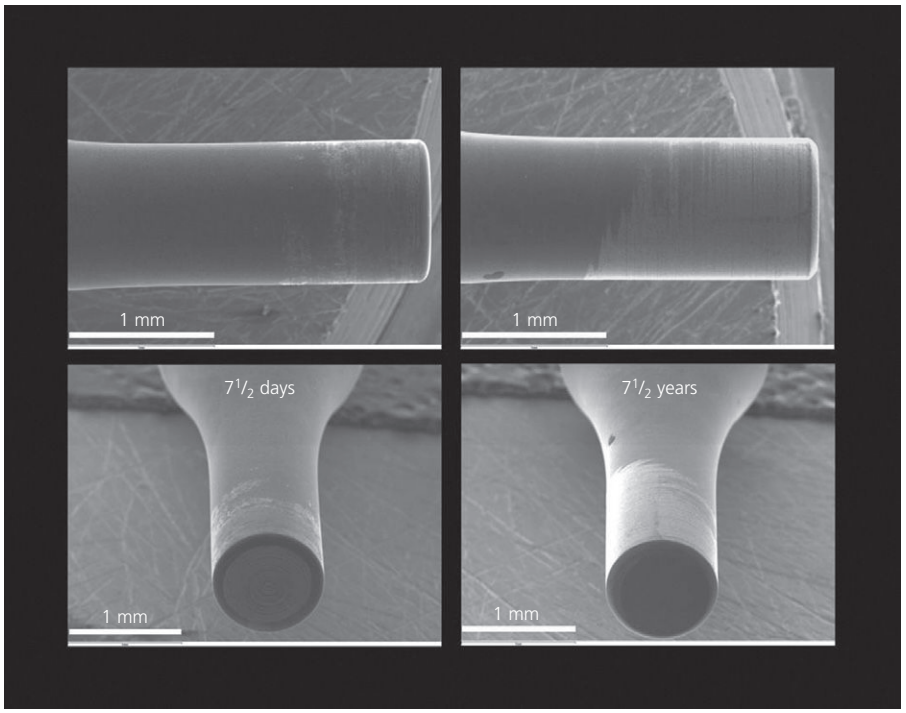

Figure 48.3. Minimal bearing wear of Jarvik 2000 is seen after implant for 7.5 years.

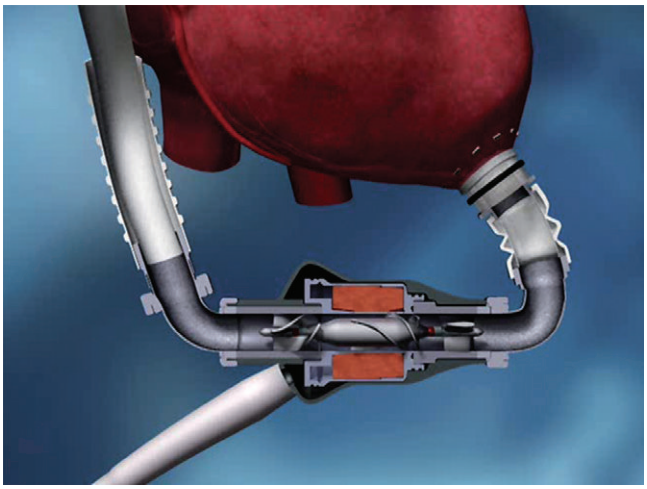

Figure 48.4. HeartMate II. Note its blood-immersed bearings.

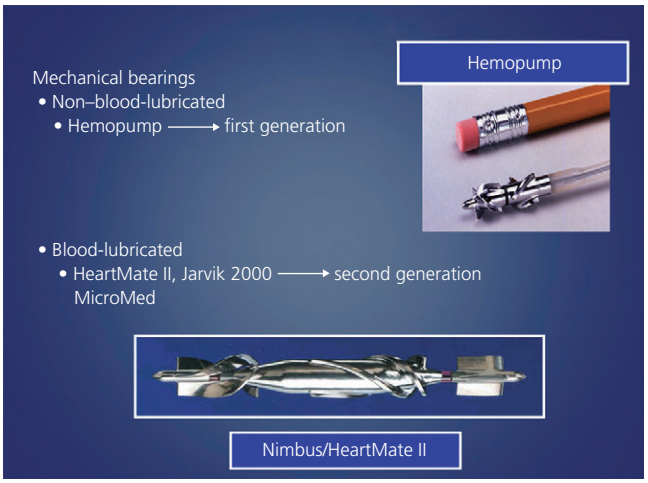

Figure 48.5. Evolution of HeartMate II from Hemopump.

Research on this was initiated in the 1980s with Nimbus and the Texas Heart Institute, and it was further developed in the 1990s by the research group at the University of Pittsburgh led by Bartley Griffith [23].

This battery-powered LVAD is connected to an external controller and batteries via a percutaneous lead. The external system controls the rotational speed, monitors system function, and provides power. The blood pump weighs 375 g and is 4 cm in diameter × 6 cm in length. The impeller, the only moving part of this device, is held in place by blood-lubricated bearings and is controlled by an electromagnetic motor embedded in the pump housing. The impeller rotates at speeds of 6000–15 000 rpm and can generate flows of up to 10 L/min. The system is normally implanted via a median sternotomy with the patient placed on cardiopulmonary bypass, although it can be implanted without cardiopulmonary bypass [24]. The inflow cannula of the device is placed into the LV cavity via a circular sewing ring at the apex or through the free wall of the LV at the diaphragm in such a way that the cannula lies parallel to the ventricular septum. This approach is similar to what we currently use to implant the HeartWare LVAD (Figure 48.6) [25].The outflow from the pump is anastomosed in an end-to-side fashion to the ascending aorta. Postoperative long-term support of patients

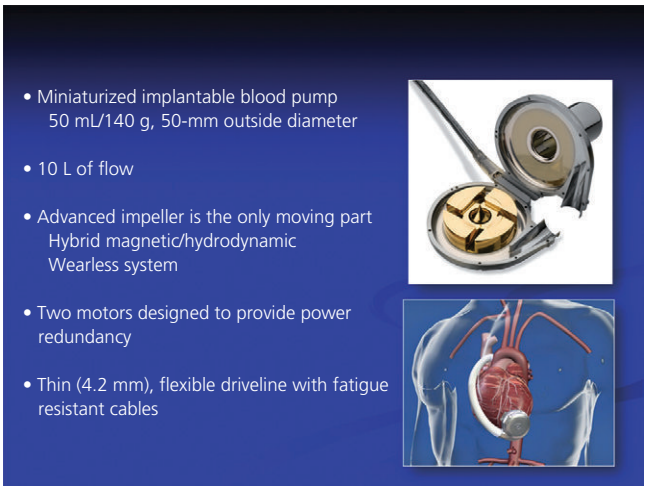

Figure 48.6. HeartWare ventricular assist system.

includes the use of warfarin to maintain an international normalized ratio (INR) of 1.5–3 [26].

Clinical trials began in the US in 2003 [23,27]. By the mid 2000s, excellent results were observed for patients receiving HeartMate II implants. In 2008, the Thoratec HeartMate II axial flow LVAD was approved by the FDA as a bridge to cardiac transplantation, leading to an increase in the number of HeartMate II LVADs implanted. A postapproval prospective study following HeartMate II recipients showed that patients receiving the HeartMate II VADs had further improvement in their survival, particularly if they received the pump earlier in the course of their illness [28]. Currently (August 2012), >11 000 patients have received the HeartMate II blood pump for circulatory support, making the HeartMate II the most widely used implantable LVAD.

Jarvik 2000

The Jarvik 2000 (Jarvik Heart, Inc., New York, NY, USA) is a small (diameter 25 mm, weight 90 g) implantable axial flow-type LVAD, which was also developed throughout the 1990s and 2000s with the support of the NIH [29]. This battery-powered LVAD is connected to an external controller and batteries via either a small percutaneous lead that can exit the abdominal cavity or via a postauricular pedestal [30]. The external portion of the system controls the rotational speed, monitors system function, and provides power. As in other axial flow-type pumps, the impeller, the only moving part of this device, is supported by blood-immersed bearings. The impeller spins at 8000–12 000 rpm. The pump can deliver up to 7 L/min of flow at physiologic pressures [31]. To address potential blood stasis issues, an intermittent speed controller was developed and implemented in 2005. This controller decreases the pump speed to 7000 rpm for 6 s in every minute. By lowering the pump speed, the ventricle is allowed to fill and then eject blood from the aortic valve. The ventricular ejection allows a washout of the aortic root, thereby preventing stasis and thrombus formation [32]. In addition, by opening the aortic valve, aortic valve fusion and resultant aortic insufficiency and stenosis may be prevented.

The Jarvik 2000 can be implanted via a median sternotomy with the outflow anastomosis to the ascending aorta (Figure 48.7). It was originally designed to be implanted via a left thoracotomy [33]. This technique avoids pressure and distortion of the outflow graft by the chest wall–pump interface and avoids repeat sternotomy at the time

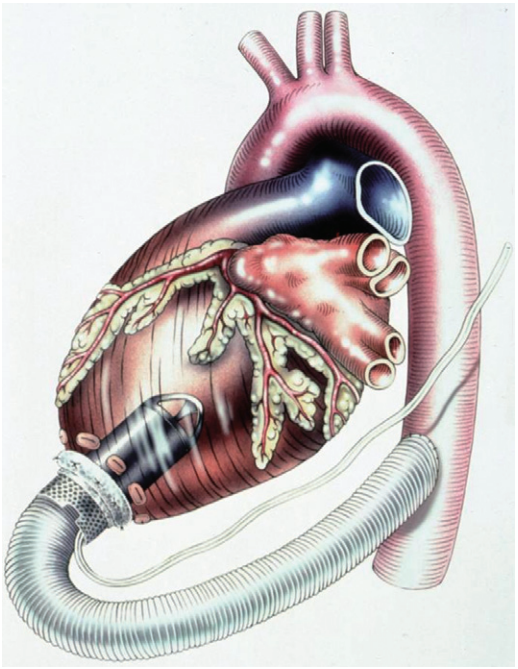

Figure 48.7. Intraventricular placement of the Jarvik pump.

of transplant. Pump implantation can be performed with or without cardiopulmonary bypass [34,35]. A unique feature of this device is that the entire blood pump can be placed into the LV cavity, thereby negating the need for a preperitonial pump pocket. By eliminating the pump pocket, the incidence of infection is greatly reduced [36]. The outflow graft from the pump with the thoracotomy approach is anastomosed in an end-to-side fashion to the descending thoracic aorta. Management of anticoagulation with warfarin depends on aortic valve opening. Another approach to pump insertion is through the diaphragmatic surface of the ventricle by a left subcostal incision. This approach is particularly beneficial in patients with prior median sternotomies, and the device can usually be placed without cardiopulmonary bypass [37]. If aortic valve opening is successfully achieved with the intermittent speed controller (Figure 48.8), INR levels are maintained in the 1.5–2.0 range. If aortic valve opening is minimal, INR ranges of 2.5–3.0 are the goal [32]. Clinical trials of the Jarvik 2000 began in April 2000. To date, the Jarvik 2000 has been used clinically for more than 360 patients according to the manufacturer [33,34].

InCor

The InCor LVAD (Berlin Heart AG, Berlin, Germany) is an implantable axial flow-type LVAD that uses magnetic levitation of the rotor with active axial electromagnetic bearings positioned at each end of the rotor. The pump weighs 200 g. The dimensions for the pump are 30 × 120 mm, and it has a displacement volume of 80 mL [38]. The system is operational at rotational speeds of 5000–10 000 rpm and can provide blood flow of up to 7 L/min [38]. The system is normally implanted via a median sternotomy with the patient placed on cardiopulmonary bypass [39]. The inflow cannula of the device is placed into the LV cavity via a circular sewing ring at the apex. The silicone outflow conduit from the pump is anastomosed in an end-to-side fashion to the ascending aorta. The pump is implanted in the chest inside the pericardial cavity to lie along the

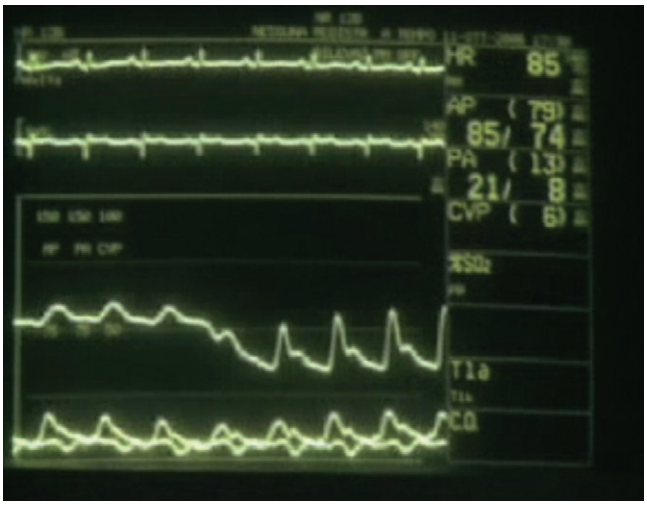

Figure 48.8. Intermittent speed control of the Jarvik pump assures aortic valve opening and is an important contribution to the field.

diaphragmatic border under the heart. A drive cable that exits the patient's right side connects the pump to a small control system that regulates and monitors the system. Batteries are used to allow for patient mobility. The blood-contacting surfaces of the InCor are coated with Carmeda® Bioactive Surface to help reduce the incidence of thrombus formation. For long-term usage, anticoagulants are administered to maintain an INR of 2.8–3.2 [40]. At the present time, this system has been implanted in over 500 patients worldwide [41].

Centrifugal-type pumps

In centrifugal-, or radial-, type pumps, blood enters the pump perpendicular to the pump outflow, is accelerated by the impeller, and is then discharged radially. Although centrifugal pumps are a little larger in diameter than axial flow pumps, the overall geometry of these pumps allows for an anatomic profile design and can fit easily into the pericardial space of a normal sized adult. Centrifugal blood pumps utilize lower rotational speeds than axial flow blood pumps (1400–4000 rpm) and do not require stationary vanes, or stators, in the pump outflow for directional stability of blood flow.

HeartWare HVAD

The HeartWare HVAD (HeartWare International, Miami Lakes, FL, USA) is a centrifugal pump designed to be implanted within the pericardial space [42]. The pump contains a rotating impeller with wide blades and imbedded magnets, a center post with imbedded magnets, and a front and rear housing, each with a sealed motor stator. When the pump is activated, the impeller begins to spin, suspended by hydrodynamic bearings and passive magnetic levitation created by the interaction of the magnets. The impeller can rotate at speeds from 1800 to 4000 rpm and can generate blood flows of up to 10 L/min. The HeartWare HVAD weighs 145 g and has a displacement volume of 50 mL. The front housing incorporates an integrated inflow cannula that allows for device placement into the LV.

The HeartWare HVAD system is normally implanted via a median sternotomy with the patient placed on cardiopulmonary bypass. The integrated inflow cannula of the device is positioned within the LV cavity via a circular sewing ring attached to either the diaphragmatic surface or the apex of the LV. Diaphragmatic placement allows the cannula to reside in the central portion of the LV, oriented towards the mitral valve and parallel to the ventricular septum (Figure 48.9) [25]. The 10-mm outflow graft from the pump is anastomosed in an end-to-side fashion to the ascending aorta. The pump is positioned within the pericardial space, eliminating the need for a preperitoneal pocket and reducing the degree of surgery required for implantation. A thin, flexible driveline exits the patient's side and connects to an external system containing a wearable controller and batteries, which monitors and controls the system as well as provides power to the system. Long-term usage requires patients to be treated with the anticoagulant warfarin to maintain an INR of 2.5–3 [43]. Over 1700 HeartWare HVAD implants have been performed to date.

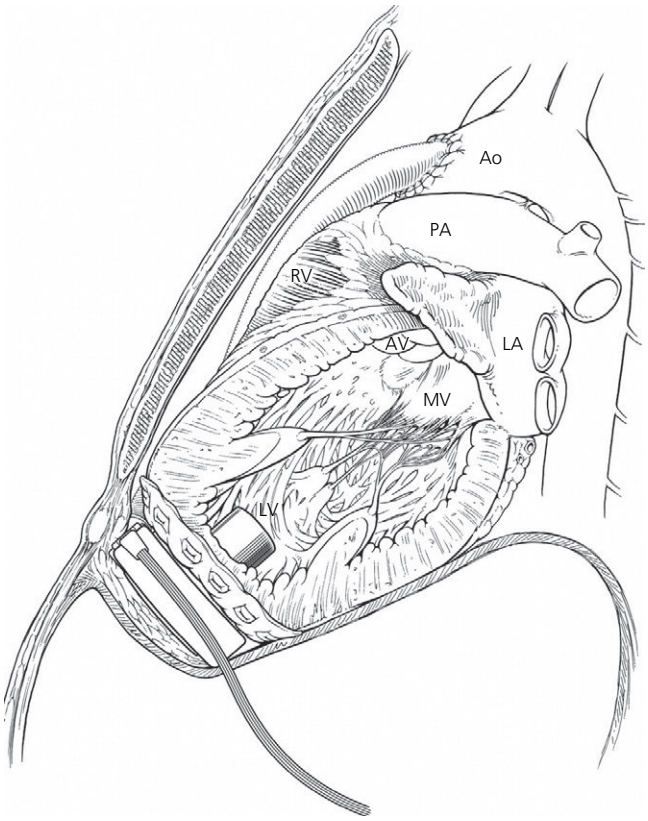

Figure 48.9. Diaphragmatic placement of inlet cannula optimizes inflow. LV, left ventricle; Ao, aorta; PA, pulmonary artery; LA, left atrium; AV, atrioventricular valve; MV, mitral valve. (Reproduced from Gregoric et al. [25], with permission from Elsevier. Copyright © 2011, Elsevier.)

EVAHEART

The EVAHEART system (EVAHEART Medical USA, Inc., Pittsburgh, PA, USA) is an implantable LVAD that combines the use of a centrifugal blood pump to generate blood flow and pressure with an extracorporeal fluid recirculating system. The pump consists of an open-vaned impeller that is mounted on a shaft and driven by a motor that weighs 420 g [44,45]. A hydrodynamic bearing provides lubrication and support for the rotating impeller shaft. Fluid from the extracorporeal recirculating system is pumped via a percutaneous channel to provide heat transfer and to prevent heat denaturation of blood proteins. The pump is connected to a controller by a percutaneous driveline. The pump can generate a

blood flow of up to 8 L/min against an aortic pressure of approximately 90 mmHg. The surfaces of the EVAHEART pump in contact with blood are coated with 2-methylacryloyloxyethyl phosphorylcholine (MPC), a polymer that mimics the vascular intima to provide a blood-compatible surface. The EVAHEART blood pump is implanted via a median sternotomy. The 16-mm polytetrafluoroethylene inflow and outflow conduits are connected to the LV apex and ascending aorta, respectively. Clinical trials assessing the EVAHEART have been ongoing in Japan and have recently begun in the USA.

Complications

Our ability to manage complications has progressed over the last decade. The use of continuous flow technology has led to a profound change in device management. The complications seen clinically with rotary blood pump LVADs largely mimic those of the earlier pulsatile LVADs. However, the frequency of adverse events from pulsatile pumps is higher in most categories.

Right heart dysfunction

RV function should be assessed during the preoperative LVAD evaluation. Chronic elevation of left-sided and pulmonary pressures can lead to RV dysfunction. LVAD support alone is sufficient to sustain the vast majority of heart failure patients as demonstrated by data from the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) [46]. The possibility for temporary right-sided support should be available at the time of LVAD implant. Most right-sided heart failure occurs during the intraoperative or the early postoperative period and can be exacerbated by the use of blood products. RV function should be assessed during implant surgery. Upon LVAD initiation, rapid unloading of the LV can cause the interventricular septum to shift from the right to the left, which can reduce the contribution of the septum to RV contraction and decrease right-to-left blood flow. Appropriate adjustment of LVAD flow can help to compensate for this RV dysfunction and can reduce the need for RV assist. A recent paper by Kirklin et al. using the INTERMACS data showed that the use of RV assist increased mortality two-fold [47]. This increased mortality is associated not only with higher blood loss but also the delay of implantation of the RV assist. If needed, the use of a temporary RV assist should not be delayed.

Although the initial development of implantable, long-term mechanical assist devices was limited to the LV, current technologies, particularly the HeartWare pump and in some instances the Jarvik, can be applied to the right side. The first implantation of the right ventricular assist device (RVAD) occurred in 2003 with a Jarvik patient who developed aggressive right-side failure shortly after implant of a VAD on both the right and left [48]. Since this implant, other cases of chronic right-sided support with implantable pumps have been applied. The largest experience has been in the German centers [49]. Excellent outcomes with this approach as a bridge to transplant have been obtained [49].

Infection

Infection is one of the major challenges to the management of the patient with an LVAD and remains one of the major causes of death to LVAD recipients [50]. Despite the decrease in pump and driveline size and the increase in experience leading to improved outcomes with the use of continuous flow LVADs [51], infections and associated sepsis continue to be major complications for patients receiving LVAD implants while awaiting cardiac transplantation [52]. Infections related to the LVAD may include the following: driveline exit site infections, which may or may not track along the driveline and from the exit site to the pump; and pump pocket infections. Complications can also occur in relation to infections not localized to the LVAD itself, such as pneumonia, sternal wounds, urinary infections, and systemic infections.

Gastrointestinal bleeding

Gastrointestinal (GI) bleeding after the immediate surgical period has been a challenge to the management of patients on chronic LVAD support since its inception. The pneumatic HeartMate as well as the HeartMateXVE did reduce the incidence of bleeding because systemic anticoagulation was not required with this technology. The Novacor pump, which required systemic anticoagulation, had an incidence of GI bleeding as high as 19% [53]. The use of continuous flow technology has been accompanied by anticoagulation. This is required not for the pump, as flow through the pump is continuous and no elements of Virchow's triad are present. However, anticoagulation is felt to be necessary, particularly for potential clot formation at the aortic root or around the base of the pump inlet. Early in the course of the LVAD experience, GI bleeding from arteriovenous (AV) malformations was observed [54]. With more widespread use of the HeartMate II pump, the number of patients encumbered by this complication increased. This problem is believed to be related to the diminished pulse pressure imparted by the use of these continuous flow pumps. It was first alluded to in 1958 by Heyde, who described bleeding from AV malformations in patients with severe aortic stenosis [55]. Its etiology remains poorly understood, but it predictably occurs in 10–15% of patients after implant. It can be addressed by decreasing the pump's rotation rate, which allows for an increase in the pulse pressure, and minimizes anticoagulation.

Summary

In the US, heart failure accounts for 34% of cardiovascular-related deaths [32,56]. Because of improved survival of patients following acute myocardial infarction combined with a population that continues to increase in age, heart failure will continue to increase in prominence as a major health problem in the US [57–60]. Long-term mechanical cardiac assistance has become an important and effective tool in the management of patients awaiting cardiac transplantation. It has been demonstrated over the last 5 years that support with rotary blood pumps can improve renal and hepatic function in advanced heart failure patients awaiting cardiac transplantation [61]. As more of these devices are put into use and clinical studies provide stronger evidence to support the safety of these devices, both the medical community and patients are becoming more open to the prospect of implanting these devices earlier in the disease process, before patients become moribund. The improved durability and reduced short- and long-term morbidity associated with the HeartMate II has reduced the need for urgent cardiac transplantation [62].

Because of the unpredictable waiting times for donor organs, which have largely resulted from the increasing numbers of patients on transplant waiting lists and from the plateau in the number of donor hearts available each year, the current use of VAD technology now far exceeds its application as a bridge to transplant. VADs are now primarily used as long-term support of the advanced heart failure patient whose ultimate goal is transplant [46]. Ideally, in the

future, with better medical as well as adjuvant therapies and more suitably designed devices, heart function will improve enough to allow device support to be removed [53].

The increased demand for mechanical assistance reflects modern medicine's success at treating various circulatory issues. Until we can conquer heart disease by finding and treating the underlying processes, VADs will continue to play a crucial role in restoring circulatory function to end-stage heart failure patients so that they may live full and satisfactory lives.

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CHAPTER 49

The Artificial Heart

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Introduction

The Syncardia CardioWest Total Artificial Heart (TAH) is the only Food and Drug Administration (FDA) approved TAH. It was approved for potential cardiac recipients who have severe biventricular heart failure. Often, these patients have multisystem organ failure and are at risk for death within hours to days. In contrast to left ventricular assist devices, the TAH replaces both ventricles of the heart. The TAH is the first implantable organ replacement with potential for use as a destination therapy. It perhaps represents a view of the future state of transplantation, where organ replacement can be achieved without the concerns of immunosuppressive morbidity and donor organ supply that dominate modern organ transplant practice. This chapter will cover the TAH, considering it separate from ventricular assist devices (covered in Chapter 48), but also within the context of bridge to transplant therapeutic modalities. As such, this chapter complements Chapters 39 and 44, which discuss the management of patients awaiting heart transplantation and the intensive care of patients with cardiac failure, respectively.

History

Early work with the pneumatic TAH included that of Akutsu and Kolff who achieved canine survival for 90 min [1]. Over the following years, work in calves provided encouraging results, and in 1964, Congress passed the National Heart Initiative supporting the creation of a TAH for humans.

Dr. Denton Cooley implanted the "Liotta TAH" in 1969 as a bridge to transplantation. His patient was supported for 64 h and then received a cardiac transplant [2]. The patient died 32 h after transplantation from sepsis. In 1981, Dr. Cooley implanted the Aktusu TAH and the patient was supported for 53 h until transplant [3]. This patient lived for 8 days after transplant but also died of sepsis. Later in 1981, DeVries, Kolff, and Jarvik at the University of Utah received FDA approval for implantation the Jarvik-7 TAH as a permanent heart replacement. Their first patient, Barney Clark, received the Jarvik-7 in 1982 and was supported for 112 days but died after a series of cerebral thromboembolic complications. The procoagulant nature of the TAH and the resultant need for anticoagulation were quickly recognized as central issues in the success of the TAH [4].

In March of 1985, Dr. Jack Copeland at the University of Arizona implanted the Phoenix TAH in 1985 to save a patient with acute cardiac graft failure [5]. The patient was supported for 12 h and then retransplanted, only to die of sepsis 33 h after the second transplant. The use of an unapproved FDA device was controversial but did result in a landmark ruling from the FDA. Since 1985, the FDA allows any physician to use an unapproved FDA device once in order to save a patient's life in a "true emergency."

Copeland and his team then implanted the Jarvik-7 into Michael Drummond on August 29, 1985 [6]. He was successfully bridged to transplantation after 9 days on the TAH and survived for over 5 years post transplant.

The success of the Michael Drummond's bridge to transplant led to subsequent use of the Jarvik-7 (100 mL) and later the Jarvik-7 (70 mL). In 1990, after a few other successful TAH implantations, the FDA decided that Symbion, the company producing the Jarvik-7, was out of compliance and closed the Symbion investigational device exemption (IDE) study that permitted implantation in the US. This paused all US programs as of 1991. In Paris at La Pitie Hospital, Szfener et al. published a report with zero neurologic complications with the Jarvik-7 in 60 consecutive patients with an anticoagulation protocol based upon anticoagulation as well as antiaggregation [7]. Many programs have adopted important features from that original protocol that emphasizes thromboelastography and platelet aggregation studies.

An effort was made to reinstate the Jarvik-7. The device was renamed the CardioWest Total Artificial Heart in 1993 and a new IDE study at five centers was launched. The first patient in that study was implanted in January 1993 at University Medical Center (UMC). She survived 186 days until transplantation and then lived for an additional 6 years. In 2002 the completed trial had included 81 patients over the 9-year period. Later, in 2002, when faced with the financial strains that have bankrupted many small artificial heart companies, and in an effort to "save the technology," the company was refounded with the name SynCardia Systems and eventually the heart was renamed the SynCardia Total Artificial Heart. The IDE study showed that the SynCardia CardioWest TAH could be used as a successful bridge to transplant for severely sick patients with biventricular failure awaiting cardiac transplantation. The data, presented in 2004 [6], resulted in the only approval by the FDA of a TAH as a bridge to transplantation. Four years later, the

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SynCardia TAH was approved for Medicare funding, and since that time, it has been funded in the US by Medicare and private insurance companies.

A total of over 1000 SynCardia TAHs have been implanted in 50 active centers equally divided between the US and Western Europe. Thirty-nine additional institutions are trained and ready to implant. Small driver consoles are now available for in hospital and out of hospital care, allowing the accumulation of over 25 patient years of out of hospital experience. Most of this experience has been in Europe. The longest bridge to transplant with this device has been 3.75 years. In the past few years, 60–70 TAHs have been implanted per year worldwide. In the first 2 months of 2012, over 20 were implanted. There has been steady growth in the number of implants since the early 1990s, followed by accelerated acceptance and use in the past 5 years.

Patient selection

Choosing among the options of left ventricular assist device (LVAD), biventricular assist device (BiVAD), and TAH implantation in heart failure patients is controversial. Patients appropriate for TAH have been shown to be those with irreversible biventricular failure, acute decompensation after cardiotomy, cardiogenic shock after acute myocardial infarction, stone heart, irreversible cardiac rejection, severe graft coronary vasculopathy, failed LVAD or BiVAD, decompensating heart failure with left ventricular thrombus, acquired ventricular septal defect, and prosthetic or incompetent native aortic valve in cardiogenic shock, or unresponsive ventricular arrhythmias. The last six of those indications are appropriate for TAH only, while the others can be appropriate for LVAD, BiVAD, or TAH. As discussed in Chapter 48, the indications for LVAD or BiVAD include left ventricular heart failure without right heart failure, and potentially reversible heart failure such as with viral myocarditis, in which heart transplantation may be avoidable.

The TAH FDA trial noted a 79% survival for bridge to transplantation with the CardioWest TAH [8]. The inclusion criteria were strict and selective:

- New York Heart Association (NYHA) class IV;
- a body surface area of 1.7–2.5 m^2;
- hemodynamic instability defined as a cardiac index of <2.0 L/min/m^2 and one of the following: systolic arterial pressure of <90 mmHg or central venous pressure (CVP) of >18 mmHg, or two of the following: dopamine at 10 μg/kg of body weight/min, dobutamine at 10 μg/kg/min, epinephrine at 2 μg/kg/min or vasopressor medications at maximal doses, intra-aortic balloon pump or cardiopulmonary bypass.

These criteria translate to the new Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) class 1—the crash and burn patient. In 2001, Copeland et al. published a retrospective study comparing the use of the CardioWest TAH, Novacor, and Thoratec as a bridge to transplantation [7]. The study demonstrated that the CardioWest TAH is the best option for patients with biventricular failure, cardiogenic shock, or multisystem organ failure, or those patients now classified as INTERMACS class 1 ("critical cardiogenic shock") or class 2 ("in progressive decline") [8].

The CardioWest TAH is designed to benefit those patients where an LVAD, right ventricular assist device (RVAD), or BiVAD is not sufficient for the severity of multisystem organ failure such as renal and/or hepatic failure. In a risk factor analysis published in 2008, the risk factors proven to be associated with LVADs such as Novacor, HeartMate I or Thoratec do not increase the risk of implantation and survival in the CardioWest TAH [9]. Control of the left and right side of the circulation is unique to the TAH. It gives the patient a predictably high cardiac output and high perfusion pressure. This may be a life-saving advantage in critical cases and may reduce postimplant morbidity in borderline cases. Also, orthotopic replacement of native ventricles and valves provides a means of salvaging patients with irreversible damage to the valves, ventricles, proximal great vessels, as well as to myocardium.

Patient selection is also dependent upon the fit of the device in the recipient's chest. If the fit is too tight, the inferior vena cava (IVC) and/or the left pulmonary veins may be compressed. Guidelines for adequate fit include the following:

- distance of 10 cm from the posterior sternum to the anterior spine on computed tomography (CT) scan at the level of T10;
- body surface area of ≥ 1.7 m^2;
- cardiothoracic ratio on anteroposterior chest X-ray of >0.5;
- left ventricular end-diastolic dimension on echocardiography of ≥ 70 mm;
- a cardiac volume on CT scan of ≥ 1500 mL.

In addition, there are some general guidelines: a large person with a normal sized heart or a small person with a large heart is most likely a good candidate, whereas a normal or small person with a normal sized heart is most likely a poor candidate. All of these factors need to be considered; with increasing experience, the size issue is recognized as very important and surgeons become proficient at using the guidelines.

One of the most difficult aspects of using the TAH is excluding patients who are at too great a risk of death or incapacitating complications. Unfortunately, in new programs the selection of the first patient is often overly optimistic and the experience ends tragically. This is reminiscent of the early experience with cardiac transplantation when the results were uniformly bad. Once stringent selection criteria were applied and contraindications were respected, survival was obtained. Besides inadequate fit criteria, there are several further contraindications: cachexia, advanced physiologic age, chronic multiorgan failure judged to be irreversible, judgment that the patient will never become a potential cardiac transplant recipient, and situations that are at high risk for massive hemorrhage (ongoing dialysis, multiple previous operations, multiple previous operations and a history of heparin-induced thrombocytopenia, failure to wean from bypass with massive bleeding, etc.).

SynCardia total artificial heart

The SynCardia total artificial heart (TAH-t) consists of two orthotopically placed 70-mL ventricles that together displace 400 mL and weigh 160 g. They are connected to lines brought out through the skin just under the left costal margin and are connected via 6-foot tubing to an external pneumatic console that controls the TAH. Each ventricle has a maximal stroke volume of about 70 mL. The large console consists of two complete pneumatic drivers (one primary and one back-up), two air tanks with enough pressurized air to last for 2 h per tank, and a panel of alarms. There are only 36 of these consoles in the world; thus, the availability of smaller more portable drivers has been long awaited and is just beginning to make an impact.

The pneumatic ventricles are lined with segmented polyurethane, including four layered diaphragms that separate the blood and air,

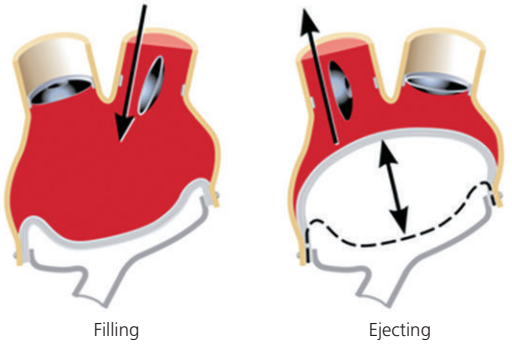

Figure 49.1. Blood flow through the SynCardia TAH-t. Blood and air are separated by a multilayer polyurethane diaphragm. Blood is pumped by air pressure and the ventricle then refills from the atrium under hydrostatic pressure.

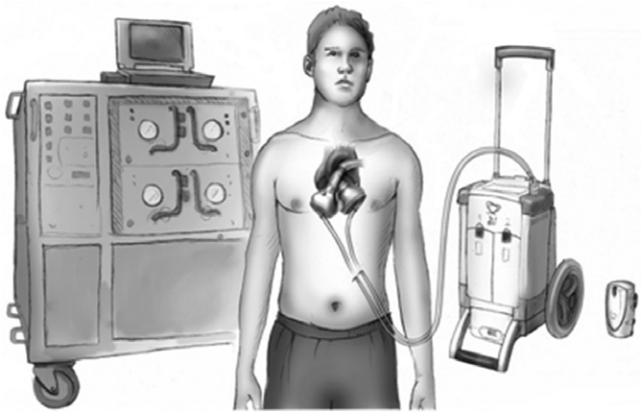

Figure 49.2. Schematic of the implanted device and three drivers. The large console on the left, "Big Blue," weighs 400 lb, the middle console on wheels, "Companion 2," weighs 55 lb, and the smallest pump on the right, "Freedom," weighs 13.5 lb.

and move in response to air pressure for outflow and venous blood filling pressure for inflow. Each ventricle has an inflow and an outflow valve. Thus, during inflow the ventricle has no air pressure on the air side and is filled through the inflow valve; and during device outflow, air pressure displaces the diaphragm, pushing blood out of the outflow valve.

The logic for running the device is very simple—partial fill, full eject (Figure 49.1). This means that the device is set to allow the ventricles to fill with 55 ± 5 mL of blood and then to completely eject that volume. The computer on the console displays ventricular air filling pressure, ventricular rate of blood filling (measured by monitoring the flow rate of air out of the ventricle), ventricular filling volume (from integration of the rate of filling over time), beat rate, output from left and right ventricles approximately every 1–2 s, and the average output over time as well as the trend over the past 8 h. Adjustments that can be made include: maximal air pressure for each ventricle, beat rate (the ventricles "beat" simultaneously), percentage systole (the percentage of the time the ventricles are in systole), and vacuum. The typical settings are: right ventricular pressure of 60 mmHg, left ventricle pressure of 180 mmHg, beat rate of 120–130/min, percentage systole of 50%, and vacuum pressure of –10 cmH_2O. This typically achieves a cardiac output of 7–8 L/min at physiologic blood pressure and a central venous pressure of 10–12 mmHg. The chosen pressure settings of the console are 30 mmHg higher than the anticipated pulmonary artery pressure and 60 mmHg higher than the anticipated systemic pressure, thus allowing for full ejection in the face of increasing vascular resistance. The vacuum never creates a negative pressure in the patient's atria. It is a negative force that is applied to the exit of air from the console (6 feet from the device), which slightly increases the rate of ventricular filling.

The external console weighs about 418 lb and was nicknamed "Big Blue" by a patient. With this console, the patient remains in the hospital until transplantation, but there are two smaller versions of the console that allow the patient to be mobile within the hospital and to be discharged home. The Companion II driver weighs 55 lb. In the US, there is currently a new portable driver for home use, called the Freedom driver. General availability awaits completion of an FDA trial in the US. The Freedom driver weighs 13.5 lb and can be worn as a shoulder case or a backpack, and it has enabled people to go home and be mobile enough to ride a bicycle (Figure 49.2).

Implantation and explantation

Implantation is very similar to transplantation. Explantation can be very difficult if the surgeon fails to "set up" for explantation at the time of implantation.

The first part of the implant operation is explanting the native heart. The native heart should be arrested and the aorta cross-clamped before there is any major manipulation of the native heart, particularly if there is any chance that mural thrombi exist on the left ventricular endocardium. The native heart is explanted just on the ventricular side of the atrioventricular groove. The annuli of the tricuspid and mitral valves are preserved, as is a circumferential 1–2-mm remnant of atrioventricular valve tissue. The purpose of this is to strengthen the base for the atrial quick-connect anastomosis. The great vessels are divided at the sinotubular junction and dissection of the great vessels is minimized to decrease postimplant adhesions.

The outflow conduits are presealed. Then, the atrial connectors and the outflow conduits are anastomosed. We usually cut the aortic conduit to 3 cm above the quick connect and the pulmonic artery to 6 cm. Prior to snapping the ventricles into place, a "neopericardium" [10] is constructed to separate the ventricles from the patient's mediastinal tissues. This prevents severe adhesion formation and facilitates explantation of the TAH at the time of transplantation. We also very loosely sling the superior vena cava (SVC), IVC, and aorta with ribbons of polytetrafluoroethylene (PTFE) or some other material to provide access for control of those vessels at the time of explantation (Figure 49.3).

Deairing and closure of the chest are major concerns at this point. The surgeon must allow the left ventricle to fill after quick-connecting to the left atrium in order to remove air from the left side. The remaining deairing techniques are similar to those in conventional cardiac surgical procedures. Closure of the chest is the time when adequate fit is confirmed. If the device is too large or there is temporarily inadequate space due to pulmonary edema, closure of the chest results in an immediate drop in pump output and reopening the chest restores output. If this is the case, the safest strategy is to leave the sternum open and to close the skin with a large Gortex patch, with the plan to reoperate and make another attempt at closure after some decrease in mediastinal inflammation and increase in pulmonary compliance.

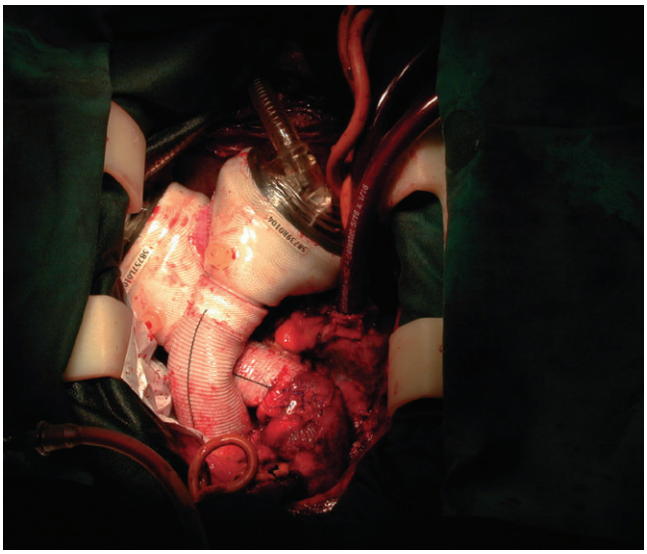

Figure 49.3. A fully implanted SynCardia TAH-t in situ prior to chest closure (viewed from the patient's head).

Anticoagulation

TAH patients are treated with anticoagulants and antiaggregants to achieve normocoagulability and moderate depression of platelet function. If TAH patients were not treated with anticoagulants and antiaggregants, the flow of blood through the device would create an imbalance by stimulating both the chemical coagulation proteins and the platelets, eventually leading to thrombus formation and increased risk of embolism. On the other hand, anticoagulation using current heparin protocols might be too much and lead to bleeding, while current aspirin protocols might not provide enough anticoagulation to inhibit platelet function, particularly in the face of the anticipated postimplantation thrombocytosis. Thus, close monitoring of coagulation tests and platelet function are very important in the titration of doses.

Our approach is based upon monitoring coagulation with thromboelastography (TEG) and platelet function with platelet count, bleeding time, and platelet aggregation studies [11]. The TEG measures the evolution of clot formation by measuring adherence and strength of the clot during its formation. As clots form in the TEG, there is a latent or enzymatic phase of stimulation by a foreign surface. The clot then becomes more adherent and stimulates platelet adhesion. After a period of minutes, the clot strength becomes maximal and then, if the fibrinolytic system is active, it is broken down.

The speed of formation of a sticky clot and the adherence of the clot to a foreign surface are variable and depend upon the excitation of the coagulation factors, the concentrations of the factors for the initial part of the TEG, and the number of platelets and concentration of fibrinogen and activity of the platelets in the later part of the TEG that describes the strength of the clot. By looking at both of these characteristics in a weighted equation, a number called the coagulation index (CI) can be calculated. This is a numerical value for defining the "coagulability" of the blood. Thus, a normal range is determined and called "normocoagulability." Anticoagulation, factor depletion, and thrombocytopenia can lead to hypocoagulability. In TAH patients, hypocoagulability correlates closely with the tendency to bleed. Stimulated coagulation factors and platelets or large concentrations of either, especially in the face of increased inflammation, lead to hypercoagulability and this is associated with a tendency to clot in the device or elsewhere (veins, diseased arteries, areas of blood stasis or turbulent flow).

The goal of anticoagulation is to make the patient "normocoagulable" according to the CI of the TEG. The beauty of this test is that it safely guides the physician in the use of anticoagulants, particularly in the first few weeks post implantation. Doses of heparin monitored by TEG are lower than they would be based on partial thromboplastin time (PTT). When using TEG, the PTT values are not even close to the "therapeutic" levels used conventionally (target PTT is 45 s). Thus, bleeding is rarely seen after the first few days following device implantation. As the patient recovers from multiple organ failure, especially from shock liver, use of oral anticoagulant therapy with coumadin may be preferable to heparin if hospital discharge and a wait of several months for transplantation is anticipated. The conventional guideline of an INR of 2.5–3.5 is reasonable in combination with antiaggregation therapy.

Antiaggregant therapy consists of aspirin and dipyridamole. Dipyridamole in fairly high doses (400–1000 mg/day) is started immediately per nasogastric tube. In selected patients it is given intravenously. Aspirin is not started until the platelet count is at least 150 000/mm^3 Initial dosing is 81 mg/day. It is gradually increased on the basis of platelet count (81–162 mg/day for each additional 150 000/mm^3 on the platelet count), bleeding time (with the aim to keep this 1.5–2 times normal), and the platelet aggregation (with the aim to preserve response to collagen, but depress response to arachidonic acid and epinephrine). As the patient recovers and can dependably take oral medication, we switch to Aggrenox b.i.d. It has been noted that the combination of aspirin and dypyridamole found in Aggrenox (20 mg and 200 mg, respectively) allows for a considerably lower dose of each agent than when aspirin and dypyridamole are administered separately..

We also use pentoxiphylline 400 mg t.i.d. for rheologic effects, prevention of rouleaux formation and decreased fibrinogen.

Postoperative complications

We have recently reported complications, or "adverse events" as they are called, for 101 consecutive patients with 24 patient years of TAH support from one center [12]. The major problems were thromboembolism, hemorrhage, and infection. There were two deaths due to central line entrapment of the tricuspid valve, a preventable event, and one death from device failure (a perforation in one of the four diaphragm layers).

Stroke

In this report, we found eight strokes (defined as neurologic deficit for >24 h). Four of these were related to the implantation and occurred within 2 days after the operation, and four occurred later during the period of support (at an average of 87 days). Two were in stable patients: one expressive aphasia that was transient and one hemiparesis; both patients were transplanted. Two were in patients with internal device infections. One stroke was fatal, three were associated with residual deficits and four patients had deficits that resolved completely within days. Thus, there were two strokes during stable support (0.02 events/patient year) and two strokes in the only two patients with internal device infections (0.02 events/patient year).

Bleeding

Bleeding requiring return to the operating room was seen in 25% of patients and occurred within 12 h of implantation in most cases. While this seems to be an important percentage, experience has shown that in very sick patients, particularly those with thrombocytopenia and liver synthetic dysfunction, this percentage is fairly constant for device implantation.

Our approach to bleeding in these patients is primarily surgical. We attempt to attain optimal hemostasis at the time of implantation, and often spend several hours waiting for adequate coagulation, controlling local bleeding with sutures and cautery as well as topical agents. We are liberal with replacement using blood and fresh frozen plasma, but reluctant to give platelets unless absolutely necessary. Platelet transfusions that may carry passenger white blood cells can cause anti-HLA antibody formation and thus limit subsequent transplantation options. We also are reluctant to use factor concentrates in this setting because of the possibility of over treatment that could result in stroke and systemic arterial and venous pathologic coagulation.

Infection

Infection in this series was defined as any positive culture or any treatment with antibiotics for a suspected infection; 64% were found to have "infection," which again is not an unexpected percentage Only a few of these infections were important, including three mediastinal infections requiring reoperation, drainage, and antibiotic irrigation. One of these patients survived.

Our approach to infections is to use limited prophylactic antibiotic therapy, usually just one or two doses of vancomycin. If infection is diagnosed, specific sensitivity-driven antibiotic therapy is given.

These three complications are intertwined, with anticoagulation increasing the risk of bleeding; reoperation and bleeding increasing the risk of infection; and infection increasing the risk of hypercoagulability and stroke.

Causes of death

In the above-mentioned most recent report of 101 consecutive TAH implants, there were 32 deaths on device support [12]. Seventy percent were in the first 2 postoperative weeks, suggesting a strong impact from the very poor condition of most patients. The causes of death were: 13 from multiple organ failure, six from pneumonia and/or pulmonary edema, five from sepsis, four from neurologic injury (one stroke, one hypoxic damage from hypotension, two intracranial hemorrhage), one from pancreatic abscess, one from small intestinal ischemia, one from disseminated intravascular coagulopathy, and one from disseminated coccidiodomycosis. This spectrum of causes of mortality is similar to that seen in mortality statistics for many types of mechanical support.

Summary

In the multi-institutional trial with SynCardia TAH-t, in which patients were carefully selected for implantation, a survival rate of 80% to transplantation and a survival of transplanted patients of 86% were found [6,8]. In our more recent report [12], using less rigorous selection criteria, the survival to transplantation was reduced to 70% and post-transplantation 1-year survival was 77%. Several large European experiences with liberal selection criteria have reported survival to transplantation of 50–70%. Regardless of the selection bias, patients in the throes of catastrophic decline who are classified as "crash and burn" with only hours to days to survive, as well as many patients who are beyond this classification and for whom there is no other solution than a total artificial heart, have survived in very significant numbers for years with TAH rescue and subsequent cardiac transplantation. Thus, this is a device that should be available in any serious end-stage heart disease program.

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CHAPTER 50

Artificial Lungs

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Introduction

There are an estimated 750 000 patients with respiratory failure in the US, of whom 150 000 die annually [1]. Originally adapted from cardiopulmonary bypass components in 1972, techniques in extracorporeal membrane oxygenation (ECMO) have evolved to improve survival and reduce morbidity from respiratory failure [2]. Artificial oxygenators use porous hollow fibers for gas exchange; bloods flows over the fibers, often with the aid of a pump, while gas flows through the fibers (Figure 50.1). Although initial interest was hampered by poor survival in two randomized clinical trials of ECMO for adult respiratory distress syndrome (ARDS), 10% survival in 1974 [3] and 33% in 1994 [4], technical improvements, including heparin coating of components, polymethylpentene (PMP) diffusion membranes, centrifugal blood pumps, and redesigned cannulas, have improved outcomes and stimulated increased clinical use [5,6]. Studies throughout the 1980s and 1990s [2,7,8] consistently demonstrated survival rates between 40% and 50%. The multicenter CESAR trial in 2009 showed decreased mortality in patients randomized to mechanical ventilation with ECMO for ARDS in comparison to ventilation alone [9]. The benefit of ECMO in acute respiratory failure was re-emphasized in the H1N1 influenza pandemic in the late 2000s as survival with ECMO was >70% [10]. Additionally, recent studies demonstrate survival when ECMO is used as a bridge to transplantation of between 50% and 80%. The success of ECMO and its extension to a variety of indications also served to spur the development of other artificial lung devices. As for most life-supporting mechanical devices, outcomes are significantly improved by the selection of candidates early in their course of mechanical ventilation and without significant co-morbidities. As the bioengineering of artificial respiratory systems evolves, improved results can be expected.

The broad acronym ECMO is generally accepted to include $ECCO_2$-R (CO_2 removal), ECLA (lung assist), and ECLS (life support). However, the original application of ECMO was for hypoxia due to decompensated ARDS, as 3–15% of respiratory failure is attributed to ARDS [2]. With use of the ARDS Network (ARDSNet) ventilator management guidelines, including low tidal volumes (<6 mL/kg), low plateau pressures, permissive hypercapnia, inhaled pulmonary vasodilators, and judicious fluid utilization, survival nears 60% [11], leaving a sizeable population with the potential to benefit from ECMO. In severe cases, this ventilatory approach may not provide adequate gas exchange and may contribute to the progression of ARDS by inducing barotrauma, alveolar damage, increased capillary permeability, pulmonary edema, and inflammation (see Chapter 45) [2].The use of ECMO to allow protective approaches to ventilation with low frequency and tidal volume provides not only lung rest and less ventilator-associated lung damage, but also has led to improved outcomes for ARDS, as revealed in the CESAR trial. This multicenter, blinded clinical trial randomized severe ARDS patients to receive conventional ventilator management with or without ECMO. The significant improvement in 6-month survival without disability (63% vs. 47%) seen in this study served to establish ECMO as a superior therapy for severe ARDS [9].

Before ECMO was used as a bridge to transplantation, it was successfully used in salvaging dysfunctional grafts following transplantation. Primary graft dysfunction (PGD) is the leading cause of early death following lung transplant, occurring after 15–35% of transplants, 7% of which historically have required ECMO support [12,13]. PGD presents as progressive hypoxia, loss of lung compliance, and eventual hemodynamic instability. Contributing factors to PGD may include ischemia–reperfusion injury, rejection, prolonged ischemic time, and perhaps massive transfusion. Although higher survival is seen in lung recipients not requiring postoperative ECMO (82% vs. 40%), studies have demonstrated that in patients weaned from post-transplant ECMO (67%), survival approaches that of no ECMO controls (80% vs. 82%) [12]. Post-transplant ECMO has no effect on long-term lung function in survivors. The ability to wean from post-transplant ECMO following PGD is significantly lower at >14 days of support, and retransplantation must be considered at this point [12]. With mortality rates between 25% [14] and 60% [12], ECMO can serve to rescue failing transplant patients by allowing lung recovery or bridge to retransplant.

As of 2005, waitlist mortality for end-stage respiratory failure patients awaiting lung transplant was 20% at 1 year and 40% at 2 years after listing, indicating a clear need for alternatives both to transplantation and bridging devices that may allow survival to transplantation [15]. As the success of ECMO for ARDS and as rescue from transplant was realized, its use as a "bridge to transplant" grew. However, its use as a bridge to transplant was belated as reported outcomes were isolated and short term. Originally, ECMO was thought to be a contraindication to transplantation due to poor outcomes (estimated at 60% in 2002) [5]. However, case reports of successful use of ECMO as a bridge to primary lung transplantation in young, acutely ill patients, often with influenza-

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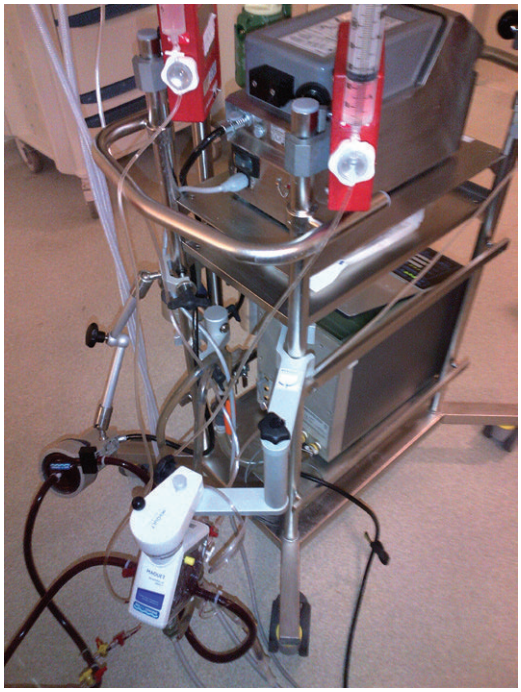

Figure 50.1. The ECMO circuit.

associated bacteria pneumonias, unveiled the potential of ECMO therapy in this application. This indication for ECMO may serve a large population of patients with irreversible respiratory failure without other organ failures who may not otherwise survive on the transplant waitlist. Patient survival is now reported to be between 50% and 80%; thus, allocation of lungs to ECMO patients is justified and common.

ECMO in support of lung transplantation

Indications for ECMO vary with the clinical urgency of respiratory failure (Table 50.1). Generally, acute or chronic respiratory decompensation without other concurrent organ failure prompts its use as a bridge to transplantation. While guidelines vary among institutions, ECMO is generally considered when Pao_2 is <50 mmHg, $Paco_2$ is >80 mmHg, and/or Pao_2/Fio_2 ratio is <1 [16]. Contraindications generally include a contraindication to anticoagulation, irreversible central nervous system (CNS) damage, sepsis, multiple organ failure, and severe peripheral vascular disease [6]. Acute renal failure, high vasopressor requirements, age, obesity, and extended duration of mechanical ventilation are relatively strong contraindications [16]. The only explicit contraindication is irreversible CNS damage. Recent data suggest that patients with chronic severe pulmonary failure anticipating transplant may benefit from early implementation of ECMO [6]. While bleeding and

Table 50.1. Indications for ECMO among different transplant centers

Center	Acute criteria	Chronic criteria
Massachusetts General Hospital (MGH) University of Pennsylvania Peter Bent Brigham Hospital Orange County Medical Center Mount Sinai Hospital NYC University of California SF University of North Carolina Karolinska Hospital, Stockholm, Sweden	Pao_2 <50 mmHg for >2 h at Fio_2 1.0; PEEP ≥ 5 cmH_2O	Pao_2 <50 mmHg for >12 h at Fio_2 0.6; PEEP ≥5 cmH_2O; maximal medical therapy >48 h
Philipps University, Marburg, Germany	A–a >525 mmHg; CT_{stat} <30 mL/cmH_2O; PIP >35 cmH_2O; extended infiltrations on chest X-ray; maximal medical therapy for >24 h (no distinction between acute and chronic criteria)	
Charité/Campus Virchow, Berlin Germany Humboldt-University, Berlin Germany Heartlink ECMO-Centre, Leicester, UK	Pao_2/Fio_2 <50 mmHg for >2 h; at PEEP ≥10 cmH_2O	Maximal medical therapy for 24–120 h; Pao_2/Fio_2 <150 mmHg; PEEP ≥10 cmH_2O; Q_s/Q_T ≥30% at Fio_2 1.0; EVLW ≥15 mL/kg bodyweight; CT_{stat} ≤30 mL/cmH_2O or recurrent barotrauma
Cochin University Hospital, Paris, France	Same as MGH with Murray score >2.5	Same as MGH with Murray score >2.5
University of Michigan	Optimal conventional therapy; Q_s/Q_T >30%; CT_{stat}<0.5 mL/cmH_2O/kg body weight; diffusely abnormal chest radiography in four quadrants	
Ludwigs-Maximilians-University, Munich, Germany	Pao_2/Fio_2<50 mmHg; at PEEP ≥5 cmH_2O for >2 h; CT_{stat}≤30 mL/cmH_2O	After 48–96 h conventional therapy, three of four criteria: Pao_2/Fio_2 <150 mmHg at PEEP ≥5 cmH_2O for >2 h; $Paco_2$ ≥60 mmHg at V_E ≥200 mL/kg; PIP ≥40 cmH_2O; CT_{stat} ≤30 mL/cmH_2O, and Q_s/Q_T ≥30%
Toronto General Hospital, Canada University of Maryland	Life-threatening cardiorespiratory compromise; progressive respiratory failure; oxygenation incompatible with life; semielective ECMO use during procedures	
University of Vienna, Austria	Pao_2/Fio_2 <70 mmHg at PEEP >10 cmH_2O for 96 h	

Pao_2, arterial oxygen; Fio_2, fractional inspired oxygen, PEEP, peak end-expiratory pressure, A–a, alveolar arterial oxygen gradient; PIP, peak inspiratory pressure; CTstat, thoracopulmonary compliance; Q_s/Q_T, intrapulmonary shunt; EVLW, extravascular lung water; VE, minute ventilation.
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thrombosis are always a delicate balance in ECMO patients, the use of heparin-bonded ECMO components has softened the contraindication to ECMO in those who cannot be anticoagulated or have a history of bleeding. Although not a contraindication, when initiated for pneumonia or sepsis, ECMO has markedly decreased survival [10].

The decision to initiate ECMO should be based on the severity of respiratory impairment and the likelihood of successfully bridging patients to recovery or transplant. Additional advancements in the management of acute lung injury, such as inhaled nitric oxide (iNO), low tidal volume ventilator strategies, and prone positioning, may improve some of the parameters and serve to restrict ECMO therapy to more severe cases of respiratory failure [2]. Additionally, as the benefits of both awake and ambulatory ECMO are realized, the timing and use of ECMO in chronic disease is evolving.

Mode of ECMO support for bridge to transplant

Clinical considerations that may be useful to determine the mode of ECMO for a bridge to transplant include hemodynamic stability, degree of oxygenation, or ventilatory failure and presence of right ventricular (RV) or pulmonary artery (PA) failure. Veno-venous (V-V), veno-arterial (V-A), or arterial venous interventional lung assist (iLA) ECMO can be considered (Figure 50.2). Minor hemodynamic decompensation secondary to hypoxia and modest RV failure can generally be stabilized with V-V ECMO, while cardiogenic shock can only be stabilized with V-A ECMO. Both V-V and V-A cannulation strategies improve systemic oxygenation; however, V-A ECMO provides the immediate circulatory support often necessary in decompensated heart failure [17]. In most circumstances V-V cannulation is preferred due to its inherent reduced risk of systemic embolization and absence of injury-related arterial cannulation. Persisting hypoxia in V-V mode is due to recirculation and/or the inability to capture all desaturated venous blood into the ECMO circuit via peripheral V-V cannulation. If severe hypoxia persists (<85% arterial saturation) after repositioning of inflow and outflow cannulas, a central V-V cannulation or switch to a V-A mode is indicated. In the absence of left heart failure, we strongly prefer avoidance of a V-A mode and centrally cannulate the right atrium (RA) for inflow and the pulmonary artery (PA) for outflow via an upper third sternotomy. Cannulas are brought out below the right costal margin to reduce infection at the small sternal incision and to enable mobility of the patient as recovery evolves. Other innovative strategies have been shown to successfully stabilize heart failure while on V-V ECMO, including the creation of an atrial septostomy. A septostomy can unload volume to the left ventricle (LV) and can deliver high saturations through oxygenated venous blood delivered to the left atrium (LA) and LV. An alternative approach is a sternotomy and central recannulation, with inflow from the RA and outflow to the PA, left atrium (LA), or aorta. Pumpless iLA, in addition to traditional ECMO, has successfully bridged patients with PA hypertension to transplant and even allowed extubation and ambulation [16,18].

Cannulation approaches generally utilize the internal jugular vein and femoral vein and/or artery. Although most cannulas can be placed percutaneously, open chest cannulation between the RA and aorta is an option during surgery. V-A cannulation carries an additional threat of distal limb ischemia due to thromboses and requires frequent assessment of limb perfusion [16]. An important consideration in cannulation placement is potential recirculation of blood through the circuit without systemic perfusion. This is prevented by maintaining distance between the ends of the in- and out-flow cannulas. Using V-A ECMO, sufficient systemic oxygenation can be achieved with the tip of a femoral arterial cannula in the lower abdominal aorta [6]. Recirculation should be suspected with high in- and out-flow oxygen content that does not correlate with the oxygenation of the patient. Another concern with femoral vessel outflow cannulation for V-A ECMO is the preferential flow of oxygenated blood to the lower extremities and abdomen, leaving cardiac and cerebral perfusion dependent on cardiac output. Some have suggested axillary artery cannulation to avoid upper body mixed perfusion and sites of severe peripheral vascular disease, and to allow ambulation [19]. Both upper and lower extremity saturations should be followed with any discrepancies treated by adjustments of both ventilatory and circuit blood flow [16]. Cannula-related complications, such as thrombosis, kinking, or malpositioning, should be suspected whenever there is a decrease in previous oxygen saturation levels, increased pressures (negative on inflow and positive on outflow) in circuit lines and hemolysis.

Routine therapeutic goals in ECMO, as defined by the Toronto Transplant Program guidelines, include oxygen saturation of >85%, Pao_2 of >100 mmHg, $Paco_2$ of 35–45 mmHg, and pH of 7.35–7.45. To prevent thrombotic complications, activated clotting times (ACTs) should be maintained between 160 and 200 s for both V-V and V-A ECMO. To prevent bleeding complications, the international normalized ratio (INR) should be <1.8 or <1.5 if bleeding is occurring and platelets should be >80 000/mm^3 or >100 000/mm^3 if bleeding is occurring [16] (Table 50.2). Diuretics are

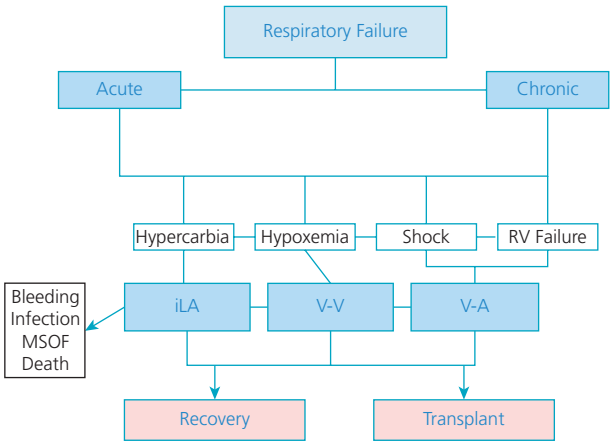

Figure 50.2. Clinical decisions in ECMO.
iLA, interventional lung assist; V-V, veno-venous; V-A, veno-arterial; MSOF, multisystem organ failure; RV, right ventricle.

Table 50.2. Goal parameters in ECMO at The University of Maryland

Hematocrit	>35%
Platelets	>20 000/μL >50 000/μL when bleeding
Activated clotting time	160–180 s
Plasma free hemoglobin	<10 mg/dL
Lactate dehydrogenase	<300 U/L
Sao_2	>85%
Pco_2	35–40 mmHg

required to maintain preillness weight. Hemofiltration is useful when necessary.

Complications of bridge ECMO

Disadvantages of ECMO include its limited duration of use and high rate of complications [15]. Complications from ECMO in patients bridged to lung transplant include renal failure (35%), pulmonary infection (52%), sepsis (41%), fatal sepsis (17%), tracheostomy (41%), digital ischemia (11%), gastrointestinal bleeding (6%), and stroke (6%) [20]. Although the development of heparin-coated components alleviates some of the need for high systemic anticoagulation, the major complication affecting mortality is bleeding. Damage to blood cells as a result of shear through the blood pump, such as hemolysis, platelet activation, and depletion and activation of white blood cells with inflammation, requires frequent monitoring (Table 50.2). Hemolysis is followed not only with cell counts but also with lactate dehydrogenase and plasma free hemoglobin levels. Other studies estimate the complication of bleeding occurs in 52%, hemodialysis in 42%, and neurologic events in 12% [13].

Outcomes with bridge ECMO

Despite affirmation of the utility of ECMO in bridging otherwise terminal respiratory failure patients to lung transplantation, reported benefits vary. The largest study examining outcomes included 51 patients throughout the US and reported a 1-year survival with ECMO bridge to transplant of 50% [21]. Additional single-center experiences from large transplant centers demonstrate higher survival rates of 80% (Hannover), 74% (Pittsburgh) [20], and 60% (Vienna) [17]. A study of two high-volume centers in Scandinavia reported 1-year survival of 75% with ECMO intention to bridge and 92% for those receiving transplant, and survival with transplant of 50% at 10 years [22]. Additionally, in this study >60% of transplants used marginal donors by International Society for Heart and Lung Transplantation Society (ISHLTS) criteria. These results suggest important considerations regarding ECMO as a bridge to transplantation. Outcomes of ECMO in transplantation are clearly dependent on the institution's experience with the technique, with higher volume centers having better outcomes and all centers' outcomes improving with time. Additionally, ECMO patients are considerably sicker and more complicated than patients presenting from home for transplant, explaining the poorer outcomes. This is also seen in the higher survival of patients bridged using CO_2 removal devices, which require hemodynamic stability (often lacking in V-V and certainly V-A ECMO patients) [17]. Interestingly, in the Vienna study, the morbidity and mortality of transplant recipients did not differ by duration of ECMO prior to transplant, which has been speculated to be a cause for variation in outcomes. While 60% survival may appear low, it is notable that of patients surviving to 3 months, 78% survived to 1 year, which was not significantly different from the 80% survival in all other lung transplant patients at that institution [17]. The US experience also demonstrated similar findings in that ECMO was a risk for early but not late transplant mortality compared to unsupported transplant patients [21]. This suggests that if moribund ECMO patients survive the initial decompensation that required initiation of support, their outcomes will be similar to those for other lung transplant patients. In a review of outcomes from Pittsburgh, a high-volume transplant center, ECMO before transplantation was associated with increased perioperative mortality, but not 1-year graft function (74%) [20]. The higher survival in this study may be augmented by the exclusion of patients transplanted with any other organ failure or systemic infection as well as use of standard criteria donors. Additional Pittsburgh findings include that ECMO patients were younger, more often repeat transplants, had longer ischemic times and higher rate of PDG, and were more likely (48% vs. 7%) to require post-transplant ECMO rescue [20]. Transplant allocation in the US has recently reclarified the goals of donating organs to patients with both highest urgency and best likely outcomes through the Lung Allocation Score (LAS). This focus serves to prioritize ECMO patients by a high score due to co-existing need for mechanical ventilation.

Novel directions in ECMO bridge to transplantation

Mechanical ventilation prior to lung transplant is a significant risk factor for complications, such as infection, ventilator-associated lung injury, deconditioning, additional organ dysfunction, as well as increased mortality [23,24]. The ability to avoid mechanical ventilation during ECMO therapy postulates improved outcomes and less morbidity when bridged to transplant. As initially reported in five awake patients with pulmonary hypertension and right heart failure, ECMO initiation earlier in the course of end-stage respiratory failure and before mechanical ventilation has demonstrated successful results. In this series, V-A cannulations were done under local anesthesia and without sedation. Two patients died from bleeding complications. Three patients breathed spontaneously, ate and drank without nutritional supplement, participated in passive and active physiotherapy, and made a full recovery [25].

An "awake ECMO" approach has been extended to broader end-stage respiratory failure patients with exciting results. The largest study examining this strategy is from Hannover and included 26 awake ECMO patients compared to 34 patients bridged with conventional mechanical ventilation [6]. Awake ECMO patients (13 V-V and 13 V-A) had significantly higher Acute Physiology II scores and lower oxygen saturations before bridging, but decreased days of mechanical ventilation following transplant and decreased mortality at 6 months. This survival advantage dropped to 43% when awake ECMO patients required intubation (most often secondary to bleeding). In addition, ICU days ($P = 0.07$) and length of stay ($P = 0.06$) trended lower in awake ECMO patients [6]. Interestingly, despite being generally sicker patients, the awake ECMO group did not have a higher mortality while waiting for lung donation. The apparent survival benefit of awake ECMO as a bridge to lung transplantation may be due to avoiding the hazards of anesthesia and mechanical ventilation. Additionally, in the setting of right heart failure, anesthesia induction and intubation carries a high risk of circulatory collapse. The ability of the awake patient to eat, drink, breath spontaneously, and participate in physical therapy likely contributes to improved post-transplant outcomes. Reported difficulties with the awake ECMO approach are the maintenance of not only oxygenation but also the respiratory comfort of severely hypoxemic patients, such as those with interstitial pulmonary fibrosis (IPF) [6].

In parallel with the development of awake ECMO, the creation of a dual lumen venous cannula for single cannulation in V-V ECMO has allowed the progression of bridging techniques to include both awake and ambulatory ECMO [26]. The dual lumen catheter (Avalon Elite Bicaval Dual Lumen Catheter) is placed

under ultrasound guidance with the proximal and distal inflow cannula tips placed in the superior vena cava and inferior vena cava (IVC), and an outflow orifice in the RA. The distal inflow cannula should be positioned far into the IVC, at or past the hepatic veins, to avoid recirculation of blood through the circuit. Outflow should be directed at the tricuspid valve under ultrasound guidance. Cannula sizes include 23-French (Fr), 27-Fr, and 31Fr, and can provide blood flow of up to 3.5–5 L/min. With the Avalon cannula, patients should be fully anticoagulated at the time of ECMO initiation and an ACT of 140–160 s maintained throughout support. In initial reports, the dual lumen catheter was able to maintain oxygenation (median Pao_2 increased from 45 to 115 mmHg), stabilize hemodynamics [median mean arterial pressure (MAP) increased from 48 to 72 mmHg], and wean 72% of patients from support, including those with chronic obstructive pulmonary disease (COPD). There were no mortalities directly associated with ECMO (55% survival), but there were two cases for whom cannulas required emergent repositioning and one case of cannula thrombosis secondary to subtherapeutic anticoagulation [26,27]. Any acute oxygen desaturation or increased oxygen pressure in inflow blood raises concern for cannula complications and the need for repositioning under ultrasound. Similar to awake ECMO, ambulation allows increased pulmonary toilet, decreased pulmonary infection, and added active physical rehabilitation, with patients being able to use treadmills and stationary bikes [27]. In addition to avoiding femoral cannulation, this technique can be useful in patients with previous vascular interventions such as IVC filters. By decreasing the length of tubing and minimizing contact with foreign materials, the dual lumen catheter may also serve to decrease inflammation and thrombosis [26]. Despite exciting initial results, no direct comparisons of single cannulation to traditional V-V ECMO have been done.

Artificial lungs for CO_2 removal

Extending ECMO beyond the indication of inadequate oxygenation, Gattinoni et al. in 1978 first conceptualized using artificial gas exchange to reduce ventilatory requirements and barotrauma through CO_2 removal [28]. They used room air through a silicone coiled membrane lung in lambs, and demonstrated reduced tidal volume, respiratory rate, peak inspiratory and end-expiratory pressures, as well as maintenance of pH, cardiac output, and oxygenation with CO_2 removal in both spontaneously and mechanically ventilated animals. Additionally, this group showed that artificial CO_2 removal can reduce airway pressures significantly enough to allow healing of induced large bronchial air leaks not successfully treated with conventional mechanical ventilation approaches [28]. This work stimulated the expansion of extracorporeal gas exchange to differing clinical scenarios and the development of new and improved devices.

Developed in 1999, the NovaLung is a pumpless PMP membrane capable of normalizing pH, peak inspiratory and positive end-expiratory pressures, as well as significantly improving $Paco_2$ and Pao_2 in severe ventilatory failure with hypercarbia and respiratory acidosis [29]. This approach is similar to ECMO in allowing lung protective ventilatory strategies, but also prevents additional barotrauma and ventilator-associated lung injury that have been identified as a risk for additional remote organ failure [30]. This device was able to remove up to 70% of excess CO_2 in adults with severe ventilatory failure, while maintaining hemodynamic stability. In initial applications as a bridge to lung transplant, 1-year survival with the NovaLung was reported at 80% in 2005 [13]. Additionally, the NovaLung has demonstrated benefit in patients suffering from ARDS following pulmonary resection, improving survival in a clinical state carrying 40–60% mortality to 71.4% [31]. iLA can be helpful in allowing decreased ventilation and sequential lung deflation in those with COPD exacerbation [10]. Use of iLA has been successfully extended to the management of severe chest trauma, ARDS, PA hypertension, pneumonia, and airway obstruction [29].

The low-resistance iLA device is implanted between the femoral artery and vein, and uses cardiac output rather than a pump to generate flows of 0.5–4.5 L/min. The lack of an artificial blood pump component in the NovaLung means less of the cardiac output (about 20%) goes through circuit, and thus allows a smaller circuit and therefore reduces the priming volume and foreign surface contact [31]. Unlike ECMO, the degree of CO_2 removal is dependent on sweep gas flow, not blood flow. While oxygenation is generally maintained by the native lung in the setting of iLA, small increases in oxygenation can be seen with the NovaLung. Contraindications to iLA include severe hypoxia, cardiogenic shock, or peripheral vascular disease [29]. Prior to iLA placement, the diameter of the femoral artery should be measured via ultrasound. An appropriate cannula size will be at least 20% smaller than the arterial diameter [31]. When both cannulas are connected and flushed with heparinized saline, the transition to iLA should be done gradually over >2 min to reduce the hemodynamic effects of the created A-V shunt. Arterial cannulation with an inflow cannula creates an additional risk of low flow limb ischemia and thus requires frequent assessment of peripheral limb perfusion. Gas flow rate is generally started at 1 L/min and titrated with blood gas results. Management principles include periodic reduction of ventilator settings to minimize ventilator-associated lung injury by decreasing rate (<10 breaths/min), tidal volume (<6 mL/kg), plateau inspiratory pressures (<20 mmHg), with an increase in positive end expiratory pressure (PEEP; >10 mmHg) in pressure control modes if necessary. Ventilatory settings can be reduced to minimal lung protective settings within 2 h of iLA initiation without hemodynamic instability. iLA is able to significantly improve respiratory parameters, decrease levels of inflammatory cytokines, and improve Murray ARDS scores. Anticoagulation to maintain a partial prothrombin time of 50–60 s will generally prevent clotting of the NovaLung membrane. Additionally, a MAP of >60 mmHg is required to maintain flow through the NovaLung and should be maintained with intravenous fluid or vasopressors if necessary. Similar to weaning ventilatory support, as respiratory function returns, gas flows can be decreased. Decannulation and removal of the NovaLung should only be considered when the patient can maintain >3 h of respiration with no sweep gas flow either with or without mechanical ventilation [31].

Paracorporeal artificial lungs

As ECMO has proven ability to sustain acute lung injury patients to recovery, rescue transplant patients with PGD, and bridge patients to transplantation, future indications for ECMO may include bridge to a paracorporeal artificial lung (PAL) device capable of extended or "destination" use as modeled in ventricular assist device therapy for heart failure [6]. Various PALs have been developed, with indications in both end-stage respiratory failure not amenable to transplantation and as a bridge to lung transplant. A PAL device has the potential to allow ambulation, rehabilitation,

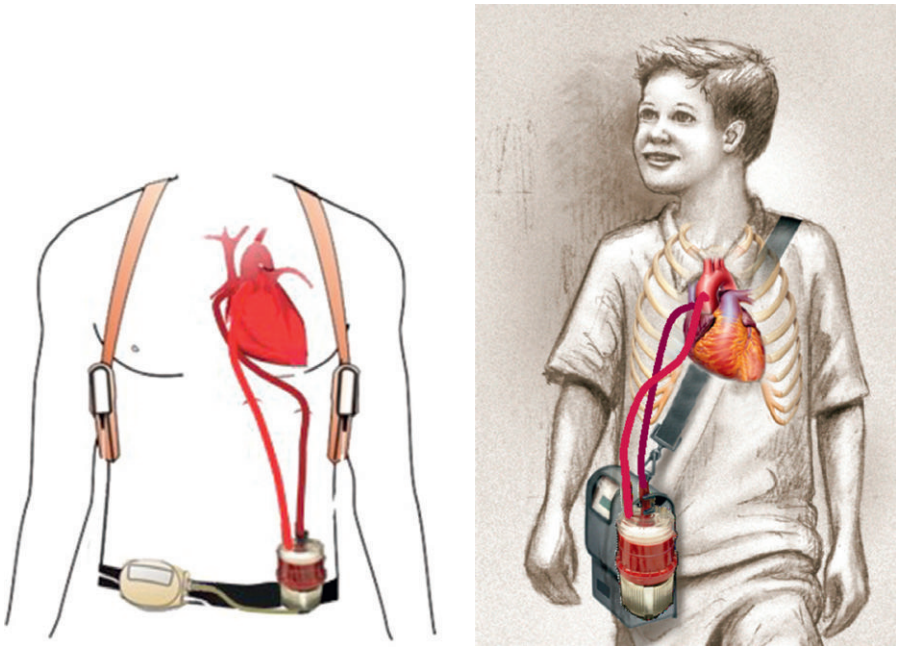

Figure 50.3. Ambulatory paracorporeal artificial lung.

and discharge from the hospital in patients who would otherwise be confined to hospital on ECMO [1].

Factors in the design of new and smaller devices must include maintenance of blood pressures across the device to prevent clotting. While this is most simply achieved with the use of a blood pump, pumpless devices using the heart to generate flow have also been shown to maintain consistent device flows [32,33]. However, the pump or external circuit itself cannot expose the heart to extensive resistive pressures as these will inevitably lead to right heart failure, as seen in animal studies of pumpless devices despite the addition of inflow compliance chambers or valves modifying the degree of cardiac output reaching the device [33]. In the setting of bridge to transplantation, perhaps the best device resistance imposed on the right heart would be that of healthy lungs [34]. Cannulation approaches must also consider the strain put on the right heart, as can happen with in-series cannulation [33]. Concerns regarding an in-parallel cannulation method are bypass of the lung's metabolic and filtering functions, as well as the threat of systemic thromboemboli from the device [32]. Shear stress through the device requires optimization, as high shear stress leads to blood cell damage, hemolysis, platelet activation, and consumption, but low shear stress leads to coagulation activation, fibrin deposition, and thrombosis in the device [32]. Activation of inflammation is a biocompatibility concern that will also require optimization before human use. Additionally, while the paracorporeal position allows device change and maintenance, the goal should be for the device to be used for the entire duration of support.

Various studies in both healthy and disease model sheep have demonstrated the capability of PAL devices to provide respiratory assist, with durability of up to 30 days [32]. Initial animal studies using PAL devices found a high incidence of right heart failure with in-series cannulation (PA-PA). Therefore, parallel (PA-LA) configurations providing lower circuit resistance may be the preferred approach to long-term PAL implantation [33]. Feasibility in long-term gas exchange was seen using PA-LA oriented pumpless systems, similar to iLA, as this technology was extended to human use [34,35]. Risks of this device, as demonstrated in animals, include embolic events and device failure, the latter corrected with relatively easy replacement [34]. These risks have improved with the use of heparin-coated components, but likely derive from the alternating use of a pumpless oxygenator in a low-pressure orientation rather than the systemic atrioventricular (A-V) orientation that is successful in iLA. Initial poor results and incidence of right heart failure with in-series pumpless devices [33] have been improved with both in-parallel cannulation approaches [34] and the incorporation of a centrifugal pump into a PAL device [36] (Figure 50.3). Using an integrated pump lung, 30-day animal studies establish the feasibility of such a device in humans. Implanted between the RA and PA, the artificial pump lung can serve to both unload volume to the right heart while maintaining total flow through the lungs and decreasing the risk of systemic thromboembolus. With a compact size and low priming volume, the integrated pump lung has demonstrated the ability to maintain oxygenation and normal end-organ function without significant hemolysis, or platelet or white blood cell activation [36]. These promising results, as well as the initial success of the pumpless PAL in patients, establish the potential for long-term mechanical respiratory support.

In 2008, initial use of a PAL device as a bridge to transplantation was reported in a patient with severe primary pulmonary hypertension, allowing survival for 62 days until donor lungs became available. Thoracic PA-LA cannulation allowed in-parallel shunting of blood at 2–4 L/min through the pumpless oxygenator (iLA), while avoiding femoral vessel cannulation. The patient was emergently placed on ECMO as a result of ARDS, right heart failure, and cardiac arrest following a cholecystectomy. The paracorporeal device was implanted after 9 days of ECMO and was associated with reversal of liver and kidney failure, and protective ventilation allowed slow normalization of gas exchange, followed

by extubation, ambulation, and thus a bridge to successful double lung transplantation [1]. This novel treatment for decompensating pulmonary hypertension and right heart failure was then successfully extended to four patients with pulmonary veno-occlusive disease (PVOD). With the same PA-LA cannulation and pumpless membrane oxygenator, patients were supported for between 8 and 21 days prior to transplantation and improvements in oxygenation, ventilation, cardiogenic shock, acidosis, and kidney and liver function were seen. Two patients were briefly supported with V-A ECMO through induction of anesthesia, one was able to be extubated with the PAL device, one required device change, and three regained full right heart function. Sepsis and multiorgan failure led to mortality in one patient following transplant; otherwise there were no complications [35]. Given the high mortality of these patients while waiting for transplantation and their reliance on V-A ECMO, these successful attempts at bridge to transplantation demonstrate a novel ability to recover the right heart function and ease refractory pulmonary hypertension using a PAL device. Our own program has been interested in ECMO for ARDS and a V-V or RA-PA central cannulation. Since 2009, we have supported 57 patients with an overall 54% survival. Of interest, eight of 11 who survived once transplanted were discharged from the hospital with an average of 53 days of ECMO support. Clearly, prolonged lung support is increasingly possible and extended bridge platforms are in evolution.

Summary

Artificial lung support has advanced significantly in the past two decades to include traditional ECMO as well as numerous novel devices to assist with oxygen delivery and CO_2 removal. These are important adjuncts to the transplant pulmonologist and surgeon seeking to provide bridge support either to transplantation, transplant recovery, or retransplantation. While not practical as destination therapy, newer devices are allowing for remarkable degrees of ambulation and rehabilitation, and it is likely that permanent artificial lung support will be an emerging option for patients with end-stage lung failure.

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CHAPTER 51

The Artificial Pancreas

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Introduction

No treatment has been shown to prevent type 1 diabetes in humans [1]. Without a means to prevent the disease, islet [2] or pancreas/kidney transplantations [3] are thought of as possible cures, but neither option is widely available as there are insufficient organ donors. Engineered cell lines [4], stem cells [5], or xenotransplantation [6] may overcome the islet shortage, but the timeline is unclear and the recipient may still require immunosuppressive therapy, which can have significant associated risks [7]. Although the benefits of good glucose control have been well documented [8–10], studies have shown that achieving the required level of control is difficult [11]. Sensor-augmented pump (SAP) therapy, leading to a closed loop artificial pancreas (AP), has thus emerged as a leading contender in the quest to reduce the burden and improve the health of individuals with this condition. Its consideration in a textbook of transplantation is appropriate as a potential future state for the field, potentially limiting the need for pancreas or islet transplantation or providing an alternative to transplantation for patients in need of glucose control.

The AP is envisioned as a fully automated system that can regulate glycemia through insulin titration based on a glucose sensor feeding information to a control algorithm [12]. This work has been at the forefront of research in the diabetes scientific community for almost four decades [13]. Early closed loop AP systems employed intravenous (IV) glucose sensing and insulin delivery methods. Miles Laboratory (Elkhart, IN, USA) commercialized the first AP in 1979 for intended use in the hospital setting and received US Food and Drug Administration (FDA) approval in 1983. The device, known as the Biostator, performed whole blood measurements by extracorporeal online analysis with an electrochemical sensor [14] and achieved glucose regulation by classical control techniques using a proportional-derivative algorithm. While the system demonstrated tight glycemic control and was extremely effective when used to perform glucose clamp experiments [15], its usefulness was limited to the clinical or laboratory environment. Other types of closed loop AP systems have emerged using the intraperitoneal (IP) insulin delivery route—one being a fully implanted system with a central intravenous glucose sensor placed close to the right atrium [16]. Although the IP system showed fast insulin kinetics, sensor latency affected control performance. The system was used in a hybrid configuration with a fully implanted IP insulin delivery pump and subcutaneous sensor [17]. An alternative to the fully implanted IP delivery pump is the DiaPort® (Roche Diagnostics, Mannheim, Germany) [18]—a port that is surgically implanted in the wall of the abdomen and is connected to an IP catheter that facilitates insulin delivery using an external pump. Although invented in 1999, this system has not been widely used, but has recently been adopted for closed loop AP studies.

Current research in the development effort toward an AP system has been mostly consumer oriented. Such a device must be suitable for ambulation and continuous wear, enabling patients to go about their daily activities. This system will likely employ interstitial fluid (ISF) glucose sensing [19] and continuous subcutaneous insulin infusion (CSII) methods. Developing algorithms for systems that measure ISF glucose and rely on CSII is made difficult by the time delays between ISF and plasma glucose [20], delays in the measuring device per se [21], and delays associated with subcutaneous insulin delivery [22]. Many of the challenges inherent in current commercially available devices have been reviewed in detail [23].

In this chapter, consumer-based AP systems are described for use outside of the hospital setting. The components of the systems include minimally invasive glucose sensing techniques in the interstitial compartment and CSII methods. Each component is described together with the particular system's performance and clinical utility. All references to the AP from this point forward will refer to subcutaneous (SC) glucose sensing and SC insulin infusion (SC-SC). The results discussed with this approach can be compared and contrasted with the results achieved through pancreas or islet transplantation, which are discussed in Chapters 107 and 61, respectively.

Glucose sensing

Minimally invasive SC glucose sensors are typically placed in the abdominal region and are used to continuously measure ISF glucose. This technology is widely believed to be the most likely to be adopted and used in the initial ambulatory systems worn by patients without supervision and outside of the clinical setting. The first commercially available continuous glucose monitoring (CGM) device approved by the FDA was the continuous glucose monitoring system CGMS® System Gold™ [24], launched in mid-1999 by MiniMed Inc., now Medtronic Diabetes. The original CGMS

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

device had the capacity to store 3 days of continuous glucose data measured with a sample acquisition time of 5 min. The data were downloaded to a PC where a software application was used to retrospectively analyze the data in a similar fashion to how electrocardiogram (ECG) signals are analyzed offline with a traditional cardiac Holter monitor. CGM was shown to capture significantly more glycemic variability. One study [25], using a CGM system developed by Roche Diagnostics, showed that 71% of hypoglycemic events were missed by subjects taking four fingerstick blood glucose samples per day and 58% of events were missed by subjects testing up to seven times per day.

This retrospective CGM technology developed by Medtronic Minimed was approved for real-time use in 2005 with the launch of the Guardian® REAL-Time [26], the first commercially available real-time CGM device. It displayed glucose values every 5 min, and provided hypo- and hyper-glycemic alerts. Efficacy of the system was demonstrated in a 12-week multicenter study [27] consisting of 156 stable type 1 patients [hemoglobin $A1_c$ ($HbA1_c$) >8.1%]: it enabled patients to decrease their $HbA1_c$ levels from 8.1% to 7.0%, with 26% of patients decreasing their $HbA1_c$ to below 6.1%. Hypoglycemia occurred once in each study arm and only in one case when the patient was wearing the device, despite a confirmatory fingerstick. The Guardian® REAL-Time system was followed by the approval of the STS™ system developed by Dexcom, Inc. (San Diego, CA, USA) for the US market. A study of that system, which included 91 patients, some with type 2 diabetes, showed that subjects using the system spent 21% less time hypoglycemic, 23% less time hyperglycemic, and 26% more time in the target range [28]. The Abbott FreeStyle® Navigator® (Abbott Diabetes Care, Alameda, CA, USA), which provides glucose samples every minute for a 5-day period and includes the FreeStyle BG meter, was introduced into the marketplace in 2008.

All of the initial studies showing the efficacy of CGM were conducted using sensor technology that has since improved. The clinical accuracy of the Dexcom SEVEN® Plus [29], the Abbott FreeStyle® Navigator® [30], Guardian®, and Paradigm® platforms [31] have been published in a number of scientific journals. However, the accuracy is improving, as are the manufacturing processes, with smaller more comfortable sensors with increased accuracy throughout the glycemic range [32] and better calibration algorithms [33,34]. Still, low error rates do not necessarily guarantee effective therapy and conversely, sensor error may not necessarily lead to unsafe therapy, as many therapy algorithms may be robust to sensor error.

The accuracy of current glucose sensors has been questioned in respect to enough accuracy for closed loop control. Several groups have already reported sufficiently accurate CGM performance to perform closed loop control. In particular, Hovorka et al. [35] demonstrated effective glucose regulation during the nocturnal period in a three-phase study that employed the Guardian® system in one phase and the Navigator® monitor in the following two phases. The study reported accuracies of 10% for the Guardian® monitor with YSI calibrations every 6 h and 12% for the FreeStyle® Navigator® meter using fingerstick calibrations. In a similar study, Castle et al. [36], also utilizing the Guardian® monitor in addition to the Dexcom SEVEN Plus system (Dexcom, San Diego, CA, USA), revealed highly accurate sensor performances during closed loop control. The combined dataset resulted in a mean absolute relative difference (MARD) of 8.7% when calibrating with YSI samples close to every 6 h.

Sensor augmented pump therapy

While no CGM technology has been approved as a replacement for blood glucose monitoring, the technology has been combined with insulin pump technology to create what is now known as SAP therapy. In this technology the sensor is still serving an adjunctive role, sounding alerts that can be verified by the user. The first major step toward the AP was the launch of Medtronic's Paradigm® REAL-Time system, which merged CGM with insulin pump therapy [37]. Although this system has all of the required components for closed loop control [38], the CGM and insulin delivery subsystems operate autonomously. Today, CGM and CSII have been successfully merged with four SAP therapy products sold worldwide—the most recent being the Animas® Vibe™ (Animas® Corp., West Chester, PA, USA), which is built upon the Animas insulin delivery technology integrated with the Dexcom G4™ CGM system with glucose sensing over a 7-day period, and the Medtronic Paradigm Veo™ insulin pump, which provides additional functionality, with increased accuracy at low glucose levels [33] enabling the addition of auto-suspend capabilities [39] that can reduce severe hypoglycemia.

Several landmark trials have successfully demonstrated the efficacy of SAP therapy. The STAR 1 trial [40] compared SAP and conventional CSII therapies in a 6-month randomized multicenter study (n = 146 pediatric and adult subjects) achieving an $HbA1_c$ reduction in both SAP cohorts (0.5% in adult). SAP therapy was compared to multiple daily injection (MDI) therapy in a number of studies, including the REAL Trend with 115 pediatric and adult subjects [41]; the Eurythmics with 83 adult subjects [42]; the ONSET study with 160 subjects aged between 1 and 16 years [43]; and STAR 3, the largest to date with 485 pediatric and adult subjects [44]. The REAL Trend study treatment arm attained close to a 1% reduction in $HbA1_c$, with the greatest improvements in subjects who wore a sensor at least 70% of the time. The Eurythmics trial over 6 months saw a reduction in $HbA1_c$ exceeding 1% with only 0.13% reduction in the control group—34% of subjects in the SAP group lowered their $HbA1_c$ to below the recommended 7%, whereas no subjects in the control arm reached this target. The ONSET trial showed lower $HbA1_c$ levels over a 15-month period for newly-diagnosed pediatric patients undergoing SAP therapy in comparison to MDI, and a lessening in the decline of C-peptide levels. Finally, the STAR 3 trial, demonstrated after a 1-year period $HbA1_c$ reductions of 0.8% (1% in adults) compared to 0.2% in the control group without increased incidence of hypoglycemia.

Paradigm Veo™ semi-closed loop system

A link between subsystems was established with the Paradigm® Veo™ insulin pump. This device was launched in 2009 in Europe and around the world outside of the US. The Veo™ insulin pump employs the same platform as the Paradigm® REAL-Time insulin pump with additional functionality that includes automatic suspension of insulin delivery beyond predefined low glucose thresholds, making it the first product that utilizes glucose sensor feedback to control insulin delivery, or in this case, suspend delivery.

This low-glucose suspend (LGS) capability is illustrated in Figure 51.1, demonstrating the functionality of the sensor in reducing the severity of hypoglycemia. This capability is not intended to prevent hypoglycemia, but to reduce the impact of any episodes missed by the user [45]. Therefore, when a low-glucose event occurs an alarm

will alert the subject and allow rescue carbohydrate to be consumed if necessary. In situations where the subject is not in a state to react to the hypoglycemic event or if an alarm has gone unnoticed, the system will respond by suspending insulin for 2 h, after which time normal basal insulin delivery is resumed.

Two examples of LGS activation are shown in Figure 51.2. In the first example, sensor glucose first crosses a hypoglycemia alert threshold between midnight and 2:00 AM, when two sensor alarms sound. However, the sensor reports glucose levels increasing back into the normoglycemic range. Sensor alarms sound again when the sensor drops below the low alarm threshold at 4:00 AM. Eventually, glucose falls below the LGS threshold, the LGS pump alarm sounds just before 5:00 AM, and insulin delivery is suspended when there is no response to the alarm. This auto-suspend function is for a 2-h period, unless there is user intervention. During this time period, glucose levels increase. With the resumption of basal insulin, glucose levels start to decrease again and a sensor low alert is triggered. Eventually, there is a second LGS activation between 10:30 AM and 11:00 AM for an additional 2-h period. Glucose concentration reaches safer levels following this activation. The effectiveness of this system has been demonstrated by retrospective analysis [46] of data collected from 935 patients totaling 49 867 days and noting 27 216 activations of the LGS feature, with 60% occurring during the afternoon or evening. The Veo™ insulin pump has demonstrated efficacy in a study of 21 patients [47] comparing the LGS functionality with a control arm with the LGS feature disabled: a reduction in the number of hypoglycemic excursions and a decrease in time spent in hypoglycemia were shown.

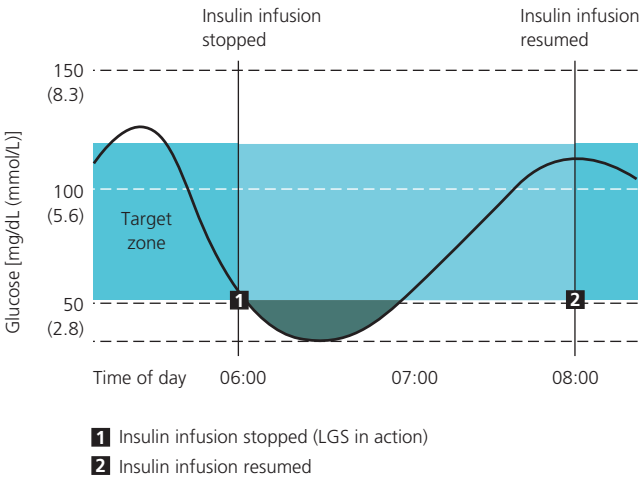

Figure 51.1. Low glucose suspend (LGS) feature of the Paradigm® Veo™ insulin pump. (Reproduced from Keenan et al.[39], with permission from PER Medical Media.)

Closed loop research

Medtronic Minimed approached the closed loop algorithm development problem by developing an algorithm emulating the beta-cell. This approach, termed external physiologic insulin delivery (ePID), was argued to be optimal based on the clinical consequences arising from the loss of various aspects of the beta-cell response [48], e.g. the loss of first-phase insulin release that often precedes type 2 diabetes. Characteristics of the beta-cell thought to be most important include not just first-phase insulin release, but also the slow rise in insulin secretion attributed to second-phase release, the cephalic phase associated with the anticipated consumption of a meal, and the effect of insulin per se to inhibit

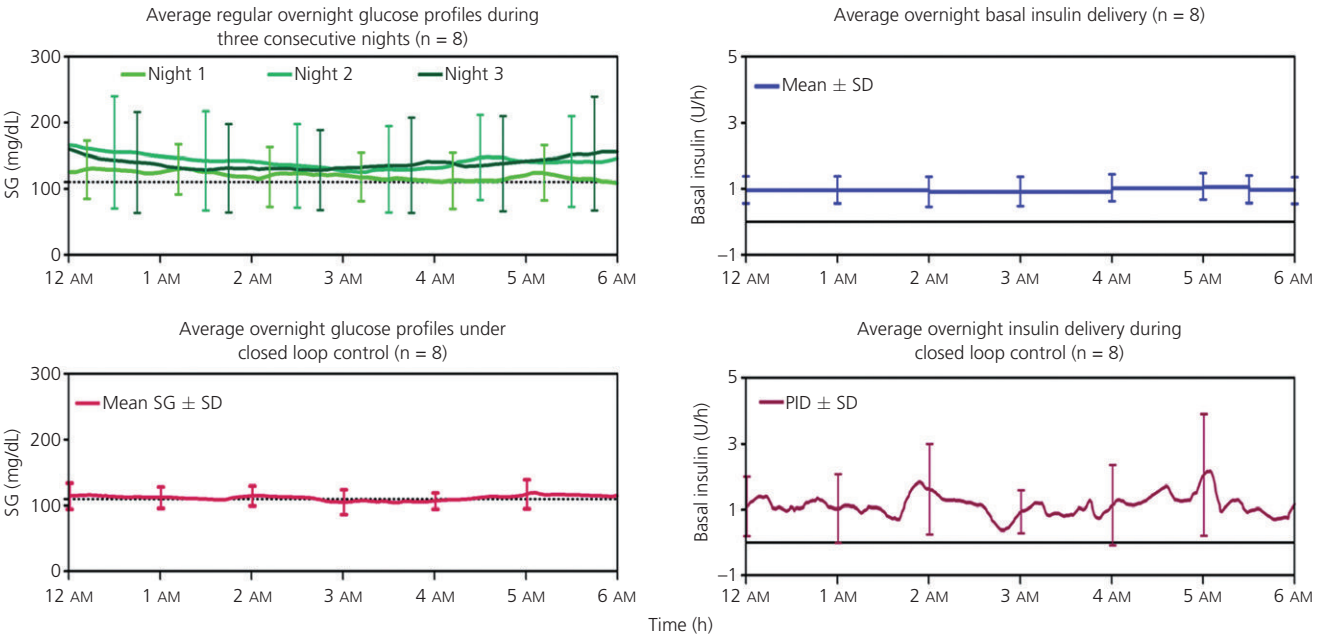

Figure 51.2. Example of the low glucose suspend (LGS) functionality of the Paradigm® Veo™ semi-closed loop insulin pump. SG, sensor glucose; PID, physiologic insulin delivery.

insulin secretion. Other putative beta-cell characteristics, such as the effect of prior exposure to glucose to accentuate the insulin response and pulsatile release, may also play significant—albeit less well understood roles—in maintaining normal glucose tolerance.

Initial attempts to model the beta-cell were dominated by the observation that the beta-cell response to an acute elevation in plasma glucose (hyperglycemic clamp) could be well described by a classical proportional-integral-derivative control algorithm. The beta-cell response when a clamp is applied is broken into three components, and the relative amount of each component is estimated by fitting the data obtained in non-diabetic individuals with healthy beta-cells [48]. Comparing classical control techniques to beta-cell physiology, the first-phase insulin release closely resembles the controller derivative component where insulin is infused at a rate proportional to the positive rate-of-change of the sensor glucose signal. The controller integral component, which is fundamentally a moving average of the difference between the sensor glucose measurement and the target or desired glucose level, mimics the second-phase insulin release or basal rate. The challenge in adapting this response into a closed loop AP utilizing SC insulin delivery was to overcome the long delays associated with the SC site. This was initially achieved by adjusting the relative ratios of each phase—slowing down the rate of rise in the integral component and increasing the relative amount of insulin delivered in the derivative component. Initial studies in a diabetic canine model showed the approach yielded stable overnight control with a reasonable meal response [49]. A subsequent study in adult human subjects with type 1 diabetes [50] also showed good overnight control (defined as percent time at 80–120 mg/dL), but generated higher than desired 2-h postprandial breakfast responses and low nadir values following breakfast.

A cephalic-like phase was introduced into the algorithm by having the user manually administer a small meal bolus in advance of an anticipated meal [51]. The modification was evaluated by Weinzimer et al. [52] in 17 adolescents under closed loop control for 34 h. Subjects who were assigned to the semi-closed loop arm ($n = 9$) received a priming bolus 15 min prior to food intake and the remaining subjects ($n = 8$) underwent fully closed loop control with no meal announcement. A significant reduction in postprandial mean glucose concentration was achieved (194 ± 47 vs. 226 ± 51 mg/dL). Overall mean glucose concentrations were 135 mg/dL in the semi-closed loop arm and 141 mg/dL in the fully closed loop arm. This study demonstrated tight overnight glucose control; however, there were two incidents of hypoglycemia in the semi-closed loop arm and one in the fully closed loop arm, all between the hours of 11:00 PM and 01:00 AM. Insulin feedback was later introduced to emulate the effect of insulin to inhibit insulin secretion, with the mechanism adapted for use with SC insulin delivery using an approach referred to as "pole placement" [53] and subsequently evaluated in a clinical study of adults with type 1 diabetes [54].

With so many choices of methods to improve the response, it becomes difficult to systematically evaluate each one via clinical protocols. One commonly used approach, traditionally employed in control systems to address this problem, is to develop a mathematical model of the system being controlled and then to use the model to evaluate the performance of proposed controller designs—a process now termed silico, or virtual, closed loop research. This requires the dynamics of the system being controlled to be well understood. A review of the metabolic literature in this area reveals that many models have been proposed, but no real consensus has emerged as to which is most appropriate [51]. Also, for any individual model, the model parameters need to be either identified from existing data or assumed using values from a variety of different sources obtained under varying conditions and patient populations. To deal with such issues, approaches that apply a series of reasonably well-accepted submodels such as the Bergman minimal model of glucose kinetics, which is a simple two-compartment model of insulin absorption and meal absorption, were combined with a model of SC–ISF glucose kinetics [54]. The combined model was then identified using the data obtained in the initial University of California, Los Angeles (UCLA) study [50]. The study protocol was then reproduced in silico (computer simulation). This yielded results virtually indistinguishable from the original in-vivo results. Based on the virtual patients ($n = 10$), we then simulated a study in which a premeal bolus was used to aid the control algorithm [55]. This resulted in a substantial lowering of peak postprandial glucose and improvement in post-meal nadir glucose. Silico models have been successfully applied to tuning control algorithms. In fact, the FDA has recognized a simulator developed by researchers at the University of Virginia and University Padova [56] to test closed loop algorithms prior to clinical studies. The University of Cambridge AP group has developed a metabolic model [57] with a number of virtual patients to test its closed loop control algorithm and sensor faults, a necessary step in achieving a reliable system for outpatient studies.

The research groups at the University of Virginia and University of Padova have been testing a closed loop controller in overnight studies with a model predictive control (MPC)-based algorithm employing a Navigator® sensor and Omnipod (Insulet Corp., Bedford, MA, USA) infusion system. This work, collaboration, and its progression have been reviewed by Cobelli et al. [58]. A US study [59] in eight subjects compared closed loop control to standard open loop control (Figure 51.3). The mean overnight blood glucose and time spent in range (70–180 mg/dL) of the closed loop control arm compared to the open loop control arm were 113.16 mg/dL versus 111.89 mg/dL and 81.69% and 96.94%, respectively. Additionally, there was significant reduction in the average number of overnight hypoglycemic events (1.63 vs. 0.13). The same system is being evaluated in two European centers [60] and one in the US that is evaluating 11 adolescents and 27 adults for 22-h in-clinic sessions that include an exercise period. Control algorithms under evaluation use a control-to-range approach with varied levels of optimization. The control-to-range method is not a fully closed algorithm and augments insulin delivery beyond predefined zones. In each implementation, time in control was increased with significant reductions in the incidence of hypoglycemia in comparison to the CSII control arm.

The closed loop control algorithms and systems described thus far have been for single hormone treatment of glycemia with insulin. The only mechanism to prevent hypoglycemia with this approach is to ensure optimal insulin delivery to a safe glucose range, thereby ceasing insulin delivery in time to avert a low glucose event. Should over-delivery happen, the only means of recovery requires human intervention. It has therefore been suggested that a fully endocrine AP should also incorporate an analog of glucagon. While a stable recumbent for the commercially available and one-time use Eli-Lilly glucagon does not exist at the present time, two groups have been successful in developing closed loop algorithms augmented with the glucagon used off-label.

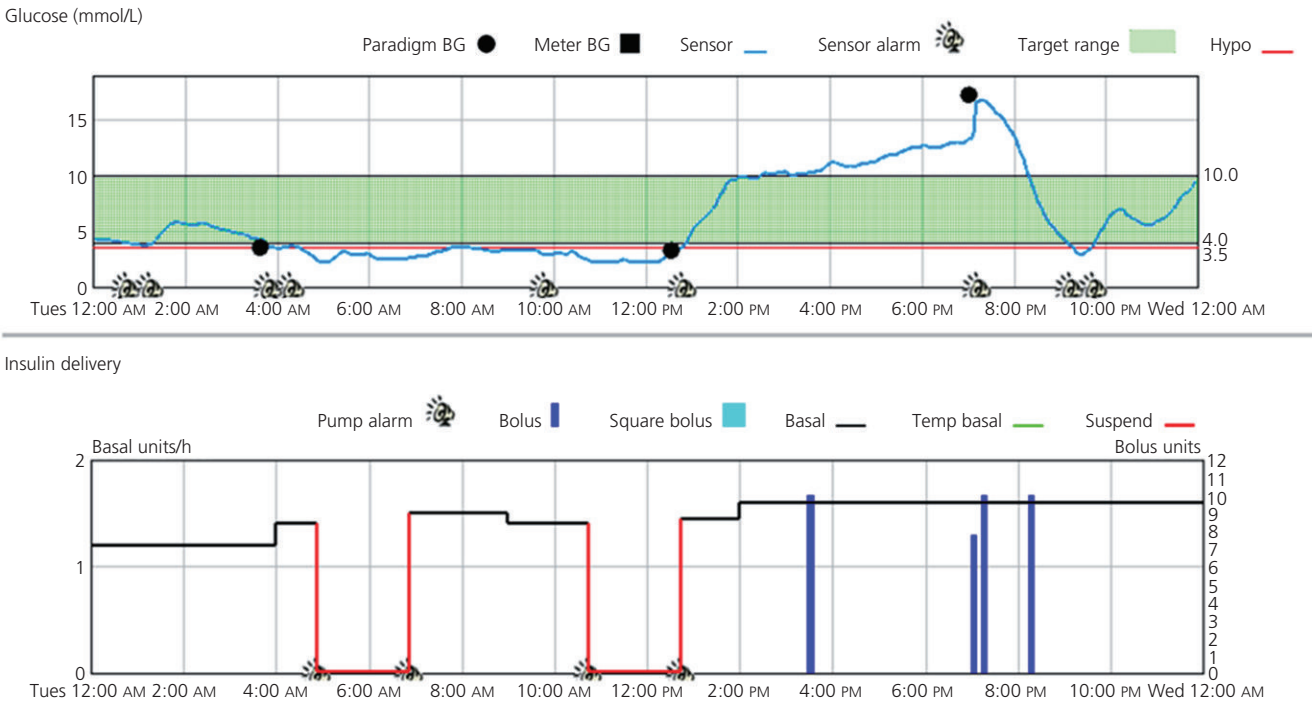

Figure 51.3. Outpatient overnight glycemia and inhospital nocturnal closed loop glucose control.

Researchers at Boston University have developed a glucagon-based controller. This system uses two Cozmo infusion pumps for dosing both insulin and glucagon. The GlucoScout® (International Biomedical, Austin, TX, USA) was used to measure plasma glucose concentrations every 5 min. The control algorithm uses MPC to calculate insulin dose and a proportional-derivative controller to determine the glucagon dosage [61]. In a recent study, 11 subjects were studied under closed loop control for 27 h during which they received three high-carbohydrate content meals [62]. A mean glucose concentration of 140 mg/dL was achieved for six subjects without the need for any intervention; however, five subjects required treatment for hypoglycemia. The control algorithm was then updated, and additional experiments with both groups produced no further hypoglycemic episodes, with a mean blood glucose concentration of 164 mg/dL. The addition of glucagon to help prevent hypoglycemia should also allow for more aggressive treatment with insulin, although the study had no insulin-only control arm; therefore, it is difficult to assess the full benefit provided by the glucagon component in achieving tighter control.

A group at the Oregon Health and Science University are using a Fading Memory Proportional Derivate (FMPD) algorithm [63] to calculate insulin and glucagon doses, basing control on either a Guardian® REAL-Time or SEVEN Plus sensor and insulin infusion with an Animas IR 1000 pump. In a recent paper [64], studies in 13 subjects, seven in whom glucagon was administered in a bolus fashion when required, and six subjects in whom glucagon was infused over a prolonged time period, were compared to insulin-only closed loop and placebo (n = 8). The addition of glucagon delivery when comparing the combined approaches to insulin plus placebo provided a 63% reduction in time spent hypoglycemic (15 ± 6 vs. 40 ± 10 min), with no significant difference in mean blood glucose concentration between the high-gain glucagon delivery and placebo (138 ± 17 vs. 131 ± 17 mg/dL). However, mean glucose was higher (157 ± 24 vs. 135 ± 16 mg/dL) for the low-gain glucagon delivery. Interestingly, the study demonstrated reduced frequency and time spent hypoglycemic with the addition of rapidly administered SC glucagon boluses, which more closely resembles physiology, than the low-gain arm with a higher total glucagon dosage.

The Cambridge AP group published randomized cross-over studies [35] that used the Guardian® REAL-Time system in one study and the FreeStyle® Navigator® CGM system in the proceeding studies, and hand entered new infusion rates into a Deltec Cozmo insulin pump (Smiths Medical, St. Paul, MN, USA) every 15 min. A total of 17 subjects underwent standard closed loop control (n = 12) or closed loop control following exercise (n = 9), and were compared with controls following meals with varied glycemic index (n = 6). The influence of each endeavor in challenging the MPC-based controller was observed during an overnight period. Median time spent in target range for open loop control was 52% versus 39% for standard closed loop, 78% versus 43% for exercise, and 53% for rapidly versus 55% for slowly absorbed meals. Throughout the 33 closed loop control nights, blood glucose only dropped twice to 3 mmol/L, both during the exercise study, in comparison to nine such events in the control arm. This algorithm was further tested with the intake of a large meal and alcohol [65] to further challenge control throughout the night. Compared to the control arm on standard therapy, time in control was increased by a median of 22% under closed loop control. In addition, the ability of the controller to maintain glucose homeostasis for a gestational diabetes cohort was evaluated in well-controlled pregnant women [66,67]. In comparison to CSII, the closed loop control arm experienced less hypoglycemia.

Closed loop systems

The previously described ePID closed loop system was developed using the Medtronic technology. The first version was developed on a laptop computer system capable of receiving glucose sensor signals at 1-min intervals (modified from the 5-min product version), calculating an insulin delivery rate based on a proportional-integral-derivative control algorithm, and transmitting the insulin delivery rate back to the pump as a series of small (0.1 U) boluses. In 2001, a fully automated closed loop was performed in diabetic canines [49] and was subsequently evaluated in humans beginning in 2003 [50], marking the first SC-SC closed loop study and initial step toward an ambulatory consumer-based closed loop AP system. The system was later introduced into the pediatric population in 2005 [52] and into the hospital ICU in 2006 [68]. Insulin feedback was introduced to emulate the effect of insulin to inhibit insulin secretion [69,70].

A number of algorithms were subsequently tested; however, the patient was always tethered to the bedside using this laptop-based system. With the ubiquitous use of smartphones today, it was a natural progression to move the control from the laptop-based system to a BlackBerry. A closed loop study [71] consisting of eight subjects was piloted in 2011. The BlackBerry houses the control algorithm and communicates with a pump and sensor system through a translator device based on Bluetooth technology. All features of the BlackBerry smartphone were enabled, and real-time glucose and insulin data were transmitted every minute to a central server. Remote monitors could log on to the server and monitor closed loop studies in real-time from anywhere in the world. This type of system is intended to provide a bridge to at-home studies. Currently, a number of similar closed loop systems are under development employing smartphone technology such as the Android and iPhone.

Nocturnal closed loop systems

To date two methodological approaches adopted for closed loop glucose control have dominated research into closed loop systems—the classical proportional-integral-derivative algorithms and the more contemporary MPC-based methods [12]. Regardless of the control approach implemented, each technique is challenged by the same disturbances, such as meals and exercise. Therefore, many studies have focused on the overnight period, which is a logical time period to study as an intermediate step toward the fully closed loop for 24-h use. Nocturnal closed loop control could provide significant patient benefit given the prevalence of nocturnal hypoglycemia, with studies reporting 55% of severe incidences occurring during sleep [72] and 75% of pediatric seizures happening at night [73]. Moreover, Buckingham et al. [74] reported 71% of nocturnal CGM alarms elicited no patient response.

Due to the multitude of control challenges inherent in any consumer-based closed loop AP system, most of which are heightened throughout the day, it is probable that the first AP will be labeled for nocturnal use only. Throughout this period when subjects are not ambulatory there are considerably fewer control disturbances to deal with, such as food intake and high levels of activity. Maintaining tight control is therefore less challenging.

Summary

The closed loop AP, with the benefit of current medical device technologies and advances in glucose sensing and insulin delivery, has been shown to achieve normal glycemia, particularly during the overnight hours. While efficacy has been demonstrated in a supervised controlled environment, before such as system can be made available to the consumer, a high level of safety must be demonstrated in outpatient studies. Current efforts have shifted from only focusing on control performance to the inclusion of fault detection and handling routines. Smartphone technology may provide the necessary bridge to outpatient studies, where a subject can be monitored in their home environment. This is a necessary step in advancing current research toward the commercialization of the closed loop AP.

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CHAPTER 52

Tissue and Organ Bioengineering

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Introduction

Broadly, the goal of regenerative medicine is to harness the body's capacity for complex development, regeneration, and healing to provide therapeutic interventions directed toward diseases of damaged, non-functional, or absent organs and organ systems [1]. Tissue engineering is a subset of regenerative medicine that seeks to build complex biologic structures that can recapitulate the form and function of natural tissues [2]. The building process may employ the cells' own capacity for self-organization into complex structures, or alternatively may involve significant structural organization in the laboratory to obtain the desired tissue configuration.

Given the success of traditional transplant medicine in the treatment of the failure of solid organs such as the liver and kidney, hollow viscera including the small bowel, components of the eye including the cornea, the heart, the airway and lungs, as well as significant recent advances such as hand and whole face transplants, the potential utility of tissue engineering might be questioned. However, as detailed throughout this textbook, transplantation is as defined by its limitations as it is by its triumphs. Indeed, all transplant therapies depend critically upon the availability of donor organs, which are limiting in all applications. Furthermore, once transplant surgery is performed, the graft is prone to rejection by the host, necessitating a lifetime of costly and morbid immunosuppressive drug therapy. Patient non-compliance with an immunosuppression regimen may result in rejection, failure, and loss of the graft, which then requires a relisting for transplant and another long course of bridging therapies. For all these reasons, tissue engineering, as a subset of regenerative medicine, is an attractive alternative, and is appropriately considered in a textbook of transplantation as a future companion or alternative therapeutic option for patients with end-organ failure.

To be successful, tissue engineering requires an interdisciplinary approach that includes input from surgeons, physicians, materials scientists, bioengineers, and others. Since the initial unsuccessful attempt by Green in the 1970s to produce cartilage by seeding chondrocytes onto bone spicules, it has become clear that the materials and scaffold used to build tissue and organs are critical to the success of the tissue engineering approach (Figure 52.1). The first true attempts at generating an appropriate synthetic scaffold from biocompatible materials were undertaken by Vacanti and Langer of Boston Children's Hospital and Massachusetts Institute of Technology (MIT), respectively, who recognized the inherent unpredictability and irreproducibility of biologically-derived materials. In the mid 1980s, they developed scaffolds based upon biodegradable polymers, including polyglycolic acid (PGA) and polylactic acid (PLA).

Tissue and organ-specific approaches

Cornea

The human cornea is a transparent tissue that is critical for vision, as it causes refraction of light rays incident to the eye for focusing and formation of an image on the retina. It is highly organized, with five layers totaling about half a millimeter in thickness. The cornea is lined on the anterior side by a stratified squamous epithelium for protection, and the inner endothelial layer regulates the hydration status of the cornea by continuously pumping fluid out and into the anterior chamber of the eye [3]. Corneal endothelium consists of a terminally differentiated cell population that cannot renew itself with age or after injury [4]. Therefore, in the setting of declining visual acuity secondary to a damaged, diseased, or aged cornea, the only curative treatment is transplantation of a full-thickness corneal allograft [5]. First performed in 1905 by Zirm [6], this procedure is highly successful in the short term, but 5- and 10-year survival of corneal grafts has been reported to be only 74% and 64%, respectively [7]. Moreover, because of the increasing survival of older adults in industrialized nations [8], demand for corneal grafts is predicted to continue to increase over the 21st century. Despite the fact that a single cornea may be divided into up to three portions using a microkeratome for transplantation of the separate components [9], the demand will almost certainly continue to outstrip supply.

Although human corneal endothelial cells (HCECs) do not undergo regeneration in vivo, they have been successfully grown in vitro. Initial isolation techniques entailed explant culture followed by selective migration of the HCECs [10,11], and evolved into a scraping method, by which the HCECs are first removed mechanically from the adjacent Descemet's membrane (DM) of the cornea [12,13]. More recently, this has been further refined into a dissection and digestion strategy, whereby the DM and its attached HCECs are isolated and digested enzymatically with collagenase, dispase, or trypsin/ethylenediaminetetraacetic acid (EDTA) [14–16]. However, this method results in the unwanted introduction of

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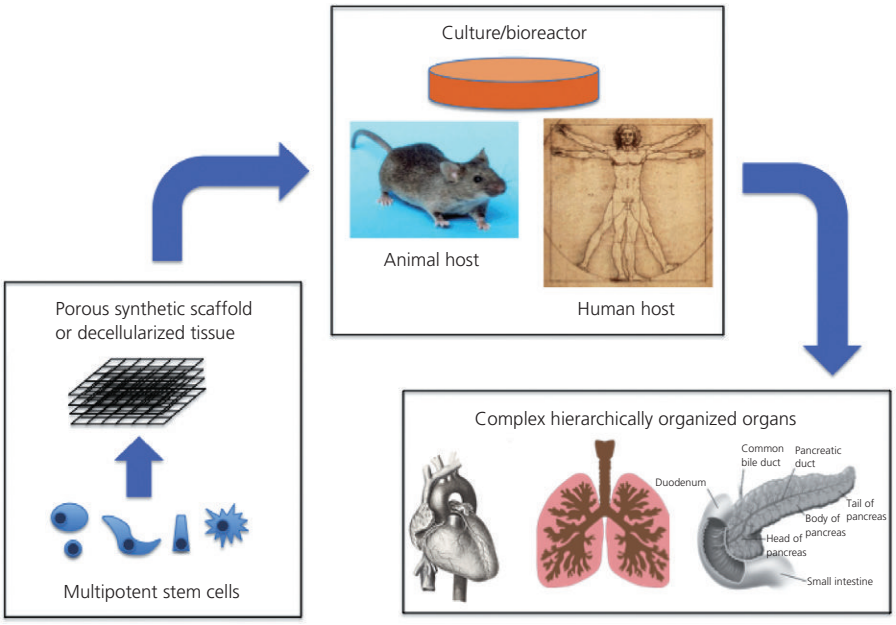

Figure 52.1. Schematic of the process for tissue engineering. In general, a biodegradable porous scaffold or decellularized native tissue is used to support the growth of a multipotent stem cell population. The loaded scaffold is characterized in vitro or implanted into an animal or human host, resulting in tissue ingrowth, proliferation of loaded cells, and the development of a complex organ.

fibroblastic stromal keratocytes into the culture, which must be suppressed with appropriate culture medium [17,18]. Contamination by keratocytes can be minimized with increased dissection skill and techniques such as securing the cornea for dissection with a vacuum suction cup [19,20]. Once isolated, the HCECs are normally grown in plates with an appropriately coated surface for attachment and proliferation. Effective coatings have included collagen [14], laminin/chondroitin sulfate [18], and the commercially available FNC coating mix (Biological Research Faculty & Facility, Inc., Ijamsville, MD, USA), which contains fibronectin, collagen, and albumin [21]. Some authors have suggested that coatings are unnecessary, however [16].

Heart

To perform its function of pumping blood to the systemic and pulmonary circuits, the heart requires a complex structure that facilitates electrical and mechanical coordination between the atria and ventricles, a reliable intrinsic pacemaker that can be regulated by neural and circulating factors, and specialized cell populations to perform these functions [22]. Cardiovascular disease remains the number one cause of death in western industrialized nations, with the majority of deaths attributable to myocardial ischemic disease [23]. Further, congenital heart defects are common, affecting approximately 1% of newborns [24]. It has traditionally been thought that the myocardium consists of terminally differentiated cells with no capacity for regeneration [25]. However, the existence of so-called cardiac progenitor cells (CPCs), that express markers such as c-kit and KI-67, and early cardiac lineage transcription factors such as GATA-4 and Nkx2.5, has been described [26].

Unfortunately, these cells proliferate poorly in vitro [27]. Therefore, other cell sources have been employed in attempts to facilitate myocardial repair and regeneration, including bone marrow-derived angioblasts [28], skeletal myoblasts [29], and neonatal rat primary cardiomyocytes [30]. In addition, stem cells from a number of sources, including embryonic stem cells, adult bone marrow stem cells, and umbilical cord stem cells, have been employed [31]. Another approach has been to place single cells from the various populations that make up the myocardium, including cardiomyocytes, endothelial cells, and fibroblasts, into culture in varying proportions to produce "cardiac organoids" with rudimentary electrophysiologic properties [32]. Some of the initial applications of these cell populations to myocardial tissue engineering have focused on the scaffold materials used [33,34]. Groups have employed a variety of materials, including collagen [35], Matrigel–collagen mixtures [36,37], polyglycolic acid [38], and alginate [39,40].

To move beyond the cell and organoid approach and optimization of in-vitro tissues, animal models for surgical intervention with tissue-engineered myocardium after infarction have been designed and studied. In rats, cardiac patch material was bioengineered from seeded alginate scaffolds that were matured within a host animal's peritoneal cavity. These patches sealed an experimentally created defect in a heterotopically transplanted donor heart in a second host in a separate operation. The patched hearts demonstrated grossly normal echocardiographic wall motion and function [41]. In a further study, the same group implanted a similar alginate material loaded with neonatal rat myocardial cells, Matrigel, and growth factors into a host rat omentum for 5 days to induce neovascularization, after which the patches were grafted to infarcted myocardium in separate host animals. There was decreased ventricular remodeling compared to controls and electrical coupling of the patch to the host animal myocardium [42]. In rabbits, another group seeded porcine submucosa with mesenchymal stem cells (MSCs), cultured it for 5 days, explanted it, and then implanted it as a free graft to the site of an experimentally created myocardial infarction, after which incorporation into the myocardial wall, decreased wall thinning, and decreased left ventricular dilation were seen relative to controls [43].

Airway and lungs

The airway and lungs serve to perform gas exchange and are intimately related to the cardiovascular system. The adult human lung transports about 10^4 L, or 10 m^3, of air per day and has an aggregate surface area of approximately 70 m^2 [44]. The trachea and mainstem bronchi are essentially conductance airways leading to the deeper airways that consist of tubular structures with varying amounts of cartilage and connective tissue. Primary disease of the trachea is rare, but it can be narrowed by mass effects, metastatic ingrowth of neoplastic processes of the thyroid, esophagus, lung, and mediastinum, or injury [45]. About 5 cm of the adult trachea can be resected with primary anastomosis, and in children, the equivalent figure is about a third of the total length. Disease of longer segments may require stenting or management with a tracheostomy. Some attempts have been made at replacement therapy of the trachea, with materials such as collagen scaffolds augmented by silicone stents or cartilaginous tubes grown in vitro [46]. However, these approaches were unsuccessful because of a lack of epithelialization that led to stricture, or inadequate mechanical strength resulting in collapse or tracheomalacia [47]. An additional problem of transplanting tissue-engineered organs is that without native blood supply, necrosis occurs, and cartilage may "melt" as the tissue is resorbed.

Because of these failures and the complexity of the hierarchical organization of cartilage and connective tissue in the trachea and mainstem bronchi, it was first believed that a synthetic scaffold approach would not succeed. Therefore, attention was turned to using the trachea itself as the scaffold. This is accomplished by decellularization, where the cellular components of the tissue are removed by washing with detergent solutions, leaving only the connective tissue skeleton [48]. In theory, all immunogenic properties of the original tissue are removed, allowing it to be used as a biologically derived scaffold supporting the patient's own cells. In a landmark paper in the *Lancet* in 2008, Macchiarini et al. successfully implanted a tissue-engineered tracheal segment to replace the left mainstem bronchus in a human patient with end-stage bronchomalacia (Figure 52.2) [49].

Given this preliminary clinical success with tissue-engineered trachea, other groups are pursuing tissue engineering of the entire human lung. The three-dimensional structure of the lung is complex and exhibits hierarchical relationships at multiple size scales. Grossly, the lungs comprise a defined lobar and segmental architecture with closely related systemic and pulmonary vascular organization. On an intermediate scale, the airways themselves undergo approximately 16 bifurcations from the level of the carina to the most terminal bronchioles. On the smallest scales, alveoli contain gas exchange apparatus in close apposition to specialized pulmonary capillaries to allow oxidation and ventilation. Therefore, engineering of the lung in toto presents a significant challenge. Diseases causing end-stage destruction of lung parenchyma include idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), cystic fibrosis, and chronic lung disease of childhood [50,51]. Although patients with these conditions can benefit from lobar or whole lung transplant, there is a shortage of donor lungs, as is the case with the solid organs and intestine. Regenerative medicine approaches to treatment of end-stage lung parenchymal disease have included both cell-based therapies and tissue engineering. However, the former is beyond the scope of this chapter and we focus here on tissue engineering.

Given the lung's complex structure, preliminary tissue engineering approaches for this organ have focused in large measure upon the scaffold upon which lung stem cells are placed [52]. Stem cells of the human lung have recently been identified and express the putative lung stem cell marker c-kit [53]. Naturally derived materials that have been used for tissue engineering include collagen [54], Matrigel [55], Gelfoam [56], and Englebreth-Holms tumor basement membrane [57]. Synthetic polymers have also been employed, including polyglycolic acid (PGA) [58], poly(lactic-co-glycolic acid) (PLGA), and poly-L-lactic acid (PLLA; Figure 52.3) [59]. Notable results from these approaches include the implantation of somatic lung progenitor cell (SLPC)-loaded PGA constructs into sheep [60]. In this work, SLPC-loaded PGA and Pluronic F-127 (PF-127) scaffolds were implanted into the right upper lobe of wedge-resected and pneumonectomized sheep. After 6 weeks, the scaffolds were noted to support some soft tissue growth, and alveolus-like structures were seen to form from the loaded PF-127

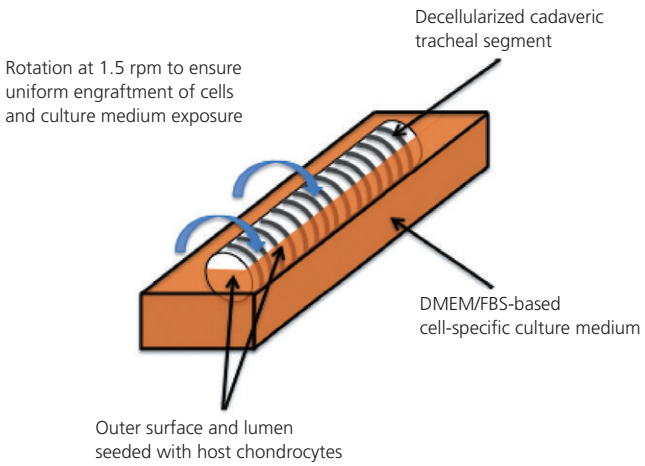

Figure 52.2. Bioreactor system for producing human tissue-engineered trachea from a decellularized cadaveric airway segment. (Adapted from [49]. Copyright © 2008, with permission from Elsevier).

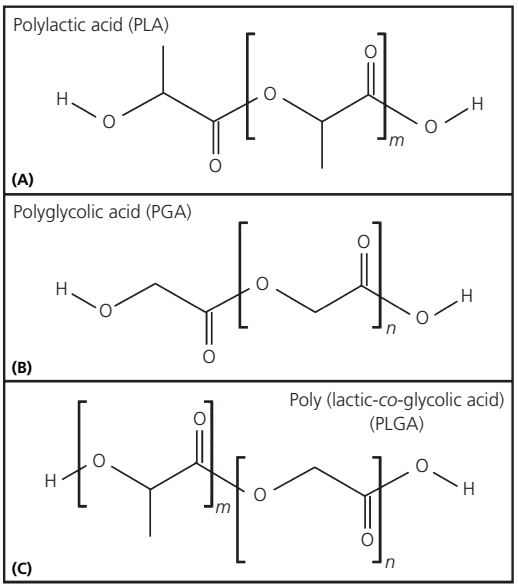

Figure 52.3. Molecular structures for biodegradable polymers commonly used in tissue engineering. (A) Polylactic acid (PLLA). (B) Polyglycolic acid (PGA). (C) Poly(lactic-co-glycolic acid) (PLGA).

scaffolds. Another study described the implantation of fetal rat-derived cell-loaded Gelfoam into the lung parenchyma of adult rats and harvest after 35–100 days. These authors noted the formation of alveolar-like structures near the edges of the implanted sponges, but fewer in the interior [55]. When fetal pulmonary cells (FPCs) were placed in Matrigel and implanted subcutaneously into the abdominal wall of a mouse host, lung epithelia and vascularization of the construct were seen [54].

Some of the most significant remaining challenges to whole lung engineering include selection of the appropriate cell population for seeding and ensuring its HLA compatibility with a potential patient, construction of a scaffold that combines structural integrity and physiologic stress–strain characteristics with cell compatibility, and the appropriate in-vitro growth factors and culture conditions to cause appropriate differentiation of the chosen precursor cell population into mature lung tissue [52].

Alimentary tract

The function of the alimentary tract, including the oropharynx, esophagus, stomach, duodenum, jejunum and ileum, cecum, and ascending, transverse and descending colon, is to transport and absorb the nutritive value of foodstuffs, which can be consumed orally or can be given as enteral nutrition via surgically placed gastrostomy or enterostomy tubes. It also assists in regulating fluid homeostasis: in an adult human, approximately 1 L of saliva, 1.5 L of gastric fluid, 1 L of pancreatic fluid, 1 L of bile, and 2 L of small intestinal fluid are produced daily, and most of the water content of this secretion is reabsorbed by the colon. When a significant portion of the intestine is lost, whether due to infection, trauma, ischemia, resection for mesenteric neoplasia, or other etiologies, short bowel syndrome (SBS) may result.

SBS is a morbid condition caused by resection of a significant fraction of the total length of the small bowel, usually >50–75%, such that its absorptive capacity is severely reduced and the patient cannot obtain sufficient nourishment from enteral nutrition [61–63]. In children, the most common causes of SBS are massive small bowel resection secondary to necrotizing enterocolitis [64] or malrotation with midgut volvulus [65]. Also, though less common, SBS can occur in adults because of multiple bowel resections in the setting of Crohn's disease [66], or after mesenteric ischemia secondary to vascular disease or trauma such as gunshot wounds [67]. Because SBS patients cannot maintain sufficient nutrition with enteral intake, they may require long-term total parenteral nutrition, which itself can be complicated in children by liver failure and cirrhosis [68]. SBS patients therefore endure significant healthcare costs, recently estimated to be on the order of $1.6 million per patient over 5 years [69]. The current standard of care for intestinal failure secondary to SBS is intestinal, liver/intestinal, or other multivisceral transplantation, but this confers only a 60% 5-year survival and consigns the patient to a lifelong course of immunosuppressive therapy [70]. Further, limited donor organ availability results in an inevitable mismatch between supply and demand as well as long waiting times [71]. For these reasons, autologous tissue engineering of human intestine from the patient's own tissue is an attractive strategy, as it could potentially offer a durable, long-term cure for SBS with none of the drawbacks of existing therapies [72].

Much of the research undertaken toward tissue engineering of the intestine has focused upon the process of normal intestinal epithelial regeneration from proliferative and stem cell populations of the intestinal crypt, the location of the intestinal epithelial stem cell niche. The intestinal mucosal layer regenerates every 3–7 days in humans. Stem cells in the crypt divide, renewing themselves as well as giving rise to transit amplifying cells that travel up the villus axis and differentiate into four mature cell types in the small intestine (enterocytes, Paneth cells, goblet cells, and enteroendocrine cells) and three in the colon (colonocytes, goblet, and enteroendocrine cells). Clevers' group in the Netherlands first identified intestinal crypt-based columnar cells as the mammalian small intestinal stem cell population in the mouse, and demonstrated that they are marked by expression of the G protein receptor leucine-rich repeat G protein receptor 5 (Lgr5) [73,74]. Other markers that have been suggested for mammalian intestinal stem cells include DcamKL-1, Bmi1, Musashi-1, CD133, and mTert [75–79].

Some of the most successful work in intestinal tissue engineering has resulted from the transplantation of organoid units, multicellular units processed from native intestinal tissue that are seeded on a biodegradable scaffold, which is then implanted in a host animal for incubation. In the processing step, which is originally attributable to Evans' lab but has since been modified [80], the intestinal stem cell niche can be preserved despite mechanical and enzymatic digestion of the native intestinal tissue, because the multicellular aggregates formed, termed organoid units, maintain the microanatomic relationship between the stem cells in the crypt and the supporting mesenchyme that surrounds them. This technique has been applied with great success to the tissue engineering of esophagus, stomach, and small and large bowel in the rat [81–85]. The technique has also been transitioned to a mouse model (Figure 52.4) [86,87], in order to employ the extensive transgenic library

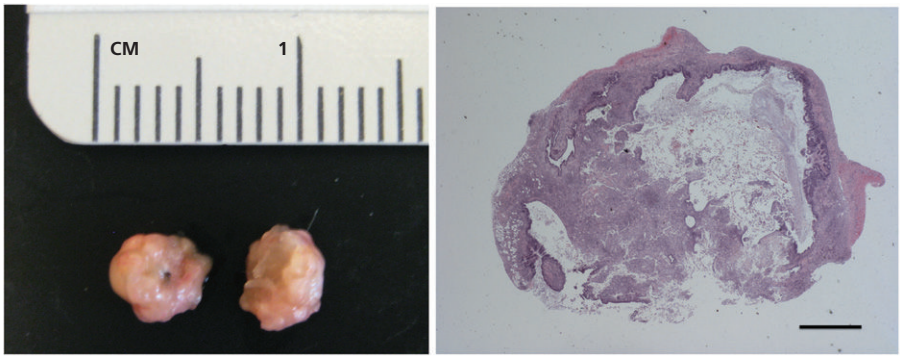

Figure 52.4. Tissue-engineered small intestine (TESI) produced in murine hosts as described in [86]. (A) Freshly-harvested TESI that has been bi-valved to show the mucosa and lumen. (B) Low-magnification H&E histology of a characteristic sample of mouse TESI. Scale bar: 100 μm.

available in *Mus musculus* to better understand the biochemistry and detailed mechanism of the organoid units/scaffold approach to intestinal tissue engineering. Similar results have also been reported in Yorkshire swine, validating this approach's feasibility in a large animal, pretranslational setting [88].

Liver

The human liver has myriad functions in digestion, excretion, detoxification, and protein production that are mediated by a structural–functional relationship of its dual blood supply, portal venous system, and network of lobules and hepatic artery/portal vein/bile duct triads. The supply–demand mismatch for this organ is significant: approximately 7000 liver transplants were performed in 2008, despite >17 000 patients being listed at that time [89]. Indications for transplantation are legion and include advanced chronic hepatic failure with Child–Pugh–Turcotte (CPT) score of >7, advanced Model for End-stage Liver Disease (MELD) score suitable for listing, acute fulminant hepatitis (viral or drug induced), biliary atresia, familial cholestasis, primary hepatic malignancy, biliary cirrhosis, primary sclerosing cholangitis, liver injury of cholestasis, and Budd–Chiari syndrome [90,91].

For adequate performance of its digestive and excretory functions, the liver must have an intact and patent connection to the extrahepatic biliary tree, as well as a functioning connection to the enteric lumen at the sphincter of Oddi in the duodenum. As with the lung, the main challenge in tissue engineering of the liver is therefore recapitulating its hierarchical structure over a wide range of length scales, from the large scale of segmental arteries, portal veins, and bile ducts, to the intermediate scale of lobules, to the smallest scale of the microscopic relationships between bile canaliculi, portal venules, and hepatic arterioles within the sinusoids. To confront this challenge, engineering approaches focus on the specific biomaterials used for scaffolding, as well as the appropriate cell populations for in-vitro expansion that also exhibit functional characteristics of the hepatocyte [92,93]. Three-dimensional microgels seeded with hepatocytes have the desirable properties of control of orientation as well as assembly into larger structures [94,95]. Another approach employs microfluidic channels, which more accurately simulate native liver sinusoids in terms of cell–cell interactions, flowing soluble factors, and three-dimensional mechanical forces, than two-dimensional systems [96]. Decellularized liver scaffolds have also been used to support hepatocytes ex vivo, which exhibited albumin and urea synthesis at levels similar to in-vitro sandwich culture, and expression of drug metabolism genes similar to normal liver tissue [97].

For source cells, both hepatocytes and a variety of stem cells have been investigated. Mature hepatocytes can be prepared either fresh or from cryopreserved tissue, but at least in the case of direct hepatocyte transplantation, cryopreserved cells engraft less well than fresh hepatocytes [98]. Hepatic progenitor cells have also been employed [99]. Stem cell approaches have included human embryonic stem cells (hESCs), which can be reliably expanded in culture [100]. Although differentiation of these cells into hepatocytes initially stops at a fetal hepatocyte stage that lacks mature expression of cytochrome P450 3A4, they can be further differentiated with the appropriate addition of cytokines to the culture medium [101]. Building upon recent advances with induced pluripotential stem (iPS) cells, stem cells that are derived from terminally differentiated somatic cells and have been "reprogrammed" to a pluripotent stem cell state [102], attempts have been made to differentiate human iPS cells into hepatocytes [103]. Finally, some work has focused on using iPS cells derived from patients with diseases including defective low density lipoprotein (LDL) receptor-mediated cholesterol uptake, misfolding of alpha-1 antitrypsin, and abnormally increased lipoprotein or glycogen storage, which can be differentiated into hepatocytes that exhibit in-vitro features of the same metabolic derangements, thereby providing patient-specific in-vitro disease models [104].

Kidney and urinary tract

The kidney is a highly specialized organ that serves to regulate overall fluid status, electrolyte levels, and blood pressure, and to excrete water-soluble toxins that are by-products of diet or metabolism into the urine. Its function is intricately related to the patency and muscular function of the ureters, bladder, and urethra to maintain normal urinary excretion. The incidence of end-stage renal disease (ESRD) continues to increase, with etiologies including diabetes mellitis, hypertension, and various glomerulopathies [105]. Although hemodialysis (HD) constitutes renal replacement therapy, it requires either catheter placement or permanent arteriovenous fistula creation [106], and results in significant complications, including derangements of serum calcium and calciphylaxis [107], cardiovascular disease [108], mechanical complications of extended indwelling catheters [109], and fistula failure and revision [110]. The alternative of peritoneal dialysis allows for increased patient independence as compared with HD [111], but confers the risk of infection and peritonitis [112] as well as dialysis catheter failure and reoperation [113].

In 2007 >90 000 US patients were on the renal transplant waitlist, while only slightly >16 000 transplantations were performed [114]. The ureters are prone to stricture from radiation, or iatrogenic trauma, and can be reconstructed using the bladder [115] or small intestine [116]. Distal to the kidney and ureter, bladder cancer resection can result in significant morbidity with the need for surgical urinary diversion [117].

As with other organ engineering approaches, the differentiation processes of stem cells, including embryonic stem cells (ESC) derived from early human embryos at the blastocyst stage [118], inducible pluripotent stem (iPS) cells [119], and MSCs, which can be isolated from bone marrow stroma [120], have been investigated in order to drive these cells toward a renal lineage. In-vitro culture systems of embryonic rat nephrons have produced branching ureteric buds and metanephric mesenchyme, which are primitive elements of the collecting system and nephron, respectively [121]. Initial efforts to make use of living renal cells incorporated them into extracorporeal synthetic scaffolds [122,123]. This approach has been applied in animals [124], as well as in a small human pilot trial [125]. However, these approaches do not constitute tissue engineering per se, the goal of which would be to construct the entire organ, or enough functioning tissue to replace the failed organ. Because of the complex three-dimensional structure of the kidney, there are significant challenges to using completely synthetic materials to develop a renal scaffold. Therefore, some work has focused upon decellularized scaffolds as candidates for cell engraftment, but to date, these experiments have been performed only in rats and monkeys [126,127]. In the rat experiments, murine pluripotent cells were placed on decellularized kidneys as a xenograft and exhibited rudimentary epithelial differentiation with primitive lumen formation. The Rhesus monkey experiments showed that fetal kidney explants could graft onto and recellularize scaffolds derived from unrelated donor kidneys.

By contrast, tissue-engineered urethras and bladders have already been applied to human therapy. Following early success using polyglycolic acid mesh scaffolds for urethral regeneration in animal models [128–130], attention was turned to bladder- and urethral-derived acellular submucosa matrices [131,132]. After success in animals, bladder submucosa constructs have been used to treat urethral strictures in humans [133,134]. Of note, these stricturoplasties were done in an onlay fashion with a non-seeded matrix, but when this approach was attempted for circumferential replacement of segmental sections of the entire urethra, postoperative strictures ensued [135]. To combat this, more recent efforts have focused upon the importance of the normal wound healing response to these engineered tissues, as well as the effect of first seeding the circumferential segments of matrix with autologous or exogenous cells [136].

Early efforts to augment the human bladder employed synthetic materials, such as polyvinyl sponge, Teflon, collagen, polyglycolic acid, and silicone. However, permanent materials resulted in mechanical failure and urinary stone formation, and biodegradable materials caused fibroblast deposition, scarring, graft contracture, and a reduced bladder volume [135–138]. In addition, when scaffolds of various materials were implanted without cell seeding, the result was preferential regeneration of the urothelial layer with an under-development of the other tissue layers [139]. More faithful reproduction of the muscularis and other layers was obtained when scaffold materials were first loaded or seeded with bladder cells [138,140]. A canine model was then developed where dogs underwent cystectomy followed by implantation of a tissue-engineered bladder prepared from bladder cell-seeded biodegradable polymers. In dogs in which tissue-engineered bladder was implanted, post-implantation bladder capacity and wall compliance were very similar to preoperative values [141]. Finally, the same approach was employed in a pilot study in human patients, but results were preliminary with only seven patients being included, and clinical studies are ongoing [142].

Extremities: bone, tendons, and ligaments

The upper extremities provide human beings with significant dexterity and function for the highly technical and skilled activities humans can perform with intact arms, joints, hands, and fingers. The lower extremities similarly provide us with the often underappreciated capacity for locomotion—walking, running, playing, competing. The axial skeleton, in turn, provides the structural support and points of attachment for the head and extremities. Therefore, since the advent of war and other sources of major trauma, physicians and surgeons have attempted to fashion appropriate prostheses for patients who have lost some of these functions. Tissue engineering approaches for bone, connective tissue, muscle, and the limbs themselves aim to completely restore these functions. Approximately 1 million orthopedic surgeries per year in the US involve bone repair for replacement, trauma, and developmental abnormalities [143]. Traditional reconstructive approaches have relied upon either autogenous or allogeneic bone grafts, and polymeric or metallic implants [144]. However, these approaches are limited by supply–demand mismatch and failures to integrate into surrounding host tissue, and allogeneic grafts in particular carry a risk of infectious disease transmission and rejection [145].

Bone tissue has a remarkable capacity for self-renewal, as it is well-known that multipotent MSCs capable of differentiation into osteoblasts are present in human bone marrow and periosteum [146]. Both skeletal development and fracture repair require a complicated, coordinated process including cell migration, differentiation, and activation, with the formation of a bone microvasculature being critical to the continued survival and regeneration of living bone tissue [147,148]. After these processes, mineralization to a solid bone structure must occur via one of two mechanisms; intramembranous or endochondral ossification [149].

Researchers have attempted to reproduce this natural process by implantation of a variety of materials, with the intent of allowing adjacent bone tissue to incorporate into and remodel the implant. A number of porous scaffold polymers, including collagen [150], PLA, PGA, and poly(lactic-co-glycolic) acid matrices, have been employed [151]. However, these polymers lack sufficient structural integrity to tolerate physiologic loads and shear stress, and have limited osteoconductivity (ability to support the attachment of native bone cells and tissue), so attempts have been made to incorporate ceramic materials such as hydroxyapapatite (HA) and calcium phosphate with PLA or PLGA [152]. When combined with human fetal bone cells, these materials result in constructs with significant osteoconductive and osteogenic potential [153]. Because of the complex spatial and temporal profile of growth factors involved in normal bone development as well as remodeling and repair, it is also thought that the incorporation of methods to deliver such factors, including bone morphogenetic proteins (BMPs), pleiotrophin (PTN), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF), may be critical for successful in-situ regrowth of bone [154].

The structures that transmit the forces and torques of human locomotion from bone to muscle and between bones are the tendons and ligaments. In the adult, these tissues have low cell density and poor regenerative capacity, and if the load on the tissue exceeds a critical value, permanent damage results [155]. In terms of material content, both tendons and ligaments consist of 55–70% water, followed by collagen (mostly type I with some type III), elastin, proteoglycans, glycosaminoglycans, and glycoproteins, such as fibronectin and thrombospondin [156]. In humans, tendon injuries can include tendinopathy or rupture, and the healing process occurs in three phases: inflammation (24 h), remodeling (3 days to 6 weeks), and modeling (6–10 weeks) [157]. Ligamentous injuries heal in a similar but slower process; the final phase is referred to as remodeling and may last for years [158].

A number of synthetic and biologic materials have been employed in attempts to reproduce the structure of tendons and ligaments [159]. Fibroblasts derived from rabbit anterior cruciate ligaments were seen to adhere and proliferate on poly(ε-caprolactone) (PCL), PLA, and PLGA two-dimensional substrates at rates similar to attachment rates to culture dish plastic [160]. To more accurately simulate the microstructure of the native tissue, others have employed a three-dimensional braided scaffold approach with PGA, PLGA, and PLLA filaments with human fibronectin coating prior to co-culture with rabbit ACL fibroblasts [161]. Although synthetics offer reproducibility in production conditions and ease of fabrication into desired shapes and sizes, they often lack appropriate functional groups for cellular attachment and can elute non-physiologic substances into the circulation [162]. Therefore, some groups have investigated the possibility of functionalizing non-degradable graft materials to improve cell ingrowth and minimize failures [163]. Other approaches to tendon and ligament engineering have focused upon natural polymerical biomaterials including collagen type I [164], silk fibroin [165], or combinations of these two [166]. In vivo implantation of MSC-seeded silk grafts as replacement therapy for resected rabbit anterior cruciate ligament

(ACL) cells resulted in substantial production of collagen types I and III and stable ligament–bone attachment, but much lower tensile strength than native human ACL tissue [167]. Lastly, preliminary data have shown that decellularized native tendons can be prepared from *Gallus domesticus* donors and subcutaneously implanted in rats to demonstrate host cell ingrowth and lack of immunogenicity [168].

Summary

From this brief survey of tissue engineering of several organs and tissues, it should be apparent that the last two decades have seen an explosion of work in this field. Though these techniques are in the preclinical stage for most organs, with the notable exceptions of the trachea and bladder/urethra, though long-term outcome data have yet to be reported, the pace of advancement and the breadth in the field vis-à-vis the types of organs for which engineering is being attempted suggest that tissue engineering may eventually allow for routine implantation, transplantation, and replacement therapy for diseased tissue in a variety of organ systems. Success of this approach will mean an improvement in the supply–demand mismatch problem and the significant immunologic barriers that still remain for transplant surgeons at the turn of the 21st century.

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CHAPTER 53

Organ Assessment and Acceptance for Standard and Expanded Criteria Donors

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Introduction

A successful transplant procedure begins with the acceptance of a suitable donor organ. While that decision is rather simple when faced with a stable, young donor with few chronic medical conditions, there has been a strong trend over the past two decades to employ organs from donors of increasing age. Thus, the process of organ acceptance has grown increasingly complex, and despite its intuitive importance in the transplant process, until recently it has been guided by few systematic or data-driven criteria. The transplant clinician is often presented with the decision at odd hours with temporal pressures and incomplete information about both the donor and the prospective recipients. The pairing of a proper organ with a proper recipient has been influenced on both sides of the equation by the progressive aging of the general population and donor pool, juxtaposed with a shortage of deceased donor organs.

This chapter will cover the criteria used to evaluate donors. Cursory attention will be given to standard criteria donation (SCD) donors, as the acceptance of these organs is rather straight forward and a decreasingly common luxury. Substantially more attention will be given to the assessment of donors who fall outside the ideal norms for donation, i.e. those designated as expanded criteria donation (ECD) donors. It will also discuss the outcomes associated with the use of these donors in general terms. Chapter 54 will specifically cover the procedures and outcomes relevant to the procurement and use of donation after circulatory death (DCD) donors. This chapter and Chapter 53 complement the organ-specific considerations outlined in the technical and perioperative management chapters for each organ.

Standard criteria donors: a shrinking source of donor organs

In the early era of transplantation, the typical organ donor was a young trauma victim with a closed or penetrating head injury. Over the past two decades, the spectrum of organ donors has changed dramatically. Among the most dominant changes in organ donor characteristics is the mean age. Data from the Scientific Registry of Transplant Recipients (SRTR) (Table 53.1) show that the percent of transplanted deceased donor kidneys from donors aged over 50 years increased by 216% between 1991 and 2011 [1]. Over that two-decade span the fraction of all transplanted deceased donor kidneys from donors aged over 50 years increased from 18.2% of 4268 donors to 33.1% of 7433 donors. Similarly, donors in the ideal age range of 6–49 years decreased from 76.2% of all donors to fewer than half at 43.4%. The reasons for the aging of the donor population relate to a number of public health interventions impacting the US population.

Among the most dominant public health victories in the US has been the concerted effort to improve automobile safety. Between 1979 and 2010 the number of automobile deaths in the US decreased from 51 093 to 35 498 [2] despite a marked increase in the US population from 225 million in 1979 to 310 million in 2010. Even in the last decade, the incidence of motor vehicle death has fallen from 15.5 per 100 000 to 10.1 per 100 000, a 50% decline. This public health achievement can be attributed to improvements in automobile crashworthiness, electronically enhanced braking systems, and increased automobile conspicuity. Similarly, the human factor has been affected by enforcement of seat belt and driving while intoxicated laws, universal driver education, and improved trauma care systems [3]. The reduction of automobile deaths can be inferred to have led to a reduction of admission to the hospital of individuals who have sustained traumatic head injury leading to the declaration of brain death and organ donation.

During this same time period, the societal diffusion of high kinetic energy weapons (missiles with high velocity or mass) has resulted in a larger fraction of victims dying at the scene of accidental or intentional gunshot wound. Notably, the number of deaths from gunshot wound has not changed in the US in the last decade, with the rate remaining equal to the current rate of deaths from motor vehicle accidents (10 per 100 000). Death at the scene from gunshot wounds to the head approaches 70%. Mortality from gunshot wounds to the head in those reaching trauma centers can be from both cardiovascular (exsanguination) and neurologic causes. High kinetic energy weapons may lead to more cardiovascular deaths at the scene. Marked improvements in the neurosurgical management of traumatic brain injury is dramatically lowering

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 53.1. Changing age of organ donors between 1991 and 2011. Note the shift to a significantly older donor pool. Donors over the age of 50 years have increased from 18.1% to 33.1% of the donor pool

Number Kidney Donors By Age
1991 and 2011

Age	1991			2011
<1	39			87
1–5	197			222
6–10	171			109
11–17	563			425
18–34	1,556	3,255 (76.2%)		2,143
35–49	965			1,987
50–64	653	777 (18.2%)		2,039
65+	124			421
Total	4,268			7,433

Data from www.srtr.org

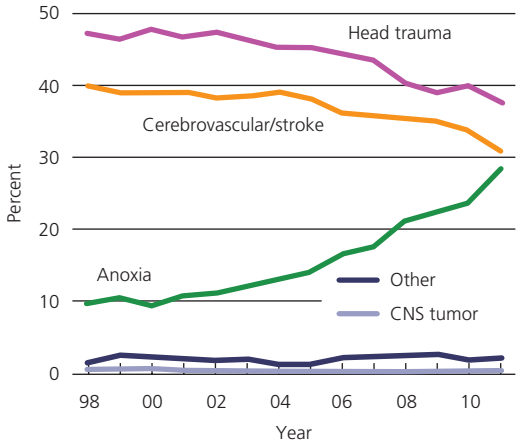

Figure 53.1. Cause of death among deceased kidney donors by year. Note the sharp reduction in the number of donors with head trauma and the dramatic increase in the number of donors with cerebral anoxia. (Data from SRTR Annual Report 2011 [1].)

mortality [4,5]. Management of cerebral edema with decompressive craniotomy combined with recognition and elimination of ongoing shock has revolutionized the care and outcome of patients with closed and penetrating head injury. Finally, it should be noted that in a study of neurosurgical trauma admissions to the University of Maryland Shock Trauma Center, 19% were over the age of 75 years, with many of these cases resulting from falls to which elderly persons are susceptible. These public health developments have led to a substantial reduction in the proportion of donors in the younger age group (Howard Eisenberg, MD, University of Maryland School of Medicine, personal communication).

Coincident with the developments in trauma incidence and management, another major public health trend has led to a rise in the number of potential elderly organ donors. Cardiac arrest is the leading cause of death in the US. Previously, recovery from cardiac arrest was considered a fortuitous event. A major public health effort has been invested in the development of organized response plans for cardiac arrest victims in public locations such as shopping malls and airports [6]. These response plans have included provision of basic instruction on response to cardiac arrest and the use of automatic external defibrillators (AEDs) [7,8]. Widespread training of the public in Basic Life Support has contributed to increased numbers of patients surviving to reach the hospital after cardiac arrest. In a landmark study comparing cardiac arrest at home or in public, survival was dramatically higher when arrest occurred in a public place. In the study, overall survival leading to hospital discharge was 7%. For witnessed cardiac arrest at home, survival for those who received cardiopulmonary resuscitation (CPR) was 10%. When in a public place, a witnessed cardiac arrest had a 20% survival; with application of a shock from an AED, survival after arrest in a public place was 42% [8]. Emergency medical services (EMS) response time was a remarkable 5.6 min for an arrest at home and 5.0 min for an arrest in public. Again, one can infer that among the non-survivors will be those who have sustained irreversible brain injury leading to declaration of brain death or those who have withdrawal of support as a consequence of advanced directives. As a consequence of organized response programs to cardiac arrest in public, increasing numbers of persons who have sustained cardiac arrest with restored circulation are now reaching hospitals. Among the eventual non-survivors, it is reasonable to infer that some may become organ donors. Thus, between 1998 and 2011 (Figure 53.1) the fraction of deceased organ donors with cerebral anoxia as a cause of death has tripled from 10% to 29% of all donors, while the number with head trauma has decreased from 48% to 38% of all donors [1].

The above considerations have characterized a donor population that is progressively aging. With further public health efforts to stem firearm-related deaths, it can be anticipated that the donor population will age further [9].

Characterization of the outcome of SCD, namely donation from those under the age of 55 years, has not been the subject of recent publications as the bulk of the recent kidney transplant literature is focused on strategies and outcomes associated with the aging and marginal donor. Characterization of the outcomes of SCD requires a focus on inferences that can be made from the current literature on ECD donors and the control SCD groups. As will be noted in the concluding section, strategies designed to preserve renal function that are deemed critical to success with ECD donors should be universally applied as even ideal donor kidneys generally function over an insufficient duration to cover a recipient's remaining lifespan.

Understanding the optimal outcome associated with the use of a SCD donor is critical. First, as utilization of higher risk donor kidneys increases, centers will need to remain mindful of outcomes with optimal donors as public perception and to some extent regulatory agencies' perception of expectations may lag behind the reality of the changed and changing donor pool. The most comprehensive review of SCD and ECD kidney outcomes is the exhaustive review by Pascual et al. [10]. This review provides a compendium of the single-center and multicenter comparisons of ECD and SCD kidneys. Nine single-center studies suggest equivalent short-term outcome between SCD and ECD kidneys. In contrast, 39 studies indicate, as is now widely accepted, that the outcome of transplants with SCD kidneys is superior to that with ECD kidneys. Importantly, 20 US and five European multicenter studies were reviewed. Each confirms superior outcome for transplants with SCD kidneys. Among these studies the most contemporary is that by Andreoni et al., which is based on United Network for Organ Sharing (UNOS) cases between 1995 and 2004. SCD kidney transplants have 1-, 3- and 5-year patient survivals of 96%, 90% and 86%, respectively; and graft survivals of 91%, 80%, and 70%, respectively. Among all the

compared series, rates of delayed graft function for SCD donor kidneys ranged from 19% to 50%.

The results of the BENEFIT trial, comparing belatacept- and cyclosporine-based immunosuppression, illustrate the outcome of transplantation in a modern well-controlled series of transplants using optimal donors. In this study, all patients received basiliximab induction, mycophenolate mofetil (MMF), and corticosteroids [11]. The variable treatments were moderate (MI) or low (LI) doses of belatacept versus cyclosporine. Only living donors or SCD donors were included. One-year graft survivals for the three groups were 95%, 96%, and 93%, respectively. The calculated glomerular filtration rate (GFR) in each group was 65, 63, and 50 mL/min/1.73 m^2. The incidence of rejection in each group was 22%, 17%, and 7%, respectively. Although this series included a few living donors, the outcome at 1 year for ideal kidneys with either a calcineurin-based immunosuppression protocol or calcineurin-free protocol can be appreciated. These findings have been subsequently extended to ECD kidneys with diminished but analogous results [12].

The remainder of this chapter will focus on ECD donors, with specific attention given to kidney and liver donors. Understanding the shifting donor demographic away from optimal kidney donors while maintaining a clear grasp of the potential best outcome with ideal kidneys positions a surgeon and transplant physician to use best judgment regarding allocation. Widespread application of nephron-sparing immunosuppression, as noted above, will be needed to obtain optimal long-term outcomes with both ideal and non-ideal donor kidneys.

Expanded criteria kidney donation

The critical organ donor shortage has led transplant professionals to seek additional sources of kidneys for transplantation. What once was considered beyond the limits of acceptable donor demographics has now become a valuable resource and has provided opportunities for many who otherwise would never benefit from organ transplantation.

The number of kidneys transplanted in the US has increased from 13 621 in 2000 to 16 899 in 2010, representing a 19.4% overall increase in absolute numbers. Much of this rise in kidney transplantation is due to a 23.4% increase in deceased donor kidney transplantation over this decade; almost twice that for living donor transplantation (12.4%). During this same time period, however, the number of candidates has increased by almost 35 000 [13]. Similar trends have been seen for other organs, indicating a general trend toward a growing gap between the need for and availability of donor organs [13]. Accordingly, efforts at increasing the numbers of transplants performed and decreasing the refusal rate for what are deemed "transplantable" organs have been intensive and the subject of controversy. It is well recognized that the organ acceptance and transplant rate is as much an overall subjective response to organ quality as it is an objective and measurable criterion. The Health Resources and Services Administration (HRSA)/OPTN-sponsored collaboratives sought to bring together transplant professionals from around the country and across various disciplines to share best practices in an effort to increase organ utilization, primarily via the use of "marginal" or ECDs [14].

The current and accepted definition for ECD stems from the Kidney Work Group at a 2001 Crystal City Meeting to Maximize the Use of Organs Recovered from the Cadaver Donor [15]. Upon conclusion, the working group recommended the development of a distinct and separate allocation system for kidneys that at the time were procured from deceased donors expected to have inferior survival compared to average, although acceptable survival compared to dialysis. This recommendation was eventually developed into a national policy utilizing the definition developed by Port et al.: kidneys from donors with characteristics associated with a relative risk of graft failure at least 70% higher than that of a reference group of non-hypertensive donors, aged 10–39 years, who did not die as a result of a cerebrovascular accident would be distributed under a distinct algorithm [6,7]. This policy was implemented on October 31, 2002 [8]. This new system was designed to decrease cold ischemia time (CIT) by expediting the placement of these organs to centers that not only were aware of the graft survival data but had also consented their recipients in advance to accept such organs for transplantation. ECD kidneys, in counter-distinction to SCD organs, are allocated by waiting time alone and without consideration for HLA matching. In addition, programs were encouraged to perform predonation cross-matching whenever possible.

Following this establishment of a definition for ECD, the number of donated kidneys that met these criteria increased by 36.3%, from 1235 in 1999 to 1683 in 2005. In this same time period, SCDs increased by 13.1%, from 6680 to 7554, reflecting a greater interest in the use of ECDs for transplantation. Currently, however, ECDs represent approximately 19% of all deceased donations in the US, a small increase from 15% in 1999 [13]. A full 40% of all ECD kidneys recovered are eventually discarded despite the implementation of this algorithm to facilitate placement. It is important to recognize that the ECD definition may significantly underestimate the extreme variability in organ quality as the relative risk of >1.7 tends to imply that all organs meeting ECD criteria have the same relative risk of failure. Some ECD kidneys demonstrate adequate long-term survival, while others may have poor outcomes [16–18].

Outcomes compared with dialysis

While a few isolated reports have suggested similar patient and graft survival using ECD kidneys compared to SCD kidneys [19,20], the vast majority of all single-center and all available multicenter and registry reports showed significantly worse 1- and 5-year patient and graft survival rates after kidney transplantation using ECD kidneys compared to SCD kidneys [18,21–23]. A review of the 2007 SRTR report demonstrated the 1-year adjusted graft survival rates for ECD-listed recipients were 83.6% for those who received an ECD organ and 90.4% for those who received a non-ECD transplant [24]. Keeping these data in context, however, Ojo et al. demonstrated in 2001 that the average increase in life expectancy for recipients of an ECD kidney compared to those remaining on dialysis and/or not proceeding to transplantation was 5 years. This benefit was found to be present for certain patient populations but did not extend to high-risk recipients [25]. Kauffman et al. noted a 62% greater 1-year death rate (14.4% vs. 9.1%) in patients aged 60 years or older who received an ECD versus an SCD transplant [26]. Further, Merion et al. studied the benefit of ECD compared to remaining on the waitlist for an eventual SCD kidney [27]. As a result of the increased mortality in the early preoperative period, the authors determined that ECD kidney recipient survival did not reach equality with that observed for SCD kidneys until 3.5 years following the surgery in regards to cumulative mortality. Consequently, ECD transplantation clearly offers a survival benefit over dialysis for almost all designated subgroups, especially those aged over 40 years, non-Hispanics,

sensitized recipients, diabetics, and those with long (>4 years) waiting time, but an SCD transplant offers additionally improved survival compared to an ECD transplant. On average, the adjusted graft survival for an ECD kidney is 8% lower at 1 year and 15–20% lower at 3–5 years after transplantation compared with an SCD kidney [7,17]. Graft outcomes are, in part, due to a recognition that single organ (as opposed to dual) kidney transplant recipients stabilize postsurgical renal function at approximately 60% of the estimated donor creatinine clearance (CrCl), and recipients of an ECD kidney experience a lower initial "baseline" and more rapid loss of function at measured time points compared to SCD [14].

Proper recipient selection

Due to the recognized difference in outcomes with ECD transplantation, various investigators have suggested targeting discrete patient populations as appropriate recipients of these deceased donor organs. SRTR data support that the majority of patients aged 60 years or older are listed for an ECD kidney transplant. Over the last 5 years, the percent of ECD recipients aged 50 years or older has increased from 55% to 81% [13]. Repeat analyses by the SRTR before and after the formal definition of ECD was established have demonstrated significant differences between the demographics of ECD versus SCD recipients, not only in age but also HLA match (ECD transplants were less likely to have zero HLA mismatch than SCD transplants) and cause of end-stage renal disease (ESRD; recipients with ESRD secondary to diabetes or hypertension were more likely to receive an ECD kidney than those whose kidney dysfunction was caused by glomerulonephritis) [17]. Blood type, race, gender, and alloantibody as determined by the panel reactive antibody (PRA) have not been associated with significant differences in ECD allocation [18]. Perhaps the most discouraging results have been seen in retransplant patients. Miles et al. examined the outcome of >9000 patients awaiting retransplantation [19]. Of the almost 3000 who eventually received a new graft, the 10% who received an ECD organ demonstrated similar survival to those remaining on the list, but with far fewer complications related to the treatment of their ESRD. Patients who received an SCD graft, however, experienced a 56% decrease in mortality. These results suggest patients who wish for retransplantation are best served with an SCD kidney if they are not highly sensitized to donor HLA.

Upon exclusion for retransplantation, the main selection criterion for patients to receive an ECD kidney is age >40 years [20]. Importantly, data would suggest there does not appear to be an upper age limit, as recipients older than 70 or 75 years have a demonstrable survival benefit when utilizing ECD donors [25,28,29]. Individual survival benefit is, and always should be, compared with waitlist mortality.

Schold and Meier-Kriesche in 2006 worked to further define appropriate recipients for ECD transplants based upon recipient survival [30]. Critical review of the SRTR data demonstrated the life expectancy for recipients aged under 40 years receiving an SCD transplant following 4 years on the waitlist was significantly greater than following an ECD transplant after 2 years (26.4 vs. 17.6 years). This same analysis did not hold true for recipients aged over 65 years (5.6 vs. 5.3 years). Conversely, diabetic recipients were found to benefit from an ECD transplant. ECD transplantation in a diabetic recipient aged under 40 years and following 2 years of dialysis demonstrated a similar life expectancy to those waiting 4 years for an SCD (9.6 vs. 9 years).

Perhaps as a direct result of the appreciable differences in graft outcomes, the Eurotransplant International Foundation, responsible for the allocation of all organs in Austria, Belgium, Germany, Luxembourg, the Netherlands, and Slovenia, established a renal allocation scheme to provide every patient on the waitlist with a reasonably balanced opportunity for a donor offer [31,32]. The Eurotransplant Senior Programme (ESP) was introduced in which organs from donors aged over 65 years are allocated to recipients similarly aged over 65 years. In its simplest terms, the ESP seeks to identify the best recipient for each available donor organ with the rationale that a kidney graft that outlives the recipient is considered a success, whereas death with a functioning graft is life years lost. The allocation scheme is solely based upon the concept of matching the metabolic demand of the transplant recipient and the excretory capacity of the donor organ. In order to obtain an acceptable degree of success, all efforts were made to minimize the degree of CIT and to reduce immunologic risk, and only non-sensitized (PRA < 5%), first transplant recipients were included. The option of dual transplantation in those in whom the donor CrCl was <70 mL/min was included. At 3 years, 64% and 67% of ESP and control grafts and patients survived ($P = 0.04$), respectively. Death-censored graft survival was also similar at 70% and 71%, respectively.

Numerous authors have described a program for evaluation and selection of appropriate patients for ECD transplantation [21,30,33–35]. In one such review, Stratta et al. described the multidisciplinary approach to the evaluation of such patients [36]. All patients undergo a comprehensive medical, psychosocial, and financial evaluation with a particular emphasis on the cardiovascular system in an effort to optimize and predict operative and early postoperative risk. During committee discussion, patients are assigned a risk assessment for the purposes of management of co-morbidities while on the waitlist as well as follow-up, and a decision is made whether or not to list the patient for an ECD donor. For patients younger than 30 years of age, they recommended against listing for ECD kidneys those who are highly sensitized (PRA > 50%), extremely obese (BMI > 35 kg/m$_2$), or retransplantation candidates. Both the patient and the referring physician may elect to decline such an option, particularly if the patient is predialysis, doing "well" on dialysis, or has a potential living donor. Patients who are either hepatitis B or C positive, and willing to accept a hepatitis B core antibody-positive or hepatitis C-positive donor, respectively, may elect to remain off the ECD list due to the relatively short waiting time for these transplants, particularly in regions where hepatitis-positive donors are more common.

Role of biopsy

There is wide variability in the histologic findings for donor kidneys from different demographic groups, such as those from aged and hypertensive donors. Age alone has a demonstrable effect on the kidney. Autopsy studies show a progressive age-related decrease in the number and size of glomeruli. Older kidneys have a reduced functional mass of nephrons that may be inappropriate for the functional requirements of recipients regardless of age [37]. In addition, grafts from marginal donors may be more sensitive to ischemic and volume insults during all phases of transplantation, resulting in a progressive and sometimes rapid decline in renal function contributing to graft failure (Figure 53.2) [17,38–40].

In a hallmark paper by Sung et al., the authors examined the factors associated with ECD kidney discards. They further evaluated the associations with the development of delayed graft function (DGF). A 6-year analysis of SRTR data demonstrated that 8% (3887) of 48 796 SCD kidneys procured during this time period were discarded versus 41% (5139) of the 12 536 ECD kidneys. ECD

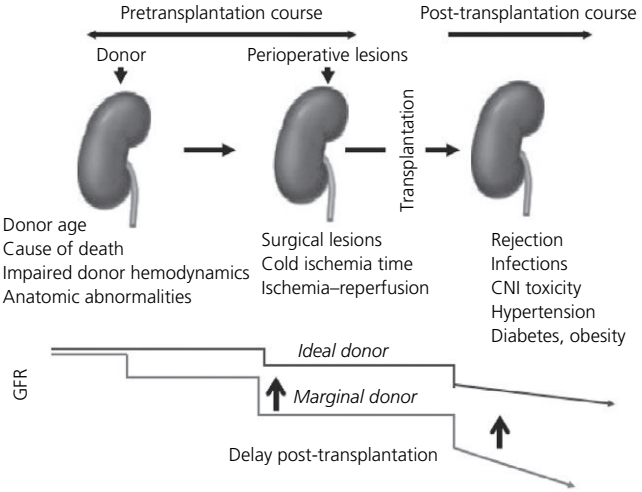

Figure 53.2. Influence of pre- and post-transplant associated factors on graft outcome from expanded and ideal donors. Similar insults are more impactful on extended criteria donor organs.
(Reproduced from Audard V, Matignon M, Dahan K, et al. Renal transplantation from extended criteria donors: problems and perspectives. Transplant International. 2008;21:11–17, with permission from John Wiley and Sons.)
GFR, glomerular filtration rate; CNI, calcineurin inhibitor.

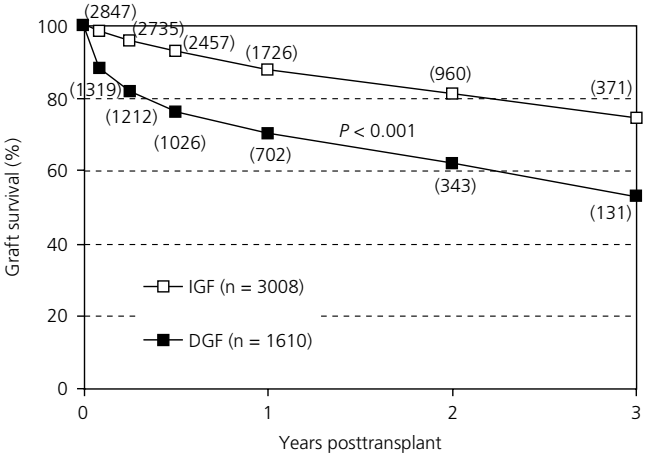

Figure 53.3. Impact of delayed graft function (DGF) on expanded criteria donation donor kidneys indicating that DGF significantly associates with worse long-term survival. Numbers in parenthesis indicate number of patients at risk at post-transplantation follow-up times (1, 3, and 6 months and 1, 2, and 3 years).
(Reproduced from Matsuoka et al. [58], with permission from John Wiley and Sons.)
IGF, immediate graft function.

kidneys were more than four times (OR = 4.35; $P < 0.0001$) more likely to be discarded as SCD kidneys using a logistic model. Among all kidneys, those that were biopsied were more likely to be discarded (OR = 2.08) [41]. Odds of discard for all groups increased progressively with increasing percentage of glomerulosclerosis (GS), from 1.5 for those organs with 0–5% GS to 16.92 for those with >20% GS. Separate analysis of ECD kidneys showed that the odds of discard increased from 0.57 for ECD kidneys with GS of 0–5% on biopsy to 7.22 ($P < 0.0001$) for ECD kidneys with GS in excess of 20%. The OR of discard for biopsied SCD kidneys was only 4.21 compared to those not biopsied. Of note, discard rates also increased significantly with the addition of each component of the ECD definition [age above 50 years, death from cerebrovascular accident (CVA), history of hypertension (HTN), serum creatinine >1.5 mg/dL] [42].

Complications

The best described complication is DGF and this may result in a cascade of complications after transplantation of an ECD organ [22,23,26,43–45]. Experimental renal allograft models have demonstrated a strong correlation between prolonged CIT, donor age, and renal allograft dysfunction. It is well established that the ischemia–reperfusion injury associated with organ transplantation contributing to DGF is associated with impaired long-term graft and patient survival. This is particularly true for transplantation of an ECD organ in older recipients. Remuzzi et al. reported the 1-year mortality rates for patients older than 60 years receiving an ECD kidney transplant increased significantly if they developed DGF compared to those with immediate graft function (7.7% vs. 18%; $P < 0.05$) (Figure 53.3) [46].

The overarching intent in the development of an allocation system for ECD organs was to decrease the CIT. UNOS data have shown that the percent incidence of DGF for an equal CIT is greater for kidneys from older donors (51–65 years) compared to younger donors [15]. Analysis of the United States Renal Data System (USRDS), however, do not demonstrate that very short intervals in decreased CIT have had an impact upon the development of DGF in ECD recipients either before or after 2002. However, efforts to significantly decrease CIT have resulted in beneficial outcome in several studies. Valcarce et al. reported the results of a prospective single-center study in which CIT was limited to <10 h (9.3 + 2.5 h for ECD and 8.3 + 3.3 h for SCD) [47]. This study found no significant differences between the two groups (ECD vs. SCD) for outcomes such as primary non-function (4.2% vs. 2%), DGF (16.7% vs. 10%), surgical complications (25 vs. 16%), or acute rejection episodes (8.3% vs. 2%). Although 1-year graft survival rate was similar between the two groups, allograft function as measured by GRF was superior for SCD kidneys (49.4 + 12.5 mL/min vs. 65.8 + 14.9 mL/min for ECD vs. SCD, respectively).

An increased incidence of death has been demonstrated from infection in recipients of ECD kidneys who are aged over 50 years, a group that also has an increased number of co-morbidities. Recipients of ECD kidneys are reported to have an increased number of surgical complications, particularly ureteral necrosis and leak. However, these complications have not resulted in a significant increase in graft loss [34,48]. Danovitch et al. reported the annual death rate for recipients of ECD kidneys was 100 per 1000 patients-years at risk compared to 48 per 1000 patients-years for recipients of SCD. Adjusted patient survival at 1- and 5-years for ECD kidneys was 90.6% and 69% compared with 94.5% and 81.2% for non-ECD kidneys [49].

Specific protocols

Grafts from marginal donors are more sensitive to the ischemia–reperfusion injury inherent in all deceased donor kidneys and have an impaired ability to repair tissue and parenchyma [38,39,50]. Strategies to prevent further loss of the initially reduced nephron mass are critical to the success of ECD kidneys. Elimination of

potential nephrotoxic exposures to the graft are important. In addition, elderly recipients of ECD grafts demonstrate an increased incidence of infections, cardiovascular complications, and malignancies. Of particular concern are the numerous reports of a higher incidence of cytomegalovirus (CMV) infection and polyomavirus-induced nephropathy in recipients of ECD grafts [42]. To that end, physicians have sought not only to reduce CIT, but also to develop specific post-transplantation protocols designed to optimize graft outcome and prolong survival while minimizing immunosuppressive complications. Very few prospective, randomized, multicenter, long-term outcome trials that include ECD recipients have been performed; even fewer randomized trials have focused solely on ECD kidney recipients.

Calcineurin inhibitors (CNIs) are nephrotoxic and may be associated with reduced short- and long-term allograft survival [51–53]. This is particularly true for ECD kidneys, with biopsies demonstrating parenchymal damage. Both CNI minimization and elimination strategies have been proposed to avoid injury. In a US study by Stratta et al. that included 101 ECD recipients, recipients received either antithymocyte globulin or alemtuzumab along with maintenance immunosuppression with MMF and steroids [20]. Tacrolimus was withheld until after the serum creatinine was <4 mg/dL and a moderate dose was instituted with tight control of trough levels. Four-year actuarial patient and graft survivals were noted to be 93% and 74%, respectively, which are far superior to those in published registry data. Several non-randomized European trials have confirmed these results in a cohort of recipients, 54% of whom had DGF [54].

CNI avoidance, however, has not met with similar success. Utilizing antithymocyte globulin and dual therapy of MMF and steroids, European investigators reported acute rejection rates of 25% and 5- year graft survival rates of 65% [55]. Using a similar protocol and recipient inclusion/exclusion criteria, basiliximab induction produced discouraging results (45% rejection rate) and recipients were ultimately converted to CNI therapy [56]. Although CNI-free, sirolimus-based therapy has found some success in SCD transplants in small single-center trials, this has not been confirmed in larger randomized trials. Other single-center, non-randomized trials have confirmed this finding in cohorts of ECD recipient [57]. Diekmann et al. reported the results of CNI-free sirolimus-based therapy in recipients of ECD as associated with "acceptable" outcomes, but with increased levels of proteinuria. Similar immunosuppressive modifications and outcomes have been reported with the use of dual ECD kidneys as well [57].

Most recently, Larsen et al. reported the results of a prospective, randomized, multicenter trial (BENEFIT-EXT) comparing the use of belatacept, a novel biologic agent for maintenance of immunosuppressive therapy, with CNI in recipients of ECD [58]. The protocol evaluated both a more intense (MI) and less intense (LI) belatacept regimen: 347 of 543 (64%) recipients completed 2 years of treatment. Patient and graft survival were similar across all groups, but renal function was improved in the CNI-free groups, with GFR 8–10 mL/min higher compared to those receiving cyclosporine. Post-transplant lymphoproliferative disorder (PTLD), specifically central nervous system PTLD, was observed more frequently in belatacept-treated patients, prompting a warning that belatacept use should be limited to Epstein–Barr virus (EBV) seropositive recipients.

In addition to specific immunosuppression protocols, some advocate aggressive intervention and control of co-morbidities in elderly recipients of ECD kidneys [36,59]. Treatment of hypertension, hyperlipidemia, anemia, diabetes, and other medical conditions to maintain blood pressure at <140/90 mmHg, fasting serum cholesterol at <200 mg/dL, hematocrit at >30%, and fasting blood glucose at <126 mg/dL is recommended. Antiplatelet therapy with oral aspirin should be administered to all patients along with a low sodium, heart healthy diet, and regular evaluation for cardiovascular risk factors/events should be routine. Regular screening for systemic or kidney-specific viral and bacterial illnesses is crucial as is early detection of presumed rejection episodes in an effort to avoid lympho-depleting antibodies in this high-risk group.

Pulsatile perfusion

As covered extensively in Chapter 25, pulsatile perfusion (PP) or "pumping" has become an acceptable method both for organ evaluation and preservation, while providing valuable information informing the transplantation decision of ECD organs. PP involves the ex-vivo hypothermic pulsatile perfusion of the donor kidney, supplying oxygen and nutrients to the organ while removing waste. During perfusion regular monitoring of renal flow and resistance provides information about kidney utility. Attempts to improve renal blood flow and correction of acidosis are important elements of the active monitoring of perfused kidneys. Perfusate often consists of standard perfusion solutions containing (or added) hydroxyethyl starch to prevent interstitial edema; adenosine to increase ATP; phosphate as a proton buffer; gluconate to decrease cellular swelling; glutathione as an antioxidant; and various other agents to reduce metabolism and balance electrolyte composition. PP is mimicked to decrease vasospasm and resistance. Decreased flow rates, increased resistance, and an increase in the calcium concentration measured during PP have been associated with DGF [60,61].

Initial studies from the 1980s failed to demonstrate a decrease in DGF or show a graft survival advantage for PP versus cold storage (CS) [62–64]. Additionally, the increased cost of PP was felt to be prohibitive, especially in the absence of an obvious kidney survival benefit. Several studies, including those performed on paired kidneys from the same donor, with one placed on PP and the other in CS found similar rates of post-transplantation dialysis [61,65].

As the numbers of ECD kidneys offered for possible transplantation continued to increase, the utility of another objective measure of kidney "health" utilizing PP began to generate increased interest. In 2003, 17.2% of transplanted kidneys were from ECD donors. Of those, 28.4% were preserved with PP. In a review of ECD-PP versus ECD-CS kidneys, Matsuoka et al. found that ECD-PP kidneys tended to come from older donors (61.1 vs. 59.8 years; $P < 0.001$) and had increased terminal serum creatinines (1.2 vs. 1.1 mg/dL; $P = 0.03$). The PP donors had a statistically increased incidence of diabetes (12.7% vs. 9.4%; $P = 0.003$) and were more likely to be DCD donors (6.5% vs. 0.9%; $P < 0.001$). Although the CS cohort experienced decreased CIT, this was under 24 h for both groups. Kidneys demonstrating increased degrees of GS (27.3% vs. 18.1%; $P = 0.002$) and interstitial fibrosis (48.5 vs. 40.5%; $P = 0.03$) were more likely to be placed on PP. The authors noted no difference in the incidence of primary non-function, but did observe a 10% higher rate of DGF in ECD-CS kidneys compared to ECD-PP kidneys (37% vs. 26%; $P < 0.001$). There was no difference noted in the incidence of rejection up to 1 year following transplantation despite the decrease in DGF. There was a decrease in DGF in the donation after brain death (DBD) versus DCD donors (34.5% vs. 54.3%; $P < 0.001$). After adjusting for all variables, PP resulted in a 49% decrease in the risk of developing DGF compared to CS (OR = 0.51; $P < 0.001$). As expected, other donor factors predictive

of DGF include DCD, history of HTN, elevated serum creatinine, and "suboptimal" donor histology. Importantly, ECD kidney transplants that experienced DGF were found to have statistically significant lower graft survival rates compared to those with immediate graft function (IGF) at both 1 (70.1% vs. 87.8%) and 3 years (53.0% vs. 74.4%; $P < 0.001$). Preservation method made no difference to survival of kidneys with IGF. Overall, graft survival for the PP and CS groups was similar.

In a separate review, Sung et al. analyzed a later cohort with a similar evaluative process and found that the discard rate for ECD-PP kidneys was 29.7% versus 43.6% for those preserved with CS [41]. Furthermore, ECD-PP kidneys with measured high resistance were associated with a higher odds ratio of discard. ECD kidneys with terminal resistance of <0.18 mmHg/mL/min and with resistance of 0.18–0.25 mmHg/mL/min were discarded 12.6% and 14.0% of the time, respectively. In contrast, ECD kidneys with terminal resistance of 0.26–0.38 mmHg/mL/min had a 25.7% discard rate and those with a resistance of >0.38 mmHg/mL/min had a 53.1% discard rate (OR = 2.50 and 7.88, respectively, compared to a resistance of <0.26 mmHg/mL/min; $P < 0.0001$). Interestingly, the authors also commented on the high variability of ECD discard rate among Organ Procurement Organizations (OPOs), which ranged from 14% to 60%. Their data showed that OPO practices, including local use of PP and ECD kidneys, affected rates of organ recovery and were significant determinants of rates of discard.

The rise in the use of ECD kidneys coincident with the rise in the use of PP by transplant programs can be explained by the need for an additional tool to determine the functional quality of the organ in question. Various measurements and especially the intra-arterial resistance can be used as further valuable data to decide on the suitability for transplantation. It can be argued that the lack of DGF in various reports utilizing PP as a preservation technique is due to the discard of many organs demonstrating poor machine perfusion parameters. Nonetheless, the use of PP in the evaluation of ECD kidneys and in the potential reduction of DGF has led many OPOs and transplant programs to utilize PP.

Dual kidney transplantation

As a result of the greater emphasis placed on the use of ECD for kidney transplantation and the paucity of organs to meet the needs of those waiting, the transplant community has focused on strategies to minimize organ discard, particularly in light of the aging donor population. Dual kidney transplantation (DKT) from kidneys procured from ECD donors has been employed in an effort to alleviate the organ shortage. Initially at the University of Maryland in 1994 [66], surgeons began to utilize both kidneys from a single donor that were often refused by other centers and would have otherwise been discarded because they were judged to be inadequate for single transplantation [67,68]. The overarching principle in DKT is that transplantation of a single kidney with nephron mass recreates experimental models that undergo progressive graft failure. These experimental models demonstrate that initial nephron mass is a determinant of chronic allograft failure. Increasing the number of viable nephron units by simultaneous transplantation of two kidneys effectively prevents the progressive deterioration in renal function that occurs in control subjects given a single kidney [69,70]. While DKT has the theoretical advantage of increasing the effective nephron mass transplanted, there is the risk that kidneys that might be suitable for single kidney transplantation (SKT) might be transplanted as DKT, thus reducing rather than increasing the numbers of transplants performed. Short-term data for DKT from both single-center and registry analyses demonstrated excellent early (1 year) survival, but found a higher incidence of primary non-function when compared to SKT [67,71,72].

A more recent paper by Tan et al. analyzed the results of DKT compared to SKT with over 8 years of follow-up of their recipients [72]. In this review, 113 patients aged 55 years or older underwent deceased donor kidney transplant with either dual (n = 39) or single (n = 72) allografts. Interestingly, the authors noted that all organs used for DKT had previously been refused by other centers prior to transplantation at their center. The most common reasons for exclusion were, as expected, donor age, donor instability, history of HTN, >20% GS on biopsy, or a combination of these factors. The determinant for DKT versus SKT was based on donor admission CrCl estimated by the Cockcroft–Gault equation. If the CrCl was <90 mL/min, both kidneys were used for transplantation into a single recipient. The lower limit for acceptance of these organs was noted to be 45 mL/min. The average donor age was greater in the DKT group (61 vs. 48 years; $P < 0.001$). There were no statistical differences between the groups in terminal serum creatinine of the donor, HLA mismatch, or CIT (15 vs. 18.5 h). Patients who received a DKT had a significantly shorter waiting time to transplantation compared to those receiving SKT (440 ± 38 days vs. 664 ± 51 days; $P = 0.002$), had similar length of stay, no differences in immediate postoperative complications such as myocardial infarction, infection or reoperation, nor in the proportion of those developing DGF or the need for the treatment of early rejection. The 5-year graft survival for DKT versus SKT was 79.5% versus 67%, and the 8-year survival was 69.7% and 59.4%, respectively (P = NS). In further subanalysis, recipients of DKT from donors having an estimated CrCl of <75 mL/min demonstrated an 8-year graft survival of 64.3%. Rejection, both acute and chronic, was the predominant cause of death-censored graft loss. Five-year patient survival for DKT versus SKT was 82.1% versus 80% and 8-year survival was 82.1% and 74.1%, respectively. The most prominent causes of death in both cohorts were cardiovascular and infectious complications. Importantly, the median 1- and 5-year serum creatinine for DKT versus SKT was 1.45 versus 1.7 mg/dL and 1.8 and 1.4 mg/dL, respectively (P = NS).

In another similar prospective case–control study, Remuzzi et al. analyzed graft survival of SKT or DKT for ECD allocated on the basis of clinical or preimplantation histologic evaluation [66]. Kidneys were considered eligible for DKT only if at macroscopic evaluation, major vascular abnormalities were excluded. Those organs with evidence of focal scarring were also excluded, due to a concern for non-homogeneous distribution of damage that could affect the microscopic view of the biopsy. A minimum of 25 glomeruli were required in order for the biopsy to be considered "complete." A biopsy-based scoring system was developed and kidneys were allocated to DKT, SKT, or discard. Twenty-four cases and 48 controls received a DKT and SKT, respectively. DKT donors and recipients were significantly older than SKT recipients. Donor CrCl was significantly lower in the DKT versus SKT recipients (54.5 ± 16.0 mL/min vs 78.1 ± 48.0 mL/min; $P = 0.03$) and no donor had clinical proteinuria. Otherwise, recipient characteristics were similar between the groups, including CIT. The incidence of DGF was identical (20.8%) in both groups and resolution of dialysis needs was rapid. Mean serum creatinine levels were comparable between the groups at all time points, with 6-month values of 1.48 ± 0.54 mg/dL versus 1.77 ± 0.93 mg/dL in DKT versus SKT recipients. At last follow-up, DKT recipients had a mean CrCl virtually identical to that for their corresponding donors, whereas those

receiving a SKT had a significantly lower CrCl than that for their donors. With reanalysis of halved donor CrCl (representing split function in a single transplanted kidney), SKT recipients had a significantly higher CrCl compared to the calculated "half" donor creatinine clearance (53.6 ± 16.2 mL/min vs. 41.0 ± 22.8 mL/min), suggesting a 30% hyperfiltration in recipients of single kidneys. These results strongly suggest a combination of both clinical characteristics and histologic criteria should be utilized concomitantly in the choice of SKT and DKT from an ECD.

Resource utilization

The preponderance of data has confirmed that ECD (and DCD) kidney recipients have a significantly higher incidence of DGF, a longer time to reach a serum creatinine below 3 mg/dL, a longer length of stay (LOS), and an increased number of readmissions for complications following the transplant procedure [23,43,45,73,74]. Therefore, it is incomplete to eliminate from discussion the financial and economic aspects often associated with ECD transplantation. In the US, the Medicare ESRD program pays >$1.2 billion/year for transplantation and is the responsible payer for approximately 70% of all kidney transplants [75].

In a retrospective analysis conducted by Saidi et al. at the Massachusetts General Hospital, investigators compared resource utilization and outcomes in adult recipients of ECD and DCD with SCD kidney recipients [76]; 271 deceased donor transplants (160 SCD, 44 ECD, 53 DCD, and 11 ECD/DCD) were analyzed. PP was routinely utilized in all ECD and DCD kidneys. The incidence of DGF was significantly higher in the ECD, DCD, and ECD/DCD groups compared to the SCD group (35.6%, 33.3%, 38.2%, and 15.1%; $P < 0.001$). The time to reach the plateau serum creatinine was significantly longer in all non-SCD groups as well. Initial LOS in the non-SCD versus SCD groups was 9.8–11 days versus 6.1 days ($P < 0.05$), respectively. The 90-day readmission rate was higher in the non-SCD groups as well. Along with an increasing incidence of DGF and longer hospital stays, non-SCD kidneys were associated with higher initial hospitalization charges. The charges for ECD, ECD/DCD, DCD, and SCD kidney recipients were $70 030, $72 438, $72 789, and $47 462 ($P < 0.001$), respectively. These differences became more significant as more readmissions were required in the care of the non-SCD recipient.

Whiting et al. merged UNOS registry data with Medicare claims data for almost 35 000 kidney transplant recipients, beginning in the pretransplant, dialysis period and projecting these same costs forward in an effort to determine a financial "break even" point for renal transplantation using ECD and high-risk recipients (HRRs) (defined as over the age of 60 years in this analysis) [77]. Utilizing this definition, there were 25 600 non-HRRs, of whom 5718 (22%) received an ECD, and 8934 HRR transplants, of whom 2200 (25%) received an ECD. When considering donor source only, recipients of ECDs had a significantly greater cumulative cost at 5 years compared to recipients of non-ECDs ($144 274 vs. $129 163 respectively; $P < 0.0001$). Similarly, when recipient risk only was evaluated, HRRs had significantly greater cumulative costs at 5 years compared to non-HRRs ($145 453 vs. $124 553, respectively; $P < 0.0001$). In analyses combining both donor–recipient groups, the payments for non-ECD–non-HRR versus ECD–HRR pairings were $121 698 versus $165 716 ($P < 0.0001$). By then plotting each transplant cost curve over time and intersecting the prospective ongoing costs of dialysis into the post-transplant period, the authors demonstrated the financial "break even" point occurs earliest with non-ECD–non-HRR pairings at 4.4 years, whereas hemodialysis does not become more costly until 13 years post-transplant for the more costly ECD/HRR donor–recipient pairs (Figure 53.4).

Although many transplant centers may fully support the utilization of ECD, it is certain that the "costs" of performing transplantation with ECD are far greater than those for SCD, making these types of organs prohibitive for low-volume centers that may not be able to invest in the infrastructure of additional staff to manage patients with DGF on an outpatient basis; as a result, the prolonged hospital admission may make use of ECD kidneys impractical. Many centers have established outpatient-driven protocols whereby recipients of ECD transplants experiencing DGF are discharged

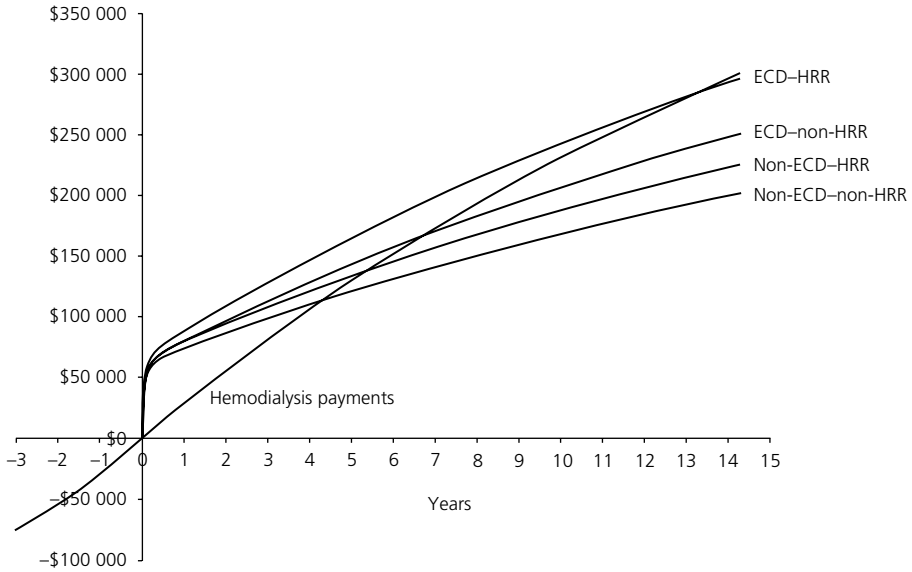

Figure 53.4. Medicare payment projections and "break even" point. This point is shown as the time point when each donor–recipient pairing crosses the projected hemodialysis payment line. Extended criteria donation (ECD) organs compound with high-risk recipient (HRR) status to substantially increase cost. (Reproduced from Whiting et al. [78], with permission from Wolters Kluwer Health.)

following cessation of their inpatient needs. Options include discharge to their pretransplant dialysis unit or to an outpatient site affiliated with the transplant institution. These strategies offset the initial costs while still providing the necessary vigilance in the early post-transplant period. The financial impact is, most certainly, another variable in some instances of organ evaluation for transplantation, and survival outcomes are critically assessed both within the institution and externally.

Expanded criteria liver donation

Similar restraints in organ availability have led transplant teams to concurrently prioritize liver allocation based upon severity of illness, along with the use of organs that were previously thought to be associated with an unacceptably high risk of primary nonfunction (PNF) or initial poor function [78]. Several donor characteristics, including age, race, height, cause of death, degree of steatosis, split versus whole grafts, and CIT have been identified to have an independent relationship with liver allograft outcomes [79].

Mullhaupt et al. in a review of the literature reported a clear association between donor age and recipient outcome, particularly for donors over the age of 60 years. African–American race was found to have a relative risk of 1.19 compared to Caucasian race. Donor height, but not weight, was also independently associated with outcomes. Cause of donor death other than trauma was associated with a 16% (CVA) and 20% (other causes) increased risk of graft failure. Further, DCD donation was associated with a 51% increased risk compared to DBD [79,80].

The gross visual assessment of the liver allograft, although fairly reliable in determining the presence of severe steatosis, has been regularly reported to be generally unreliable to detect moderate and mild degrees [75]. In addition, various tissue processing and staining techniques can affect histopathologic assessments of fatty infiltration [76]. There is, however, current agreement that even moderately steatotic grafts qualify as expanded criteria due to the increased rates of PNF (13% vs. 3% for SCD) [77,78]. Finally, >14 h of CIT has been consistently linked with preservation damage associated with prolonged postoperative course, biliary strictures, and decreased graft survival [81,82].

In an effort to counter-balance these identified donor risk factors, liver surgeons have sought to transplant these grafts with increased risk into older candidates (>50 years of age) with moderate disease severity [i.e. lower Model for End-stage Liver Disease (MELD) scores] and without hepatitis C [78,80,83,84].

Organ-specific donor risk indices

The ECD designation alone does not effectively determine donor quality as organ survival cannot be predicted solely from a dichotomous variable (SCD vs. ECD). A more graded organ evaluation with organ-specific donor risk indices has been developed to predict graft survival with various combinations of donor and recipient characteristics [81–83]. The kidney donor risk index (KDRI) developed by Rao et al. provides a continuous risk score developed with SRTR data from 1995 to 2005. The final KDRI includes 14 donor and recipient factors, each found to independently impact graft outcomes: age, African–American race, serum creatinine, donor hypertension and diabetes, cause of death, height, weight, DCD, HCV, HLA mismatches, CIT, en-bloc transplantation, and dual transplantation. The KDRI provides a continuous score which, when compared to a reference donor score of 1.00, provides the evaluator with a relative risk of allograft failure or patient death associated with the use of the organ in question. The OPTN/UNOS has developed a similar model for organ evaluation and potential allocation. The kidney donor profile index (KDPI) is a similar score based only on donor characteristics. Kidneys with the lowest KDPI represent those with the longest predicted survival time. Potential allocation of these organs to those with the longest expected post-transplant survival to maximize life-years gained has been offered as a necessary evolution in the preservation of this valuable resource [85].

The liver donor risk index (LDRI), first described by Feng et al., identified seven donor characteristics that were significantly associated with liver allograft failure: age, African–American race, height, non-traumatic cause of death, DCD, and partial/split liver. Additionally, variables of CIT and regional/national sharing were included in the final model. Allograft survival rates correlated with increasing LDRI. The authors went on to report that allografts with higher LDRIs were best suited for recipients with low MELD scores [85]. Further application of the LDRI by Maluf et al. examined the outcome of ECD livers (i.e. LDRI ≥ 1.7) and demonstrated a significant increase in relative risk of organ failure for increasing MELD score [86].

Summary

The opportunities via transplantation have supported the increased use of deceased donor organs from ECD donors. While overall graft survival outcomes are decreased compared to SCD donors, the data clearly support a benefit to transplantation versus remaining on the waitlist. As we continue to learn more about the most appropriate recipient demographics, i.e. which recipients can be expected to do well with these organs, as well as continue to develop specific follow-up protocols and immunosuppressive regimens to best care for the patient and the organ, the results may continue to improve. The use of pulsatile perfusion, discrete pathologic criteria, and dual organ transplantation has allowed for greater utilization of ECD kidneys with ongoing efforts to decrease CIT and organ discard. Similar results have been demonstrated in the use of ECD liver donors, with a more directed use in recipients of low MELD scores. As the costs for an institution performing ECD transplants are clearly increased, cost has become yet another variable in kidney allocation, program development, and resource allocation. The development and ongoing refinement of organ-specific donor risk indices has provided the transplant community with improved discriminatory tools to better assess the suitability and viability of organs from expanded criteria donors as we seek to ensure that organ utilization is balanced by ethical decision-making.

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CHAPTER 54

Organ Assessment and Acceptance for Donation after Circulatory Death Donors

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Introduction

In the US, 18 people die every day while waiting for a life-saving organ. Those who are fortunate to receive a transplant mostly receive organs from brain dead donors. With the increasing success of transplantation as life-saving therapy, the waitlist is growing rapidly and the demand for transplantable organs continues to grow accordingly. To overcome this disparity, alternate organ donors are increasingly sought. These include living donor lung transplants, marginal or extended criteria donation (ECD), and donation after circulatory death (DCD), sometimes referred to as donation after cardiac death or non-heart beating donation. DCD was reintroduced in the mid 1990s by several programs and has since contributed to a steady increase in organs available for transplantation. Kidneys, livers, pancreata, and lungs have been successfully transplanted from DCD donors, with some centers even accepting DCD organs for combined transplants [1,2] and reporting reasonable outcomes.

However, the use of organs from DCD donors has trade-offs, which manifest in higher numbers of complications that vary by the type of transplant. Since 1995, DCD donors have been classified according to the Maastricht categories [3] (Table 54.1). With few exceptions, DCD across the US are Maastricht category 3 donors, meaning that the cardiopulmonary arrest and subsequent organ procurement is in a controlled in-hospital setting. These donors are stable and expected to expire after planned withdrawal of life support, most often in the operating room, but on occasion in the intensive care unit (ICU). As a result, the ischemic insult is known and typically limited. In Europe, however, a much broader mixture of DCD donors from all Maastricht categories is being used for transplantation. These donors have less anticipatable ischemic insults and are thought to push the limit of acceptability in some circumstances. In general, the risks associated with acceptance of DCD organs are offset by a compensatory benefit to the patient in terms of more rapid organ transplantation and a reduced risk of the consequences of end-organ failure in properly selected settings. Their use thus requires that the clinician has a heightened understanding of the likely consequences of the uncontrolled arrest and procurement, and a clear insight into the risks that are assumed by the recipient of these organs. In this chapter, we will review acceptance criteria for DCD organs. We will discuss the risks and benefits of DCD transplantation as they pertain to each organ. Lastly, we will delineate the function as well as short- and long-term outcomes after DCD transplantation. Specific discussion of standard criteria donors and extended criteria donors can be found in Chapter 53. Technical details regarding the procurement of organs in a DCD scenario are found in Chapter 22.

DCD lung transplantation

Criteria for acceptance

Hardy and others described the first successful human lung transplant in 1963 using what would now be considered a DCD lung donor [4]. Shortly thereafter, brain death was defined and soon became accepted as the legal definition of death [5]. As a result, due to concerns over deleterious effects of warm ischemia time on donor organs, most organs for donation were subsequently recovered from brain dead donors [6] until DCD transplantation resurfaced in the mid 1990s due to pressures imposed by ever-increasing waitlists [7]. Since 1988, the number of lung donors has increased significantly from 33 to 1770 in 2010. As with other organs, the waitlist has continued to increase faster than the rise in donor numbers. For DCD lung donors, most programs endorse the International Society for Heart & Lung Transplantation (ISHLT) criteria for lung donor selection [8]. However, due to the additional risk that warm ischemia time poses, many centers apply the ISHLT criteria more strictly to DCD donors than to their donation after brain death (DBD) counterparts. Some centers have even more stringent criteria for acceptance of DCD lung donors. Table 54.2 lists the ISHLT criteria as well as additional criteria used by various lung transplant centers around the world for consideration of potential DCD lung donors. Puri et al. [9] have also used organs from extended criteria donors (listed in parentheses in Table 54.2).

Risks and benefits

The benefit of a DCD lung transplantation is increased availability of organs for the lung recipient population, and thus shorter waiting time for individual patients, reducing the risk of waitlist death [10]. In order to optimize outcomes after DCD lung transplantation, selection of appropriate recipient candidates is crucial. Recipients with pulmonary hypertension, idiopathic pulmonary fibrosis, or prior pleurodesis are not considered candidates for DCD lung

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

transplantation at some centers [9], either due to predictable difficulties during the recipient pneumonectomy, or for fear of further reperfusion injury to the pulmonary allograft. However, some institutions, including ours, will consider all recipients as potential recipients for DCD lung transplantation. Overall, considering the altered selection criteria utilized for DCD lungs, the outcomes of DCD lung transplantation are similar to those for DBD lung transplantation, with some important exceptions, which are discussed below.

The incidence of primary graft dysfunction after DCD lung transplantation is roughly the same as that after DBD lung transplantation at about 10–18% [9,11,12]. The need for extracorporeal membrane oxygenation (ECMO) due to primary graft dysfunction post transplant is similar if not slightly less [13]. Other metrics such as time to extubation, discharge from the ICU or hospital discharge are also similar [13]. Grade 1 acute rejection may occur more frequently in DCD lung transplant recipients (53.7%) than in DBD recipients (27.8%) [12], but in other reports the incidence of rejection is similar in DCD and DBD lung transplantation [13]. Others report rejection rates after DCD lung transplantation of 20% [11]. The rates of grade 2 or higher acute rejection are the same [12].

Airway complications after DCD lung transplantation include bronchial stenosis, bronchial dehiscence, excessive growth of granulation tissue at the bronchial anastomosis, and bronchial fistulas. Indeed, the occurrence of airway complications has been reported to be numerically doubled after DCD transplantation at 27.8% when compared to 12.8% for DBD transplantation, although this does not reach statistical significance in the small studies reported to date [12]. Nevertheless, these complications, likely the result of

Table 54.1. Maastricht categories of donation after cardiac death (DCD) donors

1	Uncontrolled	Brought to hospital dead
2	Uncontrolled	Unsuccessful resuscitation leading to death
3	Controlled	Awaiting cardiac arrest
4	Controlled	Cardiac arrest after brain death
5	Uncontrolled	Unplanned cardiac arrest in a hospitalized patient

Table 54.2. Donation after circulatory death (DCD) lung donor selection criteria

Criteria	ISHLT [8]	Spain [66]	Ontario, Canada [11]	University of Wisconsin and Belgium [12,13]	Cleveland Clinic, OH [17]	Nunez et al. [67]	Washington University, MO [9]	Melbourne, Australia [10]
Donor age (years)	<55	<55				7–50	<55 (>55 marginal)	<55
ABO	Compatible						Compatible	
CXR	Clear		Yes		Yes		Normal (infiltrate marginal)	Relatively normal
***Pao*$_2$/ABG**	>300 mmHg on *Fio*$_2$ 100% and PEEP +5 cmH_2O	*Pao*$_2$/*Fio*$_2$ >300 mmHg	Yes		Yes	>300 mmHg	>300 mmHg on *Fio*$_2$ 100% and PEEP +5 cm H_2O; (<300 mmHg marginal)	>300 mmHg on 100% *Fio*$_2$
Smoking history (pack years)	<20						(>20 is marginal)	
Chest trauma	None	None				None		
Aspiration	None							
Sepsis	None							
Prior cardiopulmonary surgery	None							None
Sputum Gram stain	Negative							
Bronchoscopy	Absence of purulent secretions		Yes		Yes	No injuries	Normal (purulent secretions marginal)	Yes
Maastricht category	All	1 or 2	3	3	3	1 and 2	3	3
Time of death/ cardiac arrest		Known				Known		Team to arrive 2 h before withdrawal
CPR initiation (min)		<15				<10		
Warm ischemia time (min)		<120	<90 If >30 ex vivo	<60n	<60			
Maximum cooling time		<240 min				<90 min		Topical cooling for up to 6 h post cardiac death optional
Inspection/ visualization		No contusion No edema	Yes		Yes	No injuries	Size matched to recipient	
Health history			No cancers, etc.			Healthy, no HIV risk factors	No pulmonary disease, negative hepatitis B and HIV (inhalational drug use marginal)	

ISHLT, International Society for Heart & Lung Transplantation; CXR, chest X-ray; *Pao*$_2$, partial pressure of oxygen in arterial blood; ABG, arterial blood gases; PEEP, positive end-expiratory pressure; CPR, cardiopulmonary resuscitation.

small vessel ischemic or thrombotic biology occurring during the circulatory arrest period, are similar to those realized in liver transplantation (discussed below) and likely reflect a real consequence of the DCD process.

Infections are also reported postoperatively, but not at higher rates than after DBD transplantation. Other reported complications include pneumonia, atrial fibrillation (41%), pericardial effusion, confusion (23%), acute kidney injury (12%), hematologic abnormalities (18%), ileus (12%), and ischemia (6%) [14], all of which likely relate more to the recipient physiology than the donor source.

As with DBD lung transplantation, primary graft dysfunction and longer cold ischemia times correlate with earlier death after DCD donation [9]. Mortality over the first 3 months after DCD lung transplantation has been reported at 18% [14].

Function of DCD and DBD lung transplants

Only two groups have described functional outcomes after DCD lung transplantation. DeOliveira et al. systematically examined the percent predicted forced expiratory volume in 1 s (FEV_1) at 1, 6, 12, 24, 36, 48, and 60 months post transplant. Lung function was the same between DCD and DBD lung recipients. Percent predicted FEV_1 was between 50% and 60% at 1 month post transplant, peaked at 6–12 months post transplant at 60–70% for both DCD and DBD lung recipients, and then remained stable for up to 48 months post transplant, after which there was a decline in the DCD group at 60 months to 40% predicted, while the DBD group stayed at approximately 60% predicted [12]. DeVleeschauwer et al. evaluated the best FEV_1 after transplant and compared DCD and DBD groups. The FEV_1 in this study also was similar; the best FEV_1 in the DCD group was 94% (range 78–106%), and in the DBD group 93% (75–112%) [13]. There was also a continuing improvement in FEV_1 after postoperative day 30 in both groups. By day 30, FEV_1 was increased by 1.58 L in DCD and by 0.84 L in DBD lung transplant recipients. Further improvements in FEV_1 were seen of up to 2.77 L on postoperative day 180 in DCD recipients and 2.31 L on postoperative day 185 in DBD recipients [14].

Chronic allograft injury in lung transplantation manifests as bronchiolitis obliterans. After DCD lung transplantation, bronchiolitis obliterans occurs in 7–20% of patients, and at 3 years in 20–50% of patients [1,12,14]. This compares to rates of 6% in DBD recipients at 1 year and 25% at 3 years. DeOliveira et al. found that the rates of bronchiolitis obliterans after DCD and DBD lung transplantation were nearly identical if DCD lung donors and recipients were carefully selected. Similar rates of bronchiolitis obliterans have been reported in Belgium by DeVleeschauwer et al., with bronchiolitis obliterans occurring in approximately 14% of DCD patients with a median onset at 328 days and in 10% of DBD patients with a median onset at 480 days, numbers that are statistically similar [13]. In a recent review, Wigfield and Love collected and summarized bronchiolitis obliterans cases from different centers after DCD lung transplantation. When added together, bronchiolitis obliterans was found in 21 of 191 (11%) patients at 3 years [15].

Long-term outcome

Data on long-term outcomes after DCD lung transplantation are rare [16], as DCD lung transplantation is a recently growing field and few programs have even small numbers of long-term survivors.

In terms of graft survival (Figure 54.1), DCD and DBD lung transplantation fare equally well, with the caveat that DCD donors are more stringently selected. Graft survival after DCD lung transplantation ranges from 69% to 83% at 1 year, from 61% to 77% at 3 years, and is 77% in the one study that reports up to 5-year graft

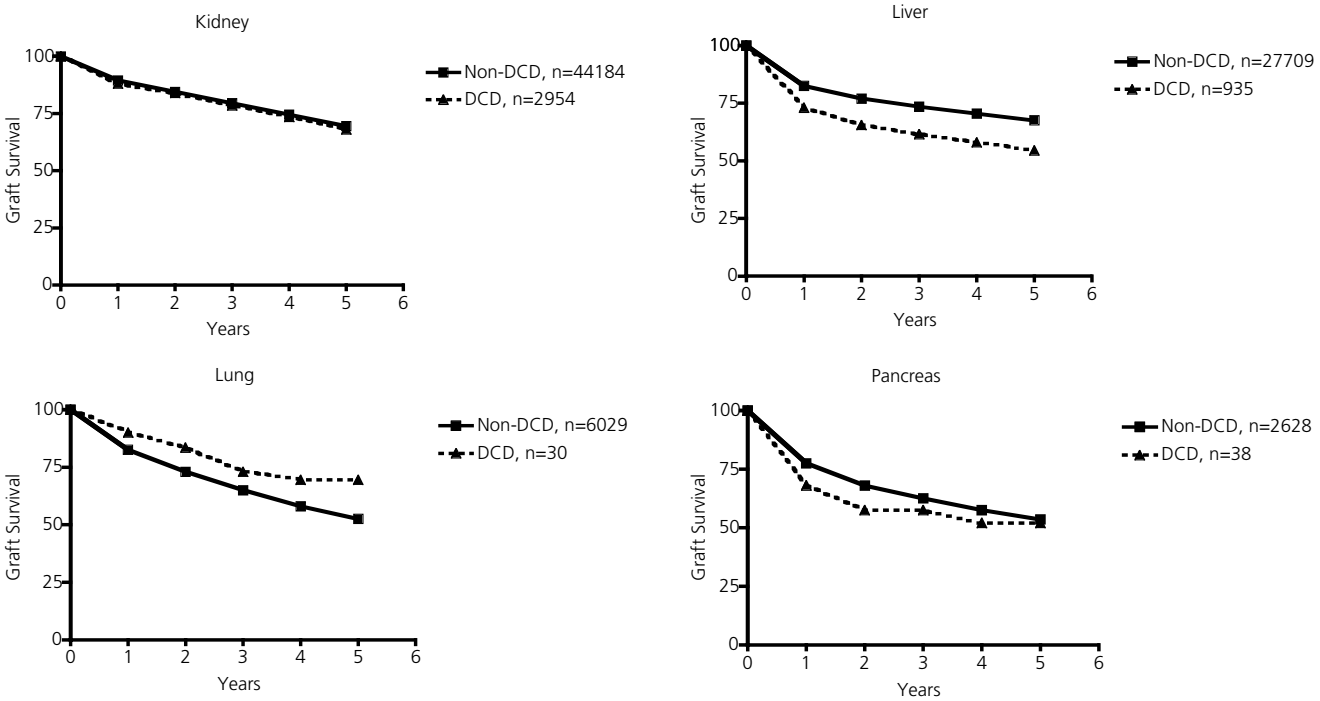

Figure 54.1. Graft survival of deceased donor organs for kidney, liver, lung, and pancreas transplant. There is no significant difference in outcomes after kidney, pancreas, and lung transplantation. Liver transplantation from donation after circulatory death (DCD) donors has statistically significant worse graft survival. (Based on OPTN data from 2002 to 2006.)

survival. This compares favorably to DBD lung transplantation, where 1-year graft survival ranges from 82% to 85%, 3-year graft survival ranges from 68% to 70%, and 5-year graft survival is reported at 60% [1,9,12–14,17].

Patient survival after DCD lung transplantation ranges from 80% to 95% at 1 year, from 69–95% at 3 years, and from 71% to 82% at 5 years. After DBD lung transplantation, the 1-year patient survival is between 78% and 96%, the 3-year patient survival is 72–91%, and the 5-year patient survival is 63–75%. Again, there are no major differences in patient survival between DCD and DBD lung transplant recipients [1,9,12–14,17].

DCD liver transplantation

Criteria for acceptance

Liver DCD donors are somewhat more common, and has been practiced and refined to a greater degree, than lung DCD donation. As such, the selection criteria have been more generally established (Table 54.3). Selection criteria for DCD liver donors vary between centers, but in general are more stringent than selection criteria for DBD livers. Most centers require a heparinized flush at cross-clamp or, preferably, systemic heparinization with high doses of intravenous heparin prior to withdrawal of life support, depending on variations in the law in each donor service area or country. A 30-min warm ischemia time is generally considered the upper limit. The most common definition of warm ischemia time is from withdrawal of all life support to cold flush or cross-clamp, but in some centers, it is defined alternatively as systolic blood pressure of <50 mmHg to flush, and then the maximum allowable warm ischemia time can be up to 60 min [18]. The conservative maximum donor age for DCD donation varies between 40 and 50 years in most centers; however, there are reports of programs successfully transplanting livers from DCD donors up to 65 years old [18–20].

The prior hospital course of DCD liver donors should preferentially be hemodynamically stable and reveal normal, or near normal, liver function tests [21]. DCD liver donors are ideally lean, with different center cut-offs being a body mass index (BMI) of <28–30 kg/m^2 or a body weight of <100 kg. Some programs prefer a history of a short hospital stay prior to donation [21–23]. Gross inspection and biopsy for hepatocyte viability and to rule out macrosteatosis or fibrosis are routinely required in some [1,18], but not all, institutions [20,21]. One study from the UK [24] recently reported a very aggressive use of DCD livers by accepting donor livers from extended criteria DCD donors. These were defined as having one or more of the following criteria: warm ischemia time

Table 54.3. Donation after circulatory death (DCD) liver selection criteria.

	Netherlands [68]	United Kingdom [18]	Spain [19]	Pittsburg [20]	Los Angeles [21]	United Kingdom [23]	University of Wisconsin [63]	Spain [69]	Boston [70]
Systemic heparinization or heparinized flush	Heparin in flush	Heparin in flush			Heparin prewithdrawal		Heparin prewithdrawal	Heparin 3 g/kg, 1.5 mg/kg every 90 min	
Warm ischemia time (withdrawal to flush unless otherwise stated) (min)	<30 and MAP <50 mmHg for <15 min	<60 and SBP <50 mmHg to flush		<20	<30	<30 and SBP <50 mmHg to flush	<30		28.6 ± 8.8
Donor age (years)	1–55	<50, now <65	<55 or <65	<60	<45	<40	<50	<60	
Liver enzymes		Good			<2 × normal	Normal			
BMI 28-30 kg/m^2 or donor weight <100 kg	<28				<30				25 ± 5.8
Length of stay (days)					<5	<5			
Gross inspection		Not fatty, perfused well			Good	Good, perfused well	Not too fatty	No steatosis, good flush, no trauma	
Biopsy (%macrosteatosis)		Yes (done in all cases)					<30–50		
Cold ischemia time (h)				<8	<8				
No history of cancer, drug abuse or other biologic risks. HIV, hepatitis B and C negative			X					X	
Normal abdominal and femoral vasculature			X					X	
Cardiac arrest <15 min			X					X	
CPR/compressor time (min)			<130					<150	
NECMO (h)			<4					<4 (flow >1.7 L/min is good)	
Maastricht category	3	3	2	3	3	3	3	1, 2, and 4	3

MAP, mean arterial blood pressure; SBP, systolic blood pressure; BMI, body mass index; CPR, cardiopulmonary resuscitation; NECMO, normothermic extracorporeal membrane oxygenation.

of >30 min (but under 60 min from systolic blood pressure of <50 mmHg to flush, or from oxygen saturation of <80% to flush), donor age of over 60 years, donor BMI of >30 kg/m^2, or cold ischemia time of >8 h. With careful selection of these donors, acceptable outcomes were achieved with graft survivals at 1 year similar to those for non-extended criteria DCD donors.

Risk factors for post-transplant graft loss, primary non-function, biliary complications, and recipient death include donor age over 40–50 years, donor weight exceeding 100 kg, donor warm ischemia time of >35 min (vs. <15 min), or prolonged donor hypotension [20,22,25]. Prolonged cold ischemia time also adversely affects outcomes [25], with each hour of cold ischemia time increasing the chance of allograft loss by 6% [20,22]. Recipient mortality is increased by increased donor weight and cold ischemia time [22]. Anticipated cold ischemia time of <8 h is required by some centers [20,21]. Organ sharing, requiring additional time for hepatic division into two usable lobes, is another potential for increased cold ischemia time and has been shown to correlate with worse outcomes after DCD transplantation. Recipient age over 60 years, renal dysfunction at transplant, and donor hepatitis C positivity also worsen outcomes after DCD transplantation [25].

Recipient selection for DCD donor livers is key to optimizing outcomes. Recipients older than 55 years have 26% higher rates of graft failure than younger adult recipients. Male recipients and African–American recipients also fare worse with DCD livers than their female and non-African–American counterparts. Retransplant recipients have a 45% higher rate of graft failure than primary DCD liver transplant recipients. Patients with metabolic liver disease also have a higher graft failure risk. Recipients with Model for End-stage Liver Disease (MELD) scores >35 have a 47% higher rate of graft failure than recipients with MELD scores between 15 and 25. Thus, liver transplant candidates older than 55 years, or who are waiting for a repeat transplant, or who have a high MELD score need to carefully consider the risk of waiting for a DBD liver versus accepting the increased risk of graft loss after DCD transplantation [22].

There are very few data in the literature to support DCD liver transplantation in children. However, Gozzini et al. have transplanted two full size and two reduced size (segment II–III) DCD liver grafts successfully into children [23].

In Spain, DCD livers have been used for transplant since 2002, and most of the DCD organs used have come from uncontrolled donors, and have been preserved with normothermic ECMO. Flow parameters on cardiopulmonary bypass have been used to determine graft viability, with good flow considered to be >1.7 L/min. Exclusion criteria have included a history of cancer, drug abuse, or other biologic risks. Donors with a criminal history or violent death have been excluded from consideration. Negative human immunodeficiency virus (HIV), hepatitis B and C virus (HBV and HCV) testing is required, as is normal abdominal and femoral vasculature. Organs with gross macroscopic steatosis have been excluded. Other criteria have included unsupported cardiac arrest for under 15 min, ventilator support, and cardiopulmonary resuscitation or compressor time of <130 min in total [19], and normothermic ECMO for <4 h in total. Donor age of under 55 years has been preferable; although in select cases, donors up to the age of 65 years have been used.

In the Netherlands, mostly Maastricht category 3 donors are used. Donor selection criteria include a donor warm ischemia time of <30 min, donor age of 1–55 years, BMI of <28 kg/m^2, and mean arterial pressure of <50 mmHg for <15 min.

Risks and benefits

The single benefit of using DCD livers for transplantation is that it ameliorates the growing waitlist and allows patients faster access to transplantation. Thus, by adding livers from the DCD donor pool, waitlist deaths are reduced. Although this indirectly provides a benefit to all patients waiting for transplantation, the benefits to the individual patient are less clearly defined. This is particularly relevant given that, compared to DCD transplantation of other organs, the risks of DCD liver transplantation are significantly increased over its DBD counterpart. Primary non-function, biliary complications, especially ischemic-type biliary strictures, hepatic artery thrombosis, and shortened graft survival remain barriers to achieving similar outcomes after DCD and DBD liver transplantation. This holds true for DCD donors from all Maastricht categories.

DCD liver donation from uncontrolled donors has been practiced most commonly in Europe; specifically in Spain. One Spanish group transplanted 10 DCD livers after maintenance of uncontrolled donors by cardiopulmonary resuscitation or compressor and normothermic ECMO. At a mean of 23 months of follow-up, five (50%) of those livers were functioning. The others had failed due to primary non-function or hepatic artery thrombosis, or were lost to death with function secondary to other complications or recurrent hepatitis C [26]. Another Spanish report described the outcomes of 16 category 2 donor livers used for transplantation [19]. There were significantly higher rates of biliary complications occurring in DCD recipients of uncontrolled livers (42%) than in DBD recipients (17%). There also were more non-anastomotic biliary strictures after DCD transplantation (25%) than after DBD transplantation (2%). These complications were ultimately associated with a three-fold higher relative risk of graft loss. A large survey of European countries practicing DCD donation has shown similar 1-year patient and graft survival after controlled and uncontrolled DCD liver transplantation [27].

Controlled DCD livers have been transplanted by several groups in both the US and Europe in efforts to decrease waitlist deaths. The costs for DCD liver transplantation exceed those of DBD liver transplantation by about 25%. This is due to higher numbers of retransplants (21% vs. 7%) and higher rates of biliary complications (58% vs. 21%). Even when retransplant costs are excluded, DCD transplants remain 20% more expensive than DBD transplants [28].

Larger database studies have reviewed outcomes after DCD liver transplantation in the US. Specifically, Scientific Registry of Transplant Recipients (SRTR) data from 1996 to 2007 showed worse patient survival in the DCD group, which did not improve with increasing experience in DCD transplantation [25]. Retransplantation is twice as common for DCD recipients (14.7% vs. 6.8%); and patient survival after retransplantation is less than survival after primary liver transplantation. Mortality after DCD transplantation is higher than after DBD transplantation, which confirms death as a risk of DCD liver transplantation.

Risk factors for poor outcomes after DCD liver transplantation include long donor warm ischemia time (>20–30 min), long cold ischemia time (>8–10 h), and older donor age (>40–60 years) [29,30]. Specific complications and risks of controlled DCD liver transplantation are delineated below.

Primary non-function

Primary non-function after liver transplantation is defined as severe hepatocyte injury [as evidenced by an aspartate transaminase (AST) of ≥3000 U/L] and the failure to synthesize clotting factors (manifest by an INR of >2.5) or clear lactate (a serum lactate

≥4 mmol/L). Primary non-function occurs 3.6 times more frequently after liver transplantation from DCD donors than after liver transplantation from DBD donors [31]. The incidence of primary non-function in recent studies lies between 0% and 12% of patients after DCD transplantation from controlled (Maastricht category 3) donors [20] compared to 1.4–3% after DBD transplantation [20,32]. A survey of Europe has shown similarly different rates of primary non-function between controlled and uncontrolled DCD donors [27]. Larger studies have shown somewhat lower rates of primary non-function (around 2.5–3%) [29,32,33]. Risk factors for primary non-function have included transplanting a male liver into a female recipient, older recipient age (>60 years), and higher recipient BMI (>30 kg/m^2) [20].

Hepatic artery thrombosis

Hepatic artery thrombosis is uncommon but quite problematic after liver transplantation. Its incidence ranges from 0% to 33% after DCD liver transplantation, with larger studies reporting lower rates (0–6%) [20,29,32,33]. A large SRTR database review showed that the incidence of hepatic artery thrombosis is similar after DCD and DBD liver transplantation [31], as expected based on previous reports [20,33,34]. One study, however, reported hepatic artery stenosis to be more common after DCD liver transplantation. Hepatic artery stenosis in recipients of DCD livers more frequently led to biliary strictures than hepatic artery stenosis after DBD liver transplantation [34]. Treatment for hepatic artery thrombosis after DCD liver transplantation frequently includes retransplantation [18,35].

Biliary complications

Biliary complications are more common after DCD liver transplantation than after DBD transplantation [30], and comprise one of the more significant issues when considering acceptance of a DCD organ. This is likely related to the adverse effects of warm ischemia time on the microvasculature of the biliary tree during the donation process. These may be similar to the issues seen in the bronchial tree in lung transplantation from DCD donors, discussed above. Thus, minimizing ischemia time may be beneficial in preventing biliary complications. Others have attempted to minimize biliary ischemia by infusing tissue plasminogen activator (tPA) in the hepatic artery during implantation, with acceptable results [36].

The incidence of biliary complications after DCD liver transplantation is between 15% and 58% [18,20,21,28,29,31–33,37]. This compares to much lower rates of biliary complications (6–21%) in DBD liver recipients [18,20,21,28,29,31–35,37]. In a multinational meta-analysis, the odds of biliary complications were 2.4 times higher after DCD than after DBD liver transplantation. Donor age over 40 years is a risk factor for biliary complications [30]. Interestingly, higher donor BMI or donor weight may increase the risk of anastomotic biliary strictures [30].

Biliary complications include a variety of problems, such as anastomotic and non-anastomotic biliary strictures, bile leaks, bile casts, biliary sludge, bilomas, biliary abscesses, and ischemic cholangiopathy, among others. Some biliary complications can be managed endoscopically, while others require repeat operations, which include a subset of retransplants [29,32]. Because more biliary complications occur in DCD liver transplant recipients, more of these patients require subsequent diagnostic and corrective procedures, which negatively impacts quality of life [32].

Ischemic cholangiopathy deserves specific consideration. It is difficult to treat, often associated with intrahepatic bilomas or biliary sepsis, and not infrequently leads to repeat transplantation. Like all biliary complications, it is significantly more common after DCD than after DBD liver transplantation; the incidence has been reported to be anywhere between 0% and 44% [28,32,33,37] after DCD transplantation, compared to around 3% after DBD transplantation [31–33]. Ischemic cholangiopathy manifests itself as non-anastomotic biliary strictures, either in isolation or diffusely. Most ischemic cholangiopathy manifests within the first 4 months post transplant [30]. Some cases can be managed percutaneously with dilations via endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC), but up to 50% eventually require retransplantation [30,33,37]. Ischemic cholangiopathy can lead to graft failure [21]. Predictors of ischemic cholangiopathy include a longer time from asystole to cross-clamp and having an African–American recipient [33]. For every minute increase in asystole to cross-clamp time, the risk of ischemic cholangiopathy increases 16% [33]. Further risk factors for ischemic cholangiopathy include cold ischemia time of >8 h and donor age over 40 years [30].

Other risks

Ischemia–reperfusion injury is exacerbated after DCD liver transplantation, and is initially manifest as aberrant laboratory parameters such as a higher peak AST [29] or a higher INR 1 week post transplant [20]. One study addressed the occurrence of a post-reperfusion syndrome during the DCD liver transplant operation, with the finding that all patients required transient vasopressor support beginning at or before reperfusion [18], and lasting through to the end of the operation in six of 32 patients.

Recovery after DCD liver transplantation is similar to that following DBD liver transplantation. Overall length of hospital stay post transplant is similar to that for DBD transplantation [20,32], although ICU length of stay was longer in DCD recipients in one study [20]. Rejection occurs with similar frequency by 90 days and 1 year post transplant [32,34].

Short-term graft survival after DCD liver transplantation is similar to that after DBD liver transplantation in some studies [32,33], but not others [30]. Two-fold higher graft failure rates after DCD liver transplantation at 1 and 3 years were seen in a meta-analysis of DCD and DBD liver transplantation [31]. Retransplantation occurs more than twice as often in DCD recipients than DBD recipients (15–18% vs. 7%), and is mostly seen in older DCD donor–older recipient pairs [20]. Worse outcomes after DCD transplantation are seen, especially when donors are older than 40 years, cold ischemia time is >12 h, and livers are shared. Similarly, recipient age over 60 years, renal insufficiency at transplant, and donor HCV status have been reported to exacerbate the risk for poor outcome after DCD transplantation. Risk factors for graft loss after DCD transplantation include non-Caucasian recipient ethnicity and retransplantation, but interestingly not necessarily older donor age [33]. Causes of graft loss have been variable, and include recurrent hepatitis C, primary non-function, hepatic artery thrombosis, and infections [21]. Recurrent hepatitis C has been seen with the same or higher frequency after DCD liver transplantation compared to after DBD liver transplantation [20].

Patient survival after DCD liver transplantation equals that after DBD liver transplantation in some studies [32,33]. Others, however, have seen an increase in 1-year patient mortality after DCD transplantation, but at 3 years, mortality was the same [31]. Patient survival is worse following DCD transplantation according to another large study, at 82% and 71% at 1 and 3 years, compared to

86% and 77% at 1 and 3 years after DBD liver transplantation, respectively. Mortality for retransplant recipients of DCD livers is even worse, with survival at 1 and 3 years of 71% and 59%, respectively. These rates are very similar to the 68% and 60% at 1 and 3 years after DBD retransplantation, respectively [25]. Causes of death are variable, including recurrent hepatocellular carcinoma, complications of retransplantation, intraoperative cardiac death, and multisystem organ failure [32,33]. Of note, splitting of DCD livers has been described and performed with some success in terms of reasonable patient and graft survival at 1 year [18].

Long-term outcome

Graft survival and retransplantation

Long-term graft survival after DCD liver transplantation is inferior to that for DBD liver transplantation (Figure 54.1). At 5 years, DCD liver graft survival is 43–53% compared to 51–68% DBD liver graft survival [19–21,30]. Only one report has described similar graft survival at 5 years (around 69% in both the DCD and DBD groups) [33]. In the very long term, over 10–20 years postoperatively, graft survival is ultimately similar at 37.5% at 10 years in both groups and 29% and 25% at 20 years in DCD and DBD groups, respectively [35]. As graft survival is worse after DCD liver transplantation, retransplantation is more common after DCD liver transplantation, with rates around 19% for DCD liver transplantation compared to only 5–7% for DBD transplantation [30,34]. Causes for retransplantation include ischemic cholangiopathy in 81%, primary nonfunction in 13%, and vascular complications in 6% [30].

Patient survival

Patient survival after DCD transplantation is similar or slightly less than after DBD transplantation, depending on the study. At 5 years, patient survival after DCD and DBD liver transplantation ranges between 68% and 77%, and 62% and 81%, respectively [19,20,30,33]. At 10 years, patient survival after DCD transplantation lies between 43% and 57% [20,30,35], at 15 years around 54%, and at 20 years around 38%. This compares to 10- and 15-year survival after DBD liver transplantation of 64–67% and 58%, respectively [20,30].

Rejection and other complications

No differences exist in long-term immunologic complications, including acute or chronic rejection [30]. One study suggests that hepatitis C recurrence is more severe after DCD than after DBD liver transplantation [38].

DCD pancreas transplantation

Criteria for acceptance

DCD pancreas transplantation for the treatment of severe type 1 diabetes with or without simultaneous kidney transplantation remains relatively uncommon worldwide. In the US, only select centers choose to transplant pancreata recovered from DCD donors [39]. In Europe, currently only the UK, Belgium, and the Netherlands perform DCD pancreas transplants [27]. There is also a case report of a DCD pancreas transplant in Australia [40]. Selection criteria for DCD pancreata are qualitatively similar to those for DBD pancreata; however, they are more stringent with regard to the quantitative aspects of selection.

Donor age ranges from 3 to 60 years [1,41], but the trend is toward more moderate ages rather than the extremes of this range, and some programs will only accept organs from adult DCD donors [39]. There can be no history of diabetes or pancreatitis. Intra-abdominal sepsis, prior pancreatic surgery, or pancreatic trauma are likewise exclusion criteria. Donors must be HIV negative and have no history of recent malignancy [42]. The presence of hyperglycemia or mild hyperamylasemia at the time of organ donation is acceptable [41]. Young donors with a low BMI who are hemodynamically stable are generally preferred if DCD pancreas transplantation is planned. Other considerations in deciding whether to proceed with recovery and transplantation of a DCD pancreas include whether the donor operation is performed in the local donor service area, whether the anticipated cold ischemia time is short, whether the same team is available for recovery, whether the quality of the flush and gross visualization of the organ is good, and whether there is a possibility of pumping an accompanying kidney in the case of simultaneous pancreas–kidney transplant [39]. The cut-off for warm ischemia time varies between centers from 20 to 45 min and depends somewhat on the course after withdrawal of support [39,41]. Warm ischemia time is variably defined as time from withdrawal of support to cold perfusion, or alternatively beginning at hypotension (systolic blood pressure <50 mmHg) or hypoxia (oxygen saturation $<70\%$) [39,41].

Risks and benefits

Overall, complication rates after DCD and DBD pancreas transplantation are similar. Rejection rates are 14% at 1 year and 19% at 5 years for DCD transplantation, which compares well to rates of 13% at 1 year and 15% at 5 years for DBD pancreas transplantation. Graft thrombosis is uncommon, typically occurring in 2–6% or recipients [1,41]. Enteric conversion is necessary slightly more often if DCD pancreas transplantation is performed initially via bladder drainage (17% at 1 year and 28% at 5 years vs. 8% at 1 year and 15% at 5 years in DBD transplantation). Enzyme leaks occur in 4% of patients in the first year post transplant and this percentage does not increase further over the following 5 years. In DBD transplantation, enzyme leaks are more common at 9% in the first year and 10% over a 5-year period. The rates of pancreatitis at 1 and 5 years post transplant are similar after DCD (9% and 9%, respectively) and DBD transplantation (9% and 14%, respectively). Pancreatic pseudocysts and pancreatic necrosis occur infrequently ($<1\%$) in both groups. Peripancreatic abscesses develop in about 8–10% of patients in both the DCD and DBD groups up to 5 years post transplant.

Function of DCD and DBD pancreas transplants

Transplantation of the pancreas occurs either as whole organ or islet transplantation. Outcomes after whole organ transplantation are discussed, followed by brief mention of outcomes after transplantation of DCD islets.

Glucose control after whole organ pancreas transplantation is similar at discharge and long-term between DCD and DBD pancreata [1,41,43]. In the long term, fasting blood glucose and hemoglobin A1c (HbA1c) are reported as nearly identical. One year post transplant, HbA1c is $5.43 \pm 0.75\%$ after DBD and $5.63 \pm 0.57\%$ after DCD pancreas transplantation [1,41,43]. Patient and graft survival are comparable after solitary pancreas transplantation and after simultaneous pancreas–kidney transplantation from DCD donors. Patient and pancreas graft survival at 12 months is 100% each, when in-situ perfusion via extracorporeal support is used in uncontrolled donors [42]. After controlled DCD pancreas transplantation, patient survival is similar at 1, 3, and 10 years after DBD transplantation (97%, 94%, and 79%, respectively) and after DCD transplantation (94%, 93%, and 87%, respectively). Pancreas graft

survival is slightly lower after DBD (87%, 81%, and 61%) than after DCD transplantation (83%, 78%, and 63%) at 1, 3, and 10 years, respectively [1]. Most patients (93%) are free from hypoglycemia at 1 year, regardless of DCD or DBD donor type [1]. Nine percent of patients in each group develop post-transplant diabetes mellitus [1]. Hospital length of stay is the same for both groups [41,43]. Pancreas graft thrombosis occurs in 13% of DCD and 6% of DBD pancreata. Causes of pancreatic graft loss after DCD simultaneous kidney–pancreas transplantation include bleeding (18%), acute rejection (9%), and chronic rejection (9%), whereas causes of pancreatic graft loss after DBD simultaneous kidney–pancreas transplantation are mostly chronic rejection and infection.

Clinical islet transplantation from DCD donors has mostly been described in Japan. Seven of eight recovered organs were transplanted with good islet yields and reasonable purity [44]. Another Japanese group later reported 34 DCD islet transplants from 64 donors into 18 recipients. Islet quality was worse with longer prearrest hypotension and longer cold ischemia time, and better with the use of Kyoto solution during recovery [45]. Markmann et al. similarly reported a lower islet yield with longer warm ischemia time [46]. While in Japan 16 of 18 recipients had immediate islet function, half lost islet function within 1 year. Still, HbA1c was improved overall after islet transplantation, and all patients eventually achieved freedom from hypoglycemia [45].

Long-term outcomes

Little data exist on long-term outcomes after DCD pancreas transplantation. DCD pancreas graft survival is 83% and 72% at 1 and 5 years, respectively, and is similar to pancreas graft survival after DBD pancreas transplantation at 89% and 79%, respectively (Figure 54.1) [41,43]. Pancreas graft survival after simultaneous pancreas kidney transplantation is 85%, 80%, and 74% at 1, 3, and 5 years after DCD transplantation, respectively [39]. Patient survival after simultaneous pancreas–kidney transplantation is 98%, 93%, and 89% at 1, 3, and 5 years after DCD transplantation, respectively [39]. Patient survival after DCD pancreas transplantation is 92% at 1 and 5 years post transplant, which is similar to the 97% and 89% survival after DBD pancreas transplantation, respectively [41,43].

DCD kidney transplantation

Of all organs, kidneys are the most frequently transplanted from DCD donors. Thus, there is more experience with DCD kidneys than with any other DCD organs. Furthermore, DCD kidneys are also most often considered for multiorgan transplants, including simultaneous kidney–pancreas transplants and simultaneous liver–kidney transplants [2].

Criteria for acceptance

In addition to the usual kidney acceptance criteria, additional selection criteria apply to DCD kidneys that vary by center; some of these are delineated here. Uncontrolled DCD donors should be reasonably young (under 60–65 years), and have no history of renal impairment, cancer, sepsis, or advanced diabetes. Warm ischemia time limits are between 40 and 120 min. Most uncontrolled DCD donors are identified in the emergency room after unsuccessful resuscitation [46,47].

In Belgium, the maximum age for DCD kidney donation is 75 years, with a warm ischemia time up to 60 min and a cold ischemia time that often exceeds 24 h due to timing of HLA typing, crossmatching, and allocation. All DCD kidneys are routinely machine preserved [48].

Farney et al. have described their optimal DCD donor as a standard criteria donor (SCD), with a cold ischemia time of under 30 h, and a kidney that is transplanted into a recipient under 60 years of age. Contraindications to DCD donation include a glomerular filtration rate (GFR) of <70 mL/min on admission, a history of cancer, HIV positivity, cold ischemia time of >45 h, and warm ischemia time of >90 min for SCD donors and >60 min for ECD donors. Machine perfusion criteria are used, with flows of <60 mL/min or resistance of >0.4 mmHg/mL/min being contraindications to transplantation of adult DCD kidneys [49].

Our own criteria for DCD kidney donation are a warm ischemia time of <120 min. However, most patients in whom support is withdrawn go on to expire and meet the criterion for donation in 60 min or less. The mean time for the few cases who expire after >60 min is 75 min. Initially, our maximum DCD donor age was 65 years; however, more recently we avoid the DCD/ECD donor unless their creatinine, creatinine clearance, and medical history are all optimal. We biopsy all DCD kidneys for Remuzzi scoring and to check for fibrin thrombi. Kidneys with Remuzzi scores of >6 are discarded.

Risks and benefits

DCD kidneys, like DBD kidneys, provide life-saving organs to patients. Waitlist mortality is reduced by using DCD kidneys for transplantation [50]. Delayed graft function (DGF), often defined as the need for dialysis within 1 week after kidney transplantation, is more common after DCD kidney transplantation than after DBD transplantation. DGF rates of 28–57% after DCD kidney transplantation, even with relatively short cold ischemia times (13 ± 5 h), are significantly higher than rates of 19–21% after DBD kidney transplantation [1,42,43,51–55]. After DCD kidney transplantation from uncontrolled donors, DGF rates of 93% have been reported [46,56]; thus, DGF rates are definitely higher after transplantation of DCD kidneys from uncontrolled donors than from controlled DCD donors [27]. The average duration of DGF is 13 ± 8 days [51]. Risk factors for DGF are donor systolic blood pressure of <60 mmHg for >20 min and donor age over 50 years [51,52]. Cold ischemia times of >30 h may also increase the incidence of DGF [49]. DGF often results in a prolonged hospital stay and more procedures [41,54,57]. A multicenter Eurotransplant study of paired DCD kidneys determined that pulsatile perfusion preserved kidneys have a shorter period of DGF than cold stored DCD kidneys, although 1-year graft survival was the same at 94% versus 95%, respectively [57]. DGF may indeed be reduced by pulsatile perfusion preservation, though not all studies confirm this [57,58]. DGF does not have a negative effect on long-term outcomes, nor does the duration of DGF affect graft survival [49,54].

Primary non-function after DCD kidney transplantation is uncommon (1–5%) [43,46,47,49,51,52], as it is after DBD kidney transplantation [55]. Lower intraoperative blood pressure ($<110/80$ mmHg) and central venous pressure (<6 cmH_2O) are risk factors for primary non-function [59].

Rejection occurs at similar or slightly higher rates after DCD and DBD kidney transplantation [1,42,54,60]. Acute rejection is reported to occur in 19–24% of patients after DCD transplantation and in 10% after DBD transplantation [42,46]. When comparing the two, others have reported higher rates of rejection after DCD transplantation than after DBD transplantation (29% vs. 16%) [53].

Rates of other complications, including renal artery stenosis or thrombosis (<2%), ureteral complications (<5%), or lymphoceles (<10%), do not differ between DCD and DBD kidneys [1,41,43,55].

Function of DCD and DBD kidney transplants

Immediately postoperatively, DGF occurs more frequently after DCD than after DBD kidney transplantation, but primary nonfunction is rare. The discharge creatinine is higher after DCD kidney transplantation than after DBD kidney transplantation (1.9 vs. 1.7 mg/dL) [43,55]. Creatinine on day 7 is also reported as higher in DCD compared to SCD or ECD kidneys [42]. DCD kidneys have comparable creatinine clearance at 112 mL/min compared to 101 mL/min for SCD kidneys and 77 mL/min for ECD kidneys [42]. Serum creatinine is reported as similar up to 10 years after DCD and DBD kidney transplantation [47,61]. Data reported from the Netherlands have suggested that DCD kidneys have a higher rate of graft loss than DBD kidney (12% vs. 6%), although it is not clear if these data are globally generalizable.

Long-term outcomes

Long-term graft survival is similar for DCD and DBD kidneys (Figure 54.1) [43,53]. Even graft survival of kidneys from DCD donors who develop DGF after transplantation is rather good at 88% at 1 year, 84% at 3 years, and 84% at 6 years [45]. Actuarial graft survival after DCD kidney transplantation is 89% at 1 year, 76% at 3 years, and 76% at 5 years. Graft failure is mostly due to chronic allograft nephropathy and death with function. Actuarial death-censored graft survival after DCD kidney transplantation is 93% at 1 year, 84% at 3 years, and 84% at 5 years. Recipients over the age of 60 years have lower 1- and 3-year graft survival at 79% and 64%, respectively [49]. DCD kidney graft survival in one report was 88%, 77%, and 44% compared to 78%, 69%, and 42% after DBD kidney transplantation at 1, 3, and 10 years post transplant, respectively [62]; and in another report was was high at 79%, 70%, and 62% after DCD transplantation and 83%, 72%, and 62% after DBD transplantation, respectively [47]. Others have also found no difference in allograft survival at 5, 10, or 15 years post transplant [55].

Patient survival is identical for DCD and DBD kidney recipients, even up to 15 years post transplant [10,17,21]. Actuarial patient survival is 93% at 1 year, 91% at 3 years, and 89% at 5 years post transplant [49]. These rates are similar to reported rates of 1-, 3-, and 10-year patient survival of 92%, 85%, and 60% after DCD transplantation [1]. Sepsis, cardiovascular disease, cancer, and gastrointestinal hemorrhage are common causes of death. Older recipients of DCD organs have lower patient survival than their younger counterparts (81% vs. 98% at 1 year, 76% vs. 97% at 3 years, and 69% vs. 97% at 5 years) [49]. Patient survival is lower in DCD–ECD recipients than in DCD–SCD recipients [49].

DCD heart and intestine transplantation

Neither cardiac nor small bowel transplants from DCD donors currently play a clinical role. As we gain more experience from preclinical studies, however, these organs may be considered for transplantation in the future.

DCD process

In general, the initial DCD process is no different from that for DBD donors (Figure 54.2). All patients with a Glasgow coma score (GCS) of 5 are referred to the Organ Procurement Organization (OPO). Also, all patients for whom a decision has been made to withdraw life support are referred to the OPO. The OPO then determines medical suitability and makes an assessment of whether the patient is likely to expire in a 2-h time frame. A respiratory drive assessment is made utilizing the University of Wisconsin (UW) DCD tool, which has been previously validated and published [63]. If a patient is on maximum ventilation or on multiple vasopressors, the tool is not performed. Our data show that when we make a decision to proceed with a DCD donor based on the tool's criteria, we recover organs 80% of the time. Families are always informed that there is a possibility that the donation may not occur. Our experience is that they are thankful that we attempted to honor their loved one's wishes to be an organ donor.

One guideline for DCD recovery of kidneys is that from Thomas et al. in the UK [64]; ICU practice determines whether or not life support will be withdrawn. Once the decision to withdraw support has been reached, potential donors are referred to the OPO in discussion with the OPO coordinator. The family is approached after the decision to withdraw support has been made by a senior ICU team member and once candidacy has been established. At this point, escalation of treatment is no longer allowed. Support is withdrawn in the ICU in the usual way and is dictated by local practice. Up to 2 h of warm ischemia time, defined as time from withdrawal of support to organ recovery, are allowed. The ICU physicians declare death after a mandatory 5-min hands-off period. The family then gets an extra 5 min with the patient before he/she is taken to the operating room. If the family requires more time, then donation is aborted. Of 100 potential donors, 29 were realized, while 30% refused donation. This process again is resource intensive and commitment is particularly required by those involved in the donation process. This protocol has been expanded to liver and pancreas DCD donation.

In Leicester, UK, 73 DCD referrals took place in 3 years, 64 from the emergency room, and nine from the wards, resulting in 24 cases (33%) and 44 recovered kidneys. Thirty kidneys were transplanted locally, eight were shipped out, and six were discarded. These DCD kidneys made up 21% of the entire transplant activity at that center. Consistent with our experience, the authors concluded that the DCD process is labor intensive and requires highly committed staff [65].

The DCD process takes resources beyond those required for DBD donation, both on the part of the OPO and the donor hospital. For example, of 56 DCD lung referrals, nine did not progress within the 2-h cut-off, and 13 needed support to be evaluated on site of these 13 lungs, only nine were transplanted. Two hospital physicians needed to be present to declare death after a 5-min wait period, ICU staff were present for withdrawal of support in either the ICU or postanesthesia care unit (PACU), and the operating room staff were tied up and ready to go [11].

Hospital relations

Currently, all hospitals in the US that have potential for DCD are required to have policies and protocols in place by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). Additionally, the guidelines of the United Network for Organ Sharing (UNOS) must be followed in DCD donor cases. Similar processes are in place in Europe, and it is likely that global adoption of DCD donation will require process adaptation to meet local practice requirements and customs. As already mentioned, DCD donor cases are more labor intensive and require more OPO

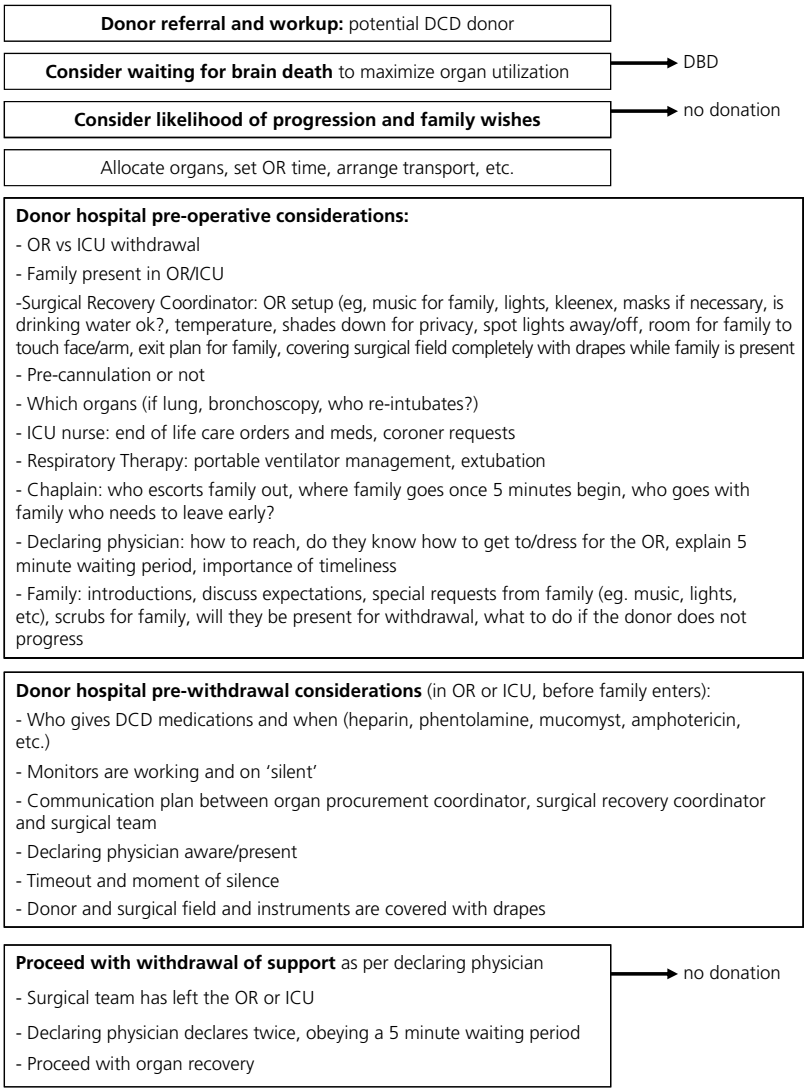

Figure 54.2. The donation after circulatory death (DCD) process.
DBD, donation after brain death; OR, operating room; ICU, intensive care unit; SRC, surgical recovery coordinator.

resources due to processes that must be communicated and followed regarding roles and responsibilities to a patient who has not yet been declared dead. We always send two OPO coordinators to DCD cases to ensure all protocols are being followed, families' needs are being met, and all personnel in the OPO and donor hospital understand their roles and responsibilities. We believe that DCD donation belongs in the continuity of care for patients at the end of life and offers the patient and his/her family the opportunity to leave a lasting legacy through donation. By caring for the potential donor and his/her family and by providing a DCD donation opportunity, we also provide desperately ill potential recipients the chance of a renewed life that otherwise might not be possible.

Summary

It is now evident that organ demand will continuously outpace organ supply. As such, additional opportunities for donor organ acquisition will remain important to best provide organ replacement for those in need. DCD donor organs are acceptable alternatives to SCD organs in many settings, especially in kidney and increasingly in liver transplantation. However, their use requires attention to risk–benefit assessment and proper patient selection, as well as a dedication to perform the organ procurements with heightened attention to established protocol and mindful of the requirement for maintaining professional decorum throughout the process.

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SECTION 5

Transplant Procedure and Surgical Technique

CHAPTER 55

Kidney Transplantation Procedure and Surgical Technique

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Introduction

As eloquently alluded to by Dr. Joseph Murray in his Nobel Prize lecture [1], advances in surgical technique, along with understanding and finding solutions to immunologic barriers, and the development of renal replacement therapies to sustain individuals with renal failure were three interrelated pathways leading to the success we enjoy today in clinical kidney transplantation. Surgical determination was an important trait held by a long list of pioneers who were responsible for the development and refinement of technical aspects of the transplant operation currently in practice today. Recent advances in imaging modalities, percutaneous and endoscopic interventions, and minimally invasive surgical techniques have also fostered significant strides forward in identifying and managing technical complications post transplantation that previously often would have required reoperation with greater potential morbidity, mortality, and risk for organ loss. Most would agree that the evolution of kidney transplantation to become the established best therapy for many patients with end-stage renal disease is the culmination of all the above contributions. This chapter will focus on technical aspects of the transplant operation as well as management of technical complications encountered following the procedure.

Preoperative considerations

Upon admission for impending renal transplantation, an updated history is obtained and physical examination performed on the intended recipient to bring to light any significant interval events or physical findings impacting the patient's health or proposed procedure since he or she was last evaluated. Specific issues to be addressed include sensitizing events, infections, and changes in the patient's cardiovascular reserve (also see Chapter 37, which covers kidney transplant list management). Laboratory and imaging studies, cardiac testing, and specialty consultations are obtained or brought up to date as indicated to ensure that the patient is a suitable candidate for transplantation—able to withstand and benefit from the rigors of the operation and long-term immunosuppression. As detailed in Chapter 89, ABO and HLA compatibility between the recipient and donor organ is confirmed. Available information regarding the donor kidney should be reviewed to confirm the appropriateness of organ quality (organ selection criteria are discussed in detail in Chapter 53). Informed consent for the procedure must be obtained from the patient or responsible party.

In preparation for the procedure itself, the surgical team should review the use of warming devices and venous thromboembolism prophylaxis, availability of blood products, and administration of preoperative antibiotics and intraoperative medications, including immunosuppressive therapy, with the anesthesia and nursing personnel prior to incision. Central venous access may be advisable in patients with poor peripheral veins or in cases where more invasive hemodynamic monitoring is indicated. Similarly, an arterial catheter may be warranted to more closely monitor blood pressure or allow frequent blood sampling. The operation is performed under general anesthesia with the patient in the supine position. An indwelling catheter is placed within the urinary bladder or conduit. Sterile antibiotic solution, with or without methylene blue, may be gently instilled to facilitate identification of the bladder in the operative field during the urinary drainage reconstruction. Special care is needed in patients with previous prostatic or urethral pathology or surgery or atrophic bladders from longstanding anuria to avoid iatrogenic injury during catheter insertion, such as false urethral passages or bladder rupture during instillation of irrigation. The authors prefer distention of the bladder by gravity drainage, as this prevents the potential for rupture of an atrophic bladder, and provides some insight into the capacity of the recipient bladder, and its implications for ureteral reconstruction.

Back-table preparation of kidney

Back-table preparation of the renal allograft is performed either previous to or concurrent with the recipient exposure. In cases where there is concern that trauma, surgical damage, or aberrant anatomy may have rendered the donor organ not suitable for transplant, it should optimally be examined by the surgeon prior to the recipient being brought into the operating room. In the deceased donor scenario, a Carrel patch of aorta is typically maintained on the renal artery, allowing a larger, technically less demanding vascular anastomosis which does not involve the renal artery ostium. This is especially desirable in the case of a pediatric donor kidney with a small artery [2] to facilitate performing the anastomosis and to alleviate concern that the ostium may be

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restricted by the suture line as the recipient and organ grow with time.

Multiple arteries are commonly encountered and there are multiple options for reconstruction. Trivial accessory arteries (especially to the upper pole) may be sacrificed without apparent negative outcomes; however, care is taken to preserve significant accessory vessels, especially those supplying the lower pole likely critical for ureteral perfusion [2,3]. Multiple arteries may be maintained on or reconstructed to form a common Carrel aortic patch on the back table to allow a single relatively straightforward arterial anastomosis in the recipient [4,5]. Conversely, when indicated, multiple renal arteries may be kept separate and anastomosed to the recipient vessels independently to prevent excessive tension, angulation, or compression of the adjacent renal vein [3]. Atherosclerotic disease of the donor aorta or at the renal artery ostium may make the aortic patch not fit for use, in which case the artery is transected at a point relatively disease free and suitable for anastomosis. Multiple arteries in the living donor kidney or other situations where there is not a suitable aortic patch may be spatulated and sewn together in "pair of pants" fashion [4] to allow a single arterial anastomosis in the recipient. Alternatively, an accessory artery may be sewn to the main artery in end-to-side fashion [3] or multiple vessels may be reconstructed to a Carrel patch fashioned from PFTE or autogenous tissue, if available, to facilitate the vascular anastomosis in the recipient. Gonadal, lumbar, and adrenal tributaries to the renal vein are controlled with clips or silk ties. Small accessory renal veins may be ligated, as renal veins typically intercommunicate via collaterals. However, if there exist two main renal veins of equal caliber draining the kidney, it is recommended to salvage both to reduce the risk of venous thrombosis and infarction [2]. The deceased donor right kidney graft optimally presents with a full cylinder of vena cava, which can be fashioned into a vascular conduit using suture or a stapler to effectively increase length of the renal vein [2,5]. This makes the venous anastomosis less technically challenging and decreases the likelihood of tearing the delicate right renal vein. Lymphatic structures in the allograft are believed to be a potential source of post-transplant lymph leakage and resultant lymphocele [3,6] and therefore are carefully controlled with ties or clips as they are encountered during the back-table dissection. Care is taken to maintain sufficient periureteral tissue and preserve the perirenal fat bordered by the ureter and the lower pole of the kidney (the "golden triangle") to reduce the likelihood of ureteral ischemia, which may predispose to subsequent urinary leak or stricture [7,8]. Figures 55.1 and 55.2 show the typical reconstruction and placement of a left and right kidney (inclusive of a vena caval conduit), respectively.

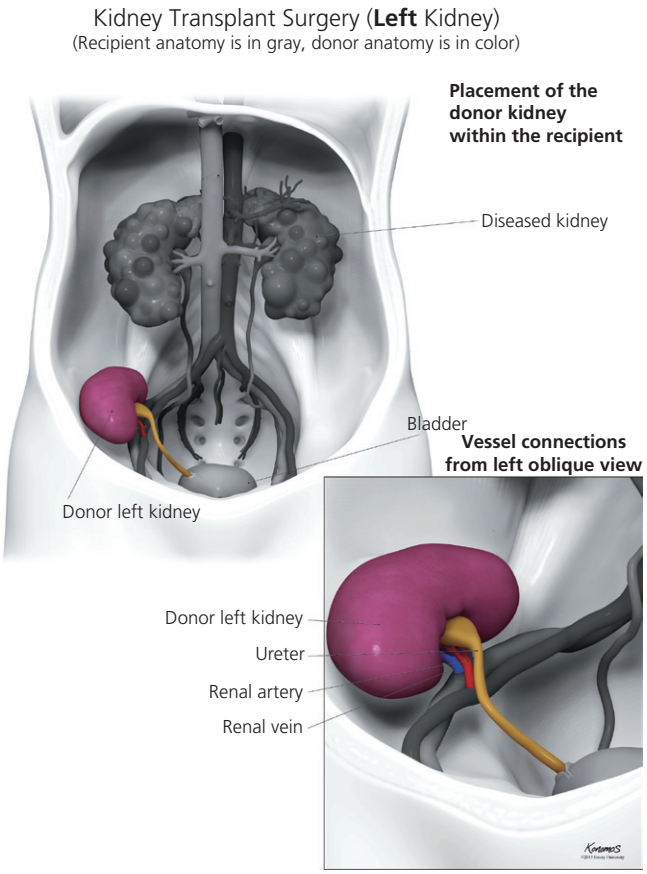

Figure 55.1. Placement of a left kidney allograft in the right iliac fossa. This is by far the most common orientation, as most live donor kidneys are left kidneys (due to the longer left renal vein). Shown are the typical relationships of the renal artery and vein to the external iliac artery and vein, respectively. The ureteral anastomosis is a standard Lich-type extravesical ureteroneocystostomy. Note that when a kidney from the contralateral side is used, the collecting system is medial/anterior, facilitating access to the ureter and collecting system in the event of a ureteral complication. The iliac vein has been mobilized laterally to accommodate a direct lie of the vein once it is anastomosed.

Incision and exposure

Typically, an oblique Gibson [4] or J-shaped "hockey-stick" [5] lower quadrant incision is made and extraperitoneal exposure obtained to prepare the external iliac artery and vein for the vascular anastomoses. The superficial inferior epigastric vessels are often ligated and divided to facilitate exposure, as is the round ligament in females [5]. In cases where the ipsilateral superior epigastric vessels have been divided recently (for example as a result of a concomitant liver transplant or recent subcostal incision), some surgeons advocate preserving the inferior epigastrics to avoid acute rectus muscle ischemia. If the donor kidney is found during the back-table preparation to have a small divided accessory artery or arterial branch requiring salvage, the superficial inferior epigastric artery may be preserved or divided with good length maintained as a potential option for revascularization [2,9]. Conscious effort is made to control lymphatic channels and nodes with cautery, clips, or ligatures as they are divided and/or resected in the process of exposing the vessels to help prevent lymph leakage and subsequent lymphocele formation [2,3,10].

As shown in Figures 55.1 and 55.2, the kidney is commonly transplanted to the recipient's right external iliac vessels, due to the relative superficial location of the right external iliac vessels and overall facility for right-handed surgeons. The left external iliac vessels may be utilized based on surgeon preference, if the right side is being reserved for a pancreas allograft, if there has been a previous transplant on the right side, or the right-sided anatomy is otherwise unsuitable [5]. Some sources advocate contralateral placement of an allograft (i.e. right donor kidney to recipient left lower quadrant and vice versa) to allow the transplant renal pelvis and ureter to lie medially, thus facilitating future urinary revision if necessary [2,4]. In general, the external iliac vein lies posterior to the external iliac artery, and, as shown in Figures 55.1 and 55.2, its

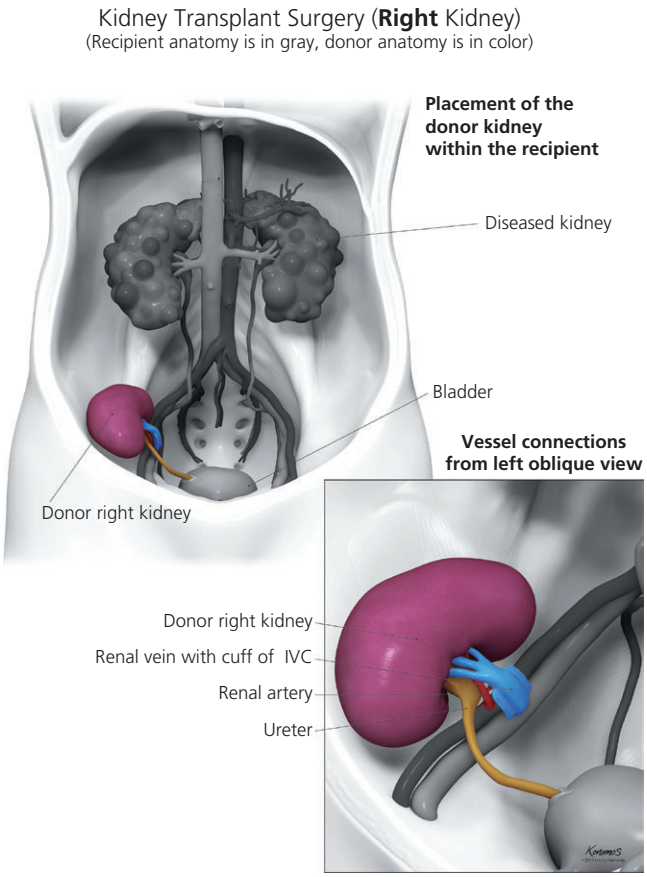

Figure 55.2. Placement of a right kidney allograft in the right iliac fossa. Shown are the typical relationships of the renal artery and renal vein to the external iliac artery and vein, respectively. The ureteral anastomosis is a standard Lich-type extravesical ureteroneocystostomy. Note that when a kidney from the ipsilateral side is used, the collecting system is lateral/posterior, hindering access to the ureter and collecting system in the event of a ureteral complication. The iliac vein has been mobilized medially to accommodate a direct lie of the vein once it is anastomosed. The right renal vein is often short. In this case, a conduit of vena cava that is fashioned by stapling across the suprarenal and infrarenal donor vena cava and using the donor's left renal vein orifice as the eventual site of the end-to-side venous anastomosis is shown.

mobilization can be varied from lateral to medial to accommodate a natural lie of the renal vein anastomosis, with contralateral placement favoring lateralization of the iliac vein, and ipsilateral placement favoring medialization of the vein. If bilateral external iliac vessels have been previously utilized, subsequent transplants are generally performed transperitoneally via a lower midline incision, to allow assessment of and access to the more proximal iliac vessels or aorta and inferior vena cava for anastomosis if necessary [5]. History of previous lower extremity deep venous thrombosis or hemodialysis access should prompt appropriate preoperative imaging to evaluate for potential iliac or caval venous stenosis or obstruction requiring intervention and affecting selection of allograft venous outflow. Transplantation ipsilateral to an active thigh arteriovenous fistula is generally avoided if possible due to concern for potential vascular complications such as venous hypertension affecting graft function. Ligation and division of the internal iliac vein may be helpful in cases with a short renal vein, deep recipient pelvis, or vertically oriented iliac vein to increase mobility of the common and external iliac veins and thereby facilitate the anastomosis [2,5].

Revascularization

Systemic heparinization is employed by some centers during the period of vascular occlusion for the anastomoses [2,4]; however, this is not universally practiced. Vascular clamps are carefully applied to obtain proximal and distal control of the iliac vessels. The allograft renal vein is typically sewn in end-to-side fashion to the recipient external iliac vein using a fine (generally 5-0) running polypropylene suture. After fashioning the arteriotomy with fine scissors or a vascular punch, the renal artery is usually anastomosed in end-to-side fashion to the external iliac artery using a running fine (5-0 or 6-0) polypropylene suture. Another option is to sew the renal artery to the recipient internal iliac artery in end-to-end fashion [2]. In cases involving multiple donor renal arteries, both external and internal iliac arteries may be utilized for inflow [5]. If the internal iliac artery is considered for inflow, its origin should be carefully examined for significant atherosclerotic disease potentially compromising allograft perfusion. The internal iliac artery should not be used if the contralateral internal iliac artery has previously been divided due to the increased likelihood of impotence, claudication, or other vascular complications [3,4]. Care must be taken while handling and suturing diseased recipient and donor vessels to avoid creating an intimal flap or dissection. In cases involving small pediatric donor organ vessels, consideration should be given to performing at least part of the anastomosis with interrupted sutures [2] to allow for growth in cross-sectional area over time.

Once the vascular anastomoses are completed, the vascular clamps are released to restore flow from and to the lower extremities as well as to the newly revascularized kidney. Hemostasis is achieved. Ideally, the kidney promptly displays a healthy pink color, good turgor, and production of urine. Initial poor allograft perfusion demands vigilance. In many cases this will be the result of prolonged preservation or other donor factors portending slow or delayed graft function. Recipient hemodynamic issues or use of vasopressors may also play a role in initial hypoperfusion. In these instances the appearance of the organ often improves over the ensuing minutes as recipient hemodynamics and perfusion are optimized and vasospasm in the allograft resolves. In some cases, the allograft may appear unusually engorged with the artery pulsatile as opposed to being characterized by a pulsating thrill or bruit. These findings suggest possible venous outflow obstruction. If perfusion to the allograft remains questionable over the course of the operation, there must be a high index of suspicion for a technical problem such as a twisted vessel, thrombosis, embolus, retractor causing vessel occlusion, flow limiting dissection (particularly at the site of a previously placed vascular clamp), or a faulty suture line. A hand-held Doppler probe is invaluable to quickly confirm the absence or presence of flow in vessels and the renal parenchyma. There should be a low threshold for taking down vascular anastomoses if necessary to eliminate the possibility of technical error or a thromboembolic event. In situations where additional complex procedures may result in prolonged warm ischemia, it may be best to re-flush the kidney with cold perfusate and perform any necessary technically demanding reconstruction on the back table. It is far better to definitively establish vascular patency including reconstructing the anastomoses than to close with a question left as to the integrity of the anastomoses.

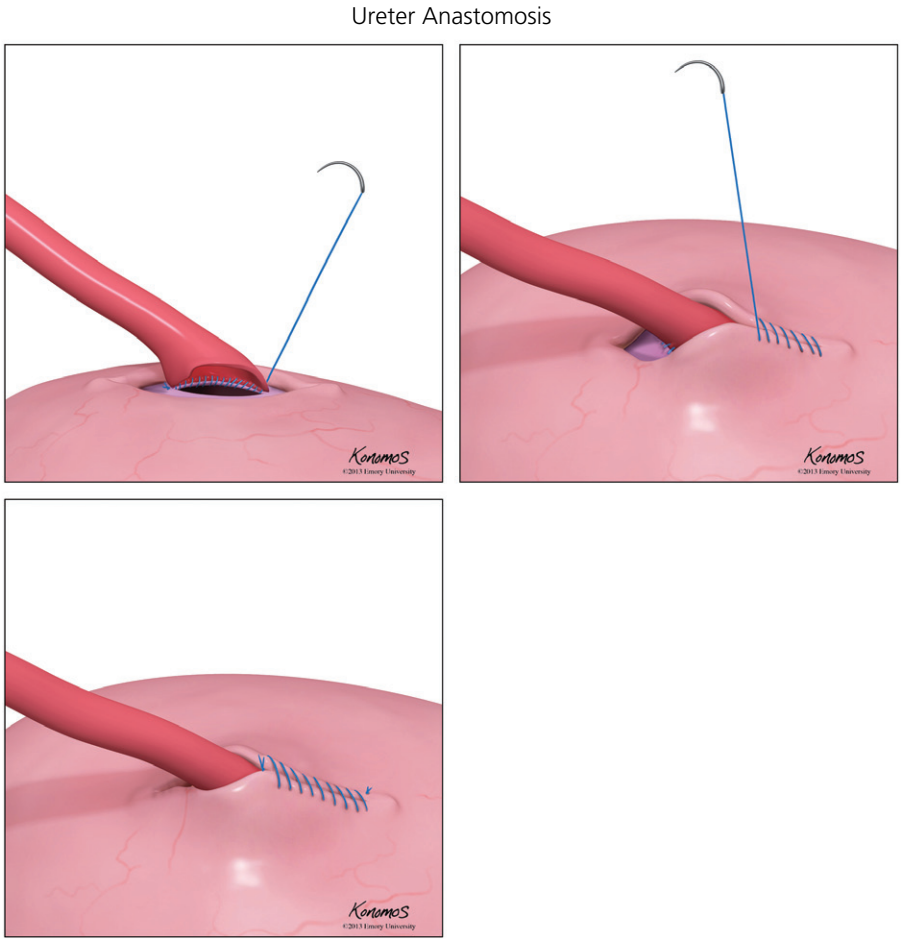

Figure 55.3. The Lich ureteroneocystostomy. This is the most common method for establishing ureterovesical continuity. After splitting the muscle of the bladder to expose the bladder mucosa, an anastomosis is created between the ureteral and bladder mucosa using a fine (5-0) absorbable suture (e.g. PDS). This anastomosis is imbricated under the approximated muscle to create an antireflux anatomy. Some centers stent this anastomosis (not shown).

Urinary reconstruction

After satisfactory perfusion of the allograft has been achieved, attention is then directed to the urinary drainage component of the operation. The type of reconstruction performed is dependent on the length and quality of the donor ureter, as well as the condition of the recipient ureter and bladder. Commonly, a Lich ureteroneocystostomy or modification of this technique is carried out (Figure 55.3). In contrast to the classic Ledbetter–Politano transvesical procedure, the extravesical Lich procedure does not require an extra cystotomy or as much donor ureteral length. As a result, the latter technique is believed to be associated with shorter operative time as well as lower incidence of ureteral ischemia, obstruction, hematuria, and urine leak [2,8,11,12]. The transplant ureter is usually routed posterior to the spermatic cord to prevent potential tenting and obstruction by this structure [2]. In general, this is easily accomplished; however, the spermatic cord can be divided to facilitate the ureteroneocystostomy without lasting adverse consequences in most cases. The ureter is trimmed to appropriate length and spatulated to allow a tension-free anastomosis to the bladder. It should be carefully inspected for adequate perfusion at its distal extent to decrease the likelihood of ischemia leading to ureteral stricture or leak. Methylene blue tinted irrigation instilled via the Foley catheter is helpful to eliminate any confusion in identifying the bladder while creating the cystotomy. Particularly in obese patients and patients on peritoneal dialysis, the peritoneum can be mistaken for the bladder resulting in a ureteroperitonostomy—a complication that should be contemplated during the bladder mobilization so as to always be avoided. The mucosa-to-mucosa ureter to bladder anastomosis is usually sewn with a fine absorbable monofilament suture (typically 5-0 PDS) in running fashion (Figure 55.3). The use of absorbable suture is important to avoid a persistent intravesical foreign body, which will be a nidus for stone formation and urinary tract infection. A double-J ureteral stent is either routinely or selectively employed based on surgeon's preference. Selective use would occur if there exists concern over integrity of the ureteroneocystostomy (or other urinary tract reconstruction) prior to completion of the anastomosis. The practice of routine prophylactic ureteral stenting is not universally accepted, but a meta-analysis of multiple randomized controlled studies and case series found that renal transplants with stented extravesical ureteroneocystostomies have significantly lower urologic complication rates than those with non-stented anastomoses [13]. Typically, the bladder detrusor musculature is reapproximated over the mucosa-to-mucosa anastomosis (Figure 55.3) to provide a potential antireflux mechanism [5,9] and prevent excess tension on the anastomosis when the

bladder is distended. Care is taken to avoid creating an excessively tight tunnel potentially leading to obstruction.

A ureteroneocystostomy allows subsequent use of the native ureter(s) should a complication requiring revision of the urinary drainage arise. Initially performing a ureteroureterostomy may be preferable in cases where the recipient bladder is extremely atrophic, inaccessible, or otherwise not suitable for anastomosis. If the donor ureter is ischemic or otherwise deemed not usable, a pyeloureterostomy may be performed. Care must be taken to preserve adequate blood supply to the native ureter in the course of mobilization in preparation for anastomosis to reduce the incidence of subsequent leak or stricture. Anastomosis involving the native ureter is often technically demanding and generally performed over an indwelling stent. If both the donor and recipient ureters appear unsuitable for use, a pyelovesicostomy is a potential consideration. To allow the bladder and a short ureter or renal pelvis to approximate without tension, it may be necessary to mobilize the bladder and perform a psoas hitch or Boari flap [2]. In cases where the donor kidney is found to have double ureters, two separate ureteroneocystostomies may be performed. If the ureters are in very close proximity or the bladder is not amenable to a second cystotomy, it may be preferable to sew the medial walls of the ureters together with absorbable monofilament after spatulating the ureters and then perform a single anastomosis to the bladder. In cases where the recipient has a history of a bladder augmentation or urinary conduit created from bowel or stomach, it is essential to give adequate forethought to the urinary drainage. Important considerations include selecting the appropriate recipient structure to which to anastomose the transplant ureter, blood supply of the augmentation or conduit, and on which side of the recipient to place the kidney. Generally, more complex urinary reconstructive procedures are performed over a protective indwelling ureteral stent.

Closure

The abdominal wall closure receives relatively little attention but is an integral part of the operation, potentially affecting long-term outcome. The position of the graft should be oriented to avoid obvious twisting, kinking, or obstruction of vessels or ureter. In cases where the recipient's extraperitoneal space is relatively small and there is concern that allograft perfusion may become compromised once the fascia is closed, consideration may be given to opening the peritoneum widely to allow the kidney to rest intraperitoneally. Drains are not routinely placed at the time of operation but may be considered in cases where there are heightened concerns for perigraft collection of blood, lymph, or urine [2,4,9].

Special circumstances

Pediatric recipients

For pediatric recipients, the exposure and vessels utilized for transplantation vary with patient size. Very small recipients weighing less than 15 to 20 kg may be best served with a transperitoneal vertical midline incision along with mobilization of the right colon to allow access to the proximal iliac vessels, distal aorta, and inferior vena cava for vascular anastomoses [14,15]. At the completion of the procedure, the kidney is allowed to rest on the psoas muscle and the ascending colon is placed back over the anterior surface of the kidney [2,5]. As recipient age and size increases, the incision should still be planned to allow the option to use the common iliac vessels, aorta, or vena cava if the need arises. If the lumen of the vascular anastomosis is limited by the diminutive size of the child's vasculature, it has been suggested to perform at least part of the anastomosis with interrupted suture to allow it to grow with the child and avoid a relative stenosis in the future [2].

Pediatric-en-bloc donor kidneys

Pediatric-en-bloc kidneys, as the name implies, are determined to be too small to be transplanted as single kidneys and are therefore maintained together as a unit on a segment of donor aorta and vena cava to allow the vascular anastomoses to be performed using these more reasonably sized conduits. Importantly, the capacity to technically perform anastomoses using pediatric kidney vessels is not at issue, as vessels of this size are well within the capability of most experienced transplant surgeons. However, small vascular anastomoses are less likely to expand and facilitate compensatory growth as the recipient and kidney grow, and experience has generally indicated that kidneys from donors less than 15–20 kg fare better in the long term when implanted en bloc rather than individually. Commonly, the suprarenal ends of the aorta and cava are oversewn and the infrarenal aspects of the aorta and inferior vena cava are sewn to the recipient external iliac vessels in end-to-side fashion [2]. The ureters may be implanted separately into the bladder or they may be spatulated and sewn together, as described previously. An alternate technique for revascularization of pediatric-en-bloc kidneys describes en bloc interposition of the donor aortic and caval segments into the recipient iliac vessels [16]. If cross-sectional area of the vascular anastomosis is limited due to small caliber of the donor vessel, consideration should be given to interrupting at least one side of the anastomosis to allow expansion of the anastomosis with growth of the graft over time [2].

Dual adult kidney transplants

Dual adult kidney transplantation, that is the placement of both kidneys from a deceased adult donor with marginal renal function into a single recipient, recently has been developed as a means of utilizing kidneys with marginal nephron mass (typically from extended criteria donors) for a patient in need of substantial clearance. This has been seen as one response to the shortage of available donor organs and resultant prolonged waiting times to transplant [17]. During this procedure, the transplantation of both donor kidneys may be carried out utilizing both right and left recipient iliac vessels through a lower midline incision via either an extraperitoneal or transperitoneal approach or through bilateral lower quadrant incisions [18,19]. More recently, increased experience with a technique of dual kidney transplantation implanting both grafts into the same iliac fossa extraperitoneally via a Gibson incision has been reported. This is reported to reduce operative time and trauma in comparison to the classic bilateral dual kidney transplant and leaves the contralateral iliac fossa intact for a future transplant if needed [20]. Outcomes from this approach have not definitively established the benefits of this over single kidney transplantation, but the utilization of "two for one" transplants maintains intuitive appeal in some centers.

Early postoperative period

In the immediate postoperative period, the Foley catheter is routinely left in place to decompress the bladder for 2–4 days. The catheter may remain for a longer period of time in patients with a difficult or tenuous urinary anastomosis, or in cases where a dysfunctional non-compliant bladder would subject the fresh

anastomosis to excessively high voiding pressures. If there is concern for a potential urine leak, a cystogram may be performed to document absence of extravasation of contrast prior to removal of the catheter. The ureteral stent in some cases is attached to the indwelling catheter at the time of transplant and is then removed along with the urethral catheter relatively early in the postoperative course. Otherwise it is routinely removed cystoscopically within the first few months post transplant. Drains, when placed, are typically removed when output has decreased to an acceptable level. Persistently high drain output should be analyzed for creatinine level to evaluate for a potential urine leak.

Technical complications

Improvements in surgical technique over the years have resulted in substantial reduction in the rate of technical complications associated with renal transplantation. The incidence of technical problems following kidney transplant is much lower than that associated with liver or pancreas transplant. However, recent improvements in immunosuppressive therapy have also brought about concomitant decreases in graft loss due to acute and chronic rejection. As a result, surgical complications remain a relatively important cause of graft loss following transplant [21].

Early postoperative problems

As with any other major abdominal procedure, the potential for early postoperative bleeding exists and should be suspected if any of the typical signs or symptoms present, such as tachycardia, hypotension, oligoanuria, excessive pain, tenderness, fullness, or mass at the operative site. Serial labs will typically reveal a decreasing hemoglobin and hematocrit, but, in cases of acute hemorrhage, may be unrevealing. As such, the diagnosis of hemorrhage is largely a clinical diagnosis and should be considered in any situation of hemodynamic instability. Potential sources of hemorrhage include a vascular anastomosis, the allograft itself, such as an uncontrolled vessel in the hilum or biopsy site, or the recipient operative field, such as a torn vessel in the retroperitoneal fat or abdominal wall musculature. Bleeding is often self-limited, not detrimental to graft function, and manageable by conservative medical measures. However, significant or persistent bleeding, as reflected by sustained hemodynamic instability, ongoing transfusion requirement, or graft compromise as a result of compression merits consideration for a return to the operating room. Commonly, an active bleeding source is not identified upon re-exploration. However, reoperation allows evacuation of hematoma to relieve compression of the allograft, vessels, and ureter, and removes a medium for potential future septic complications. An opportunity is also afforded to inspect the integrity of the allograft and its anastomoses and obtain a biopsy in cases of poor function. In general, re-exploration is far superior to exsanguination or the long-term consequences of a large undrained hematoma.

Sudden oliguria/anuria in the immediate postoperative period demands ruling out a vascular catastrophe, that is allograft thrombosis as discussed below, and should also prompt a careful evaluation of the urinary drainage system to diagnose and remedy relatively common mechanical problems such as catheter occlusion due to kinking, displacement, or clots. The patient with a malfunctioning catheter will often complain of a full bladder. Thus, any patient complaining of bladder fullness or pain should have the patency of their catheter evaluated. Hematuria is often noted in the early postoperative course. It is usually self-limited and typically resolves without intervention. Although often appearing grossly bloody, it usually does not result in substantial blood loss requiring transfusion. Hematuria is more frequently problematic due to formation of clots, which obstruct the flow of urine via the catheter and may therefore stress the newly created ureteroneocystostomy. Clots are generally manageable by gentle irrigation of the Foley catheter with sterile water or saline. In some cases it may be necessary to replace or upsize the urinary catheter. It is desirable to avoid cystoscopy, aggressive manual bladder irrigation, or unmonitored continuous bladder irrigation systems in the early post-transplant period which could lead to disruption of the ureteroneocystostomy or bladder rupture [22].

Vascular complications

Vascular complications have been reported to occur in up to approximately 15% of patients following renal transplantation and may result in significant morbidity [23,24]. They include a variety of complex problems, such as allograft arterial or venous thrombosis, transplant renal artery stenosis (TRAS), arterial dissection, arteriovenous fistula, and pseudoaneurysm.

Allograft thrombosis

Early graft thrombosis occurs in approximately 2% of renal transplants [3,25,26] and has been reported to account for more than one-quarter of all renal allograft losses within the first year [27]. It usually occurs within 10 days of transplant [21,26]. Clinical hallmarks of thrombosis include the abrupt onset of anuria and loss of function or primary non-function of the organ. However, its detection is difficult in patients who have residual native kidney function, as native urine output will continue. Venous thrombosis is more frequent, and may be manifested by an enlarged and tender allograft and occasionally graft rupture [3,21,27,28]. Arterial thrombosis may be more occult and present without pain or allograft swelling [3,27]. With progression, both artery and vein may become occluded with thrombus and the site of origin may not be distinguishable [27].

The only intervention that can definitively rule out a vascular thrombosis in time to salvage the kidney is re-exploration. Thus, any reasonable concern for arterial or venous thrombosis should prompt a discussion of re-exploration with the attending surgeon early in the evaluation. Classic presentation for thrombosis (e.g. brisk urine output that abruptly stops without evidence of catheter malfunction) should lead to immediate re-exploration without additional diagnostic maneuvers. The window of opportunity for salvage of a thrombosed kidney is brief, 1–2 hours, and time spent confirming a diagnosis usually leads to graft loss.

Prompt Doppler ultrasound is often the preferred modality for initial evaluation when renal allograft vascular patency is in doubt, but the index of suspicion is low. The relatively superficial position of the transplant and normally robust flow make it conducive to effective sonographic interrogation. Ultrasonography is generally readily available, expeditious, portable, cost-effective, and avoids ionizing radiation and nephrotoxic contrast. Ultrasound techniques are very accurate for diagnosing vascular thrombosis, but delay in their acquisition or interpretation can make their diagnostic capabilities moot. Thus, an ultrasound obtained for real concern of a vascular complication should be considered an emergent procedure. Figure 55.4 shows examples of normal and aberrant flow waves.

Sonographic findings in the case of renal vein thrombosis typically include a swollen edematous allograft and lack of venous flow.

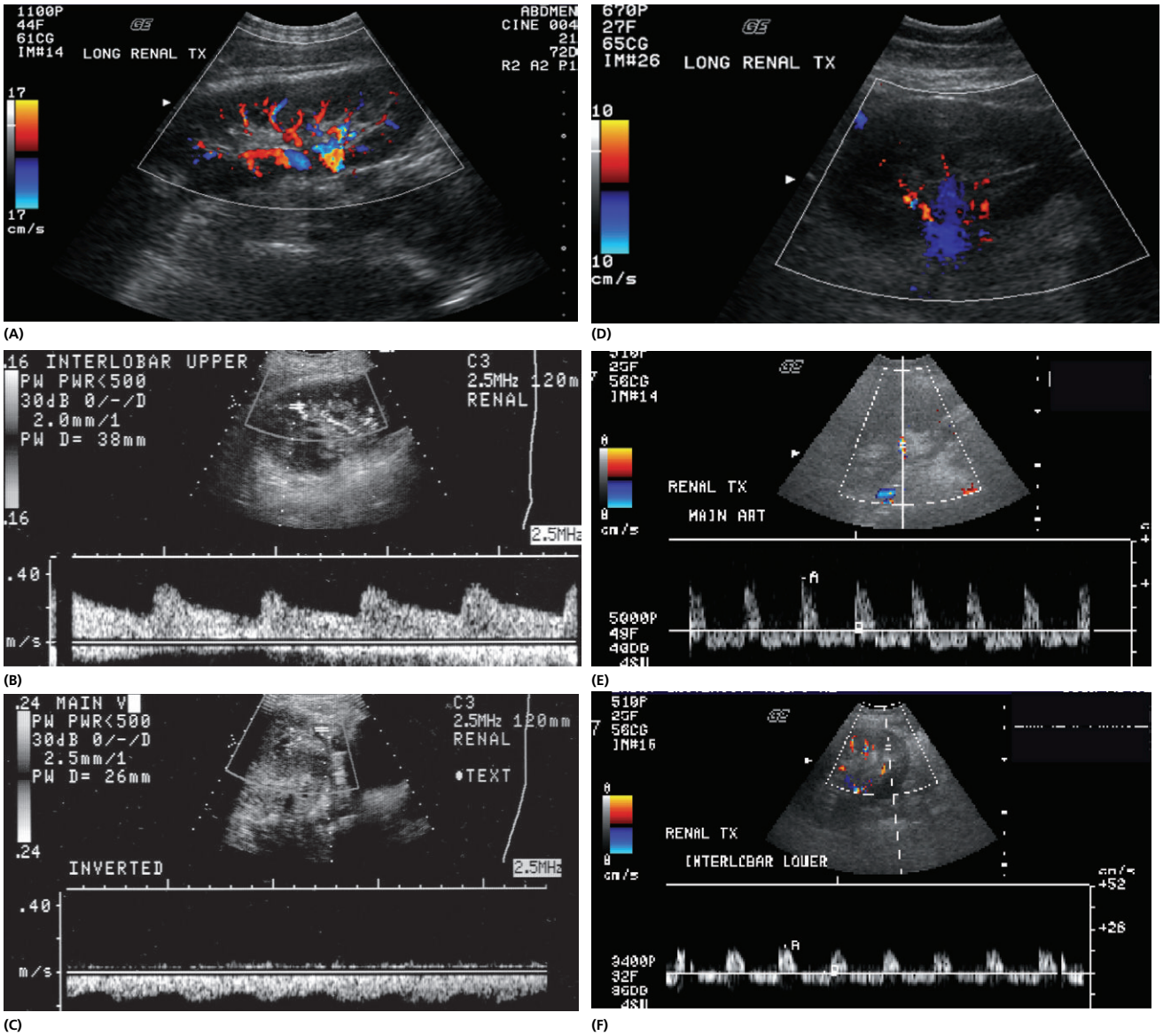

Figure 55.4. Ultrasound visualization demonstrating normal flow (A, B, and C) and the findings characteristic of venous thrombosis (D, E, and F). Note that in the normal situation, there is (A) a bright Doppler signal throughout the entire kidney, (B) a brisk upstroke of the arterial waveform with continuous flow through diastole, and (C) an easily detected continuous venous waveform. It is important to note that during venous thrombosis, (D) there may still be some flow detectable in the graft, although it is attenuated, (E) the arterial waveform may be present, but the arterial diastolic flow is reversed, and (F) no consistent waveform for the vein is seen (in this case an interlobar artery is seen with reversal of diastolic flow). Importantly, detection of flow without characterization of the proper waveforms *does not* rule out a vascular thrombosis.

Rarely, filling defects may be visible in the veins. Importantly, an arterial waveform may be present in patients with complete venous thrombosis, and thus arterial "flow" does not rule out venous occlusion. Due to outflow obstruction, the arterial waveforms show high resistive indices with reversal of diastolic flow. A proper arterial waveform should be accompanied by continuous diastolic component of flow (Figure 55.4), and in the absence of diastolic flow, venous thrombosis should be strongly considered, and with it, re-exploration. In the case of renal artery thrombosis, the allograft may appear small in size with filling defects and lack of flow in the renal artery [23]. Urgent surgical re-exploration with thrombectomy or less often catheter-directed thrombolytic techniques provide the only hope for allograft salvage when allograft arterial or venous thrombosis occurs, but in the majority of cases the organ is lost [21].

Catheter-directed thrombolytic therapy has been reported to be successful in managing selected cases of allograft thrombosis [29] but may be contraindicated in the early weeks following transplant due to increased risk of bleeding from fresh suture lines [23,24,29]. It is possible for only a branch of a renal artery or one of multiple renal arteries to thrombose, resulting in a wedge infarct or ureteral ischemia. This situation may have a more subtle clinical presentation and be manifested by poor function, hypertension, or urine leak [21]. Potential technical causes of allograft thrombosis include an intimal dissection, luminal compromise due to a twist or kink in a vessel or poor suturing technique, or extrinsic compression

such as from hematoma or lymphocele. Size mismatch between donor and recipient vessels (especially in pediatric transplants) and complex multiarterial reconstructions appear to be risk factors for thrombosis, as are hypotension, acute tubular necrosis, and rejection [26]. Often there is no technical factor or other reason found to explain this unexpected and seemingly random occurrence.

Previous reviews have reported numerous diverse markers of patients at increased risk for renal allograft thrombosis, including prior history of venous thrombosis or vascular disease, diabetes mellitus, systemic lupus erythematosus, retransplantation, use of peritoneal dialysis or cyclosporine, obesity, and inherited or acquired hypercoagulable states. Identification and better understanding of hypercoagulable conditions recently have resulted in more attention being given to thrombophilia as a potential explanation for allograft thrombosis [3,27]. The question of whether all potential transplant recipients should be screened for identified thrombophilic factors, such as factor V Leiden, prothrombin gene 20210A, or antiphospholipid antibodies, remains unresolved and specific therapeutic protocols to help prevent allograft thrombosis in higher-risk patients have not been validated in adequately powered randomized controlled trials [27,30]. It would seem prudent to at least test certain patients, such as those with lupus or previous significant personal or family history of thrombosis, who clinically appear at increased risk for allograft thrombosis for a hypercoagulable state to improve risk stratification. For patients assessed to be at high risk for allograft thrombosis, such as those with systemic lupus erythematosus testing positive for antiphospholipid antibodies or those with thrombophilia and previous thrombotic events, specific therapies, including heparin transitioned to warfarin and/or aspirin, appear to be beneficial in preventing allograft loss [27,31,32]. In the general transplant population, the incidence of renal allograft thrombosis appears to be decreased by the routine prophylactic use of low-dose aspirin [27,33,34].

Transplant renal artery stenosis

Transplant renal artery stenosis (TRAS) is likely the most common vascular complication following transplant, with a reported incidence of 1 to 23% [35,36]. The wide discrepancy in reported incidence may be attributable to variable diagnostic criteria as well as evolution of diagnostic methods over time. TRAS most commonly is diagnosed from 3 months to 2 years post transplant [3] and usually occurs at or near the arterial anastomosis [3,24]. Multiple etiologies have been reported to be responsible for TRAS, including atherosclerosis present at the time of transplant or progressing after transplant and fibrosis or intimal hyperplasia in response to vascular trauma incurred at the time of transplant. Other technical considerations include poor suturing technique, clamp injuries, flow-limiting arterial dissections or intimal flaps, kinking or twisting of the renal artery, or extrinsic compression [3,37]. A wide array of non-technical factors, such as extended criteria donors, increased donor and recipient age, delayed graft function, induction immunosuppression, ischemic heart disease [38], and CMV infection [39], have also been reported to have an association with TRAS. The clinical presentation of patients with significant TRAS reflects the effects of activation of the renin–angiotensin system [40] resulting from hypoperfusion of the renal parenchyma. Classically, refractory hypertension, edema, heart failure, an audible bruit over the graft, and allograft dysfunction have been described in association with TRAS. The diagnosis is supported by worsening of renal function with the use of renin–angiotensin system inhibitors [3]. It is noteworthy that these clinical findings may be present without TRAS being the cause. Conversely, significant TRAS may occur without any or all of these clinical manifestations being present. Therefore, imaging studies are necessary to help make the diagnosis.

Doppler ultrasound is often the initial diagnostic modality selected due to its sensitivity, ready availability, and non-invasiveness. Findings consistent with TRAS include peak systolic velocity in the transplant renal artery $>$2.0–2.5 m/s and a "parvus–tardus" waveform downstream with decreased resistive index, pulsatility index, and acceleration index and increased acceleration time intrarenally [23,24,41,42]. A velocity gradient of greater than 2 : 1 between the stenotic and prestenotic segments of the artery can also be seen in TRAS [23,24]. MRI or CT may be performed to better characterize and localize the lesion, but the risks of using iodinated contrast or gadolinium must be weighed against the beneficial information gained from the study. The gold standard investigative study continues to be conventional angiography due to the quality of anatomic definition, ability to measure pressure gradients across the narrowing, and potential for therapeutic intervention at the same setting. For TRAS detected by non-invasive techniques, close follow-up with serial ultrasound studies is reasonable if the narrowing is judged not to exceed 60%, if hypertension is adequately controlled on minimal medication, and graft function is satisfactory [3]. Data indicate that selected patients with TRAS can be medically managed without radiologic or surgical intervention and have good long-term outcomes [43]. Multicenter randomized prospective trials are lacking; therefore, recommendations for optimal mode of therapy for any particular patient with TRAS must be tempered by clinical judgment. Clinical deterioration, that is allograft dysfunction or poorly controlled hypertension along with sonographic findings consistent with significant or progressive TRAS or a stenosis of $\geq$80% on ultrasound, with or without clinical sequelae, merit consideration for angiography and intervention as indicated [3]. Angiographic findings consistent with hemodynamically significant TRAS include luminal diameter narrowing of $>$50% or a pressure gradient $\geq$10–20 mmHg across the stenosis [3,21,24].

Percutaneous transluminal angioplasty (PTA) with or without stenting is usually the primary therapeutic intervention for patients with significant TRAS. Technical success rates for TRAS-PTA are commonly reported exceeding 80% with complication rates generally under 10% [3,23,24,44–48]. Complications include arterial dissection, rupture, thrombosis, and rarely graft loss. Contrast nephropathy is another potential consequence and foresight along with careful planning are essential to limit dye load and institute renoprotective measures such as adequate volume status. Clinical success is variably measured in terms of improved renal function and blood pressure control. Although many studies report short-term improvement in hypertension and renal function in the majority of patients, long-term follow-up is limited [45–48]. Restenosis has been reported to occur in 10–60% of patients following endovascular treatment, and stenting appears to be associated with reduced recurrence of stenosis [3,47,49]. Patients with recurrent stenosis may be candidates for repeat PTA and stenting. Generally, stent placement is indicated if there is residual stenosis $>$30% or persistent systolic pressure gradient $>$10 mmHg following angioplasty, recurrent lesions, or in lesions showing high elastic recoil. Complications, such as a flow-compromising dissection or thrombosis of the renal artery, may also be amenable to percutaneous methods, including stent deployment and thrombolysis [3,23].

Surgical management may be favored for patients with TRAS due to arterial kinks, complex atherosclerotic disease, or other lesions considered poorly accessible or not amenable to PTA. These patients, along with those with recurrent or refractory lesions failing endovascular techniques, may require operative intervention as "rescue" therapy [3,24]. Options for operative repair include bypass grafting, resection of the stenotic segment with primary anastomosis, or placement of an interposition graft or patch angioplasty. Although reports claim similar or improved outcomes with surgical repair compared to endovascular techniques [37,48,49], operation for TRAS may be difficult and is associated with a rate of graft loss reported up to 20% [3,21,49]. Therefore, PTA is usually considered first-line therapy due to demonstrating reasonably good efficacy with relatively low periprocedural morbidity, while being less invasive than operation. PTA generally does not preclude subsequent surgical repair. Preanastomotic- or pseudo-TRAS occurs when a hemodynamically significant lesion proximal to the transplant renal artery (i.e. in the ipsilateral common or external iliac artery) limits flow to the allograft and presents with clinical findings similar to TRAS. Proximal TRAS has been reported to occur in up to 2.4% of recipients and appears to be associated with increasing age [23]. It is generally amenable to PTA or stent placement [24].

Postbiopsy arteriovenous fistula and pseudoaneurysm

With the increased frequency of percutaneous renal transplant biopsies in recent years has come recognition of vascular injuries associated with these procedures. Arteriovenous fistulas (AVF) and pseudoaneurysms have been reported to complicate up to approximately 15% of renal allograft biopsies [23,24,50]. Although typically self-limiting, large fistulas can become clinically relevant. An AVF may occur if an adjacent artery and vein are lacerated with resultant persistent flow between the vessels. A pseudoaneurysm may result after injury to an arterial wall. These technical complications may be asymptomatic or present with gross hematuria. A bruit may be audible over the allograft. Hemodynamic alterations in kidney perfusion due to a "steal" phenomenon from an arteriovenous shunt may result in allograft dysfunction. Less commonly, patients may present with high output cardiac failure. Enlarging pseudoaneurysms have the potential to rupture. Doppler ultrasound is usually the initial diagnostic modality to detect these lesions. Findings consistent with AVF include high velocity, low resistance flow patterns with turbulent flow outside the expected vascular boundaries, and an enlarged draining vein. Pseudoaneurysms may appear as anechoic round structures with flow which may be bidirectional [23,24]. Magnetic resonance angiography may be useful in diagnosing these lesions if sonography is inconclusive [51]. Most of these lesions may be managed conservatively [3].

It is believed that the majority (up to 70%) of AVF spontaneously resolve, usually within weeks or months [52]. Intervention is indicated for these iatrogenic complications; however, if clinical manifestations occur or if they progressively enlarge. Superselective embolization is the treatment of choice [23,24]. This usually involves angiography to identify the AVF or pseudoaneurysm followed by placement of a small catheter into the segmental or branch artery directly feeding the lesion to limit the amount of renal parenchyma infarcted by embolization. Carbon dioxide may be more sensitive than conventional contrast in detecting small AVF [23] and may be utilized to minimize nephrotoxicity . Although transcatheter embolization is usually technically successful with clinical improvement of the patient and preserved allograft survival [23,53], infarction of a clinically significant amount of parenchyma with resultant graft dysfunction is a potential complication [50,52]. Surgery is usually not favored as first-line therapy for intrarenal AVF or pseudoaneurysm as it would typically require partial or even total nephrectomy.

Urologic complications

Urologic complications, the most common of which are ureteral obstruction or urine leak, have been reported to occur in up to approximately 10–15% of transplanted patients [7,8,23,24,54]. These complications are most commonly attributed to ischemia or technical error, but may also be influenced by other factors including immunosuppressive management, inflammation, infection, and poor nutritional status [7,8]. Perfusion of the transplant ureter arises solely from the renal artery(ies) and is no longer supplemented by vessels in the retroperitoneum and pelvis. Therefore, the distal ureter is at risk for poor perfusion with resultant leak or stricture formation. Care must be taken to optimize the ureteric blood supply by preserving lower pole arteries and limiting dissection adjacent to the ureter, particularly in the "golden triangle" between the ureter and lower pole of the kidney [7,8].

Ureteral obstruction

Ureteral obstruction is commonly reported to occur in approximately 3% of transplant recipients [7,8,55], but higher rates approaching or exceeding 10% have been noted in previous series [56,57]. As alluded to previously, ureteral strictures involving the distal ureteral segment or ureterovesical junction are commonly attributed to ischemia compromising the distal transplant ureter or technical error. Ureteral obstruction may also result from other causes, including extrinsic compression from a lymphocele or spermatic cord or intraluminal calculus, clot, or foreign body. More recently, BK polyoma viral infection leading to ureteritis has been implicated in the pathogenesis of transplant ureteral strictures [7,58], and all patients with suspected stricture should be evaluated for BK viremia and/or viruria. Significant ureteral obstruction results in allograft dysfunction manifested by an elevated serum creatinine. Initial evaluation includes allograft ultrasonography, which typically shows hydronephrosis in cases of ureteral obstruction. If bladder distension is also noted, the bladder should be decompressed with a follow-up ultrasound study performed to eliminate obstruction occurring more distal to the ureter as the cause for hydronephrosis and related dysfunction. Persistent hydronephrosis with a decompressed bladder is very suggestive of ureteral obstruction. An antegrade nephrostomy is an appropriate next definitive diagnostic and temporizing therapeutic maneuver in cases of suspected ureteral obstruction. Percutaneous decompression of the collecting system relieves obstruction of the renal parenchyma and allows recovery of renal function. An antegrade nephrostogram provides a roadmap clearly delineating the location and extent of ureteral narrowing. If the obstruction has resulted in acute upper urinary tract infection, the patient should be treated with decompression and antibiotics and pyelography with contrast injection delayed until the infection has been adequately treated to reduce the risk of systemic sepsis. Occasionally, the transplant ureter may be accessed and imaged successfully in a retrograde manner cystoscopically; however, cannulation of the ureteral neoorifice on the anterior aspect of the bladder is typically difficult.

As open reoperation may be technically challenging, endoscopic and percutaneous interventions, including balloon ureteroplasty and stent placement, are commonly recommended first-line therapeutic measures for ureteral strictures. Short segment lesions

occurring distally at or near the ureterovesical junction and developing relatively early (≤3 months) after transplant are generally considered amenable to percutaneous techniques and have variable success rates reported at 50% and higher [7,24,55,59]. In contrast, longer, more proximal, and later-occurring strictures are not usually as responsive to minimally invasive techniques with success rates reported below 50% [55,59,60]. Ureteral strictures not amenable to or failing percutaneous intervention are addressed operatively to achieve long-term resolution. If adequate length of healthy ureter remains after resecting the distal strictured segment, ureteroneocystostomy may be repeated. If the bladder is of sufficient quality, it may be mobilized and surgically revised if necessary (i.e. psoas hitch or Boari flap) to allow approximation to the transplant ureter without tension. Ureteroureterostomy, pyeloureterostomy, and pyelovesicostomy are other options for reconstruction of the urinary drainage dependent on condition of the donor ureter as well as the recipient ureter and bladder. The procedure may be difficult and is facilitated by knowing prior to reoperation the orientation of the transplant renal pelvis (i.e. right or left donor kidney) and the status and proximity of native urinary tract structures.

Urine leak

Urine leak has been reported to complicate up to approximately 3–5% of renal transplants [7,8,23,24]. Patients may present with pelvic pain exacerbated with voiding, as well as swelling and tenderness over the allograft. There may be discharge of urine from the transplant incision as well as allograft dysfunction. The most common sites for urine leak include the distal ureter and ureteroneocystostomy due to failure of the anastomosis as a result of technical factors, or, more commonly, ureteral ischemia [7,23,24]. If due to technical error, the leak may often become apparent within the first 24 hours post transplant. A leak resulting from ischemic necrosis typically manifests within the first few weeks [7]. Wound drainage can be identified as urine by its elevated creatinine level relative to serum. A urine leak will result in a perigraft fluid collection on ultrasound or computed tomography and may be confirmed by sampling of the fluid. Extravasation may be noted on retrograde cystography, antegrade pyelography, or radionuclide imaging in cases of an active urine leak. Very early post-transplant leaks are often repaired surgically as reoperation is relatively straightforward in the presence of intact and identifiable tissue planes with minimal inflammation and adhesions. If the distal ureter appears well perfused, the leak may be simply repaired by placing additional sutures. Prolonged need for a nephrostomy tube, stent, percutaneous drain, and Foley catheter may be potentially avoided in these cases. If underlying ureteral necrosis is responsible for the leak, open repair is usually necessary to achieve a satisfactory long-term result. The ureter must be cut back to healthy viable tissue and an appropriate reconstruction performed. In cases where it is felt that the leak will likely heal without reoperation or the likelihood of successful reoperation to repair the leak is low, decompression and/or diversion of the urine stream is performed to reduce pressure and flow of urine across the leak to facilitate closure. Non-operative interventions typically include antegrade nephrostomy and double-J ureteral stent or nephroureteral stent placement, catheter drainage of the bladder, and percutaneous drainage of any associated urinoma. Percutaneous and endoscopic techniques are reported to be often successful in resolving appropriately selected cases of urine leak without need for open reoperation [7,60].

Lymphocele and other perigraft fluid collections

Perigraft fluid collections are quite common post-transplant, occurring in up to approximately 50% of recipients [61,62]. It is notable that the majority of these collections are inconsequential incidental findings revealed by commonly performed imaging such as computed tomography or ultrasonography. It is estimated that only 15–20% of these will be of clinical significance and require intervention [23,24,62,63]. In the absence of associated clinical findings, the vast majority may be safely observed without intervention. Principles of management for hematoma and urinoma have been previously discussed. An abscess may be suspected if the patient clinically presents with findings consistent with infection, such as fever, pain, swelling, and tenderness at the site, and imaging reveals a concerning fluid collection. Gas noted within the collection or enhancement of the wall is suggestive of infection. CT or ultrasound-guided sampling of the fluid for gram stain, culture, and sensitivity confirms the diagnosis and guides antimicrobial therapy. Along with antibiotic therapy, definitive management may include simple aspiration for small non-loculated collections. Larger infected collections may require a percutaneous drain to remain in place until output ceases and the cavity collapses. Large, complex infected collections are often best managed surgically to allow effective drainage of multiple loculations and debridement of non-viable tissue.

A lymphocele is a collection of lymphatic fluid developing in a non-epithelialized postoperative space. The source of the fluid is disrupted lymphatic channels in the operative field or from the allograft itself [3,6]. Meticulous ligation of lymphatics as they are encountered and divided in the course of exposing the external iliac vessels or alterations in surgical technique such as implantation to the common iliac vessels or placement of drains have been advocated to decrease the occurrence of lymphoceles [64,65]. Other modifiable factors, however, such as the alloimmune response as well as specific immunosuppressive agents such as sirolimus, have also been reported to play a role in their development [6,64,66]. In contrast to hematoma, urinoma, and abscess, lymphoceles are generally discovered later in the post-transplant course, usually weeks or months following surgery, with a peak incidence at approximately 6 weeks [62]. They are commonly located adjacent to the lower pole of the kidney posterolateral to the transplant ureter [3]. Large lymphoceles may cause subjective discomfort, such as pain, fullness, or urinary frequency or urgency if impinging on the bladder. Compromise of iliac venous drainage may result in lower extremity edema or deep venous thrombosis. A lymphocele compressing the ureter, transplant vasculature, or graft itself may obstruct urine flow or compromise perfusion resulting in allograft dysfunction. Clinically significant or symptomatic lymphoceles have been reported to occur in 1 to 26% of transplant recipients [10,67,68]. However, as alluded to previously, lymphoceles are often incidental findings detected on routine imaging studies and generally require intervention only if associated with symptoms or complications due to their size or location. The differential diagnosis for an elevation in serum creatinine developing shortly after ureteral stent removal in a recent transplant patient should include an obstructing lymphocele. Allograft dysfunction with hydronephrosis or hydroureter in conjunction with a fluid collection in proximity to the transplant ureter suggests a clinically significant lymphocele. Aspiration is helpful diagnostically: a lymphocele will have a creatinine level similar to that of serum in contrast to a urinoma which typically will have a markedly elevated creatinine level. Improvement in clinical symptoms or graft dysfunction

following evacuation of the fluid collection also supports the causative role of the lymphocele. Further definitive management is usually required as these fluid collections usually return following simple aspiration. A recent review reported a pooled recurrence rate of 59% following simple aspiration with individual series reporting rates from 10 to 95% [10]. Percutaneous drain placement as sole therapy to treat a lymphocele is associated with a 50% recurrence rate [10]. Percutaneous drainage with sclerotherapy has been reported to have success rates between 68 and 100% [23,24], and a recent review reported an overall recurrence rate of 31% with sclerotherapy [10]. Povodine–iodine solution has been commonly reported to be utilized as a sclerosing agent [10]. The use of a wide variety of other agents, including tetracycline, streptomycin, alcohol, bleomycin, or a cocktail of human fibrinogen, bovine protease inhibitor, human thrombin, calcium chloride, and gentamicin, as sclerosants instilled into the collection have been reported in the literature. Multiple treatments may be required to prevent reaccumulation of fluid and clinical resolution may take several weeks [10,23,24]. Some authors have expressed concern over potential nephrotoxicity associated with agents such as povodine–iodine and risk of infection associated with repeated aspiration or prolonged catheter drainage [3,10]. Operative management of clinically significant post-transplant lymphoceles is often preferred due to reported high rates of immediate clinical success with lower recurrence compared to aspiration, drain placement, or sclerotherapy [3,10]. Additionally, the discomfort and potential complications associated with a longstanding percutaneous drain are avoided. Operation consists of marsupialization or creation of a "peritoneal window" out of the lymphocele wall. This may be performed either as a minimally invasive laparoscopic procedure or as an open procedure utilizing the previous transplant incision or a lower midline incision. A recent review reported overall recurrence rates of 8% and 16% following laparoscopic and open operations, respectively [10]. The laparoscopic approach may be preferable for most cases due to decreased morbidity and lower length of stay associated with minimally invasive surgery [10]. Computed tomography is invaluable in preoperative planning to help determine the spatial relationship of the collection with adjacent vital structures such as the allograft and iliac vessels and transplant ureter to help avoid intraoperative complications. Use of ultrasonography in the operating room is also useful to positively identify the lymphocele as well as a safe point to enter it. The procedure is facilitated if the fluid collection is distended and the bladder is empty. The fenestration in the cavity wall should be at least 5 cm in diameter if possible. Tacking a mobilized pedicle of omentum in the cavity through the window has also been reported to help prevent fluid reaccumulation [3,69].

Summary

The techniques for kidney transplantation are well established, time tested, and have changed relatively little in the past several decades. Eventual success should be uniformly expected for every patient, and in large part relies on anticipation of common technical concerns with the iliac vasculature and the bladder and timely diagnosis and intervention when complications arise. A high index of suspicion is required for recognition of vascular complications within a time frame that allows for graft salvage, and a surgeon should have a low threshold for re-exploration in the rare circumstances that technical complications are suspected.

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CHAPTER 56

Liver Transplantation Procedure and Surgical Technique

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Introduction

Liver transplantation is a technically demanding procedure, made even more complex because it is generally performed in patients with portal hypertension, coagulopathy, and severe underlying illness. The surgical team must assign a premium to attention to hemostasis, avoidance of intraoperative misadventures, and expeditious performance of the surgical procedure. Clear communication among team members, including the anesthesia, surgical, and nursing components, is practiced. Use of a presurgical checklist or "timeout", preview of the surgical plan in the operating room, and review of the procedure at its conclusion add to the safety and success of the operation. Although liver transplantation is a long and complex surgical procedure, it nevertheless comprises a series of discrete steps which are detailed below. Each step must be completed satisfactorily to assure a favorable outcome for the patient.

The surgical techniques of liver transplantation have evolved considerably over the past 30 years and include better instrumentation, standardization of the operation, improved intraoperative management of fluid and blood components, improved hemodynamic monitoring, and better selection of donors and recipients. All these refinements have led to better outcomes, with the anticipated patient survival at 1 year greater than 90%, compared with 75% only 20 years ago. We herein summarize the technical approaches to the surgical aspects of adult liver transplantation that are commonly used at our center.

Back-table preparation of liver allograft

Preparation of the donor liver for implantation is a critical component of the implantation operation. Once the liver is removed from the donor by the procuring surgeon (see Chapter 19), it must be prepared to facilitate its transplantation. The procuring surgeon is careful during organ extirpation to avoid injuring the important vascular and parenchymal structures that are necessary for the operation. As a safety technique, and depending on the other organs procured, the procuring surgeon will commonly leave the diaphragm attached to the bare area of the liver, leave the aorta intact, and leave the pancreatic tissue attached to the bile duct and hepatic artery. All of this extraneous tissue must be removed during the back-table preparation.

The allograft preparation is performed in the operative suite on a separate, sterile back table (or "bench"). The donor liver is placed in an ice-lined basin along with its surrounding preservation solution. We begin by removing the diaphragmatic attachments to the bare area of the liver with scissors. Once this is accomplished, the supra- and infrahepatic vena cavae are skeletonized and small retroperitoneal perforators are secured with silk ligatures. The dissection is continued down to the hepatic vein confluence. The diaphragm is removed from the hepatic vein confluence and the phrenic veins are tied or oversewn. The adrenal vein is also tied or oversewn to prevent bleeding upon recirculation. Any remaining cardiac muscle is removed from the upper cuff of vena cava.

The caudad portion of the vena cava is then addressed. If one expects to replace the retrohepatic vena cava via the caval interposition technique, no additional preparation is necessary on the bench. If a piggyback technique is planned, the transplant surgeon must have a plan for controlling the lower portion of vena cava once recirculation has occurred. A number of strategies can be employed, including use of heavy ties or a vascular stapler after recirculation. Another popular technique is to oversew the lower vena cava cuff and place a tube into the vena cava that will be removed upon recirculation. Upon removal, the suture is then tied and the lower vena cava is controlled.

The hepatic artery is then skeletonized from the aorta up to the gastroduodenal artery (GDA). Dissection is not continued above this point due to the risk of injury to the hepatic lobar vessels. Care is taken to identify any aberrant arterial anatomy, which can be present in up to 20% of the population [1]. The most common two variants are a replaced right hepatic artery emanating from superior mesenteric artery or a replaced left hepatic artery originating from the left gastric artery. The handling of these variants will be addressed in the next section of this chapter.

The portal vein is skeletonized up to the portal vein bifurcation. Surrounding lymphatic tissue is removed and care is taken not to injure the hepatic artery or bile duct. At this point, many surgeons will place a silastic tube into the portal vein and secure it with a silk tie to facilitate flushing prior to reperfusion of the new organ.

The common bile duct is not manipulated on the bench. The vascular supply to the bile duct is variable, but it generally runs along the lateral and medial sides of the bile duct at three o'clock and nine o'clock. It is therefore crucial to leave the surrounding tissue associated with the duct to avoid bile duct ischemia [2].

If it is anticipated that either arterial or venous conduits will be necessary for the revascularization of the donor liver, a donor iliac

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

artery graft or iliac vein graft is prepared on the back table. Branches or defects are ligated or oversewn as needed. Vessel integrity is assessed by injecting heparinized saline under pressure to identify leaks. The grafts are then stored in University of Wisconsin solution on ice for future use. Vessels that are not used are stored in labeled containers in University of Wisconsin solution at 4°C.

Arterial reconstruction

The arterial anatomy of the liver is variable and thorough understanding of the common variations is required to safely prepare a liver for implantation [1].

Replaced right hepatic artery

The replaced right hepatic artery (RRHA) most commonly originates from the superior mesenteric artery (SMA) before the first jejunal perforator. Because it is not anatomically in continuity with the common hepatic artery, the vessel must be reconstructed so that a single arterial trunk can be anastomosed during the recipient phase of the operation. There are two common reconstructions for this aberrant anatomy: (1) RRHA to the donor gastroduodenal stump, and (2) SMA stump to the base of the celiac axis.

Preparation of the arterial vasculature for reconstruction is performed as described earlier. For reconstructing the RRHA to the GDA, the stump of the GDA is left patent and cut back to healthy tissue. Similarly, the RRHA is cut back to an appropriate length. Under loupe magnification an end-to-end anastomosis is performed between the two cut ends with fine polypropylene suture (Prolene; Ethicon Inc., Somerville, NJ). Care is taken to orient the main hepatic artery correctly to avoid twisting once reconstruction is complete (Figure 56.1A, option 1).

The second common vascular reconstruction option can only be performed when the procuring surgeon provides the RRHA on a segment of SMA. In this case, the SMA and RRHA are skeletonized up to the level of the common bile duct. The celiac axis is then cut back to an appropriate length and the SMA stump is sewn to the cut end of the celiac axis with Prolene suture (Figure 56.1A, option 2). It is imperative to ensure that both the common hepatic artery and the RRHA are oriented correctly to avoid twisting. In addition to these two common techniques, the RRHA can also be sewn to the splenic artery stump. The choice of splenic versus GDA stump for implantation of the RRHA depends on the lumen diameter, the length of the vessels, and other anatomic considerations.

Replaced left hepatic artery

The replaced left hepatic artery (RLHA) most commonly originates from the left gastric artery. For this reason, it is imperative to identify the artery in the gastrohepatic ligament, if present, during the procurement operation. The procuring surgeon should leave the left gastric artery intact and supply the benching surgeon with all of the tissue from the lesser sac of the stomach. This technique ensures that the RLHA will remain unharmed.

On the bench the common hepatic artery is skeletonized up to the GDA. Attention is then focused on the left gastric artery, following it until the RLHA branches off toward the umbilical fissure. The distal end of the left gastric artery beyond the RLHA is tied with silk ligatures and the remaining small branches of the RLHA are tied. Gentle flushing of the RLHA can identify small branches that need to be controlled with silk ties. Diligence on the bench will prevent bleeding issues during arterial recirculation. The RLHA is usually not shortened, even though it may seem redundant (Figure 56.1B).

There are other less common anatomic variants that may be encountered and each should be addressed individually. The main focus of back-table vascular reconstruction is to allow for a single

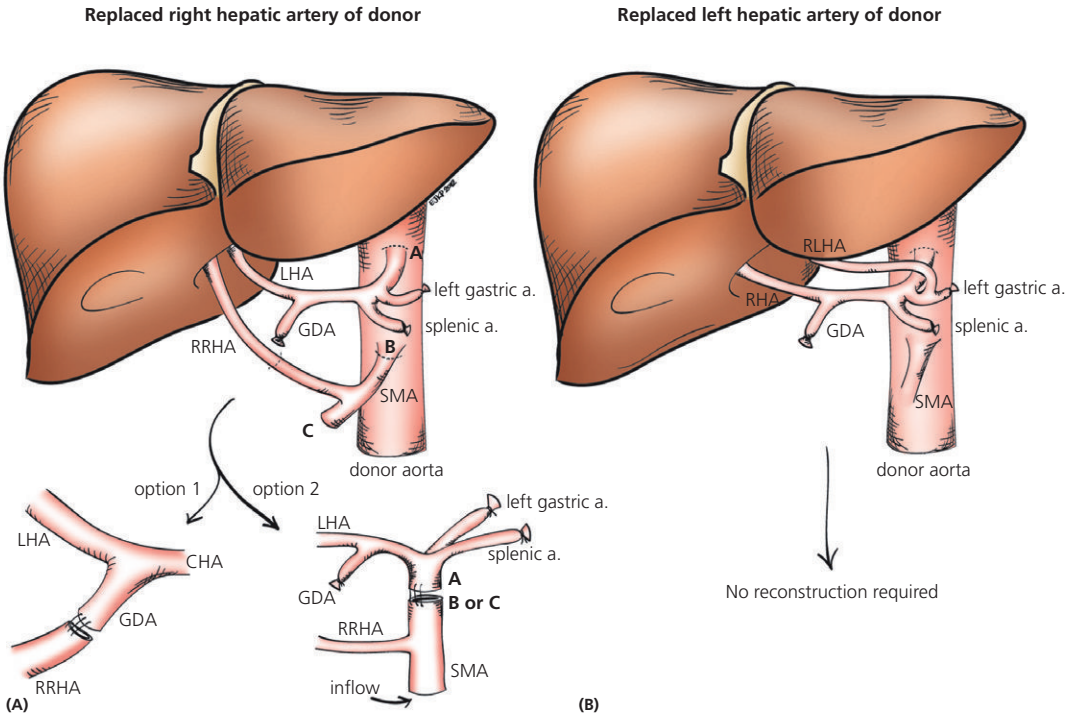


Figure 56.1. Arterial anatomy of donor liver with reconstruction options. RRHA, replaced right hepatic artery; LHA, left hepatic artery; GDA, gastroduodenal artery; SMA, superior mesenteric artery; CHA, common hepatic artery; RHA, right hepatic artery; RLHA, replaced left hepatic artery.

arterial anastomosis to be performed during the hepatic implantation. This anastomosis should be large enough to ease implantation and to avoid thrombotic complications.

Recipient hepatectomy

Control of bleeding

The recipient hepatectomy is generally considered the most difficult aspect of liver transplantation and the technical aspects of this portion of the surgery will significantly influence the patient's postoperative course and overall outcome. Particular attention is given to minimizing blood loss and the need for blood product transfusion, as this parameter significantly affects hemodynamic stability, renal function, pulmonary function, immune function, and cost.

Patients with severe portal hypertension tend to bleed considerably during hepatectomy, and this is exacerbated by the underlying coagulopathy due to liver failure and thrombocytopenia due to splenomegaly and splenic sequestration. Approaches to minimizing blood loss include maintenance of a low central venous pressure (CVP) during the procedure or at least until liver reperfusion is complete. Bleeding tends to be less when the CVP is <10 mmHg or as low as the patient will tolerate while maintaining hemodynamic stability. A cell saver may be used to allow for the re-administration of autologous blood aspirated during the case. While cell savers may minimize the need for allogeneic blood transfusion, it should be noted that cell saver blood must be treated with either heparin or citrate, which results in additional metabolic demands on the liver to clear these anticoagulants. The degree of care taken in dissection, with meticulous attention to hemostasis at all times, can greatly impact the need for blood transfusion. Technical aspects include liberal use of electrocautery and argon beam cautery, although not at the expense of thermal injury to blood vessels and biliary structures.

Pharmacologic approaches to reduce bleeding include the use of aminocaproic acid (Amicar; Xanodyne Pharmaceuticals, Inc., Newport, KY) for patients with evidence of fibrinolysis. Aprotinin is used in some practices to reduce blood loss prophylactically, although it has been associated in some cases with diffuse thrombosis [3]. Studies suggest that tranexamic acid may be used either prophylactically or as an alternative to Amicar. In our practice we avoid the use of these agents except in the event of fibrinolysis. In patients with fibrinolysis a loading dose of 5 g of Amicar is given and continued as an intravenous infusion of 1 g/h until resolution of clot lysis. A hemoglobin level of 8 mg/dL at the end of the liver transplant procedure provides adequate oxygen-carrying capacity and reduces the viscosity of blood such that hepatic artery thrombosis is less likely. Plasma and platelets are administered only if there is clinical evidence of excessive bleeding. The international normalized ratio (INR) likewise is not corrected unless there is evident bleeding, because the INR is also a useful means to monitor liver synthetic function post-transplant. The thromboelastogram has been used to measure the intraoperative coagulation status of patients and may be useful to guide the appropriate administration of blood products [4].

Hepatectomy

The abdomen is opened through a bilateral subcostal incision (chevron), often with a vertical midline extension up to the xiphoid process as needed for exposure of the suprahepatic inferior vena cava (IVC). The umbilical vein is doubly ligated and divided. An alternative incision is a right subcostal "hockey-stick" incision, which avoids extension to the left of midline. A potential advantage of this incision is improved wound healing compared with a T-shaped incision. A strong retractor is placed beneath the right and left costal margins, which are retracted cephalad to expose the liver.

The left triangular ligament is taken down with cautery, taking care to avoid the phrenic veins and left hepatic vein as well as injury to the stomach or spleen. The gastrohepatic ligament is inspected for a replaced left hepatic artery and divided with electrocautery. If present, the replaced left hepatic artery is doubly ligated. Adhesions to the liver from previous surgery or due to peritonitis are taken down with electrocautery. This may take considerable time depending on the severity and density of the adhesions. Care is taken to avoid injury to the transverse colon and duodenum. The cystic duct and artery are ligated and divided if the gallbladder is present. The porta hepatis is exposed and the peritoneal surface and lymphatics overlying the vascular structures and common bile duct are divided. The left and right hepatic arteries are dissected with care to avoid intimal injury or dissection. These structures are doubly ligated and divided adjacent to the liver.

The common bile duct is divided adjacent to the liver, and care is taken to preserve the blood supply of the remaining recipient common bile duct. The portal vein is then dissected free of surrounding connective tissue and lymphatic tissue such that it is ready to be cross-clamped. At this point, the right triangular ligament is taken down with electrocautery, taking care to avoid diaphragmatic injury or injury to the right adrenal gland. The dissection can remain anterior to the right adrenal gland and it is not necessary to ligate its main vein entering the IVC. When dissecting near the IVC, care is taken to avoid injury to the right hepatic vein.

Piggyback technique

The caudate lobe is dissected off the retrohepatic IVC by ligating and dividing all accessory hepatic veins. This is aided by rotation of the operating table from side to side. The caval dissection continues cephalad toward the main hepatic veins. Exposure of the upper IVC may be aided by division of the portal vein as the last structure holding the hilum down. After portal vein division adjacent to the liver, the liver parenchyma can be elevated off the IVC more easily. Again, care is taken during the piggyback dissection of the IVC to avoid injury to the right, middle, and left hepatic veins. When the liver is attached only by the major hepatic veins, these can be cross-clamped with a large Satinsky vascular clamp or a Klintmalm clamp and the liver excised, cutting close to the liver parenchyma to leave as much hepatic vein length as possible. With the liver excised and removed, the retroperitoneal bed and IVC can be inspected for hemostasis prior to implantation of the donor liver.

Caval interposition technique

The infrahepatic IVC is dissected enough to be encircled with a vessel loop or umbilical tape at the level of the caudate lobe and cephalad to the right renal vein and right adrenal vein insertions into the IVC. The suprahepatic IVC is encircled with a red rubber Robinson catheter by passing a clamp or finger behind the IVC at the level of the diaphragmatic hiatus. The advantage of using a rubber catheter is that the posterior jaw of a Klintmalm or similar clamp can be placed within the open end of the catheter, which is then gently withdrawn while guiding the clamp into position around the suprahepatic IVC. The infra- and suprahepatic IVC are clamped with large vascular clamps and the patient is checked for

hemodynamic stability by communicating with the anesthesiology team. If the patient tolerates caval clamping, the IVC is divided both above and below the liver as close to the liver parenchyma as possible. This will maximize the length of IVC remaining for implantation of the donor liver.

Venovenous bypass technique

This method was developed in order to prevent the hemodynamic insult associated with portal vein and IVC clamping [5]. It requires a trained perfusionist, and a BioMedicus (Medtronic Inc, Minneapolis, MN) or equivalent centrifugal pump with the necessary associated tubing. All tubing is primed with normal saline. A "Y" configuration to the inflow tubing is prepared to allow both the portal vein and infrahepatic IVC to be cannulated for inflow. A single outflow cannula to the upper body is placed via either an axillary or jugular vein. Catheter placement may be achieved percutaneously by Seldinger technique or by an open cutdown [6]. Large-bore catheters, such as modified chest tubes, are used for access. The infrahepatic IVC is usually accessed via the femoral or greater saphenous vein. The catheter is advanced cephalad into the IVC. The portal cannula is then placed and secured with an umbilical tape. Great care is exercised to avoid air entry into the circuit as this could result in air embolism. Small air bubbles can also be removed by the addition of a bubble detector to the circuit. All air is removed from the primed tubing and flow is initiated using the centrifugal pump. Optimal flow rates for venovenous bypass differ from patient to patient, but are generally in the 2–3 L/min range. Additional advantages to venovenous bypass include the ability to warm the patient by using a heat exchanger in the circuit, the ability to administer volume and blood rapidly, and the ability to perform hemofiltration via the same circuit, which adds the potential for rapid volume adjustments.

Disadvantages of venovenous bypass include longer operative times, consumption of blood and platelets by the circuit, the potential need for a cutdown for venous access at the groin, axilla, or neck, as well as the associated surgical complications (i.e. lymphocele formation or lymphedema).

Implantation of the whole-organ hepatic allograft

There are numerous techniques and styles in use for hepatic implantation, each with certain advantages and disadvantages. It is important for the liver transplant surgeon to be familiar with many of them because certain techniques may be superior in specific situations. However, here we will discuss the basic steps for liver implantation: vena caval outflow, portal vein inflow, hepatic arterial inflow, and bile duct reconstruction.

Vena caval outflow

Donor vena cava interposition

In the "classic" approach to liver transplantation, the recipient retrohepatic vena cava is excised along with the native liver. This method requires interposition of the donor vena cava in the recipient bed (Figure 56.2A,B). The suprahepatic caval anastomosis is performed first. The anastomosis is performed with 3-0 double-armed Prolene suture. Corner sutures are placed on the left and right and the vessel edges are everted to provide intima-to-intima approximation. A third traction suture is placed in the posterior wall in the middle of the anastomosis to pull the posterior wall anteriorly and provide excellent visibility. The liver is then placed in the hepatic fossa. It is helpful to place an icy laparotomy pad behind the right lobe to support it in the appropriate position. The suture on the patient's left is tied and the posterior wall is sewn

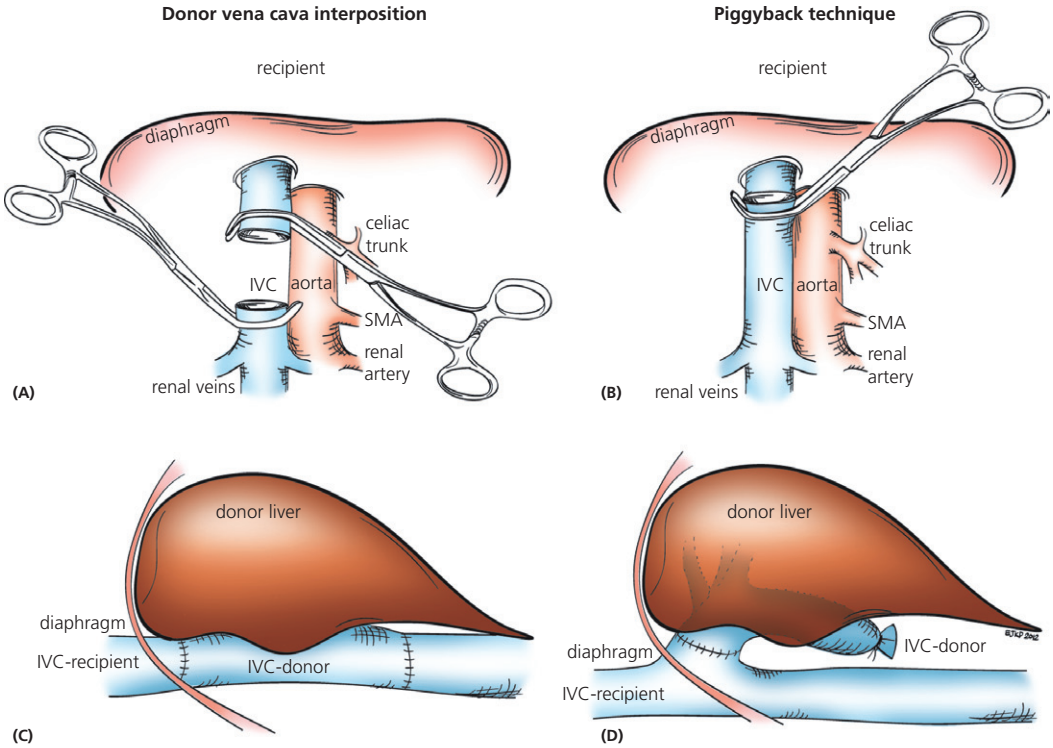


Figure 56.2. Management of vena cava in whole-organ liver transplantation. IVC, inferior vena cava; SMA, superior mesenteric artery.

from inside the vessel from left to right, ensuring good intimal apposition. The middle traction suture is excised when the suture line reaches it. The suture line is carried around the right corner anteriorly for a short distance. The anterior wall is then sewn left to right with the remaining tail, and the suture is secured leaving a 1-cm "growth factor" to prevent purse-stringing and narrowing of the anastomosis [7]. The remaining corner suture is then secured.

The allograft is flushed via the portal vein with 1 liter of ice-cold lactated Ringer's solution containing 10% human albumin, and effluent is evacuated via the graft infrahepatic cava. This serves to remove metabolic wastes that have accumulated during the cold storage interval and washes out the preservation solution. This is particularly critical if the organ has been preserved with University of Wisconsin solution, as this contains a high concentration of potassium that can cause cardiac arrest upon reperfusion. The infrahepatic vena cava anastomosis is performed in an end-to-end manner using 4.0 Prolene sutures using the technique described for the suprahepatic anastomosis. The flush is done prior to completing the infrahepatic caval anastomosis.

Piggyback technique

In the "piggyback" technique the recipient suprahepatic, retrohepatic, and infrahepatic vena cava is completely preserved and only the hepatic veins are clamped (Figure 56.2C,D). This obviates the need for complete clamping of the inferior vena cava. However, this technique results in a more time-consuming hepatectomy since the short hepatic veins must be meticulously ligated [8–10]. A large hepatic venous outflow tract is created by forming a single ostium from the left, middle, and right hepatic veins, which is anastomosed to the graft suprahepatic vena cava (Figure 56.2D). Similar to the caval interposition technique, corner sutures are placed while everting the vessel edges, and a middle traction suture is placed in the posterior wall. The liver is then placed in the hepatic fossa, the left corner suture is secured, and the posterior wall is sewn from left to right ensuring sound intimal apposition. The suture line is carried around the right corner anteriorly as above. Careful suture placement is critical in this location as outflow obstruction is a common pitfall, which can be exceedingly difficult to correct. The middle traction suture is excised. The anterior wall is sutured left to right using the remaining tail, and secured leaving a 1-cm growth factor. The allograft is flushed with 1 liter of ice-cold lactated Ringer's containing 10% human albumin via the portal vein that is evacuated via the graft infrahepatic cava. The donor infrahepatic cava is oversewn or secured with a vascular stapler.

A variation on the piggyback method is the side-to-side cavocavostomy [11]. In this technique, the recipient vena cava is side-clamped longitudinally with a large Satinsky or similar clamp. This results in only partial vessel occlusion. A single ostium is created from the recipient middle and left hepatic veins. It is often helpful to oversew the right hepatic vein for proper alignment, but alternatively it can be included in the anastomosis. The cava is then slit longitudinally for several centimeters from the single ostium, inferiorly. The posterior wall of the donor vena cava is then slit inferiorly for a similar distance. Corner sutures are placed at the superior and inferior-most aspects of the ostia, once again everting the vessel edges for sound intimal approximation. The liver is placed in the right hepatic fossa and rotated to the patient's right. An icy laparotomy pad may be required to support the liver and provide tension-free approximation. The right side of the anastomosis is performed first from the inside of the vessel. A traction suture is placed midway between the cephalad and caudad corners on the posterior (right) wall to provide alignment and excellent visualization. The inferior-most suture is secured and the anastomosis is sewn caudal to cranial. The suture line is carried around the cephalad corner and the middle traction suture is excised. The left wall is then sewn caudal to cranial and the tails secured. No growth factor is required. The remaining superior stay suture is secured. The allograft is then flushed with 1 liter of ice-cold lactated Ringer's containing 10% human albumin via the portal vein, which is evacuated via the graft infrahepatic cava. The donor infrahepatic cava is then oversewn or secured with a vascular stapler.

Portal vein inflow

End-to-end portal venovenostomy

Ideally, portal inflow is provided via the recipient's native portal vein. This can be accomplished in the vast majority of cases because portal vein thrombectomy can generally be performed in the presence of most organized clots. The recipient portal vein is controlled with clamps and the vascular clamp "flashed" to flush any fresh thrombus. The donor portal vein should be cut short to prevent redundancy while recipient portal vein length should be preserved, should future retransplantation be necessary. If there is a size discrepancy between donor and recipient portal veins, the smaller vessel can be spatulated for good size-matching. The anastomosis is then performed with double-armed 6-0 Prolene suture. Corner sutures are placed laterally and the vessel edges are everted to ensure good intimal apposition. A traction suture may be placed in the middle of the posterior wall to aid in alignment and visualization. The suture on the patient's left is tied and the posterior wall is sewn from inside the vessel from left to right, ensuring good intimal apposition. The suture line is carried around the right corner anteriorly for a short distance. The middle traction suture is excised. The anterior wall is sewn left to right with the remaining tail, and the suture is secured leaving a growth factor, which is approximately 75% of the diameter of the vessel [7].

At this point the liver is generally reperfused. The suprahepatic vena cava clamp is removed first to allow retrograde filling of the liver with blood and to check for bleeding from the anastomosis, followed by the infrahepatic vena cava clamp (if the caval interposition technique was used). Any significant hemorrhage is controlled. The portal vein clamp is then removed gradually. Portal inflow is modulated by finger compression while observing the patient's EKG tracing for ST wave changes on the electrocardiogram as well as blood temperature. Portal inflow should be slowed if significant EKG changes are noted or if blood temperature drops precipitously. In cases where there is any question about the quality of portal inflow (i.e. if a portal thrombectomy or jump graft is performed) it is preferable to perform the arterial anastomosis prior to reperfusion. This will ensure hepatic perfusion should portal vein inflow be inadequate.

Pre-existing portal vein thrombosis

Patients with suspected portal vein thrombosis should have preoperative imaging studies so careful operative planning can be carried out. In the majority of patients with portal venous thrombosis, a portal thromboendvenectomy can be performed [12]. Note that thrombectomy is performed *before* beginning the implantation, as it is crucial to have a plan for adequate portal inflow before removing the donor liver from cold storage. In most cases the portal vein thrombus extends down to the splenic vein confluence. The portal vein is dissected circumferentially down to the splenic confluence for secure control. The coronary vein and small pancreatic venous

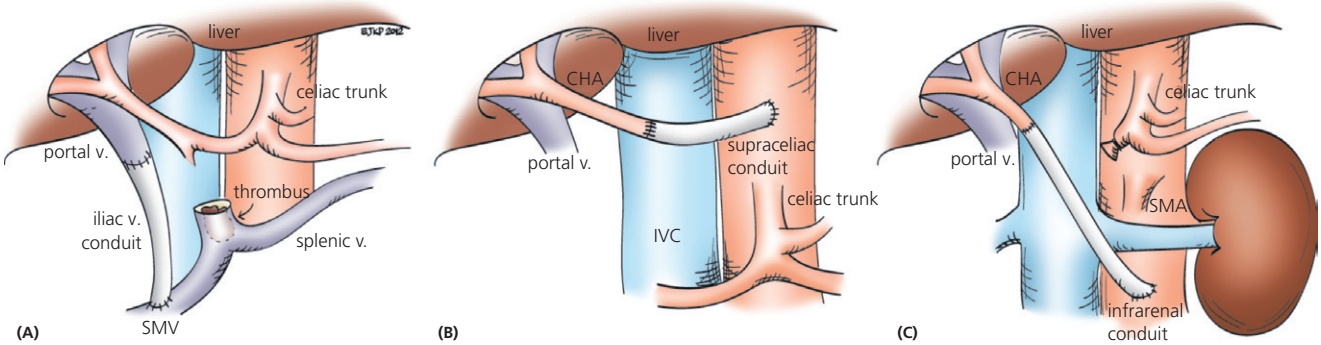


Figure 56.3. Portal venous and hepatic arterial conduit configurations. SMA, superior mesenteric vein; CHA, common hepatic artery; IVC, inferior vena cava; SMA, superior mesenteric artery.

branches are carefully ligated to prevent avulsion and unnecessary bleeding. An eversion thrombectomy is then performed, which can be aided with a Penfield elevator. The thrombus is then grasped with a Kocher clamp and pushed gently inward to peel the clot back, prior to removing. In most cases the entire clot can be removed in this manner. Extreme caution is used to prevent further injury to the portal vein. An end-to-end portal venovenotomy is then performed as above during the liver implantation.

If a thrombectomy cannot be performed safely, a portal venous jump graft should be used to ensure adequate inflow [13]. Again, this should be done prior to beginning hepatic implantation. A long segment of donor iliac vein should be prepared during the back-table preparation of the liver in anticipation of a portal jump graft. The superior mesenteric vein (SMV) is identified at the base of the transverse mesocolon to the right of the superior mesenteric artery. The vein is dissected distally beyond the area of thrombosis and skeletonized for several centimeters to accept a vascular clamp. The SMV is slit longitudinally and the iliac graft spatulated gently to allow the graft to lie directed cephalad. An end-to-side anastomosis is performed using 6-0 Prolene suture. The graft is then tunneled through a hole in the transverse mesocolon on a convenient side of the middle colic vessels. It is passed anterior to the pancreas toward the porta hepatis. An end-to-end anastomosis is then performed to the allograft portal vein in the standard fashion (Figure 56.3A). In cases with an inadequate SMV or in a reoperative abdomen, large portal collaterals can be used for portal venous inflow as a last resort. It should be noted that these collateral vessels are extremely friable and should be handled with the utmost caution.

In a small number of cases, none of the above procedures can be safely performed. Detailed preoperative imaging is mandatory in such cases and creative solutions can sometimes be achieved. For example, left renoportal anastomosis has been reported in cases of portal vein thrombosis with large splenorenal shunts [14]. In cases of complete splanchnic venous thrombosis, portocaval hemitransposition [15] or portal vein arterialization [16] has been described. Reports of the long-term outcomes of such techniques are sparse.

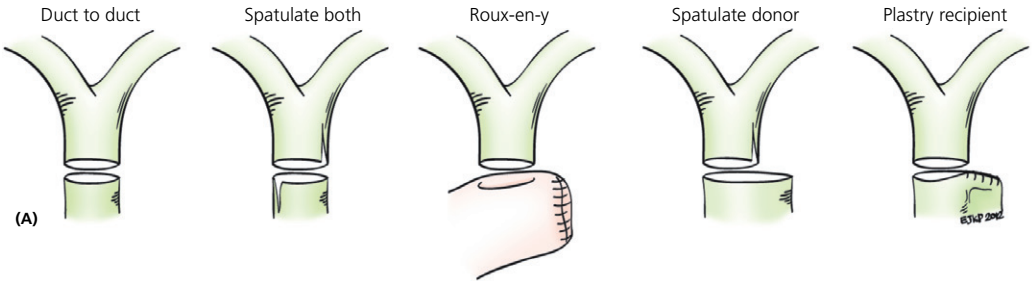
Hepatic arterial inflow

The recipient hepatic artery is carefully dissected proximal to the gastroduodenal artery. The distal gastroduodenal artery is doubly ligated and the common hepatic artery is clamped with a vascular clamp. A branch-patch is then fashioned from the recipient's GDA confluence. On the donor liver, a Carrel patch is cut from the aortic cuff. If the donor aorta is diseased, the anastomosis should be performed using the donor celiac trunk or a more distal site without vascular disease. It is our general practice to leave the donor vessel of adequate length so the vessel avoids tension on the anastomosis and does not kink. An end-to-end anastomosis is performed using either running or interrupted 6-0 or 7-0 Prolene suture [17,18]. If the gastroduodenal artery is unusually large, or if preoperative imaging suggests celiac stenosis, the GDA should be preserved and the anastomosis performed to the recipient's proper hepatic artery or a branch patch that is fashioned from the recipient's left and right hepatic arteries. Alternatively, an arterial conduit can be used to ensure adequate arterial inflow.

If the recipient hepatic artery is unsuitable for implantation due to diminutive size or poor quality, an aortic conduit using donor iliac artery should be performed. This can be done either in the supraceliac [19] or infrarenal [20] positions. Once again, it is preferable to place the aortic conduit prior to beginning hepatic implantation to minimize the time that the liver is without arterial inflow.

The supraceliac aorta is most accessible after the donor hepatectomy and prior to allograft implantation. The esophagus is retracted to the left and the diaphragmatic fibers overlying the supraceliac aorta are divided with electrocautery. Paraesophageal varices must be handled with extreme caution. The aorta is encircled with a vessel loop and skeletonized from the diaphragm for a short distance inferiorly. The aorta can be clamped with a side-biting clamp or two straight vascular clamps, as needed. An aortotomy is made and an end-to-side anastomosis is performed to the donor iliac conduit with running 5-0 Prolene suture. The conduit is clamped with a vascular clamp and the aortic clamps slowly released. Graft flow is assessed by briefly flashing the vessel before flushing it with heparinized saline and reclamping the graft. An end-to-end anastomosis is later performed to the donor hepatic vasculature in the standard fashion (Figure 56.3B).

The recipient's infrarenal aorta is accessed by retracting the small bowel to the patient's right. The descending colon is not mobilized because the aorta is approached by incising the overlying retroperitoneum. One should be vigilant for large collaterals, and meticulous hemostasis should be obtained. The aorta should be carefully dissected cephalad to the left renal vein. This often requires dividing the ligament of Treitz and retracting the fourth portion of the duodenum to the patient's right. The aorta is encircled with a vessel loop and clamped with a side-biting clamp or two separate vascular clamps as needed. The anastomosis to the donor iliac conduit is performed in the same manner as the supraceliac conduit. The conduit is tunneled cephalad to the lesser sac through the transverse mesocolon and in a retrogastric, antepancreatic position.



(B)

Donor	Recipient	Reconstruction
small	small	1) duct-to-duct 2) spatulate both 3) Roux-en-y
small	small	1) spatulate donor 2) plasty recipient 3) Roux-en-y
large	large	Roux-en-y
large	large	Duct-to-duct

Figure 56.4. Options for bile duct management.

Alternatively, the graft can be tunneled to the patient's right by performing a Kocher maneuver and placing the graft posterior to the head of the pancreas and duodenum. An end-to-end anastomosis is later performed to the donor hepatic vasculature in the standard fashion (Figure 56.3C).

Measuring hepatic artery and portal vein blood flow

Vascular complications are the most catastrophic in the field of liver transplantation. To date, there is no uniformly accepted way to predict which grafts are at risk for portal vein or hepatic artery complications. Available to the surgeon at the time of operation is the Medistim Ultrasonic flow probe (Medistim ASA, Norway). These probes allow the surgeon to measure the flow of both the portal vein and/or hepatic artery in real time. In one study, a flow rate of $<$240 mL/min of hepatic arterial flow was a significant predictor of graft failure [21]. Interestingly, the rate of biliary complications was not influenced by hepatic arterial flow. With regard to portal flow, this same study demonstrated that portal flow $<$1300 mL/min negatively affected graft function in a univariate analysis, but this trend was not found in multivariate analysis. To date, there is no universal practice among surgeons, and a prospective trial would need to be performed to fully understand the role of flow measurement and graft outcomes.

Bile duct reconstruction

After portal venous and hepatic arterial perfusion has been established, a cholecystectomy is performed. The bile duct is then prepared for anastomosis by trimming the length and ligating the cut edges of the lateral and medial vessels with fine suture for hemostasis. It is advantageous to retain as much of the recipient duct's length as possible, and to shorten the *donor duct* to prevent redundancy. This is due to the fact that the blood supply to the distal donor duct is now entirely derived from the donor liver and it is crucial to avoid ischemia to prevent biliary anastomotic complications. This is most often achieved by transecting the duct above the cystic duct insertion. One should be cautioned that this is not possible in the case of a very high cystic duct insertion or a very low inserting posterior right hepatic duct. If the common hepatic duct is transected above the cystic duct insertion, the cystic duct should be excised completely to prevent mucocele formation. With a high cystic duct insertion, the cystic duct can simply be ligated and preserved, as long as there is free drainage into the common bile duct to prevent mucocele formation.

With typical-sized donor and recipient ducts, a simple end-to-end anastomosis is performed using running or interrupted 6-0 polydioxanone suture (PDS; Ethicon Inc., Somerville, NJ). In the case of small ducts or slight to moderate size mismatching, one or both ducts can be spatulated to prevent narrowing of the anastomosis [22]. In cases with a small or normal donor duct and an extremely large recipient duct, a portion of the recipient duct can be oversewn with 6-0 PDS suture and an end-to-end anastomosis performed to the remaining opening in the standard fashion (Figure 56.4A,B). Under most circumstances, we do not routinely use biliary stents. However, if a stent is thought to be useful, one is fashioned out of a short segment of an appropriate diameter silastic catheter with additional side holes cut in it. We do not use T-tubes and feel that their associated complications outweigh their usefulness [23] given the advances in modern endoscopy and interventional radiology [24,25].

In the case of a very large donor duct and small recipient duct, or with an unsuitable recipient duct, a Roux-en-Y hepaticojejunostomy is performed. All patients with a diagnosis of primary sclerosing cholangitis are reconstructed with a Roux-en-Y in order to excise a maximal amount of recipient common bile duct down to the level of the pancreatic head and to avoid postoperative stricture. Others have reported selective use of duct-to-duct anastomosis in such patients [26]. For liver retransplantation, a duct-to-duct reconstruction or Roux-en-Y may be used, depending on the quality of the recipient duct [27]. To form a Roux limb, a segment of proximal jejunum is selected approximately 30 cm distal to the ligament of Treitz and transected with a gastrointestinal stapler. The transection point is based upon the vascular arcades and the ability of the cut end to reach the porta hepatis. The distal staple line is oversewn with interrupted 3-0 silk Lembert sutures. A 40-cm Roux limb is then created and a two-layer, hand-sewn, end-to-side jejunojejunostomy is performed. The inner layer is sewn with running 4-0 PDS or vicryl suture and the outer layer is sewn with interrupted 3-0 silk Lembert sutures. Alternatively, this anastomosis can be performed with staplers, but this is not our preferred method. The Roux limb is then tunneled through the transverse mesocolon to the porta hepatis. A small enterotomy is made near the end of the Roux limb and a hepaticojejunostomy is performed using full thickness, interrupted 5-0 or 6-0 PDS suture. The Roux limb is then tacked to the transverse mesocolon and the mesenteric rent is closed with interrupted 3-0 silk sutures.

Closure

If an umbilical hernia is present, this may be repaired from within the abdomen using #1 PDS suture. Closed suction drains are placed in the dependent portions of the upper abdomen and a drain may be placed adjacent to the bile duct anastomosis. The fascia is closed using #1 PDS suture and the skin edges are reapproximated with surgical staples.

Summary

A remarkable amount of progress has been made in the field of liver transplantation since the first successful procedure in humans was done in 1967. Liver transplantation has evolved from an experimental procedure with high morbidity and mortality into a relatively standardized procedure with excellent long-term outcomes. This rapid progress is due to great strides in surgical instrumentation and techniques, immunosuppressive protocols, preoperative imaging, postoperative management, and critical care medicine, to name a few. The techniques described have been in routine use at our center with excellent outcomes and continue to evolve to make liver transplantation a safer and more universally available technology.

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CHAPTER 57

Living Donor Liver Transplantation Procedure and Surgical Technique

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Introduction

Soon after deceased donor liver transplantation (DDLT) became a standard treatment for patients with end-stage liver disease (ESLD), the supply of deceased donor liver grafts was outstripped. In order to overcome the rarity of deceased child donor liver grafts, reduced-size liver transplantation was developed [1]. Split-liver transplantation then allowed transplantation for one child and one adult recipient [2], and subsequently for two adult recipients using one liver graft [3]. In situ splitting further shortened the cold ischemic time and lessened the inevitable graft rewarming of various degrees during the back-table procedure [4]. These technical innovations paved the way for living donor liver transplantation (LDLT). With extensive experience in liver resection for tumors and reduced-size/split-liver transplantation, after the first successful LDLT using a left lateral segment from an adult to a child in 1989 [5], the ethical justification (also discussed in detail in Chapter 138) and technical success of this procedure resulted in its widespread application worldwide [5–7]. This chapter will cover the key factors contributing to a successful recipient procedure for LDLT. Specifics of the live donor liver donor procedure are covered in the companion Chapter 24. Technical aspects of deceased donor liver transplantation can be found in Chapter 56.

The greatest impact of LDLT in adult patients has been in Asian countries where cadaveric organ donation was uncommon or nonexistent [8]. LDLT using left lobe grafts for children [7,9] and adults [10] proliferated in Japan, although this procedure has not become widespread due to the inability of these relatively small-sized grafts to meet the metabolic demands of all adult recipients. To overcome the inadequate graft volume and poor results in the adult recipients with left lobe grafts, transplantation with a right lobe liver graft was introduced for adult recipients in 1996 [11]. Although this method rapidly led to the worldwide use of adult LDLT, right lobe hepatectomies are associated with a greater surgical risk for live donors than left lobe hepatectomies; they are also associated with increased morbidity and mortality rates owing to the reduced volume of remnant liver in the donor [12].

In LDLT, donor safety is of paramount importance and cannot be compromised regardless of the implications for the intended recipient. Moreover, right lobe graft without reconstruction of hepatic venous drainage to the right anterior section has often led to right lobe graft congestion and failure [13]. Although the graft size is critical for successful outcomes, the importance of uniformly successful venous drainage of the anterior section of the right lobe has been regarded as crucial for maximizing graft function. As a result, modified right lobe graft, that is right lobe graft with reconstruction of middle hepatic vein tributaries or extended right lobe graft (right lobe graft with the middle hepatic vein trunk) have been recognized as the preferred grafts based on consideration of the donor and recipient factors. Not all potential donors can donate their right hemiliver because safe donation is possible only when the estimated remnant liver volume is more than 30% [14]. If the volume of the right lobe in potential donors is more than 70% of the volume of the whole liver or if a large-size recipient requires more graft volume than the expected liver graft volume from a single donor, dual-graft LDLT may be an alternative in which two grafts from two donors are transplanted into one recipient.

Perioperative considerations

Compared with deceased donor liver transplantation, aggressive postoperative care of adult LDLT recipients is crucial in order to achieve the best possible outcome. The degree of portal hypertension is a crucial intraoperative risk factor for adult living donor liver recipients.

The favored skin incision of the recipient is a bilateral subcostal incision with midline extension or an inverted T-shape incision. The surgical technique used for recipients is based on whole liver resection with preservation of the inferior vena cava (IVC) [15]. In contrast to DDLT, LDLT is a more sophisticated operation and requires much more careful and delicate dissection of the hilum as high as possible and while leaving a long length of individual structures. Live-donor partial liver grafts have a much smaller hepatic artery, hepatic vein, and portal vein, making them technically more challenging. This usually allows for a variety of different reconstruction options because of the numerous variations in liver anatomy. For technically successful LDLT, we need to satisfy the following four conditions: (1) adequate graft volume in order to avoid small-for-size syndrome, (2) adequate outflow in order to avoid congestion, (3) adequate portal inflow to enhance graft regeneration, and (4) a secure bile duct anastomosis to avoid biliary leak (Figure 57.1) [16].

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

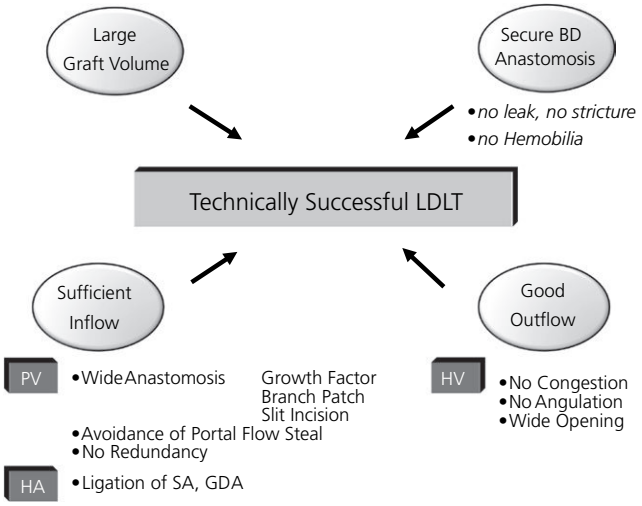

Figure 57.1. Interrelationship between graft volume, hepatic inflow and outflow, and bile duct anastomosis for determining technically successful, living donor liver transplantation (LDLT).

Operative procedure

Recipient hepatectomy

At the time of surgery, the surgeon must be well aware of the related anatomies of the recipient and donor, according to the expected graft type, and must also have a specific plan for how to manage the recipient's anatomical problems, including the hepatic artery (HA), portal vein (PV), hepatic vein (HV), and bile duct (BD). In addition there must be a plan for how to maintain adequate portal inflow without portal flow steal in the presence of large portosystemic collaterals, huge splenomegaly with an enlarged splenic artery, and a small graft size with less than a 1.0 graft-to-recipient weight ratio (GRWR).

At the time of the surgery, the recipient is placed in a supine position on the operating table. The abdomen is prepared and draped for a bilateral subcostal incision with midline extension or for an inverted T-shape incision; simultaneous preparation and draping of the left groin and thigh may also be required in order to access the autogenous great saphenous vein (GSV), which has a thick wall (used for bench work on the back table), and for graft implantation in the recipient. Electrocautery is commonly used to divide the subcutaneous tissue and to extend the incision through the fascia. At this point, division of the transverse wound should be performed earlier than the longitudinal wound, and a combination of electrocautery and ligature might be necessary so as to decrease bleeding from the wound as the courses and diameters of collateral veins developed in the abdominal wall of cirrhotic patients are longitudinal and often sizable. The xyphoid process should be removed in order to provide optimal exposure of the liver and some of the distal part of the sternum may also need to be removed on division of the longitudinal wound. The abdomen is entered by ligating and dividing the falciform and teres ligaments, although is it preferable to preserve large paraumbilical veins without interruption of their flow until completion of the total hepatectomy so as to decrease bowel edema and the amount of bleeding if paraumbilical veins are the main collateral routes draining the hypertensive portal flow.

The falciform ligament is divided toward the hepatic veins. The retractors are then placed so as to provide optimal exposure of the liver. Before beginning the perihepatic dissection, the condition of the intra-abdominal cavity should be checked and it should be decided whether the preoperatively planned tasks, including splenic artery ligation, splenectomy, isolation of portosystemic collaterals, etc., will be accompanied or not. Usually, splenic artery ligation or isolation of the splenorenal shunt causing portal flow steal is undergone at the initial stage because edematous changes of bowel and mesentery after liver graft implantation might hinder those procedures. Other tasks, such as isolation of other collaterals and ligation of portosystemic collaterals, are performed at a later stage, mainly before or after engraftment. Using electrocautery, the coronary ligament is taken down and the superior and anterior surfaces of the three major HVs are exposed. The left triangular ligament is then divided in order to mobilize the left lobe of the liver; likewise, the right triangular ligament and the peritoneal attachments on the right lobe of the liver are divided. During those procedures, the detachment should be performed along the avascular plane. However, we had better often peel off the hepatic capsule in some part by thorough cauterization of both the diaphragmatic and hepatic surfaces and ligation of sizable diaphragmatic vessel so as to reduce intraoperative bleeding when perihepatic collateral vessels or dense adhesions are present, such as in salvage liver transplantation. When it comes to division of the adrenal attachment from the posterior liver, incision of the peritoneum overlying the anterior surface of the IVC and division of adjacent several short hepatic veins should be done. Then, mass ligation with reinforcing sutures for the right adrenal attachment, including the adrenal vein, is performed under the guidance of the operator's left index finger positioned behind the adrenal gland and above the IVC, after which division of its attachment to the liver with thorough cauterization on the hepatic side and suture ligation of the adrenal vein on the IVC side are necessary. When hemostasis of the divided adrenal gland is not complete and is difficult, 3-0 Prolene matrix sutures using a pledget protective patch has been helpful in our clinical experience. After adrenal detachment, hemostasis of the perihepatic area is easy and there is not much bleeding before dissection of the retrohepatic IVC is begun. The next procedure, that is dissection of the hepatic hilum, is often done before dissection of the retrohepatic IVC, considering the usual bleeding tendency and/or the technical difficulty related to the encircling of the IVC by the caudate lobe, especially in the setting of a hypertrophied caudate lobe or Budd–Chiari syndrome, etc. Dissection of the hepatic hilum and/or encircling of the infrahepatic suprarenal IVC before dissection of retrohepatic IVC can be helpful to facilitate the next procedures with less bleeding and to control brisk bleeding from the torn short hepatic veins.

When copious bleeding and/or dense adhesion are expected during liver mobilization, particularly in salvage LDLT patients who underwent previous major hepatectomy or in secondary biliary cirrhosis patients who underwent repeated surgery for biliary problems such as intrahepatic duct stones and/or bile duct strictures, we first dissect the hepatic hilum after preparation of the venovenous bypass through the inferior mesenteric vein in order to reduce bleeding.

The main technical principle of dissection of the hepatic hilum is to preserve implantation options by maintaining the length and integrity of all hilar structures. In particular, meticulous dissection of the hepatic artery to obtain a sufficient length and adequate diameter is very important in order to match with the small hepatic artery opening of the partial liver graft without tension and to avoid intimal dissection of the recipient hepatic artery, such as is often

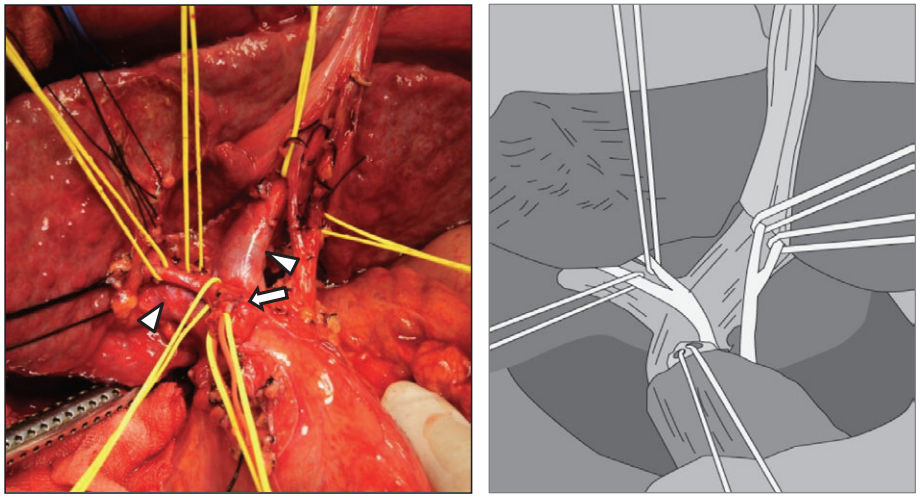

Figure 57.2. Operative view and schema for dissection of the hepatic hilum in the recipient. Second-order branches of the hepatic artery (HA), including the right anterior and posterior HAs, as well as the middle and left HAs, are dissected and taped by a yellow vessel loop. Divided bile ducts at the proximal site of the right and left hepatic ducts, indicated by a white arrow, are retracted by the yellow and red vessel loops. Finally, the portal vein (white arrow head) is dissected up to the right anterior, posterior, and left portal branches, and the main portal vein is mobilized down at least 2 cm in length and toward the pancreatic side.

encountered during hilar dissection of a recipient with portal hypertension. In addition, the available alternatives are not easily achievable because of its small diameter of usually less than 3 mm, in contrast to the diameters of BD and PV. Cholecystectomy is commonly performed first and then hilar dissection begins from the left side. When cholecystectomy is performed, the cystic duct is divided close to the neck of the gall bladder in order to preserve as much of its length as possible in case the cystic duct is needed for duct-to-duct anastomosis of the two BD openings of the liver graft. The left hepatic artery (LHA) is isolated first at the left hilum and then dissected up to the umbilical portion of the left hilum in order to get enough length and also to get the branch-patch of hepatic segment 2 and 3 HAs to accommodate the often larger graft hepatic artery. When right hemiliver implantation is planned, this step is a type of insurance procedure and it is important for a surgeon to proceed following dissection in a comfortable situation. Dissection of the middle and right HA should be performed with preserving the periductal connective tissue encompassing the axial periductal microcirculation in order to avoid post-transplant biliary complications related to ischemia. The right hepatic artery (RHA) should be freed up to the anterior and posterior branches so as to overcome size disparity between the graft and the recipient HAs. Division of HAs without ligation in the recipient side is better than ligation of both sides in order to obtain longer hepatic arteries and to avoid intimal injury. The divided ends of a recipient's HAs are left under microclamp to avoid intimal injury, and are declamped at a regular time interval (usually every 15 minutes) in order to avoid thrombus formation and propagation. In contrast to DDLT, the left and right HAs should not be dissected down toward the proper HA and gastroduodenal artery because previous dissection of the hilum alone is enough for reconstruction of smaller hilar structures in a partial liver graft. In addition, this procedure might be harmful for preserving the blood supply to the bile duct.

Dividing the site of the bile duct should be decided by the size and number of ductal openings of the graft. Pre- and intraoperatively, there should be communication between the recipient and donor surgeons about the cholangiogram. If multiple ductal openings are expected in the graft, the Glisson tissue containing the ducts in the recipient should be divided at a high level of the hepatic hilum in order to create multiple ductal orifices. Arterial bleeding from the cut end of the Glisson tissue ensures an adequate blood supply to the bile duct in both the recipient and the donor. Considering that more than two bile duct openings of the graft after ductoplasty requires at least one hepaticojejunostomy, more than three bile duct openings in the recipient except for the right anterior, posterior, and left duct openings, are usually unnecessary. As a result, the reported techniques for obtaining many bile duct openings in order to maximize the anastomotic options [17–19] seems not to be practical.

The last structure in the hilum to be further identified is the PV. All surrounding tissue of the PV, including lymph nodes, are ligated and divided. The PV is then circumferentially mobilized and, if possible, traced toward and beyond the takeoff of the right anterior, posterior, and left portal branches; all posterior and lateral branches to the caudate lobe are then ligated and divided for full-length mobilization. Regardless of the extent of the proximal dissection, the PV is mobilized down at least 2 cm in length from the portal bifurcation toward the superior margin of the pancreas (Figure 57.2).

The recipient can now be placed on portal bypass if this option is chosen for the remaining hepatectomy and liver graft implantation. During anhepatic phase in LDLT, portal bypass is usually not the preferred procedure because most recipients tolerate portal clamping without hemodynamic instability due to maintaining caval flow, and construction of hepatic and portal vein anastomoses requires less than 60 minutes. Even though portal bypass is not used, bowel edema soon subsides after portal reperfusion. LDLT using a right lobe graft, excluding extended right lobe, left lobe, and dual-lobe LDLTs, does not require systemic bypass because the piggyback technique allows partial clamping of the vena cava with a side-biting clamp and without hemodynamic instability. If much bleeding is expected during retrohepatic dissection of the IVC in

the presence of severe inflammation of the retrohepatic space or hypertrophied caudate lobe encircling the IVC, selectively portal bypass is be good option in order to facilitate dissection without much bleeding.

As the next procedure, the gastrohepatic ligament is divided. At the time of the left liver or dual-graft LDLT, however, the LHA coming from left gastric artery should be dissected as long as possible for arterial reconstruction of the implanted liver graft. The ligamentum venosum is then isolated beside the origin of the left hepatic vein (LHV) and is divided in order to easily obtain a sufficient length of the trumpet-shaped LHV and middle hepatic vein (MHV) stump. The peritoneal attachments on the left side of the IVC along the caudate lobe are divided using cauterization and ligation all the way up to the origin of the LHV. The caudate lobe of the liver is completely detached from the IVC using a left-side approach in order to enhance the retrohepatic dissection as much as possible. The right lobe of liver is then peeled off the IVC by ligating and dividing all retrohepatic veins entering the IVC, although this procedure can be tedious. If brisk bleeding occurs, infrahepatic IVC clamping sometimes helps to control the bleeding with suture ligation. After this procedure, the liver is fully mobilized and attached only by the HV pedicles and the PV.

Communication with the donor surgeon is important in order to share information regarding the anatomical variation of the liver graft and the recipient throughout the recipient's procedures, including the presence and size of the inferior right hepatic vein (IRHV) and the distance from the right hepatic vein (RHV), the size and number of BD openings, PV and HA, etc., because this information can help us to achieve adequate preparation for successful engraftment. Before removal of the recipient's liver, an autologous GSV is retrieved from the groin, most commonly on the left side, because the right groin is usually used for placement of the femoral artery and vein cannulation by the anesthesiologist. Procured GSV is dilated by hydrostatic pressure and bisected, and is mainly used for plasty of the donor HV by reconstruction on the back table.

Considering the harvest time of donor graft and the duration of the bench procedures, the diseased liver is removed as late as possible in order to reduce the length of the anhepatic phase. The PV is usually divided at the right and left branches, although sometimes at the main PV in order to obtain a Y-graft for reconstruction of two PV openings of the donor graft. HVs are divided individually using a vascular clamp. Stapling of the HV with an endovascular stapler is not preferred because the recipient's HV openings should be used for anastomosis with graft HVs after venoplasty.

Anhepatic phase

Recipient side

The PV and rarely HV are often dissected from explanted liver to obtain an autologous vessel graft used for venoplasty in the recipient or reconstruction of the MHV branches as a vascular conduit at the back table if cryopreserved iliac vessels or autologous GSV of a sufficient size are absent or short of length.

After removal of the recipient's liver, meticulous bleeding control should first be performed. A frequent bleeding site during this phase includes the retrohepatic dissection area, and particular care should be given to the inferior phrenic artery and the adrenal gland because they are difficult to expose after engraftment, and bleeding from those sites often occurs during the postoperative course.

Optimal venous outflow is critical for the success of LDLT. Making a wide HV orifice in the recipient in order to accommodate the corresponding donor HV is an essential preparatory step for the engraftment.

For anastomosis of the reconstructed MHV tributaries of the right lobe (RL) graft without the MHV trunk (modified RL graft), the septum between the recipient's middle and left HV is usually divided and a single opening is made using unification venoplasty. Several Allis clamps are placed at the end of the middle and left HV stumps, after which the previously applied clamp is removed so as to facilitate deep placement of the side-biting vascular clamp to the RHV and including the anterolateral wall of the IVC.

The inappropriate ventrodorsal matching of the graft–recipient RHV anastomotic site was found to be a significant risk factor for the development of RHV stenosis. Technical refinements to reduce this risk factor include widening of the recipient RHV orifice [20]. For RHV venoplasty adding the caudal ventral incision to the IVC, a large, side-biting clamp placed deeply on the RHV, and including the anterolateral wall of the IVC, is necessary. We perform an L-shape incision or a separate, longitudinal incision of the RHV with IVC caudally as well as a wide patch-plasty using bisected autologous GSV. These methods result in acceptably low incidences of early onset RHV stenosis (0–2%) [20].

For reconstruction of large, short, hepatic veins (SHVs), deep and secure side-clamping of the IVC is required in order to prevent unnecessary tension during anastomoses. Therefore, it is usually necessary to extensively dissect the more than right-half circumference of the suprarenal IVC, and some branches of the right adrenal vein to the vena cava need to be divided [21].

Back-table procedures

The donor graft is removed, after which the graft is brought to the back table for flushing with 4°C cold preservation solution, such as histidine–tryptophan–ketoglutarate (HTK) or University of Wisconsin (UW) solution. The graft weight is measured before perfusion in order to determine the GRWR. The graft is flushed through the PV until effluent from the HV is clear. The preservation solution is also used to flush the bile duct clean. The HA is carefully flushed so as to avoid intimal injury without insertion of a catheter into the HA using hydrostatic pressure alone. We prefer to use 2 liters of HTK solution for these procedures.

As preparation for the liver graft implantation, vascular reconstruction may be required. Tributaries of the graft's MHV require reconstruction with the autologous GSV, PV, dilated umbilical vein, and/or cryopreserved iliac vessel. The RHV of the right hemiliver graft and the trunk of the middle and left HV of the left hemiliver graft may require venoplasty with previously mentioned vessel grafts in order to prevent hepatic vein outflow obstruction (Figure 57.3). When a single or multiple major SHVs are present, we propose venoplasty guidelines for major SHV reconstruction (Figure 57.4) [21]. If two, separate or piggy-nose PV orifices (right anterior and posterior branches) in the right hemiliver graft are present due to type III or II PV variants, the recipient's Y-graft of PV bifurcation prepared from the recipient's hilar dissection can be used to make a single PV opening with adequate length of the neck for simple and safe anastomosis during engraftment [22,23]. Unification venoplasty of two PV openings or a pig's nostrils-shaped PV opening alone can result in intraoperative difficulty during anastomosis and/or postoperative PV stenotic complications because of the weak PV wall of the liver graft and the configuration mismatch between the liver graft and the recipient PV. When the recipient PV sometimes cannot be used for the Y-graft due to severe PV steonosis caused by thrombosis, circumferential fencing of the

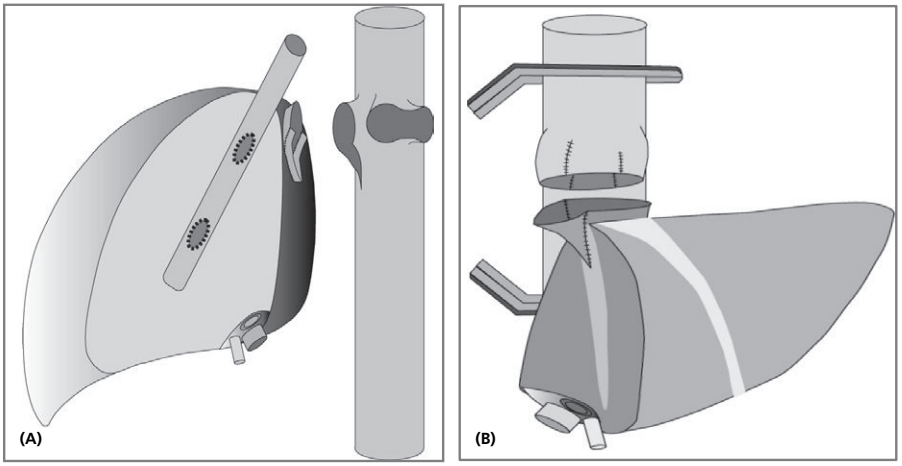

Figure 57.3. Hepatic venoplasty to create a large outflow in order to prevent its obstruction. (A) In the right lobe living donor liver transplantation (LDLT), a caudal incision and augmentation patch-plasty of the right hepatic vein (RHV) is performed in the graft. Corresponding to the enlarged RHV opening, the caudal incision of the recipient's RHV is extended to the IVC. (B) In left lobe LDLT, a common hepatic opening is made after division of the septum in the right, middle, and left hepatic veins in the recipient. Corresponding to the large common hepatic vein opening, the right-corner of the middle hepatic vein is incised and augmentation patch-plasty is performed in the graft.

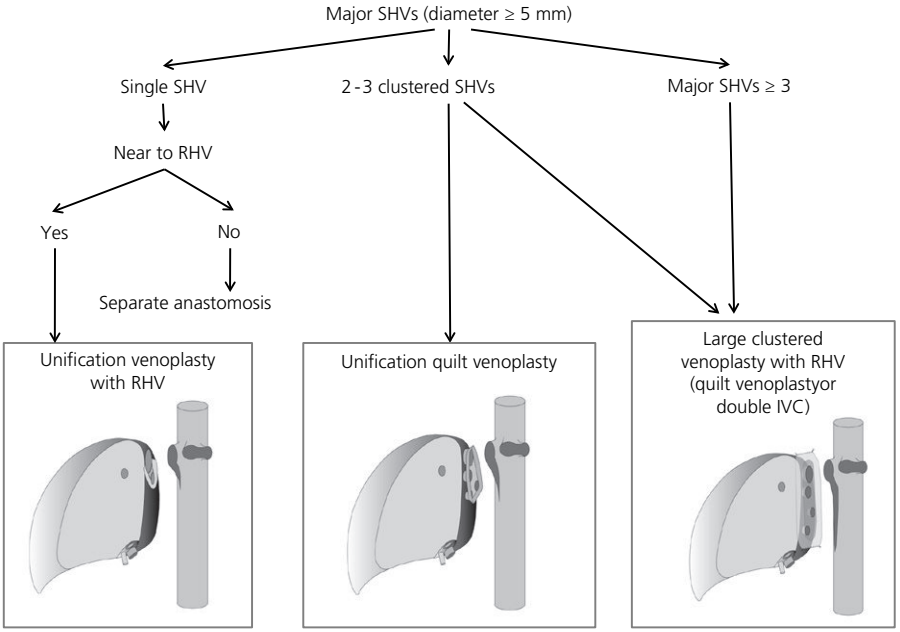

Figure 57.4. Current institutional guidelines for reconstructing a short hepatic vein (SHV) with respect to the main right hepatic vein (RHV). Quilt venoplasty is a complex patch-plasty procedure used to unify multiple SHVs without the application of a niche to the SHV stump.

PV using autologous bisected GSV or Y-graft from a cadaveric, fresh iliac vein can be used as an alternative. Likewise, if two arteries are present in the graft, arterial reconstruction can be performed using a Y-graft and including the appropriate right and left hepatic artery [24]. However, this Y-shape HA preparation may require wide dissection around the bile duct and can often result in biliary stricture. In addition, severe size discrepancy between segmental HAs (right anterior and posterior branches) of the donor and enlarged right and left HAs of the recipient, due to longstanding portal hypertension, are often encountered. Therefore, after portal perfusion we perform two, separate HA anastomoses under a microscope and using mostly the recipient's lobar or sectoral HAs, as, therefore, the recipient HA is dissected as high as possible in order to obtain a long length and size-matched HA.

If more than two separate orifices of the bile ducts are not too far apart or pig's nostrils-shaped orifices are present, unification ductoplasty can be performed in order to create a large single opening.

These back-table reconstructions may require up to 2 hours of warm ischemia time in the complex anatomy of HV, PV, and BD. Therefore, the procedures should be done by submerging the liver graft in 4°C cold preservation solution inside an ice-packed jar.

After completion of the back-table procedures, the liver graft is brought to the recipient room in a submerged state in the preservation solution and packed in ice.

Graft implantation

Hepatic vein anastomosis

Optimal venous outflow is critical for the success of LDLT for both maximal liver graft function and graft regeneration. In this respect, we consider not only the anastomosis itself, but also the positioning of the liver graft. Therefore, the graft should be placed in an orthotopic position, and care should be taken to consider the final position of the graft after the abdomen is closed.

Outflow problems can occur, however, because regeneration of the liver graft may result in a change of its position. Venoplasty of the recipient's HV extended to the IVC wall to make an oval-shaped, wide orifice with adequate length of the neck may help to minimize the outflow complications even though slight torsion of the anastomosis occurs and causes outflow stenosis. Because of this, the recipient HV should be maximally incised longitudinally, and then attached like a fence using an autologous vein patch with a thick wall. The most common and useful graft material is bisected GSV, with bisected recipient's PV being the next choice. For proper alignment at the time of the HV anastomosis, two 5-0 non-absorbable sutures with double needle arms are placed at the cephalic and caudal ends of both the graft and the recipient's HVs. All stitches are placed halfway between the two corner stitches on the anterior and posterior aspects of the donor and recipient HVs, respectively. The posterior wall is sewn, after which the anterior wall is sewn toward the middle. Filling the inside of the HV with heparinized saline is done in order to remove air bubbles before closure of the anastomosis. Venting of the liver graft on reperfusion is usually not necessary in LDLT when HTK perfusion solution is used as it has low potassium content.

Portal vein anastomosis

As mentioned above in Figure 57.1, adequate portal flow is essential for successful LDLT. The PV anastomosis is performed using the recipient's PV trunk or with PV bifurcation to avoid redundancy of the PV anastomosis. Occasionally, the recipient right or left PV branch is used due to its better size match or more favorable alignment than the PV trunk. The PV anastomosis must be constructed without tension, redundancy, or twisting. Before beginning PV anastomosis, the recipient PV should be opened to remove any possible thrombus newly formed during clamping. The preferred suture material is 6-0 Prolene, and the anastomosis is generally performed in a running fashion and incorporation of a sufficient "growth factor" [25].

Recipients with PV thrombosis, once thought to be an absolute contraindication to the already technically challenging living donor procedure, have undergone successful transplantation with partial grafts from living donors [26–28]. In recipients with severe PV thrombosis and who cannot undergo a thrombectomy and/or PV plasty to enlarge the diameter of PV, mesenteric or renoportal interposition grafts are necessary using a cadaveric, fresh iliac vein or a polytetrafluoroethylene (PTFE) vascular graft [29]. Patients who require caval transposition or arterializations of the PV, or both, are at significantly higher risk for morbidity and mortality and are, perhaps, inappropriate candidates for LDLT because adequate portal inflow is mandatory for partial liver graft regeneration.

With a GRWR $<$0.8%, small-for-size syndrome accompanied by hyperbilirubinemia, portal cholestasis, delayed synthetic function, and intractable ascites are more common than with a GRWR $\geq$0.8%. Portal hyperperfusion can cause excessive shear stress against sinusoidal cells of a partial liver graft with a small graft weight, and which is known to be the primary cause of the small-for-size syndrome [30–32]. Several kinds of portosystemic shunts have been described which attenuate portal venous over-perfusion and protect the small-for-size graft from excessive PV flow and pressure [33–35].

However, there is a fatal drawback of portosystemic shunts being seriously considered. It is the portal flow steal phenomenon to the transplanted liver graft that may result in graft dysfunction or even graft infarction in extreme situations and due to insufficient portal inflow to the implanted graft during the early postoperative period of 1 or 2 weeks [36]. Although many transplanted recipients recover without lethal complications, they might have delayed graft dysfunction or failure accompanied by hyperammonemia, hepatic encephalopathy, hyperbilirubinemia, etc. resulting from portal flow steal. To deal with those potentially fatal complications, perioperatively precise hemodynamic monitoring and interruption of pre-existing, large, portosystemic collaterals in an appropriate time are essential (Figure 57.5) [30,37]. However, if and when the collaterals should be interrupted remains a controversial and poorly studied question because there might be other significant problems to be solved, such as acute rejection, vascular complications of PV inflow or HV outflow, an existing small-for-size graft, etc. Other surgical techniques used to reduce portal hypertension are splenic artery ligation and splenectomy. Splenic artery ligation decreases the portal pressure without procedure-related complications during the early post-transplant period in small-for-size graft LDLT [38]. Although splenectomy must be an effective method to decrease portal pressure but has only rarely been recommended for chronic end-stage liver failure patients due to the risk of serious post-splenectomy infections [31]. However, in our clinical experience, concomitant splenectomy is a feasible procedure and does not provoke postsplenectomy sepsis. As for the portal flow steal phenomenon, it is not an issue limited to small-for-size grafts with GRWR $<$0.8% having undergone hemiportocaval shunt, but a common issue in the field of LDLT using partial liver grafts with GRWR $\geq$0.8% and having sizable spontaneous portosystemic collaterals [36,39].

At our medical center, the minimum graft volume should be more than GRWR $\geq$0.7% or $\geq$0.8% in a recipient with or without portal hypertension, respectively, in order to avoid small-for-size graft syndrome. When the graft volume is less than the minimum criteria, the preferred method is not a small-for-size graft LDLT added by hemiportocaval shunt, but a dual-graft LDLT to give adequate graft volume to the sick recipient and to ensure a successful postoperative outcome [40–43]. In contrast to DDLT using a whole liver graft having a large volume, basically most partial liver grafts in adult recipients are relatively small-for-size grafts that need rapid regeneration during the early postoperative period, even though LDLT is performed using more than a GRWR $\geq$0.8% liver graft. Therefore, the portal steal phenomenon through the pre-existing, sizable portosystemic collaterals may often result in graft dysfunction or failure. Intraoperative interruption of sizable portosystemic collaterals, possible routes of portal steal phenomenon during the postoperative period, is an essential step in adult LDLT. Surgical ligation is the first choice; however, as it is limited by complicated courses and the deep-seated location of portosystemic collaterals in many instances, we introduced intraoperative cine-portogram for complete interruption of sizable collaterals by

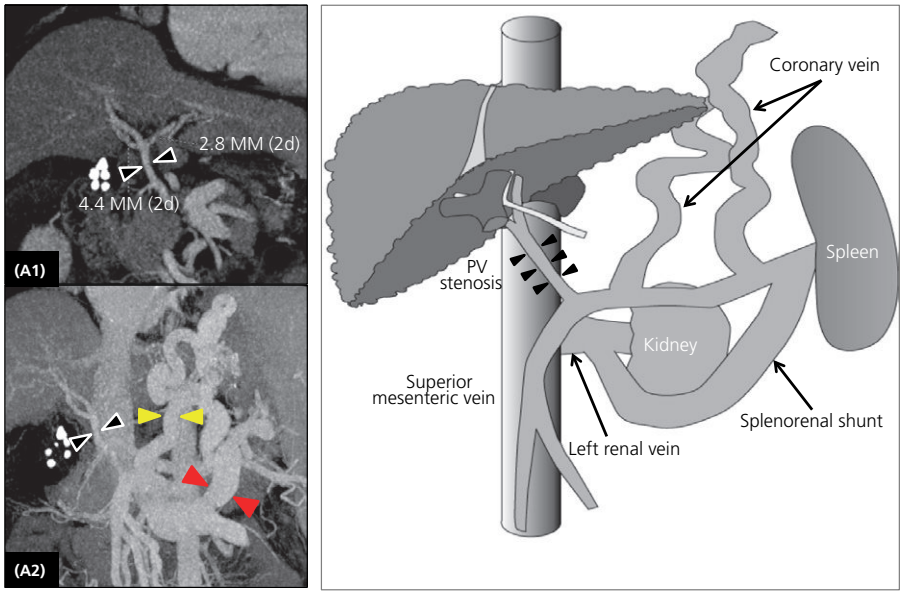

Figure 57.5. Preoperative evaluation of the splanchnic vein anatomy including the portal vein (PV) and collaterals, as seen on the recipient's three-dimensional computed tomography scans (3-D CT). Severe PV stenosis (black arrowheads) in (A1) and (A2), the coronary collateral (yellow arrows), and the splenorenal collateral (red arrowheads) in (A2) are visible. (B) The schema shows severe PV stenosis (multiple black arrowheads) and enlarged coronary and splenorenal collaterals draining splanchnic blood flow into the left renal vein and inferior venal cava.

obtaining the correct information regarding the portosystemic hemodynamics and by monitoring the completeness of surgical ligation or the further "coil embolization" of the deep-seated and surgically inaccessible, large portosystemic collateral veins. Intraoperative cine-portogram is important not only as a diagnostic tool but also as a therapeutic tool for portal vein stenosis and/or thrombus, commonly accompanied by large portosystemic collaterals, by placement of endovascular stent or balloon dilatation (Figure 57.6) [39,44].

Hepatic arterial anastomosis

Arterial anastomosis of DDLT can be performed either before or after reperfusion of the liver graft. In LDLT, arterial anastomosis is performed after reperfusion in most cases because the donor hepatic artery is thin, small, and short, the anastomosis is tedious and often time-consuming work and requires great attention until completion. The diameter of the donor hepatic artery, particularly in Asians, is less than 3 mm in more than three-quarters of the donors [45,46]. These small anastomoses are generally performed in an interrupted fashion with 9-0 or 10-0 Prolene sutures using an operating microscope. Introduction of microsurgical reconstruction technique instead of surgical loop magnification has resulted in a decreased HA complication rate [45]. The preferred recipient's HA is usually the counterpart HA to the donor HA, although selection of the recipient's HA is decided primarily by size matching between the donor and the recipient HA. When there is size disparity, the branch patch technique using small branches of the donor or recipient HA is useful for wide and tension-free anastomosis [47,48]. In addition, the length, status of the arterial wall, and feasibility of stable positioning during anastomosis are also important factors for choosing the anastomotic artery.

In many LDLT centers, a separate microsurgical team consisting of plastic surgeons performs the arterial anastomosis. However, they often experience technically challenging circumstances because the operating field is quite different from their familiar static field [49,50]. Recently, as transplant surgeons have performed HA anastomosis at large-volume LDLT centers, the clinical outcome has improved compared to that of the previous era [51].

In many cases of left hemiliver graft, and a few cases of right hemiliver graft, multiple donor hepatic arteries are present. Whether all accessory vessels require reconstruction or not remains debatable [52–55].

With pulsatile back bleeding from the smaller artery after anastomosing the main hepatic artery of the graft, assuming there is no patchy discoloration of the liver graft, ligation of the smaller arteries was recommended by Ikegami et al. [52]. Some form of hilar or intrahepatic communication can provide good pulsatile backflow, which is not normally demonstrable on angiography unless they are actively functioning as collaterals [56]. All hepatic arteries, including replaced and accessory arteries, however, are essentially necessary arterial inflows because hepatic arteries are end vessels that supply a specific area of the liver. In addition, it remains somewhat unclear what impact a smaller ligated artery, in the presence of good pulsatile backflow, has on the arterial blood supply to segmental bile ducts [55]. Therefore, we prefer to reconstruct all hepatic arteries to reduce possible HA complications, particularly related biliary complications that are the Achilles' heel of LDLT as they use first (lobar; right, left) and second (sectional; anterior, posterior, middle, left lateral), or even third (segmental) order branches of the recipient HA in consideration of the size match between them. When there are few HA inflow vessels for graft HAs due to the size disparity, the recipient's right gastroepiploic artery, cystic artery, and right hepatic artery can be useful alternatives because those arteries are of a relatively small caliber. If reconstruction of all HAs is technically difficult due to their small caliber (less than 1 mm in diameter) and/or poor operative vision related to a short HA stump positioned at the posterior side of the reconstructed PV, intraoperative Doppler ultrasound can be helpful to

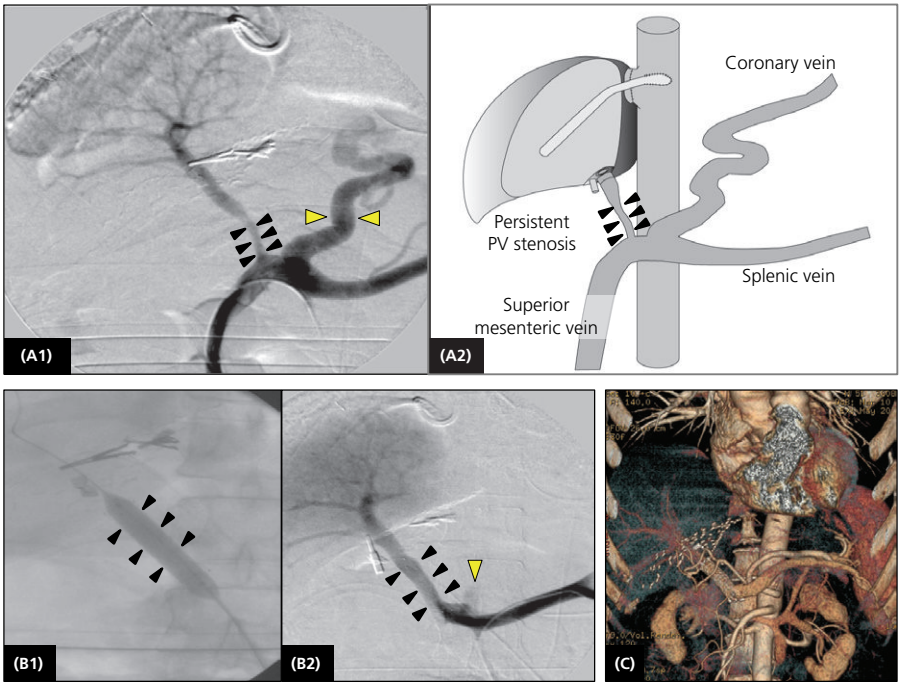

Figure 57.6. Importance of intraoperative cine-portogram. Intraoperative cine-portogram (A1) and the schema (A2) after engraftment showed severe remaining PV stenosis in the intrapancreatic portion (multiple black arrowheads) and portal flow steal through the coronary vein (yellow arrowheads) regardless of PV plasty using the bisected saphenous vein and left renal vein ligation to interrupt steal through the splenorenal collateral. Balloon dilatation (multiple black arrowheads) in B1 and subsequent PV stenting (multiple black arrowheads) in B2 was performed. The final intraoperative cine-portogram (B2) revealed the absence of PV stenosis and portal flow steal through the coronary collateral (yellow arrows). A follow-up CT scan at 27 months (C2) showed a patent PV stent and disappearance of all of the collaterals with no evidence of portal hypertension.

alleviate additional reconstruction by confirming sufficient arterial flow patterns in all segments of the liver graft.

When a right hemiliver graft has two hepatic arteries, direct anastomosis of the two donor HAs with the previously mentioned various HA branches of the recipient has been our standard approach. These anastomoses are technically more demanding, but the most important thing is meticulous and careful dissection of the recipient's hepatic hilum in order to obtain multiple branches of an HA having a small caliber and long stump length. Alternatively, the recipient proper HA, with the bifurcation into the left and right HAs, is divided as a Y-graft and anastomosed to the two HAs of the graft on the back table under optimal conditions [57]. This approach has two potential disadvantages. First, removing the recipient's proper HA may devascularize the proximal bile duct, thus requiring the creation of a Roux-en-Y biliary reconstruction. Second, it requires three anastomoses including on the back table (two) and in the recipient (one).

Compared to the donor HA, when the recipient hepatic artery is slender from intimal hypertrophy after multiple episodes of transarterial chemoembolization but maintains good pulsatile flow, the branch patch technique using segmental branches of the HA can be useful for size-matched HA reconstruction. When the extended intimal dissection occurs after hilar dissection of the recipient or a destroyed HA is present related to previous transarterial chemoembolization or HA thrombosis occurs after LDLT, the right gastroepiploic artery can be commonly usable for alternative HA inflow [58–61]; the reasons being that the right gastroepiploic artery is easy to perform dissection on, is free from limitation of its length, it is frequently enlarged as a compensatory mechanism, and it is feasible to perform anastomosis with a sizable graft HA. The right gastric artery, gastroduodenal artery, left gastric artery, splenic artery, inferior epigastric artery, internal iliac artery, sigmoid artery, inferior mesenteric artery, radial artery, and saphenous vein are also useful for interposition grafts for extra-anatomical HA reconstruction [62–68]. Arterial conduits may be preferred to a saphenous vein interposition graft because saphenous vein grafts tend to develop pseudoaneurysms and have an unsatisfactory long-term patency rate [65]. However, when saphenous vein graft for HA reconstruction in LDLT must be used, we prefer a saphenous vein harvested from the ankle area in order to reduce the possible rate of complications because it has a thick and strong wall and its caliber is usually well matched to that of the graft HA. Occasionally, an interposition graft from the infrarenal aorta using a fresh cadaveric artery or GSV is necessary for the arterial reconstruction when the recipient hepatic artery is thrombosed or obliterated all the way to the origin of the celiac axis.

Biliary reconstruction

Biliary complications in LDLT are more frequent and intractable compared to those of DDLT once considered the Achilles' heel of LDLT [69,70]. The two most probable causes are the small-sized and/or multiple graft-duct openings and the insufficient blood supply to the biliary anastomosis from donor and recipient hilar dissection. The segmental ducts are small, generally only 2–4 mm in diameter, and are cut flush with the hilar plate of the graft when multiple bile duct openings from liver graft hepatectomy come out. Even though there is only one bile duct opening of the graft, its diameter is only about half that of a common bile duct. These

biliary reconstructions of LDLT are technically more demanding than those of DDLT. As a consequence, the incidence of biliary complications, including leaks and strictures, is significantly higher in LDLT.

In our experience, 32% of right lobe and 12% of left lobe grafts required reconstruction of multiple ducts [71]. During the minimum 32-month follow-up period, the cumulative calculated 5-year biliary complication rate was 20.2%. The anastomotic leaks occurred within the first month, with an incidence of 2.9%, and biliary strictures developed after a median period of 6 months or after a mean period of 11.7 ± 11.8 months, and with an incidence of 17.8%. In other series, the overall incidence of biliary complications in recipients ranged from 8.4 to 40.6% [17,19,71–77]. The studies published since 2008 have shown a promisingly dramatic drop in the overall incidence of biliary complications in recipients (8.4–12.8% since 2008 versus 24.3–40.6% before 2008) [78]. However, the long-term studies, with more than a 2-year minimum follow-up period for a large patient population of more than 200 LDLT recipients, have shown at least 20% biliary complication rates [71,72]. In some medical centers, a separate and well-rested microsurgical team performs the biliary reconstruction in order to decrease the rate of biliary complications [77].

The standard biliary reconstruction of living donor grafts was initially the Roux-en-Y hepaticojejunostomy with or without a stent. A Roux jejunal loop must be of sufficient length so that the biliary anastomosis can be created without any tension.

In preparation for the hepaticojejunostomy, if only one donor bile duct needs to be anastomosed, a small stab incision is made smaller than the diameter of the donor bile duct [79]. If a serosa-splitting technique is used, the mucosa is tacked to the serosa with fine absorbable sutures [80]. Full-thickness bites should be taken about 5 mm away from the bile duct edge in order to decrease the risk of stenosis. The stitches should also not be placed too close together in order to reduce the risk of ischemia and subsequent stricture [79]. The posterior wall can be reconstructed using a 6-0 monofilament interrupted or with continuous sutures. From five to eight interrupted sutures of 6-0 absorbable monofilament, which depend on the diameter of the graft bile duct, are used for the semicircle of bile duct anastomosis at our medical center.

On completion of the posterior wall, an internal or external transanastomotic stent is placed in order to reduce the rate of biliary complications in patients undergoing LDLT [71,81,82]. Closure of the anterior wall is then always performed using interrupted sutures. If an external stent is used, a Witzel tunnel is created.

Currently, compared with Roux-en-Y hepaticojejunostomy, duct-to-duct anastomosis is recognized as a simple and the favorable method for adult LDLT and it is currently a standard technique because of its theoretical advantages [71,72,76,83,84]. Duct-to-duct anastomosis is believed to preserve the physiological sphincter of Oddi and, consequently, prevent reflux cholangitis and contamination by the intestinal contents. It also decreases the operative time and allows good access for the endoscopic management of postoperative biliary complications. In view of the occurrence of biliary strictures, however, duct-to-duct anastomosis has a higher risk of biliary stricture than Roux-en-Y hepaticojejunostomy [19,71,84]. In particular, duct-to-duct anastomosis involving a small-sized duct less than 4 mm in diameter has been found to be a significant risk factor [71].

Confirmation of the viability of the donor and recipient bile ducts before reconstruction of duct-to-duct anastomosis is important in order to reduce biliary complications, and the viability is decided by the presence of pulsatile arterial bleeding from the cut ends of the BD [17]. We prefer to prepare the recipient duct opening one and one-half times larger than the size of the fully expanded graft duct opening in order to reduce the possibility of anastomotic strictures. Anastomosis techniques for duct-to-duct anastomosis are similar to those for hepaticojejunostomy with a single-layer closure.

It also remains controversial whether internal or external transanastomotic biliary drainage is beneficial for reducing the rate of biliary complications. Liu et al. reported duct-to-duct anastomosis without a biliary stent in right lobe LDLT; and their rate of biliary complications was similar or superior to the rates in previous studies using biliary stents. A probable hypothesis is that the stent, itself, is a foreign body and will thus induce inflammation and subsequent stricture formation [85]. However, transanastomotic biliary stenting in LDLT is still a generally preferred procedure because it maintains the biliary flow despite swelling of the anastomosis and is an easily accessed route for cholangiography in case there is a suspected leak or stricture [71,75,76,81,84]. We prefer external stents to internal stents despite the several disadvantages, among which is that a long waiting period is necessary until external stents can be safely removed, thus preventing leaks at the insertion site. External stents are more effective for reducing biliary complications, including leaks and strictures, than internal stents, and, in particular, life-threatening bile leaks from the anastomosis have almost disappeared with the routine use of external biliary drainage.

The reconstruction of multiple graft ducts is sometimes a real challenge of LDLT when graft ducts are tiny, thin-walled, and prone to ischemia. Multiple, biliary anastomoses are definitely adverse factors that can lead to biliary leaks and stenosis [73,86–88]. When the two ducts are adjacent, unification ductoplasty may be applicable. Although this strategy can facilitate the feasibility of a single anastomosis, this artificial manipulation also increases the risk of bile-duct stump ischemia [87,88]. For double graft ductal openings, separate duct-to-duct, separate hepaticojejunostomy, or a combined duct-to-duct and hepaticojejunostomy were chosen depending on the circumstances. A compromise can be made between duct-to-duct and hepaticojejunostomy under unfavorable bowel loop conditions, such as marked edema, peritonitis-induced thickening, or shortened mesentery [71]. For duct-to-duct anastomosis for more than one bile duct opening, right and left hepatic ducts or more distal segmental ducts can be used. In addition, the cystic duct can be successfully used for duct-to-duct anastomosis [89], particularly when bile duct openings of the liver graft are widely apart, such as the right posterior or accessory duct arising from the common hepatic duct and which is unable to be reconstructed without hepaticojejunostomy (Figure 57.7). Before reconstruction of the cystic duct anastomosis, straightening the spiral valve of the cystic duct should be done by probing and dilatation with a metal probe until it is entirely passable. We routinely insert an external stent into a duct-to-duct anastomosis using the cystic duct in order to keep the lumen open and to avoid obstruction caused by fibrous tissue replacing the anastomotic site [89,90].

All biliary anastomoses should be fashioned tension free and, at their completion, should be checked for leakage. We use a dye test with methylene blue to detect leakage at the completion of the biliary reconstructions. Routine completion cholangiography should be performed when an externalized stent is placed. Leaks are best identified and corrected during the surgery [76].

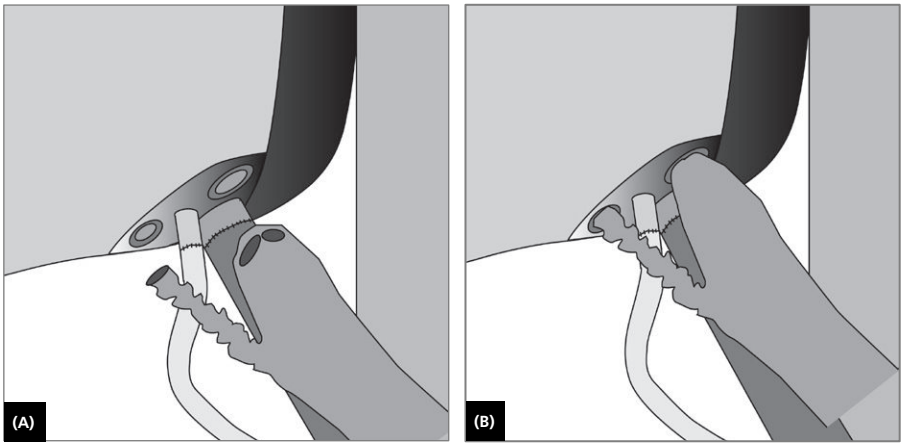

Figure 57.7. Duct-to-duct biliary reconstruction using a cystic duct. When the separate two ducts of the right-lobe graft are more than 1 cm apart and the size- and length-matched cystic duct is available in the recipient (A), we perform duct-to-duct biliary reconstruction (B).

Biliary complications adversely affect the recipient's quality of life and can occasionally even cause graft loss and death [85]. Avoiding biliary complications is very important, although currently there is approximately a 20% complication rate seen on the long-term follow-up results after LDLT and this seems to be inevitable despite the many types of preventive measures. Therefore, intraoperative and postoperative careful management to reduce or treat expected biliary complications is essential in order to prevent the worst outcome caused by bile leak or stricture.

Closure

Once all of the anastomoses are completed with satisfactory results, hemostasis should be assured at the cut surface of the liver graft and all of the recipient's dissection sites, including the perihepatic dissection area (e.g. inferior phrenic artery, adrenal gland, and perirenal space) and the hepatic hilum because postoperative bleeding is not an uncommon complication in LDLT. When we insert closed suction drains, puncture sites of the abdominal wall should be selected at a relatively avascular aponeurotic area lateral to the rectus muscle, in order to avoid injury to epigastric vessels. If bleeding is visible from the peritoneal side of the drain puncture site, we perform a two-thirds, circular stitch around the puncture site using absorbable suture materials and taking care not to bite the drain catheter. After complete hemostasis of the abdominal wall muscles, the abdominal fascia is closed with both absorbable monofilament sutures, in a running fashion, and with additional, interrupted reinforcement sutures.

Graft-type-specific operative procedures

Right lobe graft without middle hepatic vein trunk (right lobe or modified right lobe graft)

Initially, a right lobe graft with MHV trunk (extended right lobe graft) was used for adult LDLT by a Hong Kong group because it can assure optimal drainage of the anterior sector, thus avoiding venous congestion, and it offers better graft function even for a larger recipient [11]. However, LDLT using an extended right lobe graft poses the question of undue risk in the living donor and may be an important ethical issue regarding the aspect of donor safety [91]. We use extended right lobe graft only on the following two scenarios. First, donor age less than 35 years, with no fatty change and congestion-free remnant left liver volume $\geq$30% of the original liver volume. Second, congestion-free remnant left liver volume $\geq$35% of the original liver volume if the donor age is >35 years or mild fatty change is up to 30% [92]. As an alternative graft type, right lobe graft without MHV is commonly used for adult LDLT. However, MHV tributaries draining the anterior sector (Couinaud segments 5 and 8) had not been reconstructed before 1998, when SG Lee introduced use of a modified right lobe graft in which sizable (>5 mm) MHV tributaries are reconstructed using interposition vessel grafts into the recipient's hepatic venous system in order to prevent congestion of the anterior sector [93]. Before the introduction of MHV reconstruction in right lobe graft LDLT, severe congestion of the anterior sector immediately following surgery developed in two of five recipients and was followed by prolonged, massive ascites and severe hepatic dysfunction in early right lobe (RL) LDLT. One recipient died of sepsis with progressive deterioration of graft function 20 days post transplant [94]. Inomata et al. also reported a relatively poor outcome, that is 73% survival rate, after LDLT using a right lobe graft without MHV reconstruction [95]. After application of a modified right lobe graft, the 6-month recipient survival rate improved from 71% to more than 90% at our institution [96]. One study compared the regeneration rate of RL liver graft to three techniques: (1) without MHV trunk preservation or MHV reconstruction; (2) without MHV trunk preservation but with MHV reconstruction (modified right lobe graft); and (3) with MHV trunk preservation (extended right lobe graft). That study showed that if the MHV trunk is not retained, MHV reconstruction should be performed in 85% of all RL LDLT [97].

However, when functional mass of the right lobe graft is adequate (high GRWR) and the MHV tributaries are small (<5 mm in diameter), it means that RHV functions as the major outflow of the anterior sector and right lobe graft without reconstruction of MHV tributaries may be sufficient to assure good graft function [93]. Other researchers have suggested that the lack of anterior sector regeneration is resolved by compensatory regeneration of the posterior sector and that graft congestion in the sector does not affect overall graft regeneration, particularly if the GRWR is sufficient [98,99].

For implantation of a modified right lobe (MRL) graft, all sizable (>5 mm in diameter) MHV tributaries are preserved and

temporarily occluded by tying over a short rubber band or performing Hemolock clamping for future reconstruction during donor hepatectomy. After procuring the RL graft, the temporarily occluded MHV tributaries are opened and flushed with preservation solution at the back table until the perfusate is clear. The initial interposition graft material used for MHV reconstruction was the recipient's GSV after hydrostatic dilatation or two sheets of the GSV. In this situation, the anastomosis between MHV tributaries and autogenous vein grafts was performed using a continuous 6-0 Prolene suture. However, the size disparity between the small caliber of the single-lumen GSV graft and the larger MHV tributaries from segments 5 and 8 created a near occlusion [93]. As an alternative autologous vein graft to overcome this size disparity and to increase the long-term patency, that is to provide a relatively large-diameter recipient left portal vein, several other vein sources can be used, including paraumbilical vein, hepatic vein, superficial femoral vein, external iliac vein, or internal jugular veins [93,100–103]. If homologous vein grafts, such as iliac vessels, femoral vessels, and IVC or aorta, can be harvested from cadaveric organ donors, those are the most preferred vessel grafts for MHV reconstruction at our institution because they do not require time-consuming dissection of recipient tissue and are large and of a long enough length to be used for any variant type of MHV branch [104]. However, the recent increase in the number of adult LDLTs, coupled with the relatively limited number of cadaveric vessel allografts, has led us to search for new vessel substitutes [43]. Thin-walled expanded PTFE grafts were used for MHV reconstruction. The patient and graft survival rates after transplantation of an MRL graft were comparable to those of the extended right lobe (ERL) graft, although their patency rates appeared to be insufficiently low [105]. Ringed PTFE grafts have been preferred at our institution as they increase the patency rate, and they showed a high 6-month patency rate (75.3%) comparable to that of the cryopreserved iliac vein (76.6%). As a result, ringed PTFE graft can also be a good alternative interposition vessel graft when another sizable vessel allograft is not available [106].

In the recipient's side, the interposition vein grafts are anastomosed to the recipient's middle and left HV common opening or directly to the IVC by a continuous 6-0 or 5-0 Prolene suture after the liver graft was reperfused by portal blood flow. The recipient's middle and left HV trunk is the preferred site for anastomosis of interposition vein grafts for several reasons. First, its course and configuration of the anastomosis are more natural due to the use of orthotopic normal anatomic structures and the technical feasibility. Second, the outflow orifice of the interposition vein graft is exposed closer to the negative intrathoracic pressure system and thus promotes hepatic vein outflow drainage more smoothly [107].

Right lobe graft with middle hepatic vein trunk (extended right lobe graft)

Regarding the aspect of unimpeded outflow of the right lobe graft to avoid parenchymal congestion, the right lobe graft with MHV is the best type of right lobe graft if donors are corresponding to the previously described two occasions [92]. In particular, when the GRWR is not sufficient, that is less than 1.0%, in the presence of severe portal hypertension, an ERL graft may have an important role in reducing the small-for-size syndrome by securing a reliable venous outflow.

The Hong Kong group has been a proponent of the ERL graft from the beginning of LDLT. Their technique has evolved over time. In their initial experience, the MHV was anastomosed end-to-end to the ostium of the middle and/or left HV of the recipient [11]. The finding that medial rotation of the graft after reperfusion or later during regenerative growth could in certain cases cause compression of the MHV, caused a change in their initial reconstruction technique. The new technique consists of the construction of a triangular-shaped, common orifice of the right and middle HV of the graft. The distance between the ostia of the right and the middle HV of the graft can be eliminated by dissection of the interposed parenchyma, and horizontal suturing of the vertical incision can be performed on both adjacent walls [108]. In recipient side, circumferential control of the IVC is necessary during HV anastomosis. Several lumbar and phrenic veins must be ligated and divided before IVC is completely mobilized from the diaphragm down to the level of the right adrenal vein. The presence of an inferior RHV greater than 5 mm in the graft calls for freeing of the IVC more inferiorly for vascular anastomosis. When the liver graft is ready for implantation, the IVC is cross-clamped cranially and caudally to the hepatic veins. The longitudinal orifice of the RHV is enlarged by incising transversely across the anterior wall of the IVC corresponding to the transverse dimension of the common hepatic vein orifice in the graft. The cranial and caudal flaps are excised so that a large triangular opening is created and is matched with that of the graft [109]. The height of this triangular opening with the base on the right side and the apex on the left should be no longer than half of the IVC circumference in order to prevent its stricture. The implantation starts with the hepatic vein anastomosis in a triangular fashion using 5-0 polypropylene sutures. After the hepatic vein anastomosis is completed, the portal vein of the liver graft is clamped using a bulldog vascular clamp, and the IVC circulation is restored before PV anastomosis for maintenance of hemodynamic stability [110].

They insist that the described hepatic venoplasty technique converting the RHV and MHV to a single triangular cuff by venoplasty, shortens the caval cross-clamping time, does not necessitate the use of venovenous bypass, and ensures excellent venous drainage [11]. However, their method has several shortcomings. First, venoplasty to create a single, wide-open triangular cuff without using an interposition vein patch between the RHV and the MHV might cause undue tension on the liver parenchyma and thus result in ischemic portion when there is a wide distance between the RHV and the MHV. Second, PV anastomosis under clamping at both recipient's and donor's side, so as to reduce the cross-clamping time of the IVC, is not feasible because the graft PV is sometimes divided too short to clamp for fear of PV stenosis in the donor side. Third, the distance of the apex between the triangular cuff of the graft hepatic veins and the triangular opening of the recipient IVC during anastomosis is far away because there is no patch plasty and also the absence of vessel wall redundancy on both sides. As a result, the weakened vessel wall of the anastomosis might be torn from excessive tension, as indicated in their recent report [111]. Fourth, the adverse effect of venovenous bypass is not so worrisome. On the contrary, it allows decompression of the splanchnic and retroperitoneal circulations and prevents major hemodynamic changes during the anhepatic phase. It, therefore, provides more time for us to prepare the recipient IVC such as cavoplasty and to perform venous anastomosis without having to hurry.

At our medical institution, venovenous bypass is used for implantation of ERL grafts. The RHV and MHV of the graft is converted into a common cuff resembling a dome using the quilt venoplasty technique with the autologous saphenous vein, portal vein patch, and sometimes with homologous cryopreserved cadaveric veins.

At the same time, the orifices of the recipient's RHV, MHV, and LHV are opened altogether to make a large orifice in the recipient's IVC. The bisected saphenous vein segment is sutured to the middle and left sides of the common orifice in order to make a fence for thick-walled, tension-free anastomosis during reconstruction. This method is freely applicable to the extended right lobe grafts with preservation of the proximal MHV branches at the donor's remnant liver side because the corresponding defect at the graft MHV can be covered by the enlarged vein cuff [112].

Recently, an ERL graft leaving the proximal MHV to preserve the drainage veins of segment 4 to the MHV in the donor's remnant liver has been commonly used due to consideration of the donor's safety [99,113,114]. In this situation, the gap between the graft's MHV and the recipient's hepatic veins is wider than a conventional ERL graft, and separate anastomosis of the MHV to the recipient's middle and left HV trunk using an interposition vein graft such as a modified RL graft without venovenous bypass or a large single anastomosis after making a redundant common cuff using quilt venoplasty under venovenous bypass, can be performed for reconstruction of the MHV (Figure 57.8).

Left lobe graft with and without the caudate lobe

In adult-to-adult LDLT, a left lobe graft has a limited role because the graft volume is not sufficient to avoid the small-for-size syndrome in many patients. However, as long as the graft size is greater than 40% of the recipient's standard liver volume in small-body-sized recipients, a left lobe (LL) graft is still a useful graft for adult-to-adult LDLT [115,116]. Although the caudate lobe is a small part of the whole liver, its volume is not negligible in the partial liver graft and provides a 6 to 12% gain in LL graft weight [117,118]. When the pretransplant recipient's physical condition is good and without cirrhosis such as metabolic disorders, including citrullinemia or familial amyloid polyneuropathy, the minimally successful graft volume might be decreased to as low as 0.6% GRWR or 30% of the recipient's standard liver volume [119].

Considering the relatively small-sized graft volume, large hepatic vein outflow is essential for a functionally perfect graft after transplantation into the recipient. During total hepatectomy we mobilize the retrohepatic IVC from the retroperitoneal attachment. To ensure adequate hepatic vein outflow, venoplasty of the hepatic veins of the liver graft should be performed using an autologous, bisected GSV segment. The venoplasty technique is used to make a wide, single orifice with a sufficient length of the hepatic vein stump. The right side of the MHV only or both the right side of the MHV and the left side of the LHV are incised longitudinally and the bisected GSV segment is attached to the hepatic vein for venoplasty. In the recipient's side, the orifices of the recipient's RHV, MHV, and LHV are completely opened, and venoplasty making an adequate-sized, large orifice is performed in order to accommodate the enlarged hepatic vein orifice of the graft (Figure 57.9). Venovenous bypass under clamping of the supra- and infrahepatic IVC is a necessary step for the recipient's venoplasty and engraftment (Figure 57.10).

When the left lobe with the caudate lobe graft is used, complete revascularization of the caudate lobe may contribute to full graft regeneration [120]. The caudate hepatic vein resected with a cuff of the IVC, which resembles a Carrel patch, is first reconstructed, after which the enlarged hepatic vein orifice of the graft is anastomosed

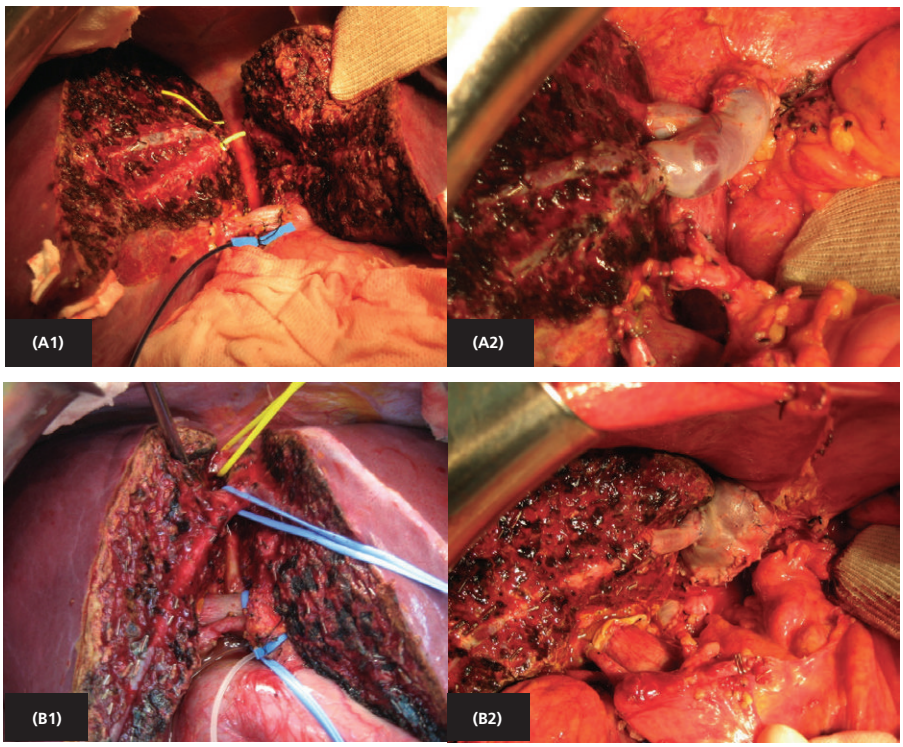

Figure 57.8. Extended right lobe graft leaving the proximal middle hepatic branch of segment 4 in the donor's remnant liver. Two openings, including the distal trunk of the middle hepatic vein and segment 8 of the middle hepatic vein branch (V8), are expected to come on procurement (A-1, B-1). Separate anastomosis of the MHV to the recipient's middle and left HV trunk using interposition vein graft such as a modified right lobe graft (A-2) or a large single anastomosis after making a redundant common cuff using quilt venoplasty under a venovenous bypass (B-2) can then be performed.

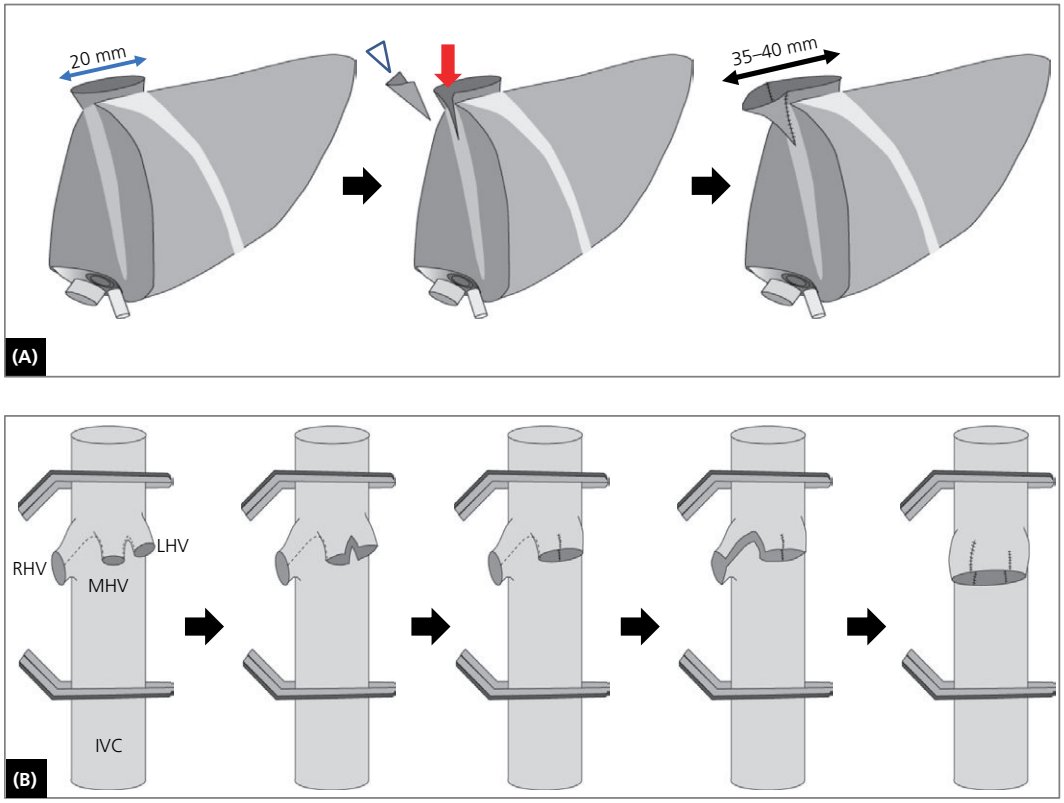

Figure 57.9. Hepatic vein plasty to create maximal outflow in left lobe, living donor liver transplantation. (A) When the common opening of the middle and left hepatic vein in the graft is small, that is approximately 20 mm in diameter, the right side corner of the middle hepatic vein is incised (white arrowhead) and patch-plasty using the bisected great saphenous vein (red arrow) is performed at the back-table to create an enlarged, common opening of approximately 35–40 mm diameter. (B) In the recipient's side, all three of the hepatic vein orifices are completely opened, and venoplasty is performed to make a large common orifice for accommodation of the enlarged hepatic vein orifices of the graft.

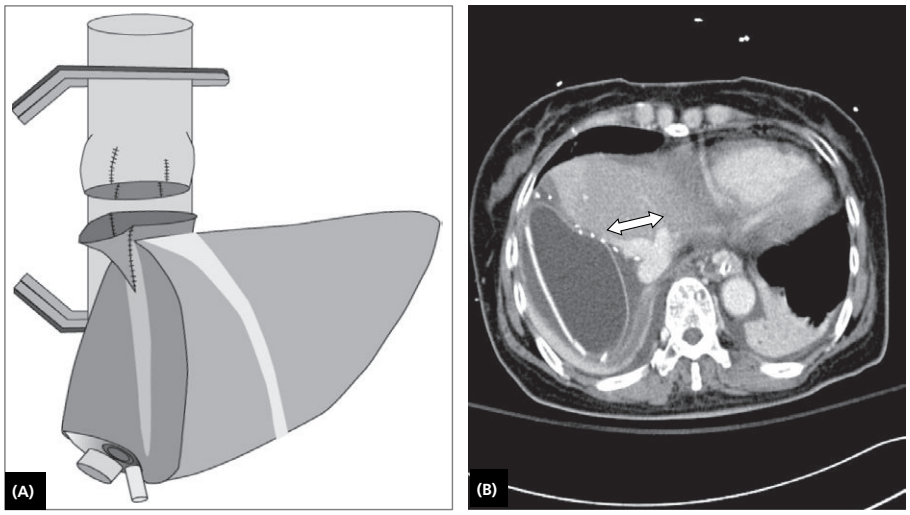

Figure 57.10. Reconstruction of the hepatic vein in left lobe, living donor liver transplantation. (A) Anastomosis between the donor and recipient's maximally enlarged, common hepatic veins is performed by clamping the supra- and infrahepatic inferior vena cava and by venovenous bypass. (B) Follow-up CT scan obtained on postoperative day 7 showed wide hepatic vein outflow and tissue expander filled with saline solution to prevent torsion of the hepatic vein anastomosis until there was adequate regeneration of the implanted graft.

to the large common opening of the recipient's hepatic veins. When the orifice of the caudate vein is located close to the left and middle hepatic veins, the caudate vein with the IVC cuff can be made into a single opening with a common orifice of the left and middle HV, and a single HV anastomosis is sufficient for the outflow reconstruction [121]. However, we have seldom observed any large caudate vein in such a location during harvesting of hundreds of left lobe grafts [117]. An isolated caudate PV originating from the main PV is present in 13% of recipients and reconstruction is necessary for a large, that is more than 5 mm, one [122]. However, during donor hepatectomy in our series, a large, isolated caudate PV was very rare and we only chose whether to meticulously excise the caudate PV branch as a common PV opening or to ligate it. Considering the reconstruction of the hepatic artery, all hepatic arteries of the left lobe graft might not be able to be reconstructed [52] because accessory hepatic arteries usually communicate with the original lobar arteries in the hepatic hilum [56]. However, we reconstructed all of the hepatic arteries of a liver graft when the size- and length-matched inflow arteries were present because individual hepatic arteries are basically end arteries and we occasionally encountered abnormally elevated liver enzyme and an ischemic area of implanted left lobe graft during the immediate post-transplant period when unreconstructed HA is present.

Reconstruction of the caudate duct can be performed in a single anastomosis when closely approximated or in a separate anastomosis when widely apart [17]. However, it is an optional procedure, not an essential procedure at our medical center. Instead of a preference for caudate duct reconstruction, we give special attention to determining the bile duct division site in order to avoid sizable, separate caudate ducts with exploration of the biliary tree, including the ducts of segments 9 and 1 through the cystic duct and using a 1.5-mm coronary artery dilator [117].

Dual-graft living donor liver transplantation

Partial liver graft from a single donor, regardless of whether it is a left or right lobe graft, cannot often meet the metabolic demands of a large recipient or of a critically ill recipient with portal hypertension. As an alternative approach in order to avoid the small-for-size graft syndrome and also to provide donor safety based on the established donor selection criteria, dual-graft LDLTs have been performed since 2000 at our institution and recently at many other hospitals as well [42,123–127].

During the recipient's surgery, both the right and left branches of the PV and the hepatic artery are dissected free from the surrounding tissue and as high as possible so as to obtain enough length for future bilateral vascular anastomosis. During the total hepatectomy, the recipient's IVC should be mobilized from its retroperitoneal attachment because venoplasty of the recipient's hepatic veins and graft implantation are performed under clamping of the supra- and infrahepatic IVC. Venovenous bypass is necessary in most cases in order to maintain stable hemodynamics and to avoid mesenteric congestion during the anhepatic phase.

Before implantation of liver grafts, venoplasty of hepatic veins in the recipient and/or liver grafts should be performed to make wide outflow orifices with a thick wall and a long cuff. The recipient's RHV is enlarged and elongated by longitudinal incision at the inferior corner and fencing with the bisected GSV. The middle and left HVs are converted to a single opening by division of the septum, and are then enlarged and elongated by a transverse incision at the right corner and fencing with bisected GSV. At the back table, venoplasty of hepatic veins of the liver grafts can be performed considering the size match between the recipient's and the graft hepatic veins. The methods are the same as those of the single-graft LDLT mentioned previously. The venoplasty is important so that the surgeon can perform engraftment without difficulty during the surgery and can also avoid postoperative outflow disturbance.

Engraftment procedures using two left liver grafts are as following. First, a 180° rotated left liver is placed into right upper quadrant space, and its hepatic vein anastomosis to the recipient RHV is performed. Second, an orthotopically positioned left liver is anastomosed to the common opening of the middle and left HV. Third, the PV anastomosis of the orthotopic graft is performed to the recipient's left PV. Fourth, a left-sided (orthotopic) graft is reperfused earlier in order to avoid bowel congestion and reduce the ischemic time. Before the procedure proceeds, a vascular clamp is applied to the recipient's RHV to prevent regurgitation of caval flow into the right-sided (heterotopic) graft after release of the caval clamp. Vascular clamps to the vena cava and the main PV are then removed together under clamping of only the recipient's right PV. Fifth, bile duct anastomosis of the right-sided (heterotopic) graft to the recipient's bile duct, is performed in a duct-to-duct fashion earlier than PV anastomosis because the bile duct comes to lie behind the PV and the hepatic artery from the reversed hilar structures of the graft. Sixth, the PV anastomosis is performed between the right-sided graft and the recipient's right PV, and is reperfused after removal of the vascular clamps on the recipient's RHV and right PV. Seventh, hepatic artery anastomoses are performed and finally a Roux-en-Y hepaticojejunostomy to the left-sided (orthotopic) liver is performed. As additional procedures, a tissue expander filled with saline solution (from 200 to 450 mL) should be inserted underneath the right-sided (heterotopic) graft to relieve undue tension on the hilar anastomoses because the left liver graft is always too small to replace the right upper quadrant space after total hepatectomy in the recipient (Figure 57.11).

Engraftment procedures using both right and left liver grafts are a type of combination of two single-graft LDLTs using right and left liver grafts, respectively, because both grafts are positioning orthotopically. First, the right liver graft is placed into the right upper quadrant space and reconstruction of the hepatic veins is performed. The interposition graft of the MHV tributaries is anastomosed to the anterior wall of the IVC below the recipient's MHV. Second, the left liver graft is placed orthotopically and its hepatic vein and PV are reconstructed sequentially. Third, anastomosis between the recipient's right PV and the graft PV is performed, after

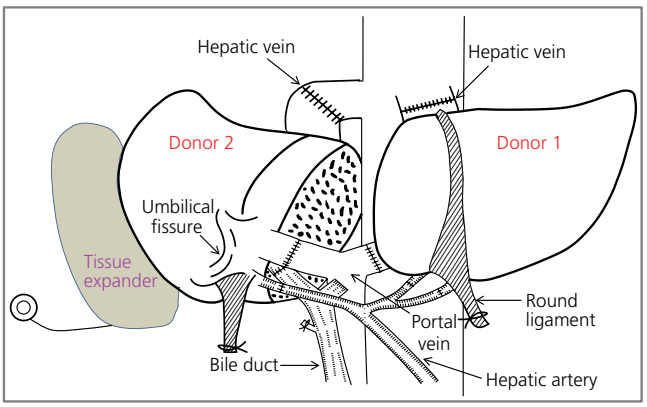

Figure 57.11. Living donor liver transplant using dual, left lobe grafts.

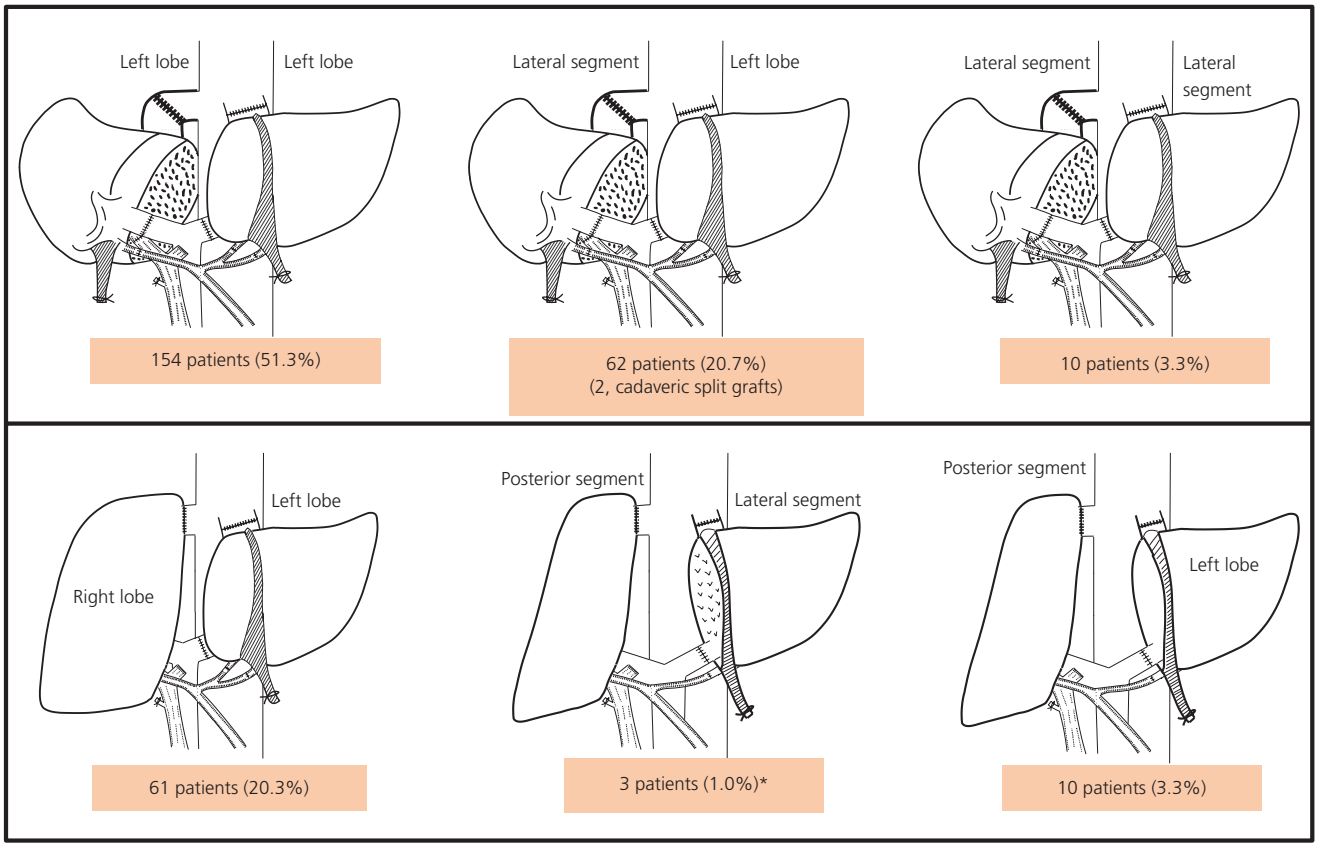

Figure 57.12. Various strategies employed for dual-graft living donor liver transplantations. Taken from an experience of 300 cases of dual graft transplantation (from a total adult living donor liver transplantation experience of 2227) performed from March 2000 to February 2011 at the Asan Medical Center.

which both liver grafts are reperfused at the same time. Fourth, after completion of the hepatic artery anastomoses, biliary reconstruction to both grafts is performed using Roux-en-Y hepaticojejunostomy only or a combination of the recipient's bile duct and Roux-en-Y hepaticojejunostomy.

If the available single largest graft cannot meet the recipient's metabolic demand, a dual-graft LDLT using left or right liver grafts from two living donors can expand the application of adult-to-adult LDLT by satisfying the GRWR of the recipient. The mean GRWR with dual left lobe LDLT (median 0.95%; range 0.59 to 1.25%) approaches that of right lobe transplant (median 0.98%; range 0.64 to 1.29%).

Most dual-graft LDLTs, particularly those using two left liver grafts, have been performed to secure the donor's safety rather than the recipient outcomes. The prevalence and the severity of donor complications were lower than those of single, right lobe graft LDLTs [92]. The most common complications in dual-graft recipients were biliary strictures (28%) and hepatic venous outflow obstruction of the heterotopic, right-sided left liver graft (15%). Hepatic vein obstruction did not occur in orthotopically positioned, both left-sided left liver and right-sided right liver grafts. This might be related to the progressive compression of the hepatic vein anastomosis by the regeneration of a heterotopically positioned left liver graft. In contrast to auxiliary, partial, orthotopic liver transplants (APOLTs), the two liver grafts in dual-graft LDLT do not enlarge competitively but do so cooperatively during the regeneration period [128].

At our medical center, of the 2227 adult LDLT recipients, 300 (13.5%) underwent dual-graft transplants from March 2000 to February 2011 (Figure 57.12). Urgent, dual-graft transplants were performed for fulminant hepatic failure in 18 patients (6.0%) and for acute-on-chronic liver failure in 44 patients (14.7%). The incidence of biopsy-proven acute rejection is similar to that in single-graft, living donor recipients (17%), three-quarters of whom developed acute rejection simultaneously in both of the grafts. The inhospital mortality rate was 7.0% (21 patients), and there was a status I and 2A patient comprise of 52% (11 patients). The overall survival rate and the incidence and severity of long-term complications between dual-graft and single-graft recipients were similar. Infrequently, unilateral graft atrophy developed in recipients of two left lobes, but this did not affect their liver function or survival.

LDLT with dual left liver grafts is technically complex and elaborate, but this procedure can help solve problems related to small-for-size grafts and can help to expand the indications for LDLT and for split-liver transplants in adult recipients.

Right posterior sector graft

A right posterior sector graft (RPS) can be a good alternative to a full right lobe graft when it satisfies the minimum volume requirement for the recipient, and which has been set at 40% of the recipient's standard liver volume [129,130] and also being larger in volume than the left lobe [131,132]. Technically, procurement of the RPS graft is the most demanding because it requires the longest time of parenchymal transection and also hilar dissection [130].

Sugawara et al. indicates that there are no exclusion criteria in RPS graft procurement, from an anatomic point of view [133]. However, considering the 45–50% biliary complication rates, including leakage and stenosis, more refined donor selection criteria for procurement of an RPS graft might be necessary in order to reduce complications in the recipients. The procurement of an RPS graft at our medical center is selectively performed so as to reduce surgery-related complications after consideration of anatomical variations including the PV, hepatic artery, and bile duct. When the left lobe volume is disproportionately small (<30% of whole liver volume) and there is type II or III PV, successful RPS graft procurement is likely [132].

In the recipient surgery, meticulous hilar dissection as high as possible should be performed in order to obtain a size-matched hepatic artery with a small caliber (1–2 mm in diameter) and/or bile duct openings for duct-to-duct anastomosis. For successful engraftment, the preparation and implantation procedures are basically the same as those for right lobe implantation. When the PV of the RPS graft has a short stump and/or a weak wall, making a fence to the graft PV using a bisected GSV can be useful in order to perform safe and wide anastomosis. When the hepatic artery of the graft has too short a stump to rotate under clamping of a metallic double micro clamp, posterior wall repair should be performed first with interrupted suturing.

Summary

Living donor liver transplantation is now applied worldwide, although it remains a technically challenging procedure to be performed only by the most experienced of teams. Donor selection is critical to insure the safety of the donor, particularly to ensure that they are left with adequate (at least 30%) hepatic volume, and careful assessments of the size of the donated hepatic lobe relative to the needs of the recipient must be undertaken preoperatively. If the volume of the right lobe in potential donors exceeds 70% of the volume of the whole liver or if a large-size recipient requires more graft volume than the expected liver graft volume from a single donor, dual-graft LDLT may be an alternative. The surgical technique for recipients is based on whole liver resection with preservation of the inferior vena cava. For technically successful LDLT, the following four conditions must be satisfied: (1) transplant a large-volume graft to avoid small-for-size, (2) good outflow to avoid congestion, (3) adequate portal inflow to enhance graft regeneration, and (4) secure bile duct anastomosis to avoid biliary leak.

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CHAPTER 58

Heart Transplantation Procedure and Surgical Technique

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Introduction

The fundamental surgical procedures used in modern cardiac transplantation are all based on the technique of the vascular anastomosis developed by Alexis Carrel in the 1890s. At the turn of the century, Carrel first applied his anastomotic technique to organ transplantation, including cervical heterotopic heart transplants in dogs [1]. After a period of relative inactivity in the field, Vladimir Demikhov in Moscow began experimenting with intrathoracic heterotopic heart transplant and heart–lung transplantation in dogs. By the late 1940s, he had performed successful orthotopic heart transplantation in dogs, although most of his work was not published in English until the early 1960s. By that time, Lower and Shumway at Stanford had refined the techniques of orthotopic heart transplantation in dogs and reported significantly longer survival than that obtained by previous groups [2]. Utilizing the orthotopic techniques developed by the Stanford group, Christiaan Barnard performed the first successful human heart transplant on December 3, 1967 in Cape Town, South Africa [3]. Since then, the most common method of transplantation has been implanting the donor heart into the orthotopic position; however, other procedures, including heterotopic and heart–lung transplantation, have proven useful in certain situations [4]. While the most significant overall advance in heart transplantation since 1967 has been the advent of effect immunosuppression, the most notable evolution in the technique of heart transplantation has been the switch from biatrial to bicaval anastomoses of the vena cavae.

By way of a disclaimer, there are many different ways to preserve, procure, and implant human heart allografts. The techniques described below are those employed at the Massachusetts General Hospital. They have contributed to the excellent short- and long-term outcomes observed in our recipients. However, techniques can and do vary greatly between institutions with similar excellent outcomes.

Donor operation

Excellent medical care of the donor in the ICU and meticulous surgical technique during procurement are arguably the most important parts of the overall transplant procedure for the following reasons. First, to diminish the risk of early graft dysfunction, the heart must be taken in a high energy state. This can only be assured by the procuring cardiac surgeon carefully managing/optimizing the donor's blood pressure, cardiac output, volume status, metabolic parameters, and hematocrit. Second, because this period is when the donor is accepted or rejected, all patient data that bear on donor suitability must be assiduously obtained, evaluated, and incorporated into the decision-making process. Third, the procuring teams, especially the heart and lung teams, must coordinate their respective intraoperative plans so as to avoid putting any organ (and ultimately, any recipient) at risk. This requires collegial communication before surgery begins. Finally, the graft must be adequately preserved, safely explanted, and expeditiously transported to the transplanting facility to assure an optimal outcome. More general discussions on the conduct of the donor procedure are available in Chapter 21.

Preparation

The initial steps of donor–recipient matching are discussed in Chapter 39. Before leaving for the donor hospital, the heart team must ensure that all necessary items are packed. We typically carry a cooler with 2 liters of crystalloid cardioplegia (Plegisol) for use as preservation fluid. The specifics of cardiac preservation are discussed in Chapter 26. The cardioplegia is surrounded by bags of frozen saline and transported in a cooler. We also bring along items that are not universally available, such as an aortic root cannula, Prolene sutures, tourniquets, and tubing with necessary adaptors for the cardioplegia. Our local Organ Procurement Organization (OPO) provides additional cardioplegia and a sternal retractor. Upon arrival at the donor hospital, pertinent medical records, echocardiograms, chest radiographs, and angiograms are reviewed. Patient identification and ABO compatibility are verified. The medical condition of the donor is reassessed because rapid changes in physiology are not uncommon. It is important to verify proper monitoring capabilities. At a bare minimum, the donor should have a large-bore peripheral intravenous line as well as arterial and central venous monitoring lines. Goals of management in the donor operating room are maintaining a systolic arterial pressure over 100 mmHg, a central venous pressure of 8–12 mmHg, a PaO_2 of >95 mmHg and a hematocrit of ≥ 30. To minimize ventricular stimulation and energy expenditure, we avoid β agonists and

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instead use phenylephrine as the vasopressor of choice. However, volume is usually adequate to treat transient hypotension.

The various surgical teams should become acquainted with each other and should clarify procedural steps, especially those involving competing anatomical interests. In the case of concomitant left lung retrieval, the heart and lung surgeons should work together to plan how and where to divide the pulmonary veins so as to leave adequate tissue for both implanting teams. The same holds for dividing the main pulmonary artery and to a lesser extent, discussing division of the inferior vena cava with the liver surgeon. A few minutes of collegial conversation before the operation can prevent mistakes, save time, and avoid heated arguments during the operation.

Surgical technique

After the requisite "time out," the donor is draped from neck to groins, and the multiorgan retrieval operation is begun with a generous midline incision. Strict adherence to hemostasis is important as even slow oozing from the exposed sternal surfaces can accumulate with time. To this end, the sternal marrow is completely sealed with bone wax. To avoid compromising the abdominal exposure, the sternal retractor is oriented with the crossbar toward the head. The pericardium is opened and suspended with silk stay sutures. The inferior right pericardium is divided down to the level of the inferior vena cava to improve visualization and provide for the egress of blood into the right thorax when the inferior vena cava is later vented. The heart is inspected for ventricular dysfunction, distension, coronary atherosclerosis (both by visual inspection and manual palpation), anatomic variations, evidence of trauma, and proper size match for the intended recipient. Palpation of an aortic thrill may indicate valvular pathology. The final decision on donor suitability is made and communicated back to the recipient team along with the anticipated clamp time.

Although dissection can proceed in a variety of ways, we typically proceed in clockwise fashion starting with the superior vena cava and left innominate vein, which are fully mobilized. The azygos vein is identified posteriorly and ligated distally. The preserved azygos vein takeoff can be used to enlarge the donor super vena caval orifice if necessary. If the lungs are being procured, the plane between the superior vena cava and aorta is developed and the relatively avascular pretracheal tissue is divided longitudinally between the innominate vein and pulmonary artery (see Chapters 21 and 59). The aortopulmonary window is then opened to prevent pulmonary artery injury during subsequent aortic clamping. Dissection around the aortic root is avoided to prevent injury to the coronary arteries. The inferior vena cava is circumferentially dissected.

Cannulation of the ascending aorta is delayed until all teams are nearly ready to proceed with aortic clamping. At that time, the patient is systemically heparinized (300 units/kg). The preservation solution is then removed from the cooler and hung in a pressure bag at the head of the table. For the initial flush, we use cold Plegisol. It requires the addition of 10 mL of 8.4% sodium bicarbonate solution before use. A cardioplegia catheter is placed in the ascending aorta and is secured with 4-0 Prolene as it will be retained for use during implantation. If lungs are to be procured, an additional cannula is placed through a purse-string suture in the distal main pulmonary artery at a point mutually acceptable to both heart and lung teams. All neck lines are withdrawn to above the innominate vein by the anesthesiologist. Once the preparatory steps are completed by all teams, heart procurement proceeds rapidly. The priorities for the cardiac team are adequate decompression of the right and left ventricles and optimal administration of preservation solution. The following steps are performed in a rapid but controlled sequence:

1. If the lungs are to be procured, 500 μg prostaglandin E_1 is injected into either the pulmonary artery adjacent to the cannula or into the right atrial appendage.
2. The superior vena cava is ligated at its confluence with the innominate vein.
3. If lungs are to be procured, the left atrial appendage is divided or the inferior wall of the left atrium incised to vent the left heart. If lungs are not being harvested, the left inferior pulmonary vein is divided at its pericardial reflection.
4. The intrapericardial inferior vena cava is partially transected and blood vented into the right chest.
5. Once adequate decompression is verified, the aortic cross clamp is applied as far distally as possible (preferably at the innominate artery origin).
6. One liter of cardioplegic preservation solution is infused with 100–150 mmHg pressure applied to a pressure bag surrounding the cardioplegia container.
7. Simultaneously, the pericardium is filled with cold saline slush.
8. The heart is observed for adequate aortic root pressure, prompt arrest, blanching, and the absence of ventricular distension.

After the cardioplegia infusion is completed, donor cardiectomy is performed. The posterior wall of the inferior vena cava is divided freeing the right atrium inferiorly. The superior vena cava is divided at its confluence with the innominate vein. If the lungs are not being procured, the pulmonary veins are simply transected at their pericardial reflections. If the lungs are being procured, the heart is retracted to the left, and the interatrial groove is incised and extended superiorly onto the left atrial dome and inferiorly behind the inferior vena cava. The apex is then retracted cephalad bringing both inferior pulmonary veins into view. The incision is then continued from right to left parallel to the atrioventricular groove and then anterior to the left pulmonary veins. Lastly the superior left atrial wall is divided as the heart is retracted even more cephalad and anteriorly. It is imperative that the pulmonary vein orifices be frequently reassessed during the left atrial division with supervision by both the lung and heart teams. In particular, on the left side of the left atrium, there is often a limited distance between the left pulmonary veins and the donor left atrioventricular groove. Sufficient left atrial cuff must remain to permit implantation without compromise or kinking of the circumflex coronary artery (see Chapter 59). The aortic arch branches and proximal descending aorta are divided. Finally, the main pulmonary artery is transected at its bifurcation. The donor heart is then taken to the back table for additional preservation and preparation for transport (see below).

Preparation and transportation of the allograft

Preparation at donor institution

On the back table, we infuse 500 mL of University of Wisconsin (UW) solution into the clamped aortic root and use the remaining solution as transport medium. The graft is inspected for anatomical abnormalities, including a patent foramen ovale, and/or surgical damage. The cardioplegia cannula is left in place for later use. The heart is placed into a sterile plastic bag with UW preservation solution, deaired, and tied with an umbilical tape. Ice is not placed directly in contact with the graft. This bag is placed into another

bag of saline ice, which is then placed in a plastic container that is wrapped with the final bag and put into a cooler for transport. The recipient team is notified of the cross clamp time, condition of the organ, and estimated time of arrival at the recipient hospital. See Chapter 26 for additional details on cardiac preservation.

Preparation at recipient institution

At the recipient institution, the heart is sterilely removed from the packaging and placed on the back table for final preparation. The procuring surgeon performs this as the recipient cardiectomy is being completed. ABO matching is visually confirmed. The great vessels are separated. The left atrial cuff is prepared by connecting all four pulmonary vein orifices and incising the left atrial free wall longitudinally to create a single large orifice. If a patent foramen ovale is present or the left atrial appendage was opened to vent the left heart, they are closed securely. If a biatrial anastomosis is planned, the superior vena cava is doubly ligated 1–2 cm above the sinoatrial node, and the right atrium is opened from the lateral inferior vena cava toward the base of the right atrial appendage away from the sinoatrial node. For a bicaval anastomosis, the superior vena cava cuff is trimmed at the level of the azygos vein orifice. This can later be used to enlarge the anastomosis as mentioned above. The donor aorta and pulmonary artery are not trimmed in length until later. At this point, the donor heart is brought to the operating table and perfused with cold, oxygenated, blood cardioplegia.

In an effort to expand the donor pool, some authors have reported repair techniques for a number of donor heart defects. However, the functional advantage of extensive repairs must be weighed against the expense of ischemic time. Mitral valve repair has been reported [5–7] but should be undertaken only if there is a high degree of confidence that such repair is necessary and that a durable functional result can be obtained. Aortic valve repair has been reported as well but should be performed by surgeons with experience with these repair techniques [8]. Tricuspid valve incompetence should probably be repaired by ring annuloplasty because it has been shown that recipients with even moderate tricuspid regurgitation after implantation have decreased survival [9]. Whether all donor hearts should undergo tricuspid valve annuloplasty is controversial (see below) [10]. Closure of ventricular septal defects has been reported [11] but right ventriculotomy should be avoided given the propensity for compromised right ventricular function following transplantation.

Recipient operation

Timing

Coordination of the transplant process begins before the procuring team leaves to travel to the donor site. The recipient should be brought to the operating room 2 hours in advance of the estimated start of the recipient operation and lightly sedated for insertion of monitoring lines. The procuring team communicates with the recipient team prior to aortic cross clamping to synchronize the timing of donor heart explantation and recipient cardiectomy so that the recipient team is ready in advance of the donor team arrival. This avoids increasing the ischemic time while waiting for the completion of cardiectomy. With current techniques, ischemic times should not exceed 6 hours (in adults) and should be kept to 4 hours or less if possible, particularly in high-risk transplants (i.e. suboptimal donors or recipients with increased pulmonary vascular resistance).

Preoperative preparation of the recipient

Recipients are often on warfarin or are coagulopathic from a combination of medications and hepatic congestion from long-standing heart failure. In this case, we administer vitamin K (10 mg) intramuscularly or by very slow intravenous infusion over 30–60 minutes. Given their extensive cardiac history, recipients may have had previous documentation of heparin antibodies. It is important to review previous hematology input regarding the use of heparin for cardiopulmonary bypass. It has been shown in patients with ventricular assist device (VADs) that if heparin is avoided during the bridge to transplant and preoperative antiheparin/platelet factor-4 antibody (HPF4) titers are low or absent, re-exposure during the transplant procedure is not associated with an increase in perioperative complications [12]. However, if HPF4 titers are elevated, options include use of a direct thrombin inhibitor such as bivalirudin or use of heparin with intraoperative plasmapheresis or administration of a glycoprotein II_BIII_A inhibitor [13–15]. When patients are on argatroban, we typically stop the infusion 4–6 hours before surgery. We give our patients mycophenylate mofetil (MMF) 1000–1500 mg orally on call to the operating room and high-dose intravenous corticosteroids upon induction of anesthesia [16]. Likewise, broad-spectrum prophylactic antibiotics and antifibrinolytics are administered just prior to induction of anesthesia. We routinely place a pulmonary artery catheter, arterial line, large-bore venous line, and transesophageal echocardiography probe.

Recipient operation

Preparation

Permanent pacemakers and/or implantable cardioverter-defibrillators need to be prepped in the field. After sternotomy, the patient is heparinized for bypass (initial dose of 300 IU/kg). Aortic cannulation is performed as distally as possible on the ascending aorta to provide as much length as possible for implantation. Bicaval venous cannulation is performed with right angled metal cannulas, again as far peripherally as possible. It is helpful to place the purse-string sutures such that the tourniquet is on the side of the cannula opposite the atrial suture line in order to preserve as much length as possible and minimize subsequent distortion of the anastomosis. When the procuring team arrives, cardiopulmonary bypass is initiated. Once on bypass, we systemically cool to 28°C to prevent early rewarming of the donor heart as it lies within the pericardial cavity during implantation. All central venous lines are withdrawn into the superior vena cava. Once satisfactory initiation of cardiopulmonary bypass is confirmed, the aortic cross clamp is applied, caval tourniquets are tightened, and the recipient cardiectomy is performed.

Cardiectomy

The aorta is transected at its sinotubular junction. The recipient cardiectomy then progresses in an approximately clockwise progression. First, a right atrial incision is made parallel to the atrioventricular groove through the appendage and into the dome of the left atrium. The right atrial incision is continued inferiorly toward the medial aspect of the inferior vena cava. The superior vena cava is divided just above the cavoatrial junction. Recipients often have leads traversing the superior vena cava, which are pulled toward the heart and cut as short as possible with heavy scissors. The aorta and pulmonary artery are then divided just distal to their respective semilunar valves after the aortopulmonary window is developed (if not done previously). The initial incision into the dome of the left atrium is continued in two directions. First, the interatrial septal

component is continued through the fossa ovalis and inferiorly toward the atrioventricular groove, joining the previous right atrial incision just medial to the inferior vena cava. It is then continued parallel to the atrioventricular groove just cephalad to the posterior mitral leaflet. The other component of the incision is continued over the left atrial dome, between the base of the left atrial appendage and left pulmonary veins to join the previous incision along the atrioventricular groove, thus completing the cardiectomy. As in the donor cardiectomy, great care is taken to preserve an ample cuff of left atrium near the pulmonary vein orifices. This is the ideal time to obtain hemostasis of the posterior pericardial contents, including cauterizing the free edges of the left atrium. Subsequent bleeding in this area will be difficult to correct once off bypass. Some surgeons place a vent through a right superior pulmonary vein purse-string suture to evacuate all warm pulmonary venous return.

Implantation

There is considerable variety in the sequence in which the allograft may be implanted, which allows the surgeon to tailor the strategy to the needs of each operation and to limit ischemic time as needed. Because we favor the bicaval technique, our default anastomotic sequence is as follows: (1) left atrium, (2) inferior vena cava, (3) pulmonary artery, (4) aorta (with subsequent removal of aortic cross clamp), and (5) superior vena cava. If there are concerns about an unusually prolonged ischemic time, the sequence is modified as follows: (1) left atrium, (2) aorta (with subsequent removal of aortic cross clamp), (3) pulmonary artery, (4) inferior vena cava, and (5) superior vena cava. The disadvantage of the latter sequence is that coronary venous return can partially obscure the pulmonary artery and inferior vena cava anastomoses. During the entire procedure an iced gauze covers the right ventricle and the operative field is flooded with carbon dioxide to limit the collection of intracardiac air. Note that an everting left atrial suture line is preferred to keep bare atrial muscle from being exposed inside the left atrium. In general, the pulmonary artery anastomosis is not tightened and not tied down until the end of the operation so that air can be expelled from the right heart. As with all open cardiac surgery, care must be used to ensure that the left heart has been well de-aired. Even a small air embolus down a right coronary artery during weaning from bypass can be a major issue, although usually temporary. If the cross clamp is removed early in the implant sequence, the heart should be continuously monitored to ensure that the left ventricle is not being distended.

Bicaval versus biatrial techniques

The right atrial anastomotic technique must be determined prior to implantation because this influences the cardiectomy procedure and back-table preparation. The most significant technical advance since the early era of heart transplantation is use of the bicaval method of systemic venous return. Before the turn of the century the traditional biatrial technique was used almost exclusively. A recent review of the United Network for Organ Sharing (UNOS) database showed that nearly two-thirds of transplants in 2007 utilized the bicaval technique [17]. That review of nearly 21 000 recipients over 10 years showed an increased incidence of permanent pacemaker placement in the biatrial group (odds ratio 2.6) with a significant survival disadvantage at 30 day and long-term follow-up (biatrial OR for mortality 1.17 and 1.11, respectively), which supported similar findings reported nearly 10 years previously [18]. The bicaval technique is also advantageous in that it preserves right atrial morphology as well as sinoatrial node and tricuspid valve function [19,20]. Tricuspid regurgitation is not well tolerated in the setting of right ventricular dysfunction, which is often seen after heart transplantation [21]. Additionally, the bicaval technique is associated with decreased frequency of permanent pacemaker implantation [18]. However, the caval suture lines are at risk of anastomotic stenosis, both early and late, which is not the case with the biatrial technique. The primary advantage of the biatrial technique is its relative technical simplicity, both in terms of the dissection necessary to perform the anastomoses and the anastomoses themselves. However, the biatrial anastomosis puts the sinoatrial node at risk of injury, and redundant atrial tissue may adversely impact atrial hemodynamics and contribute to an increased risk of atrial arrhythmias in the postoperative period [22–24]. The presence of two sinoatrial nodes with the biatrial technique can present difficulty in determining the pattern of sinoatrial node function and atrioventricular conduction during the postoperative period. A meta-analysis of 41 papers on this topic found significant benefits for the bicaval technique in terms of early atrial pressure, tricuspid valve regurgitation, return to sinus rhythm, and even perioperative mortality. However, long-term outcomes were less disparate between the groups [25].

Bicaval orthotopic technique

Left atrial anastomosis

Prior to implantation it is occasionally necessary to trim the donor and/or recipient left atria to prevent the creation of an excessively large cavity that may be at risk for thrombus formation [26] (Figure 58.1A). A long 3-0 polypropylene suture is started adjacent to the recipient left superior pulmonary vein and donor left atrial appendage (Figure 58.1B). The site of the recipient left atrial appendage and the donor left atrial appendage can be used to ensure the rotational orientation of the heart. The first several stitches are placed with the heart ex vivo; it is then placed within pericardium. The suture line is continued first clockwise, second counterclockwise, with forehand technique as much as possible. A running simple stitch can be used if there are deficient atrial cuffs but we favor a running vertical mattress technique, everting and excluding the thrombogenic muscle layer of atrial cuffs. In the area of the circumflex coronary artery, care is taken to avoid deep sutures. It is important to periodically reassess the position of the inferior vena cava to ensure that it is brought into proper position for subsequent bicaval anastomosis. The knot is tied near the middle on the right side of the interatrial septum.

An alternate to the traditional bicaval technique, the so-called bicaval total technique, involves two left atrial anastomoses, one to a right pulmonary vein cuff (including the superior and inferior pulmonary vein orifices) and the other to a left pulmonary vein cuff similar to those used for lung transplantation. In this case, the recipient cardiectomy would include removal of all atrial tissue except bilateral pulmonary vein cuffs. For implantation, the left pulmonary vein cuff is sewn first with the heart retracted to the right followed by the right cuff anastomosis with the heart retracted to the left [27].

Inferior vena caval anastomosis

A 4-0 polypropylene is run along the posterior onto the anterior wall (Figure 58.1C). To minimize the risk of stenosis, a portion of the recipient right atrium is retained near the inferior vena cava to allow for a patulous anastomosis. As in all our anastomoses, an interrupted stander suture is placed at the 3 and 9 o'clock positions. Deep sutures are avoided in the region of the donor coronary

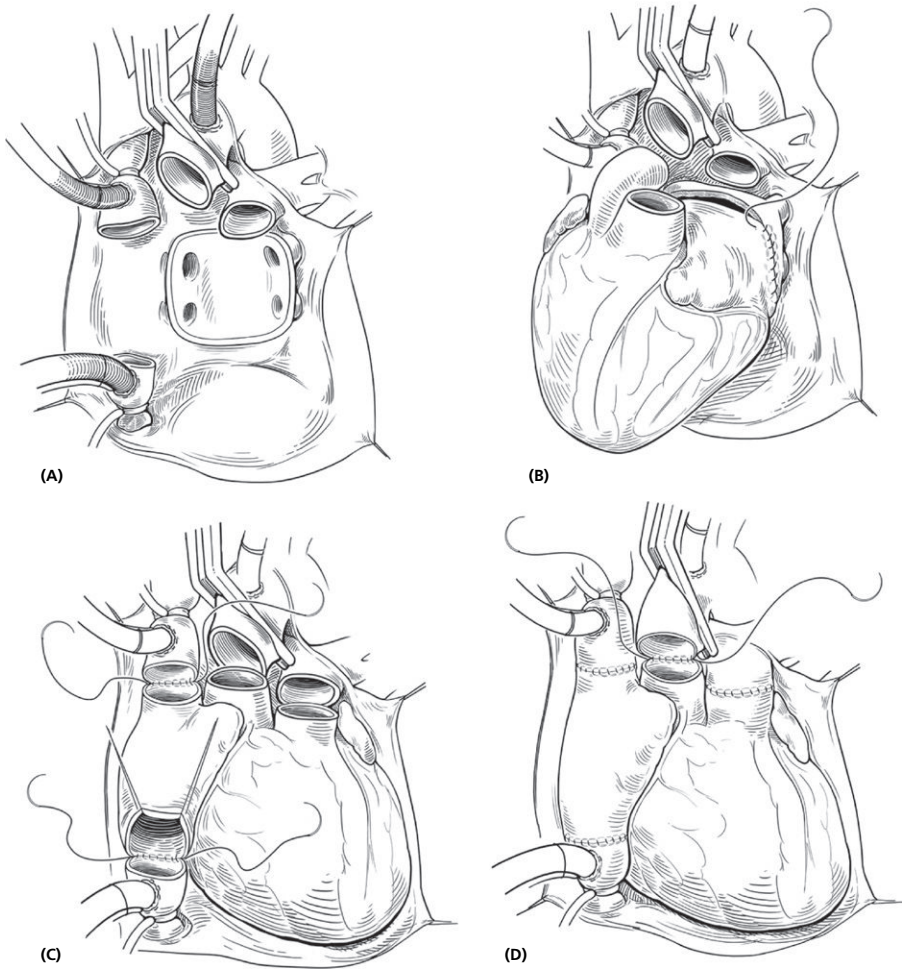

Figure 58.1. (A–D) Progressive steps detailing heart transplantation utilizing the bicaval anastomotic technique.

sinus. This anastomosis may be facilitated by femoral venous cannulation and use of vacuum assisted venous drainage with a clamped or open inferior vena cava [28]. On infrequent occasion, if the recipient has a persistent left superior vena cava draining into the coronary sinus, the recipient coronary sinus and associated tissue in the left atrioventricular groove can be preserved. When the interior vena cava anastomosis is done, the recipient side includes the interior vena cava orifice and the coronary sinus orifice. In this situation it is usually necessary to separately cannulate.

Pulmonary arterial anastomosis

Excess pulmonary artery must be trimmed aggressively because this anastomosis is particularly prone to kinking or telescoping, especially with a large donor heart. For the same reason, care must be taken to assure that the geometry of the donor and recipient pulmonary arteries are well aligned. The anastomosis is sewn with a continuous 4-0 polypropylene that is tied to interrupted stander sutures at 3 and 9 o'clock. It is advantageous to trim the donor pulmonary artery short rather than the recipient vessel as it brings the anastomosis closer and improves access if repair sutures are required. Note that excessive length of the resulting new pulmonary artery can produce invagination and a supravalvular gradient.

Aortic anastomosis

As with the pulmonary artery, the aortae are trimmed to avoid extra length with the possibility of subsequent kinking. This anastomosis is typically performed the same way as the pulmonary artery anastomosis (Figure 58.1D). Rewarming of the patient is commenced. After the suture line is completed, the heart is deaired and the aortic cross clamp is removed. Spontaneous contractions will usually commence soon after clamp removal. Continuous aortic root de-airing is performed by placing the root vent on suction.

Superior vena caval anastomosis

This anastomosis is performed with attention to fine suture technique to avoid purse-stringing. We use a running suture on the posterior wall and a simple 5-0 Prolene interrupted sutures on the anterior wall (Figure 58.1C). It is imperative to leave the recipient superior vena cava as long as possible during cardiectomy so that if the donor superior vena cava is small, the recipient superior vena cava can be sewn directly to the cavoatrial junction. If this anastomosis is performed after the removal of the cross clamp, the donor superior vena cava can be clamped to prevent blood from obscuring the field. The completed transplant is depicted in Figure 58.2, which details the anterior and posterior view of a heart transplanted using the bicaval anastomotic technique.

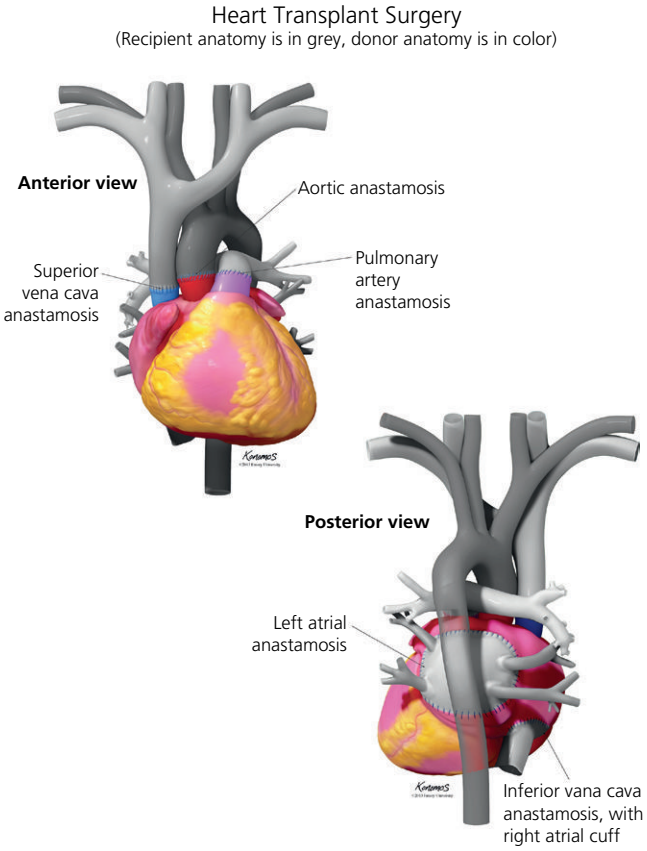

Figure 58.2. Completed anastomoses, anterior and posterior view, following performance of the bicaval anastomotic technique.

Biatrial orthotopic technique

The implantation begins with the left atrial anastomosis as in the bicaval method (Figure 58.3A,B). The right atrial anastomosis is usually performed after the left atrial anastomosis is completed; however, to decrease ischemic time, the aortic anastomosis may be performed before the right atrium. A 4-0 polypropylene suture is started near the superior end of the interatrial septum and is run clockwise around the inferior vena cava, which is the most difficult area of the anastomosis given the relatively small remnant of recipient atrium near the inferior vena cava (Figure 58.3C). Next, the superior end is sewn counterclockwise and the sutures are tied along the free wall of the right atrium. It is imperative to use caution when placing sutures near the donor sinoatrial node. The pulmonary arterial and aortic anastomoses are performed as described above (Figure 58.3D). No matter which technique is used, 1 liter of cold, oxygenated, blood cardioplegia is given antegrade after the completion of each anastomosis.

Separation from cardiopulmonary bypass

Once all anastomoses are completed, the caval tourniquets are released and high-level suction is applied to the aortic root vent. With flow through the heart, more vigorous de-airing maneuvers are performed. Gentle ventilation and massage of the heart, as well as turning the table right and left, may be used to expel air from the ventricles and great vessels. Ventricular fibrillation is promptly defibrillated during this and subsequent portions of the procedure to avoid distention of the left ventricle. The pulmonary artery catheter is then readvanced into the pulmonary artery with the surgeon's help. In cases of a prolonged ischemic time, the graft is allowed a period of reperfusion before separation from bypass. Inotropic support, usually Milrinone (0.5 μg/kg/min), is begun at this time. A heart rate above 100 beats per minute is preferred because the allograft recovering from cold ischemia is more dependant on heart rate than stroke volume to maintain cardiac output [29]. This reperfusion period is an ideal time to inspect all suture lines and place repair sutures as needed. Before separating from bypass, transesophageal echocardiography is used to evaluate for retained intracardiac air, valvular and ventricular function, gradients across the caval and pulmonary arterial anastomoses, and patency of the pulmonary veins.

Right heart dysfunction of varying degrees is a common problem following heart transplantation. Therefore, pulmonary artery pressures and right ventricular function need to be carefully and frequently assessed. The principles used to manage right heart dysfunction include: (1) minimizing right ventricular afterload, (2) optimizing RV performance, including both the free wall and the interventricular septal contributions, and (3) maximizing the systemic pressure available to perfuse the right ventricular free wall. Early right heart dysfunction is best treated by simply raising the systemic perfusion pressure. However, the threshold to institute pulmonary vasodilators should be low. The initial agents of choice include inhaled epoprostenol or nitric oxide, given their selective action. The most reliable agent to start with is inhaled nitric oxide. This should be started at an inhaled dose of 10–20 ppm. Mild hyperventilation to a P_{CO_2} in the range of 30–35 mmHg and maintenance of oxygen saturation >95% are useful adjuncts to minimize pulmonary vasoconstriction. Central venous administration of vasodilators such as prostaglandin E_1, prostacyclin, or nitroglycerin, or inotropes such as milrinone or dobutamine may be instituted as an alternative [30]. If a stenosis is suspected at the pulmonary artery suture line based on a palpable thrill or echocardiographic findings, the pressure gradient should be documented directly. A gradient of ≥10 mmHg is an indication for revision [31]. Ultimately, if right heart failure persists despite all attempts to reverse it, a mechanical assist device is indicated [32].

Particular attention is paid to the tricuspid valve after separation from cardiopulmonary bypass. Tricuspid regurgitation is encountered more frequently after biatrial technique, likely due to geometric distortion of the right atrium [33]. The severity of tricuspid regurgitation as determined intraoperatively correlates with poor late survival, even with mild regurgitation. Although tricuspid regurgitation is related to right ventricular dysfunction, these findings suggest a possible role for concomitant tricuspid valve repair at the time of transplantation [9]. A series prospectively evaluated 60 bicavally transplanted grafts randomly assigned to receive (n = 30) or not receive (n = 30) prophylactic de Vega tricuspid valve annuloplasty prior to implantation. The authors reported a short- and midterm survival benefit of tricuspid annuloplasty with significant reduction in the severity and number of patients with significant tricuspid regurgitation at 1 year. Because of these benefits and the fact it is inexpensive and quick to perform, they routinely perform this procedure during preparation of the donor heart for transplantation [34].

Once adequate rewarming has taken place, cardiopulmonary bypass is terminated and satisfactory hemodynamic and echocardiographic findings are achieved, aortic root suction (air venting) is terminated, and protamine is administered. The aortic root vent is then removed. The caval canulas are removed and the purse-string sutures snared. If there is no evidence of protamine reaction

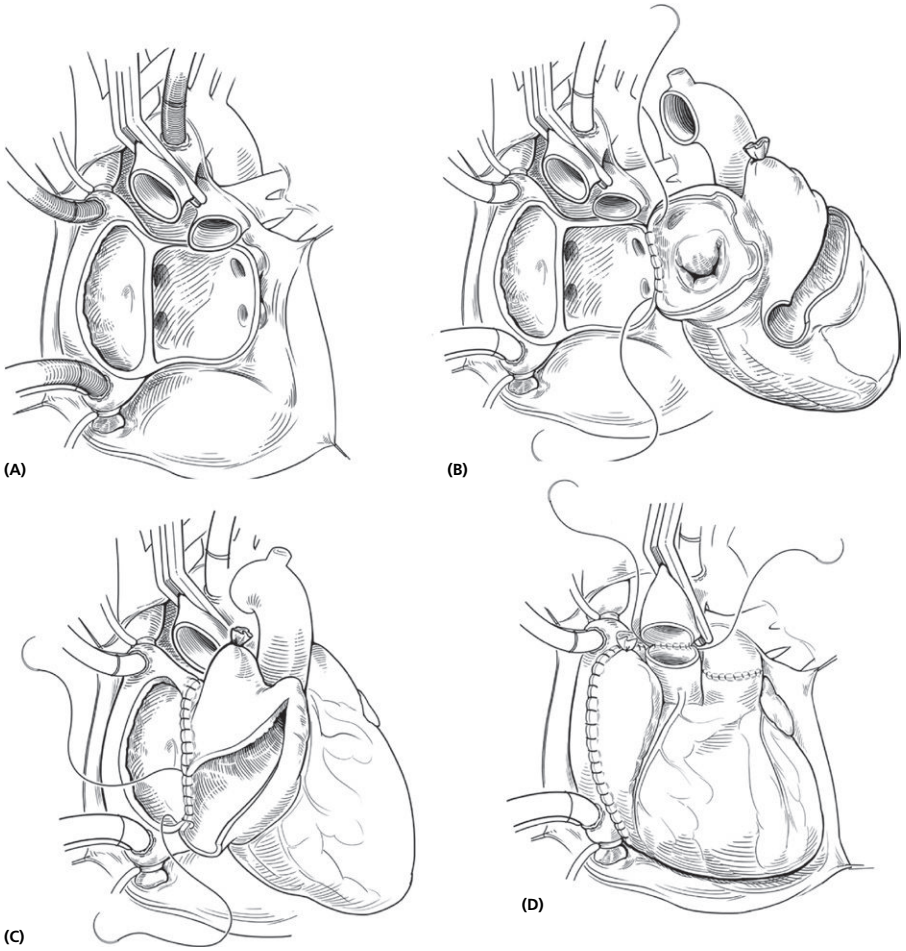

Figure 58.3. (A–D) Progressive steps detailing heart transplantation utilizing the biatrial anastomotic technique.

after 50% of the neutralizing dose is administered and all volume is returned from the pump, the aortic cannula is removed.

Drains are placed in every thoracic cavity that is opened during the procedure. There is usually a large pericardial space relative to the allograft. In addition to the usual drains, a Blake drain or similar drain is often placed in the posterior pericardium and/or VAD pocket for long-term use. Temporary atrial and ventricular epicardial pacing leads are placed. Some form of atrioventricular block occurs in approximately 10% of procedures; however, only 2% have complete heart block [35]. Use of the bicaval technique is associated with lower rates of temporary pacing postoperatively [36] and permanent pacemaker implantation [17,18,25]. For patients with a previous automatic implantable cardioverter defibrillators/permanent pacemakers, these are usually removed once coagulopathy has been corrected. Because this step is not essential at this time, it may be performed at a later date if there are concerns regarding coagulopathy or stability. The sternum and soft tissue and skin are closed in the usual manner after obtaining adequate hemostasis and hemodynamics.

Special situations

Redo sternotomy/redo heart transplant

Patients with biventricular failure and a distended right ventricle are especially at risk during redo median sternotomy. In this instance, it is prudent to place external defibrillator pads and access the central circulation for cardiopulmonary bypass through the femoral artery and vein before beginning the procedure. Once sternotomy is accomplished, care is taken to avoid injury to the right ventricle and previous bypass grafts if present. Manipulation of bypass grafts is avoided to prevent embolization and hemodynamic compromise. The priority is to expose a site for aortic cannulation, then dissect out the superior and inferior cavae for bicaval cannulation. Occasionally cardiopulmonary bypass may be required in order to decompress the heart, limit blood loss, and facilitate gaining exposure.

Given the median survival of cardiac allografts, it is anticipated that many recipients will eventually undergo retransplantation [37], especially when the primary transplant was performed at an early age as is common for congenital heart disease [38]. Although technically very similar to orthotopic heart transplantation as outlined previously, there are a few considerations unique to retransplantation. First, patients typically undergo this operation after an extended period of chronic allograft rejection. Unlike most redo cardiac surgeries involving a single inflammatory episode, redo cardiac transplantation for chronic rejection is complicated by longstanding inflammation and notoriously dense scarring and bleeding during dissection of the allograft. Dissection and exposure needed for recipient cardiectomy may require additional time, which may affect coordination with the donor operation. Every

effort is made to remove all of the previous allograft including all anastomoses, trimming back to native tissue. Outcomes for retransplantation for graft coronary disease are similar to those for primary transplantation; however, those for acute graft failure are quite poor [39].

Explanting ventricular assist device

With the number of cardiac donors being relatively constant, an increasing number of patients are bridged to transplantation with a ventricular assist device with good post-transplant survival [40–43]. This is discussed in detail in Chapter 48. Strategies to employ during assist device placement that facilitate future transplant include:

1. directing the outflow graft toward the right atrial gutter to prevent injury during sternotomy;
2. covering the outflow graft with an extra layer (usually GorTex because there is often insufficient pericardium to use for this purpose;
3. constructing the outflow anastomosis as proximal as possible on the right lateral ascending aorta thereby preserving as much aorta as possible;
4. leaving the aortopulmonary window as undisturbed as possible to facilitate dissection and exposure later;
5. placing a protective barrier over the right ventricle (i.e. GorTex, CorMatrix, etc.) to prevent injury at the time of resternotomy;
6. avoiding use of pledgets, thus avoiding associated scarring and fibrosis.

Based on these strategies, there are several considerations unique to the recipient with a ventricular assist device in place. The location of outflow graft must still be documented before resternotomy as it can shift position. Manipulation of the left ventricle needs to be minimized to decrease the risk of embolization of mural thrombus, which is more likely to form in this population. This can be assessed by transesophageal echocardiography. The device needs to be turned off and the outflow graft clamped immediately prior to institution of cardiopulmonary bypass to prevent massive functional aortic insufficiency across the device. Because the outflow graft is sewn to the ascending aorta, cannulation and clamp placement must be more distally than usual. Cannulation of the aortic arch using the Seldinger technique is useful in these cases. Alternatively, femoral or axillary arterial cannulation may be used.

Heart transplantation after congenital heart surgery

Heart transplantation after congenital heart surgery is a recognized treatment for long-term failure of corrective or palliative procedures. While patients with congenital heart disease have historically contributed relatively few patients to the adult heart transplant population, this is certain to change in the near future with the improved survival and scope of anatomy that can now be treated. There are a number of considerations that pertain to this population. First, pulmonary vascular disease is common and may necessitate heart–lung transplantation (see below). Second, patients with failing Fontan circulation are quite ill, often presenting with multiorgan insufficiency (especially renal and hepatic) and nutritional deficiency from protein-losing enteropathy. Third, finding a suitable donor may prove very difficult and, in some cases, impossible as the patients can be highly sensitized. Fourth, postoperative care can be difficult because of their predisposition to bleeding, infection, and pulmonary hypertension and the presence of aortopulmonary collaterals resulting in a significant left-to-right shunt [44].

The technical aspects of these transplants can be quite complex given the unique reconstructive needs, especially after single ventricle palliation [45–48]. As a result, the great vessels are procured in an extended fashion and are not trimmed until implantation. In some cases prosthetic material is required to complete the reconstruction. Alternatively, native tissue that might otherwise be discarded (such as the descending thoracic aorta or portions of the great vessels) may be harvested for use as a patch or other conduit. The transplanting and procuring surgeons must be familiar with the situs, vascular anatomy, and previous operations of the recipient.

Redo sternotomy can be particularly treacherous in the setting of dense adhesions, cardiomegaly, and the substernal position of the great vessels or conduits. Pre-emptive groin access or even cannulation should be considered in this population [49]. Chronic cyanosis associated with this population increases the possibility of collateral vessels, especially in the posterior mediastinum, which increases the risk for hemorrhage [50].

One of the most common scenarios is that of a failing Fontan reconstruction. Indications for transplantation in this group are protein-losing enteropathy, intractable atrial arrhythmia, and systemic ventricular dysfunction [51]. Again, ample pulmonary artery and vena caval length (including innominate vein) are procured; a segment of descending thoracic aorta may also be obtained for complex reconstructions of the pulmonary artery. During the recipient cardiectomy, the Fontan pathway is deconstructed, either by resecting the extracardiac bypass graft or the intra-atrial baffle. In either case, a generous rim of remnant superior vena cava and interior vena cava is preserved. After the Glen pathway is taken down, the pulmonary arterial circulation can be reconstructed by a variety of methods, including patching or interposition grafting [48,52]. Significant time may be required for the recipient cardiectomy and reconstruction required to establish anatomy compatible with donor heart implantation. It is of utmost importance to coordinate the cross-clamp time at the donor institution with the recipient team to account for these complexities and minimize ischemic time as much as possible. The possibilities for recipient anatomy and reconstructive techniques are numerous and beyond the scope of this chapter. Transplants for complex congenital heart disease should be deferred to surgeons with significant experience in the field of congenital heart surgery. In general, results for heart transplantation in this group are comparable to those without congenital heart disease, with 1-year survival of approximately 85% and 10-year survival of approximately 60–70% [50,53,54]. Given the young age of most of these recipients, retransplantation is likely to be needed in the future.

Heart–lung transplantation

Combined heart–lung transplantation has decreased in frequency over the last two decades as the indications for lung transplantation have broadened [55]. However, there remains a very select group of patients, many with congenital heart disease [56], who have combined pulmonary vascular disease and end-stage heart failure for which this combined transplant is the best treatment option [57]. The initial dissection and exposure of the heart–lung block is similar to those of isolated heart and lung donor procedures as described in other chapters. Briefly, in addition to typical exposure for the donor cardiectomy, the trachea is exposed by dividing the posterior pericardium between the aorta and superior vena cava. Dissection around the trachea is minimized to preserve its laterally based blood supply. Cardiectomy involves division of the superior and inferior vena cavae, placement of the aortic cross clamp, and

administration of preservation solution in the aortic root, and amputation of the tip of the left atrial appendage to prevent left heart distension by perfusate. Maximal aortic and caval procurement is paramount, particularly if a bicaval anastomosis is desired. One liter of cardiac and at least 3 liters of pulmonary preservation solutions are administered followed by division of the aorta. Next, the inferior pulmonary ligaments are divided, followed by tracheal stapling and division with the lungs partially inflated to prevent atalectasis during transport. The pericardium around the pulmonary veins is divided. The trachea is dissected free from the esophagus and posterior mediastinum with attention to meticulous hemostasis of the heart–lung block. The heart–lung block is prepared in a similar fashion to orthotopic heart transplantation. The right atrium or both cavae are prepared depending on the type of systemic venous anastomosis that is planned. Potential sources of bleeding from the mediastinum of the heart–lung block must be identified and controlled. The recipient is placed on total cardiopulmonary bypass using aortic and bicaval cannulation. The recipient cardiectomy is performed as described above. Given the size of the surgical field and considerable bleeding risk, hemostatic technique during dissection and exposure is mandatory. The right atrium and cavae are prepared as dictated by the planned type of systemic venous anastomosis. The recipient pneumonectomy follows. After the pleura are opened sufficiently to allow for removal of the lungs, the inferior pulmonary ligaments are divided and generous pericardial windows posterior to the phrenic nerves is created. The hilum of each lung is dissected out and stapled followed by removal of the lung. Next, the right pulmonary artery is removed and the carina is circumferentially exposed and divided. Hemostasis of the posterior mediastinum is meticulously ensured. Residual left atrial tissue may be oversewn. The heart–lung block is placed within the chest by inserting the lungs through the pleuropericardial windows posterior to the phrenic neurovascular bundle. The tracheal anastomosis is performed first using either a running or interrupted suture technique. Lateral tracheal stay sutures are placed to minimize tension at the level of the anastomotic sutures. Next, the right atrial anastomosis is performed as in the biatrial technique; however, a bicaval technique may be employed. The aortic anastomosis is performed last. The anastomotic sequence may be modified based on the needs of the operation. Donor ischemic time can be minimized by performing the aortic anastomosis after the tracheal anastomosis with removal of the aortic cross clamp prior to atrial anastomosis.

Summary

Improvements in heart transplant techniques have contributed to excellent 1-year survival of heart allograft recipients. However, the graft half-life for heart recipients remains a short 11 years [58]. After 5 years, cardiac allograft vasculopathy (30%) and malignancy (23%) account for most cardiac recipient deaths [58]. These sobering statistics emphasize that despite meticulous surgical technique, the limitations of chronically administered immunosuppression on long-term survival are profound, and they make clear the need for tolerance strategies that achieve long-term graft survival and prevent chronic rejection without the use of long-term immunosuppression. Similarly, as with all solid organ transplantations, the shortage of available donor organs remains the primary limitation to the greater application of heart transplantation. The development of expanded organ sources through xenotransplantation [59], heart organogenesis [60], and ex vivo perfusion systems [61] will greatly impact the future numbers of heart transplants performed. Regardless of the source of suitable donors, standardizing and implementing operative techniques as described above should provide for excellent patient outcomes.

Acknowledgments

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CHAPTER 59

Lung Transplantation Procedure and Surgical Technique

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Introduction

To address the higher rate of rejection and mortality in lung transplantation, clinical practice has undergone remarkable changes, such as increased immunosuppression and strategies to address non-alloimmune injury, including gastroesophageal reflux (GER) [1]. These protocol changes, along with astute recipient selection, meticulous technical performance of organ procurement and implantation, and refinements in perioperative care, have led to a significant improvement in patient and graft survival over the last two decades [2]. However, new and innovative approaches to lung transplantation are still needed to further improve short- and long-term outcomes. This chapter will outline the techniques preferred at our center. Alternative techniques described in the literature, but that our center does not routinely employ, will also be presented. Details of lung procurement and preservation/resuscitation can be found in Chapters 21 and 26, respectively.

Anesthetic details

Recipient management during lung transplantation continues to be one of the most challenging endeavors for any anesthetic team [3]. Respiratory compromise and hemodynamic perturbations are frequent and require experienced, cardiothoracic-trained anesthesia staff for optimal outcomes. In addition to appropriate large-bore venous access, radial and pulmonary artery catheters are routinely placed to aid in continuous hemodynamic monitoring. Frequently, the radial artery line functions poorly due to patient positioning for bilateral anterior thoracotomies; therefore, we routinely place a small-bore femoral artery cannula as well. Other regularly utilized monitoring devices include cerebral oximetry measurements and transesophageal echocardiography (TEE). TEE allows for evaluation of cardiac function and filling during the operation, in addition to interrogation of most vascular anastomoses at the end of the case. Postoperative analgesia is typically accomplished with a thoracic epidural that is placed after transplant in the ICU, but prior to extubation.

We typically use a left-sided double-lumen endotracheal tube (ETT). Occasionally, in smaller recipients an adequate-sized double-lumen ETT cannot be placed and the operation will ensue on cardiopulmonary bypass (CPB). We prefer to avoid attempts at maintaining single lung ventilation via a single-lumen ETT due to airway compromise from tube dislodgement or occlusion. Regardless of the selected airway, flexible bronchoscopy with suction is essential. This allows for one to place the ETT correctly, as well as maintain its position and patency throughout the operation. Bronchial manipulation during the procedure will inevitably lead to tube migration with subsequent inadequate ventilation and requires intense vigilance on the part of the anesthetic team.

Effective communication among all the components of the operating room team remains an essential part of successful lung transplantation. Notifying the anesthesia team prior to cardiac retraction allows for pre-emptive administration of vasoactive drugs. This can ameliorate team angst and avoid over-reactive interventions. Minimization of intravenous fluids, coinciding with generous inotropic support (e.g. epinephrine), helps to support recipient hemodynamics during tenuous periods of the operation. This in turn contributes to fewer problems with pulmonary edema following implantation. Red blood cell volume and clotting factors should be closely monitored and replaced as needed. This remains particularly important in patients with significant bleeding, as well as those with hepatic congestion from pulmonary hypertension or right ventricular dysfunction.

Patient positioning and choice of incision

With a few notable exceptions, our center preferentially performs bilateral sequential lung transplantation via a fourth intercostal space, anterotransternal thoracotomy incision (i.e. clamshell). For this, the patient is placed in the supine position with arms slightly abducted and supported anteriorly on padded arm boards, with the elbows flexed. A thermal warming pad is placed posteriorly, while a warming blanket covers the recipient's lower body anteriorly from the umbilicus down. Groin access is not necessary as vascular access for extracorporeal support can be attained in the chest. The incision for a female patient requires placement just below the inframammary crease and typically provides for acceptable aesthetics after transplantation. This requires breast flaps to be developed bilaterally, followed by retraction of breast tissue superiorly, and then pleural space entry through the 4th intercostal space. The mammary arteries are routinely divided bilaterally between vascular clips and then the sternum transected with the sternal saw. Of note, other groups have reported a significant number of sternal complications, including malunion and override, after transecting the sternum. Although the incidence of sternal complications

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remains low at our center, less than 5%, the procedure can be performed through sternal-sparing bilateral anterior thoracotomies in many patients without sacrificing adequate exposure. Those recipients with restrictive lung disease and small chest cavities, pulmonary hypertension, or requiring concomitant cardiac procedures typically require a clamshell incision.

Recipient explantation

Following the transverse sternotomy, division of the mediastinal pleura occurs superiorly to the level of the mammary veins and inferiorly to the pericardium. The pericardium does not require opening; however, difficulty exposing the left pulmonary veins for the anastomosis later may require opening of the pericardium and anterior retraction of the apex of the left ventricle for better visualization. Prior to extensive opening of the chest, the 4th intercostal muscles are divided just above the 5th rib in a posterior direction. The overlying serratus anterior and latissimus dorsi muscles should be mostly spared. For those patients with septic lung disease or previous thoracic procedures, a tedious lysis of adhesions may then ensue. This is oftentimes the most time-consuming portion of the operation. Electrocautery dissection can be safely used for the majority of this dissection, but extreme caution should be maintained in the vicinity of the phrenic nerve, along the mediastinum. Adhesions near the phrenic nerve should be sharply divided. Occasionally, the adhesions will be so dense near the phrenic nerve that dividing the overlying lung tissue with a stapling device is required. In patients with restrictive lung diseases and smaller chest cavities, a heavy silk figure-of-eight stitch placed in the fibrous dome of the diaphragm and retracted caudally and laterally through an inferior intercostal space enhances exposure.

After the lungs are mobilized from the pleural attachments bilaterally, attention can be directed towards the hilar dissection. Ideally, most of the hilar dissection on both sides will be carried out prior to either pneumonectomy. This will minimize the time between reperfusion of the first lung and implantation of the contralateral lung. The decision of which pneumonectomy to perform first depends on several important factors. If there is an appreciable difference in native lung function, then typically the worse lung is replaced first. However, the donor lung quality must also be taken into account, as significant unilateral contusions or consolidation may preclude single lung ventilation on the transplanted lung without CPB or extracorporeal membrane oxygenation (ECMO) support. If no appreciable difference in recipient or donor lung function is noted, then the more technically difficult side should be done first in order to decrease the time during which only one transplanted lung is being perfused. This is typically the left side.

The recipient pneumonectomy can initially be performed outside of the pericardium. An endo-GIA stapling device with vascular loads is utilized to staple and divide the pulmonary artery (PA) at, or just distal to, its first branch. Similarly, the superior and inferior pulmonary veins are taken separately prior to the confluence in the left atrium. The bronchus can also be divided with a stapling device at the level of the upper lobe take-off. The pericardium will need to be opened circumferentially and intrapericardial dissection then ensues. This includes developing Waterston's groove (interatrial groove) on the right side, as well as separating the pulmonary artery from the roof of the left atrium and the posterior left atrium from the pericardium. These maneuvers facilitate placement of the left atrial clamp. The right and left pulmonary arteries require mobilization proximally. On the right side, this involves dissection between the right main PA and the superior vena cava (SVC). On the left side, careful attention is focused on avoiding injury to the recurrent laryngeal nerve. Electrocautery dissection is minimized along the superior aspect of the left PA. The posterior hilum contains abundant lymph nodes and bronchial artery circulation that can be the source of bothersome bleeding after the lung is implanted. Attention towards hemostasis posteriorly prior to implantation is most efficient. Likewise, extensive removal of a bulky subcarinal lymph node station can optimize exposure and avoid potential future anastomotic complications. The vagus nerve runs adjacent to this area in the posterior hilum and should be avoided in order to minimize gastrointestinal motility problems post transplant.

After the intrapericardial dissection is complete, but prior to allograft implantation, the most posterolateral two figure-of-eight #1 Maxon pericostal sutures are placed in preparation for closure of the chest. Here we take advantage of optimal exposure prior to lung placement in the pleural cavity. Similarly, a large-bore thoracostomy tube is placed in the costovertebral gutter, via which a small-bore suction catheter is placed for continuous removal of excess cold slush fluid during the remainder of the case. Oftentimes a 24-french Blake drain is also placed in the inferior sulcus to assist with fluid removal.

Single versus bilateral lung transplantation

Many complications, including infection, hyperinflation, shunting, and malignancy, occur due to the native lung. Likewise, there is a well-documented long-term survival benefit of bilateral orthotopic lung transplantation (BOLT) compared to single (SOLT) [4,5]. Therefore, our center preferentially performs BOLTs for most of our recipients. However, there are subsets of patients and certain clinical situations in which a SOLT is preferred. In those situations, multiple options exist for the initial incision. For a right SOLT, our preferred incision is an anterolateral thoracotomy. This approach allows for easy access to the hilum for implantation, as well as the aorta and right atrium in the event that central cannulation is required. In left lung transplantation, the anterolateral thoracotomy can occasionally limit exposure of the left atrium for the pulmonary vein anastomosis. This can be particularly problematic in pulmonary fibrotic patients, and in this situation we will oftentimes approach via a standard muscle-sparing posterolateral thoracotomy. If concomitant cardiac procedures are required, then a median sternotomy typically provides optimal exposure for both portions of the operation.

Allograft implantation

There are very few differences between the details of left and right lung implantation. However, exposure of the left atrium for the pulmonary venous anastomosis can be challenging at times and we will discuss way to enhance that visualization in the following text. Heparin is administered prior to implantation of the first lung with a dose of 80–100 IU/kg and goal activated clotting time (ACT) can range from 200–300 seconds, depending on surgeon preference.

The hilar structures have been dissected as described above and seen in Figure 59.1. Long silk sutures are placed through the staple lines of the PA and each pulmonary vein branch and then these vascular pedicles are retracted anteriorly after a Rummel tourniquet is placed over each stitch to protect the phrenic nerve during retraction. The Rummel tourniquet can be fashioned from a simple red-rubber catheter. To aid in the exposure of the left hilum, a heavy

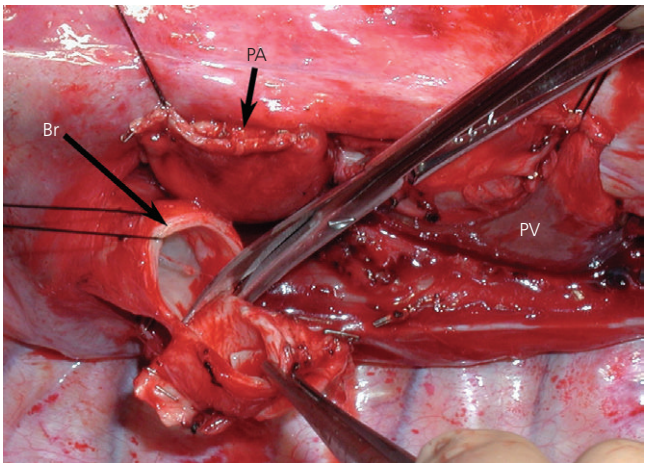

Figure 59.1. Dissected hilar structures of the lung allograft. The stapled bronchial cuff is being resected in preparation for the bronchial anastomosis. Br, bronchus; PA, pulmonary artery; LA, left atrium. Source: Hartwig et al. 2004 [6]. Reproduced by permission of Wolters Kluwer Health.

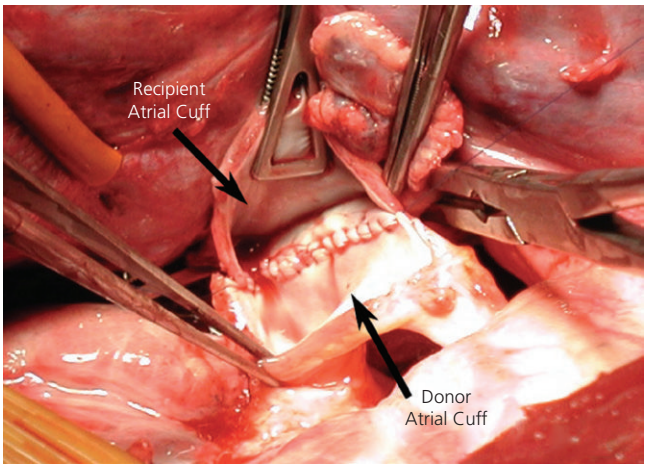

Figure 59.2. Left atrial anastomosis. A Satinsky vascular clamp is placed on the body of the recipient's left atrium while the pulmonary veins are anastomosed using a running 5-0 polypropylene suture to complete the posterior wall first, followed by the anterior wall.Source: Hartwig et al. 2004 [6]. Reproduced by permission of Wolters Kluwer Health.

silk retraction stitch is also placed inferiorly on the pericardium, posterior to the phrenic nerve, and anterior to the inferior pulmonary vein. The silk suture is similarly passed through a heavy-duty Rummel tourniquet, thus allowing the heart to be safely retracted upward and to the right to provide improved exposure during further dissection of the hilar structures and implantation of the donor lung.

The airway is the first anastomosis to be performed. The recipient mainstem bronchus is first sharply cut with a scalpel just proximal to the upper lobe bifurcation. A silk retraction stitch can then be placed several cartilaginous rings proximal to help with maneuverability of the airway. The membranous portion has a tendency to retract after transection and it should be made slightly longer than the cartilaginous side. The bronchial lumen is suctioned clear of secretions and on the left side the ETT may need to be slightly retracted. The pleural cavity should be irrigated well with antibiotic solution, followed by placement of the initial 4-0 polydioxanone suture (PDS) at one corner of the recipient membranocartilaginous junction. Iced laparotomy pads can then be placed posteriorly in the pleural cavity and the donor lung is orthotopically placed on these pads. Additional topical ice slurry maintains allograft hypothermia during implantation.

A key component to the bronchial anastomosis is preservation of the membranous-to-membranous and cartilaginous-to-cartilaginous apposition. The posterior, membranous portion of the anastomosis is performed first in a running, end-to-end fashion with the 4-0 PDS. Upon reaching the opposite membranocartilaginous junction, a transition stitch from the outside of the smaller bronchus to the inside of the larger bronchus is performed. Usually the recipient bronchus is slightly larger, and this allows a small intussusception to occur along the anterior, cartilaginous portion of the bronchus. The intussusception is typically one cartilage ring in depth and the anterior row of suture is also performed in a running fashion. During completion of the bronchial anastomosis, the anesthesiologist should be suctioning the airway clear from above and preparing to manually inflate the donor lung with room air. Following completion, the anastomosis is submerged in water and evaluated for an air leak, while the lung is gently inflated.

The PA anastomosis follows the airway. The recipient main PA is occluded proximally with a small Satinsky vascular clamp, followed by removal of the staple line. The donor PA is also trimmed to an appropriate length. Typically, this is approximately 1.5 to 2 cm proximal to the first arterial branch, but accurate measurement is often best done at this time. Poor estimation of donor and recipient PA length can lead to excessive PA tissue and kinking after completion of the anastomosis. We use a 6-0 polypropylene suture to perform the PA anastomosis. This is initiated in one corner and done in a running fashion beginning with the posterior row. Preservation of native alignment is also crucial for the vascular anastomoses, and the anterior and apical segmental branches of the donor lung can assist with proper orientation. After completing the anastomosis, a second vascular clamp is placed distally on the donor PA while slowly removing the more proximal Satinsky clamp. This tests the PA anastomosis without premature reperfusion of the donor lung. Minor bleeding is generally seen from needle holes, but additional time spent on hemostasis is unnecessary as this will eventually stop. However, larger areas of bleeding from the anastomosis should be repaired at this time.

The distal PA clamp remains on while the pulmonary vein–left atrium anastomosis is performed. A large Satinsky vascular clamp is placed on the body of the left atrium, while the previously stapled native pulmonary vein stumps are retracted laterally with Pennington clamps. The staple lines are then excised and the left atrial tissue bridge between the superior and inferior pulmonary veins orifices is transected. This creates a large common orifice for the recipient left atrial cuff. A Pennington clamp placed on the anterior edge of this cuff with anterior and medial retraction provides optimal exposure for subsequent sewing (Figure 59.2). Occasionally, the left pulmonary venous anastomotic exposure remains less than ideal in spite of the previously placed retraction stitch on the edge of the pericardium anterior to the left pulmonary veins. In that situation, the pericardium can be opened and the apex of the heart positioned anteromedially with either moistened sponges or a suction retractor designed for off-pump coronary surgery. Hemodynamic pressures may be hindered during this retraction and small bolus administration of inotropes is often necessary during this anastomosis.

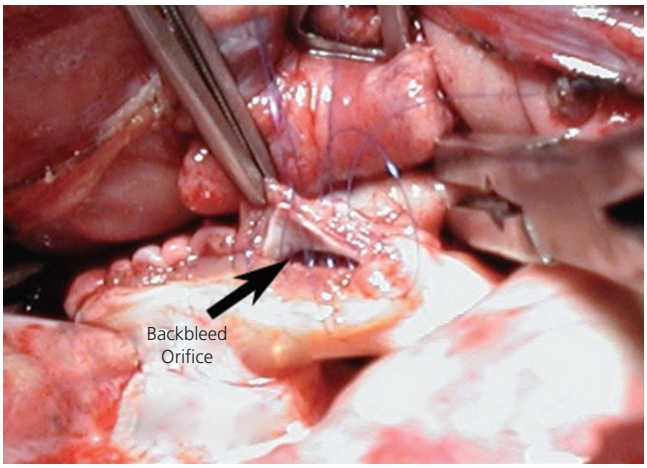

Figure 59.3. De-airing the left atrium. The last several throws of the pulmonary vein cuff anastomosis remain loose anteriorly and the vascular clamp on the PA is partially released to remove air from within the pulmonary vessels. Then the Satinsky clamp on the left atrium is loosened to force out any residual air from the left atrium. Source: Hartwig et al. 2004 [6]. Reproduced by permission of Wolters Kluwer Health.

Orientation of the superior and inferior pulmonary veins is critically important to a technically adequate anastomosis. Also, the vein cuffs should appose endothelium-to-endothelium by excluding most of the muscular tissue from the suture line, while focusing on the intimal layer. The running suture line is performed analogously to the PA, with initiation in one corner of the anastomosis and completing the posterior row of running sutures first, followed by the anterior walls. A 5-0 polypropylene suture is typically used for the pulmonary vein anastomosis, and we ask anesthesia to administer 25 grams of mannitol and 500 mg of methylprednisolone prior to completing the vascular anastomoses on each side.

Reperfusion

The period of allograft reperfusion should be carefully controlled in order to protect the graft from primary graft dysfunction (PGD). With the left atrial clamp still in place, the last several throws of the pulmonary vein cuff anastomosis remain loose anteriorly when the vascular clamp on the PA is only partially released (Figure 59.3). This allows a small amount of warm blood to flow through the donor lung in order to remove air from within the pulmonary vessels and initiate re-warming. At this time, the large Satinsky on the left atrium is then loosened to force out any residual air from the left atrium as well. After an adequate de-airing has occurred, the 5-0 suture is secured, the venous anastomosis is complete, and the left atrial clamp can be completely removed.

Low-pressure reperfusion of the allograft is achieved via incremental de-ratcheting of the PA clamp over 10 to 15 minutes. During this time, inspection of the three anastomoses, hilar dissection, and donor lung occurs with hemostatic intent. Of note, during this period of early reperfusion, hypotension is often encountered. Communication with anesthesia prior to opening of the clamp is critical. Hemodynamic perturbations at the time of reperfusion have been attributed to an autoinfusion of prostacyclin (PGI_2) or alprostadil (PGE_1) that may be residing in the graft from procurement, a redistribution of blood flow through the newly perfused graft, or an air embolus. If the hypotension is secondary to residual vasodilators within the graft or a simple redistribution phenomenon, the observed hypotension should be self-limiting. However, during this time the anesthesia team will likely need to provide temporary inotropic support and modest volume resuscitation.

Initially, the allograft should be gently inflated manually on room air. This should not occur until the lung has fully re-warmed and the airway has been cleared of residual bloody secretions. After complete removal of atelectasis with hand ventilation, the lung can be placed on the ventilator. Pressure control mode is generally preferred, with positive end-expiratory pressures of 5–8 cm of H_2O, inspiratory pressures less than 18 to 20 cm of H_2O, and minimal FiO_2—preferably less than 30%. With the pleural spaces widely open, as in the setting of a clamshell incision, inspiratory pressures tend to be lower than those needed following closure of the chest. Loop diuretics and inhaled nitric oxide are utilized if pulmonary artery pressures are elevated, early pulmonary edema is evident, or if systemic oxygenation is not adequate. PA pressures will ideally be normal by the time the PA clamp is completely removed. Abnormally elevated PA pressures at this time may indicate a technical problem, poor preservation, or early reperfusion injury and should precipitate a rapid assessment of the vascular anastomoses.

For the contralateral side, most of the preliminary dissection will have already occurred and the pneumonectomy should proceed rapidly. One may notice compromised oxygenation when single lung ventilation occurs on the newly implanted lung while the contralateral lung is deflated. This should be greatly improved once the deflated native lung creating the shunt is removed. The pericardium is opened, if it hasn't already, and the intrapericardial dissection, hilar preparation, and subsequent implantation ensue as described above. During this portion of the case, technical problems or early primary graft dysfunction may precipitate inadequate ventilation or oxygenation. In this situation, implementation of venovenous extracorporeal membrane oxygenation (VV ECMO) can be rapidly performed by dual cannulation of the right atrium [7]. Details of this are described below. Figure 59.4 depicts the final appearance from anterior and posterior views of a bilateral lung transplant.

Closure

Single lung transplant performed through a thoracotomy incision can be closed in standard fashion. Proper closure of the clamshell incision is critical to avoid subsequent incisional complications after transplant. The anterior aspect of the clamshell opening is re-approximated with three sets of #5 wires. One simple wire is placed in the midline of the sternum and then one figure-of-eight wire secured on each side of the midline with the lateral aspect placed lateral to the mammary pedicle. All three of these wires are placed through the sternum. Occasionally, there will be a significant space between the 4th and 5th ribs when the sternum is brought together. This can be avoided by removing a small transverse section of sternum with the sternal saw prior to closure. The remainder of the clamshell incision can be closed with #1 polyglyconate sutures. The most posterior of these pericostal sutures have been previously placed just after the native lung was explanted on each side.

Prior to closure, additional bilateral pleural drainage tubes are placed anteriorly. If concerns of excess drainage exist (i.e. after CPB), an additional 24-french Blake drain can be placed in the inferior sulcus. Also, we preferentially drain the submammary space in women with smaller Blake drains. The pectoral fascia,

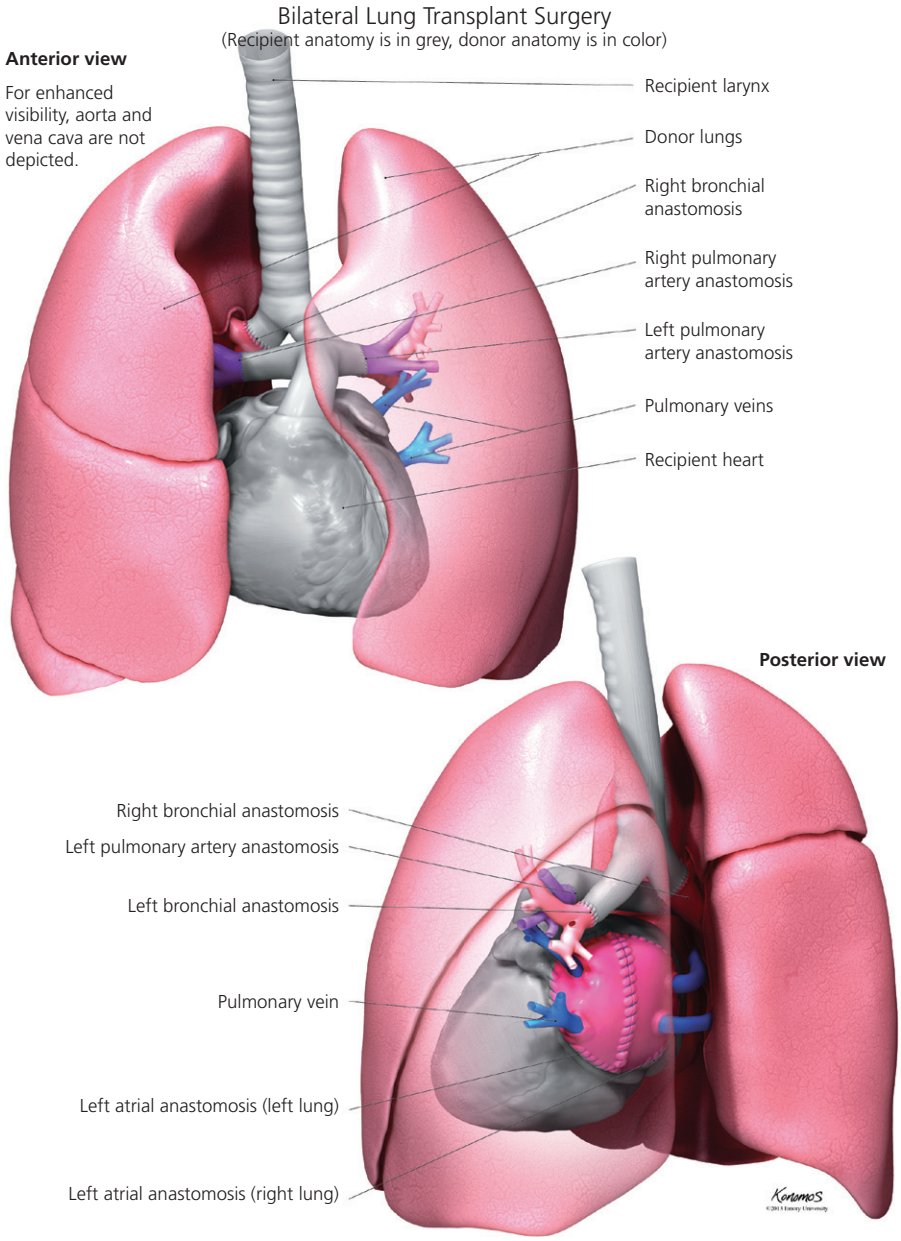

Figure 59.4. Anterior and posterior view of a completed bilateral lung transplant.

subcutaneous tissues, and dermal layers are then re-approximated with absorbable suture, followed by metallic skin clips.

The chest tubes are typically placed to suction; however, if the implanted lungs lack sufficient volume to adequately fill the pleural cavities, over-distention of the lungs may occur on suction. If this occurs, stretch injury and subsequent allograft dysfunction will develop. Marked differences in tidal volumes when the chest tubes are on and off suction will alert the surgeon to this development. After chest closure, exaggerated increases in inspiratory pressures with diminished tidal volumes may herald over-sized allografts or significant early pulmonary edema. Pneumoreduction can be performed at this time, but our preference is a priori removal of excessive lung volumes during the back-table preparation if possible. Typically, a simple right middle lobectomy and contralateral lingulectomy will be sufficient in these instances. On rare occasions, early development of pulmonary edema may require the chest to be left open.

Following the application of sterile dressings, the double lumen ETT is exchanged for a single lumen ETT. Flexible bronchoscopy is performed prior to leaving the operating room and serves multiple purposes. First of all, the bronchial anastomoses are directly inspected for patency. Torsion, or malrotation, of the airways will manifest as crescent shaped narrowing of the distal airways. Residual bloody secretions are removed and any developing PGD may be heralded by the development of frothy pulmonary edema.

Cardiopulmonary bypass

For the most part, CPB can be avoided during bilateral lung transplantation and we implant approximately 90% of our lung allografts

without the heart–lung machine. However, there will be occasion for both a priori, as well as unplanned, utility of CPB based upon recipient status and certain technical factors. Clinical scenarios typically requiring CPB include: (1) concomitant intracardiac procedures such as atrial/ventricular septal defect closures, (2) valve repair, (3) severe pulmonary hypertension, (4) small recipient airway precluding double-lumen ETT placement, (5) previous contralateral pneumonectomy, and (6) fragile or inadequate left atrial cuff tissue. In this last case, cardioplegic or fibrillatory arrest facilitates an anastomosis without the need for vascular clamps.

Cannulation strategies for performing lung transplantation on CPB vary by exposure. Standard midline, clamshell, and right thoracotomy incisions all lend themselves to central cannulation of the right atrium with a dual-stage venous cannula and the ascending aorta for the arterial return. Left single-lung transplants on bypass can typically be performed with a long 15 to 23-french femoral venous cannula and a standard aortic cannula in the descending aorta. If the groin is not easily accessible when CPB is needed, the left pulmonary artery can be cannulated proximally in the chest for venous return. However, when performing a left single-lung transplant, we will typically leave the ipsilateral groin prepped and in the sterile field in case vascular access is needed. If there is a high likelihood of needing CPB, a small-bore vascular cannula can be placed in the femoral vein and/or artery prior to initiating the procedure. Either of these can then be re-wired for a CPB cannula if necessary.

While CPB can be initiated at any time during the operation if necessary, our preference is to perform as much as the dissection as possible, including unilateral pneumonectomy, prior to going on bypass. When implantation occurs on CPB electively, both pneumonectomies are performed, and bilateral intrapericardial dissections are completed prior to beginning implantation. Following this, the posterior thoracotomy sutures and pleural drains are placed, and both lungs are implanted as described above prior to reperfusion of either allograft. The first lung that is implanted is packed in ice slush during implantation of the second lung. Methylprednisolone (1 g) and mannitol (50 g) are administrated prior to reperfusion. While the lungs are ventilated with room air, gradual reperfusion is initiated starting at 5–10 mmHg of mean PA pressure and increasing by 5 mmHg every 5 minutes until normal systemic pressures are achieved. Reperfusion of the lungs with oxygenated, hypocarbic blood may be especially advantageous to maximize pulmonary vascular recruitment and parenchymal recovery, while pulmonary artery pressures are easily controlled during reperfusion on CPB.

Extracorporeal membrane oxygenation

Extracorporeal membrane oxygenation (ECMO) is a crucial component of any lung transplant surgeon's armamentarium [8,9]. This physiologic adjunct can be used both intraoperatively when single-lung ventilation proves inadequate, as well as postoperatively in the management of severe primary graft dysfunction (PGD). The most common reason in the operating room to take advantage of ECMO is in the setting of transplantation of a severely affected pulmonary fibrotic patient in whom the native lung is not capable of fulfilling the need for single-lung ventilation. Oftentimes one can predict this prior to transplant based upon baseline oxygen requirements, chest radiographs, and nuclear pulmonary perfusion studies. In these instances, VV ECMO can be instituted percutaneously with cannulation of the right internal jugular vein with the dual-lumen ECMO cannula (Avalon™, Maquet Holding, Rastatt, Germany). An alternative cannulation strategy includes via the right femoral vein with a venous catheter (Bio-Medicus, Medtronic, Minneapolis, MN) and the left internal jugular vein with a pediatric arterial cannula (Medtronic, Minneapolis, MN). Cannulas are placed percutaneously using a modified Seldinger technique over a guide-wire following serial dilatations. The optimal placement of circuit in-flow and out-flow ports are determined by the level of recirculation noted in the system and are initially guided by TEE or fluoroscopic assistance. The venous cannula is typically near the inferior vena cava–right atrial junction, while the "arterial" return cannula is in the right atrium. The dual-lumen ECMO cannula if used should help minimize recirculation, as there are distal and proximal ports in the IVC and SVC for venous return, and an "arterial" in-flow port placed in the right atrium and directed towards the tricuspid valve. The ECMO circuit consists of a hyaluron-based heparin-coated 3/8″ tubing with a Quadrox D Poly-methylpentene (PMP) oxygenator and Jostra Rotaflow pump (Maquet Cardiopulmonary, AG, Germany).

In rare circumstances, the initial allograft may suffer immediate dysfunction and be incapable of adequate ventilation or oxygenation during implantation of the second lung. In this and other intraoperative situations, central cannulation can occur for VV ECMO, and systemic heparinization and full bypass can usually be avoided. If accessible, the right atrium can be double cannulated with the venous cannula placed near the IVC–right atrial junction and the "arterial" return cannula directed towards the tricuspid valve.

Potential pitfalls

From the beginning of the lung transplantation experience, primary graft dysfunction (PGD) has significantly impacted early survival [10]. Through the use of extracellular-based pulmonary perfusion solutions, the addition of retrograde pulmonary perfusion to lung preservation, minimizing the oxygen exposure at the time of reperfusion, controlling reperfusion pressures, and by using oxygen free-radical scavengers, the incidence of severe PGD has decreased from 15–25% to as little as 5%. PGD causes or significantly contributes to the majority of early post-transplant deaths. Unless reversible causes can be identified, treatment has primarily been supportive with optimization of ventilator parameters, inotropic support, and nitric oxide. Recently, we have shifted to a strategy of early institution of VV ECMO when recipients develop severe pulmonary edema and require a Fio_2 over 0.60. During VV ECMO support, a protective ventilatory strategy is used including minimal oxygen (Fio_2 0.30 or less) and low-pressure ventilation (*positive end-expiratory pressure* [PEEP] 10 cm of H_2O and peak inspiratory pressures less than 25 cm of H_2O). Initially, this can be done without systemic heparinization, until the surgical team is satisfied with hemostasis. Subsequent modest anticoagulation can occur with activated clotting times of 180–200 seconds typically being adequate. Weaning from VV ECMO involves discontinuing membrane gas flow and increasing ventilatory parameters as needed. No increase in anticoagulation is required for VV ECMO weaning. Using this strategy, patients are usually weaned from ECMO within 3 to 5 days of support, as pulmonary vascular resistance decreases following institution of ECMO and pulmonary capillary leak resolves. The 30-day survival in patients requiring VV ECMO at our institution approaches 90%.

Although PGD remains the most common reason for the development of severe allograft dysfunction, the development of marked hypoxia, pulmonary edema, elevated pulmonary artery pressures, and poor compliance requires immediate investigation for reversible causes. Asymmetric pulmonary edema or crescent-shaped airways on bronchoscopic evaluation may be indicative of mechanical problems. Most importantly, anastomotic or mechanical etiologies, especially venous outflow problems, need to be identified and corrected immediately. With respect to venous outflow obstruction, correction later than 4–6 hours will be unlikely to achieve reasonable allograft recovery. Interrogation of the anastomoses in the operating room includes visual inspection to assess for torsion or kinking, as well as TEE to assess for turbulent or absent pulmonary vein flow, left atrial anastomotic quality, the presence of intraluminal clot, and direct measurement of pressures across the anastomosis. TEE should provide optimal evaluation of all pulmonary veins except for the left lower, which is sometimes difficult to see with echo.

Pulmonary vein thrombosis can occur in the acute setting early after transplant and can mimic the clinical appearance of PGD. Prevention of pulmonary vein thrombosis should be emphasized by strict adherence to proper procurement techniques, a technically sound intimal-to-intimal anastomosis, and systemic anticoagulation in high-risk individuals. If discovered in the operating room, immediate revision of the anastomosis should be performed. It may be possible to treat non-occlusive thrombus with thrombolytics and/or anticoagulation, although the risk for life-threatening hemorrhage is clearly highest during the early postoperative period. Oftentimes the safest management strategy includes inspection and treatment in the operating room.

Cardiac complications can usually be identified by TEE, including left-sided valvular abnormalities, left ventricular failure, intracardiac shunts, and tamponade. However, TEE does not provide adequate assessment of the pulmonary artery anastomoses. Therefore, we routinely obtain a quantitative perfusion scan within the first few hours after the transplant. Lobar or greater perfusion defects on the perfusion scan often necessitate further assessment, most preferably by operative exploration. Computed tomography or conventional angiography can also be utilized to assess the pulmonary arterial vasculature. On rare occasions, PA kinking can be better managed endovascularly with a stent.

Uncommonly, early graft dysfunction can also be caused by humoral lung injury from circulating antidonor antibodies. These antibodies are identified via a cross-match of donor and recipient sera. Treatment of donor-specific antibodies is multimodal, including plasmapheresis or exchange, intravenous immunoglobulin (IVIG), and anti-B-cell therapies such as anti-CD20 (rituximab).

Summary

There has been encouraging progress in lung transplantation over the last two decades, with improvements in graft survival occurring with each new era. The most impressive changes have been in early outcomes, with 30-day and 1-year patient and allograft survival now comparing favorably to those of liver and cardiac transplantation [11]. Much of the enhanced patient outcomes can be attributed to astute recipient selection, meticulous technical performance of organ procurement and implantation, and refinements in perioperative care [12]. Unfortunately, as with all solid organ transplantations, the shortage of available donor organs and the development of chronic allograft injury remain the primary limitations to a greater applicability of lung transplantation. Without question, the development of expanded organ sources through xenotransplantation [13], lung organogenesis [14], and ex vivo perfusion systems [15,16] will greatly impact the future numbers of lung transplants performed. Regardless of where suitable donors are discovered, standardizing and implementing operative techniques as described above should provide for appropriate patient outcomes.

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CHAPTER 60

Pancreas Transplantation Procedure and Surgical Technique

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Introduction

The prevalence of type 1 diabetes in the United States is estimated to be approximately 2 000 000 individuals, with over 35 000 new cases diagnosed each year [1,2]. Currently, there is no practical artificial endocrine pancreas (i.e. a mechanical insulin-delivery device coupled with an automated glucose-sensory apparatus) that is capable of administering insulin with the degree of control necessary to produce a near-constant euglycemic state without risk of hypoglycemia. Persons with type 1 diabetes are resigned to manually regulate blood glucose levels by various forms of exogenous insulin administration. Consequently, patients with diabetes exhibit wide excursions of plasma glucose levels from hour to hour and day to day. Since severe hypoglycemia is life-threatening, glucose control must err on the high side. Therefore, patients must live with chronic hyperglycemia and elevated hemoglobin A1c (HgbA1c).

The only treatment that reliably produces sustained euglycemia, normalizes hemoglobin A1c levels, and has been rigorously documented to influence the progression of some secondary complications include β cell replacement therapy with the immediately vascularized whole-organ pancreas transplant. Currently, islet transplantation is a very promising experimental procedure for select patients with type 1 diabetes.

A successful pancreas transplant obviates the need for exogenous insulin therapy, normalizing hemoglobin A1C levels for as long as the graft functions. Pancreas transplantation also has the added physiological properties of proinsulin and C-peptide release not possible with intensive insulin therapy [3]. Through improved metabolic control, many secondary complications of diabetes are markedly improved, or reversed. Several seminal studies have documented these benefits, including improvement in diabetic neuropathy [4], autonomic neuropathy-associated sudden death [5], diabetic nephropathy in both uremic and non-uremic patients [6,7], and stabilization of visual acuity [8]. A successful pancreas transplant significantly improves quality of life [9] and life expectancy [10,11]. This chapter will provide an overview of the critical aspects of the pancreas transplant procedure, including patient preparation, organ procurement and reconstruction, implantation, and perioperative management.

Pancreas transplantation categories

Approximately 1100 pancreas transplants are performed annually in the United States. Sixty-five to 70% involve a simultaneous pancreas and kidney (SPK) transplant for patients with type 1 diabetes and chronic renal failure. The uremic patient with diabetes is an excellent candidate for an SPK transplant from the same donor because the immunosuppressive medications that are needed are similar to those for a kidney transplant alone and the surgical risk of adding the pancreas is low. The benefits of adding a pancreas transplant to ameliorate diabetes are profound—transplantation saves lives [10,11].

The second category for pancreas transplantation consists of patients with type 1 diabetes who have received a previous kidney transplant from either a living or deceased donor. This group accounts for approximately 20% of patients receiving pancreas transplants. The important consideration is that of surgical risk, since the risk of immunosuppression has already been assumed.

The third category for pancreas transplantation is the non-uremic, non-kidney transplant patient with type 1 diabetes. In this situation one assesses the risk of immunosuppression to be less than the risk of diabetes treated with conventional exogenous insulin. Some of these patients with diabetes have extremely labile disease associated with frequent emergency room visits and inpatient hospitalizations for hypoglycemia or diabetic ketoacidosis. Other patients have significant hypoglycemic unawareness that results in unconsciousness without warning. For select patients this state can be a devastating problem that affects their employment and their ability to keep a driver's license and creates concern about lethal hypoglycemia while asleep.

More detailed discussions of pancreas transplant outcome can be found in Chapter 107.

Preparative aspects of pancreas transplant surgery

Timing

The efficient coordination of pancreas allograft allocation to a specific patient relative to the timing of actual surgical organ procurement has important implications for an efficient transplant

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

process that minimizes cold ischemia time. Effective communication between the procurement and transplant teams and the organ procurement organization is essential. Determining donor HLA typing, infectious serologic results, and HLA cross-match results will permit the ideal situation of allocating the deceased donor pancreas (plus kidney with SPK transplant) prior to operative procurement of the organs. This sequence of events has several advantages: (1) it will allow the transplant center performing the pancreas transplant the choice to also procure the pancreas; (2) it will allow patients to be admitted to the hospital and the inpatient evaluation process to begin simultaneously, rather than sequentially, to the procurement of the organs; and (3) it will minimize the cold ischemia time of the pancreas prior to implantation. Pancreas allografts do not tolerate cold ischemia as well as kidney allografts. It is ideal to revascularize the pancreas within 24 hours from the time of procurement cross-clamping. Finally, it will also allow identification of 0 antigen mismatched donor–recipient pairs to be identified prior to procurement which will minimize cold ischemia time if the organs need to be transported across country.

Preoperative transplant assessment

The pancreas transplant recipient is admitted to the hospital, re-evaluated, and a final decision made whether or not to proceed with surgery. The re-evaluation process is similar to that for kidney-alone transplant recipients, emphasizing work-up for acute medical issues that might contraindicate surgery. More detailed discussions of the initial patient evaluation and wait list management can be found in Chapters 32 and 41, respectively. There are several special considerations for the diabetic patient. Careful management of diabetes pretransplant is important for patients not allowed eat or drink prior to surgery. A bowel preparation is performed for patients that will undergo enteric drainage of the pancreas transplant.

Review of the cardiac evaluation is the most critical aspect of the preoperative evaluation. Virtually all patients will have undergone an extensive cardiac evaluation consisting of chest X ray, ECG, stress testing, and usually coronary angiography. Many patients will have been treated by coronary artery intervention to diminish the perioperative risk of the transplant procedure and to prolong the duration of life post-transplant. Patients who have experienced long waiting periods prior to pancreas transplantation should have their cardiac status carefully re-assessed.

Autonomic neuropathy is prevalent and may manifest as neurogenic bladder dysfunction, gastropathy, and orthostatic hypotension. Neurogenic bladder dysfunction is an important consideration in patients receiving a bladder-drained pancreas transplant. Inability to sense bladder fullness and to empty the bladder predisposes to urine reflux and high postvoid residuals. These problems may adversely affect renal allograft function, increase the incidence of bladder infections and pyelonephritis, and predispose to graft pancreatitis.

Impaired gastric emptying, gastroparesis, is an important consideration with significant implications in the post-transplant period. Patients with severe gastroparesis may have difficulty tolerating the oral immunosuppressive medications that are essential to prevent rejection of the transplants.

The combination of orthostatic hypotension and recumbent hypertension results from dysregulation of vascular tone. This condition has implications for blood pressure control post-transplant, especially in patients with bladder-drained pancreas transplants that are predisposed to volume depletion. Careful assessment of post-transplant antihypertensive medication is important.

Diabetic retinopathy is a nearly ubiquitous finding in patients with diabetes and end-stage renal disease. Blindness is not an absolute contraindication to transplantation since many blind patients lead very independent life styles. Although rarely a problem, it should be confirmed that a patient with significant vision loss has an adequate support system to ensure help with travel and immunosuppressive medications.

Lower-extremity peripheral vascular disease is significant in patients with diabetes. Uremic, diabetic patients are at risk for amputation of a lower extremity. These problems typically begin with a foot ulcer associated with advanced somatosensory neuropathy. The risk is further complicated by sensory and motor neuropathies in patients with longstanding diabetes. Vascular disease may have implications for the rehabilitation post-transplant and is an indicator for potential risk for injury to the feet and subsequent diabetic foot ulcers.

Mental or emotional illnesses, including neuroses and depression, are common. Diagnosis and appropriate treatment of these illnesses is important to consider because of the important implications for ensuring a high degree of medical compliance.

Donor pancreas procurement

Identification of suitable deceased organ donors for pancreas transplantation is an important and often underappreciated determinant of outcome. Table 60.1 lists the common contraindications for pancreas transplantation. Misjudging the quality of the donor organs will have significant adverse consequences post-transplant. The transplant operation begins with organ procurement.

Donor pancreas organ evaluation

The criteria determining an appropriate donor for pancreas transplantation are more stringent than for kidney or liver donors. Deceased pancreas organ donors are typically between the ages of 10 and 60. The lower age limit does not relate to the metabolic efficiency of the pediatric endocrine pancreas to regulate blood sugar control in an adult. Rather, the lower age limit of a donor pancreas reflects the anticipated small caliber of the splenic artery, which may preclude successful construction of the arterial Y-graft needed for pancreas allograft revascularization.

Regarding upper age limits, the use of pancreata from older donors has been associated with increased technical failures due to graft thrombosis, a post-transplant pancreatitis, and decreased pancreas graft survival rates [12]. The body weight of the deceased

Table 60.1. Contraindications to pancreas transplantation

1	Omission of consent for organ donation
2	Incompatible blood group
3	Donor HLA class I and/or class II antigen generating a positive immunological cross-match
4	History of type 1 or type 2 diabetes mellitus in donor
5	Donor viral infectious disease of HIV, hepatitis B, and/or C
6	Significant bacterial and/or fungal infection of the donor
7	Significant and prolonged donor hemodynamic instability
8	History of previous donor pancreatic surgery
9	Intra-abdominal trauma to the donor pancreas
10	Cancer

From [19] Kaufman DB. Chapter 47. Complications of Pancreatic Transplantation, pp. 640–655. In: Complications in Surgery, 2nd edition. Ed. M. Mulholland and G. Doherty. Lippincott Williams & Wilkins, 2011. Reprinted with permission from Wolters Kluwer.

organ donor is an important consideration. Obese donors >30 kg/m^2 body mass index (BMI) are frequently found not to be suitable pancreas donors. Obese patients may have a history of type 2 diabetes, or the pancreas may be found to be unsuitable for transplantation because of a high degree of adipose infiltration of the pancreas. Further, retrospective analyses have identified a number of donor-related factors that correlate with worse outcome following pancreas transplantation, including BMI >30 kg/m^2 [13]. Finally, a pancreas donor risk index (pDRI) has been developed to inform organ acceptance decision making, which considers ten common donor variables and one transplant factor (ischemia time) as factors associated with an increased risk of allograft failure [14].

Importantly, pancreata from relatively older donors (age 55 to 65) and obese organ donors are associated with very successful islet isolation recovery required for islet transplantation. Therefore, application of β cell replacement therapy should be considered for nearly all deceased organ donors.

Donor hemodynamic stability and need for inotropic support is an important consideration. Hemodynamic stability has more influence on the anticipated function of the kidney allograft than it does on initial endocrine function of the pancreas allograft in the case of an SPK transplant. Deceased donors who have experienced a significant period of cardiac arrest or who require high doses of prolonged inotropic support frequently exhibit slow deterioration of renal function, which may result in delayed renal allograft function in the SPK transplant recipient.

The most important determinant of suitability of the pancreas for transplantation is direct examination of the organ during surgical procurement. The experience of the procurement team is important. During procurement, this judgment regarding the degree of fibrosis, adipose tissue infiltration into the parenchyma, trauma, and specific vascular anomalies can be made. The important vascular anomaly that must be accurately evaluated during procurement is the presence of a replaced or accessory right hepatic artery originating from the superior mesenteric artery (SMA).

The use of marginal and donors with circulatory death (DCD) for pancreas transplantation has been reported [15]. If the pancreas is deemed suitable, there is the added consideration of the effect of delayed kidney graft function in a uremic SPK transplant candidate. The use of marginal and DCD donors for pancreas-alone transplantation is a decision made on a case-by-case basis.

The use of living related and unrelated pancreas donors has also been described wherein a distal pancreatectomy is performed for a segmental pancreas transplant [16]. Anecdotal cases of combined live donor partial pancreatectomy and nephrectomy for SPK transplantation have also been reported [16]. These procedures are not widely performed and are mostly of historical note. Finally, the combined use of simultaneous deceased donor pancreas and living donor kidney transplantation has been successfully applied [17].

Procurement of the pancreaticoduodenal splenic allograft

There are several standard surgical methods for procurement of the pancreas for transplantation. The general principles are similar irrespective of the specific techniques utilized. The pancreas must be procured with an intact vascular supply that does not compromise the vascularity of the liver. The pancreas is procured with the spleen and duodenum intact. The organ is perfused with preservation solution and cold stored. The donor iliac vessels are obtained for revascularization of the arterial supply and sometimes the portal vein.

There are two general methods of organ procurement. Many programs prefer to perform an en bloc removal of the liver and pancreas together and separate the two organs at the backbench. Other programs prefer to perform a more deliberate dissection of the pancreas and liver by mobilizing the relevant vasculature prior to preservation. The liver and pancreas are separated in situ. The relevant components of the in situ procurement process are briefly described.

1. long midline incision (± cruciate incision);
2. mobilization of ascending colon, control of infrarenal aorta, and identification of superior mesenteric artery;
3. control of supraceliac aorta;
4. identification of hepatic artery (ligation of gastroduodenal artery), splenic artery, portal vein, and division of common bile duct;
5. identification of replaced and/or accessory left and right hepatic arteries;
6. exposure of the anterior aspect of the pancreas for visual and manual inspection;
7. mobilization of the spleen by division of short gastric vessels and dissection of its ligamentous attachments;
8. mobilization of head, tail, and body of the pancreas;
9. NG tube positioning into the proximal duodenum and irrigation of antibiotic solution;
10. removal of NG tube and division of the proximal duodenum just distal to the pylorus;
11. heparinization of the donor and infusion of intra-aortic (± intraportal) preservation solution;
12. division of proximal jejunum, middle colic vessels, and superior mesenteric vessels distal to the pancreatic uncinate process;
13. division of celiac, SMA, splenic arteries, and portal vein;
14. procurement of liver, pancreaticoduodenosplenic allograft, and kidneys;
15. procurement of donor iliac vessels;
16. closure of incision.

Additional discussion of pancreas procurement in the context of a multiorgan procurement from a standard criteria donor or a donation after circulatory death (DCD) donor can be found in Chapters 21 and 22, respectively.

Back-table preparation of the pancreaticoduodenal allograft

The back-table preparation of the pancreaticoduoenal allograft for transplantation requires careful and meticulous surgical technique to ensure a properly revascularized pancreas with adequate duodenum and minimal extraneous fibrotic and adipose tissue (Figure 60.1). The quality of the back-table surgical procedure has the greatest impact on the immediate outcome of the transplant surgery.

The pancreaticoduodenosplenic allograft has previously been procured from the deceased donor by the procuring surgeons and placed in preservation solution and packed according to UNOS guidelines in a cold storage container for transportation to the transplantation center. The container with the allograft is brought into the operating room and the UNOS identification number and donor blood group cross referenced with the pancreas transplant recipient. A basin filled with ice is covered with a plastic wrap that is filled with fresh, cold preservation solution. The organ is then removed from the transport container and placed in the chilled preservation solution. Two surgeons typically partner to perform the back-table operative preparation.

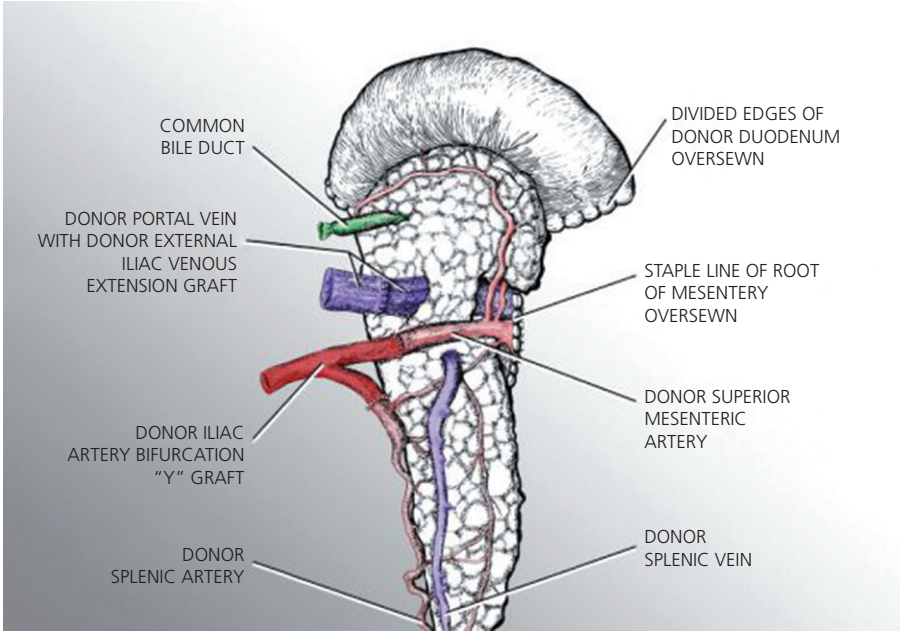

Figure 60.1. Anatomy of the pancreaticoduodenal allograft for back-table reconstruction. Illustration by Simon Kimm MD, Department of Urologic Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY.

Visual inspection of the allograft is critical prior to making the final decision to proceed with transplantation. The organ should be evaluated for the degree of parenchymal adipose infiltration, fibrosis, or palpable nodular lesions Pancreata with heavy infiltration of adipose tissue are relatively intolerant of prolonged (>18 hours) cold preservation and the potential of a high degree of saponification due to reperfusion pancreatitis following revascularization. These organs may be more suitable for islet isolation.

Inspection for parenchymal, vascular, and duodenal injuries, as well as vascular anomalies, should be conducted in a disciplined fashion. The divided small bowel mesentery should be inspected to ensure that it has not been divided excessively short, encroaching on the pancreatic parenchyma and compromising the intraparenchymal vascular supply. The pancreatic portal vein must be carefully inspected to ensure that it has not been cut excessively short, damaging the joining splenic vein. This can occur when the portal vein is put on stretch during liver procurement and over aggressively divided to generate excessive length for the liver transplant team, typically when the procuring surgeons are told that the liver recipient is undergoing a re-transplant. Rescue of a short (2 mm) portal vein can be accomplished with a portal venous extension graft of donor external iliac vein.

An important vascular anomaly that must be skillfully managed during procurement and re-evaluated on the back table is the occurrence of a replaced or accessory right hepatic artery originating from the SMA. The presence of a replaced right hepatic artery is not a contraindication for the use of the pancreas for transplantation under most conditions. Experienced procurement teams will be able to successfully separate the liver and the pancreas either in situ or on the back table, without sacrificing quality of either organ for transplantation.

A few important caveats determine if this maneuver is possible. The replaced right hepatic artery must be dissected to the junction with the SMA. If the replaced right hepatic artery traverses deep into the parenchyma of the head of the pancreas, requiring extensive parenchymal dissection, this circumstance may preclude the pancreas for transplantation (however, it may be suitable for islet transplantation). In such circumstances, another alternative is to divide the replaced right hepatic artery outside the pancreatic parenchyma, not violating the pancreas. Such arteries can be safely reconstructed for the liver transplant without injuring the pancreas, but this requires experience with this specific reconstruction.

The SMA is divided distal to the origin of the replaced right hepatic artery, preserving a short length of SMA with a carrel patch for the liver graft. Occasionally, there is a large inferior pancreaticoduodenal arterial branch vascularizing the head of the pancreas that originates proximal to the origin of the replaced right hepatic artery. The inferior pancreaticoduodenal vessels are critical to vascularization of the head of the pancreas because the gastroduodenal artery is routinely ligated during the process of hepatic artery mobilization for the liver transplant. In the case of a very proximal origin of the inferior pancreaticoduodenal artery, dividing the SMA at the appropriate location for liver procurement would significantly impair vascularization of the head of the pancreas and preclude its use for transplantation.

Evaluation of the arterial vascularity of the pancreaticoduodenal allograft can be tested on the back table by several methods: (1) injection of Renografin into the SMA or Y-graft and obtaining an X ray; (2) intra-arterial injection of fluorescein, with visualization using a Wood lamp; and (3) performing a methylene blue angiogram.

The duodenum should be carefully inspected to rule out a serosal injury. Many groups will open the duodenum at the distal corner staple line, to irrigate and drain the contents into a separate container, then close the opening. Some programs routinely culture the fluid and a small piece of duodenal tissue.

The main principles of back table allograft preparation follow. First, the spleen is removed from the pancreas tail with secure ligatures on the large splenic vessels. The use of the vascular stapler is also acceptable and may be more efficient. Next, it may be useful

to cut the tie off the common bile duct (CBD) and place a cannula (5F feeding tube) temporarily to identify the location of the ampulla by manual palpation. A marking pen can be used to designate its location to accurately ensure its center position as the proximal and distal duodenum are shortened to an appropriate length (the tube is removed and the CBD re-tied). The first portion of the duodenum is dissected a couple centimeters distal to the pylorus and divided proximal to the ampulla with the stapler. The third portion of the duodenum is dissected off the mesentery until adjacent to the pancreas and stapled. Next the staple lines are inverted with 3-0 nylon or silk suture as interrupted stitches. It is important to carefully and liberally invert the corners of the duodenum to avoid its protrusion and eventual breakdown and leakage.

The mesenteric vessels protruding through the pancreas uncinate may have been stapled or individually ligated by the procurement team. The staple line on the root of the mesentery is over sewn with a running 3-0 monofilament suture for reinforcement. The middle colic vessels are secured.

The inflow arterial system is routinely reconstructed. The donor splenic artery and the SMA are identified and prepared for reconstruction by removing surrounding ganglionic tissue. The reconstruction requires use of the donor iliac arterial complex: a Y graft of common, internal, and external arteries.

The iliac arterial and venous complex is a critical inclusion. The arterial complex should be carefully inspected to determine if injury at the internal–external junction has occurred (usually by excessive retraction during procurement). The Y-graft is modified by dividing the internal and external iliac arteries to an appropriate length tailored for the end-to-end anastomoses on the splenic and superior mesenteric arteries of the pancreas allograft, respectively. Typically 7-0 monofilament suture is used as a running stitch, but interrupted stitches would be appropriate, especially if a pediatric donor pancreas is being transplanted. Conversely, if a sufficient length of splenic artery can be mobilized, it is possible to perform a direct end-to-side anastomosis to the superior mesenteric artery.

Occasionally, the pancreas allograft is obtained without a simultaneous liver procurement. In this circumstance the procurement team will return the pancreas allograft on an aortic patch with complete celiac and SMA and intact gastroduodenal artery. In these cases the Y-graft reconstructions are not necessary so long as the celiac and SMA arterial inflow are preserved. Exceptional duodenal graft perfusion will be observed following recipient graft arterial revascularization.

Some groups routinely modify the outflow portal venous vascular system as well. The portal vein is carefully mobilized to allow for appropriate length and determination if a short portal venous extension graft utilizing donor external iliac vein would be useful. A portal vein extension is easily accomplished using donor external iliac vein, aligning in the direction of blood flow using 5-0 or 6-0 monofilament suture as a running stitch.

Following vascular reconstruction, some groups infuse the pancreas Y-graft with 100–200 mL of fresh, cold, preservation solution to examine for parenchymal vascular leaks, which can be repaired prior to recipient revascularization. It is important not to use the solution in the basin for flushing since small particulate matter will be introduced into the organ and increase risk of vascular compromise and thrombosis. Finally, the preservation solution should be exchanged for fresh solution and plenty of ice resupplied in the basin underneath it. The total operative time for the back-table work can exceed 3 hours in a complicated case.

Pancreas transplantation surgery

Preparation

Optimizing the outcome of the pancreas transplant begins prior to the patient entering the operating room (OR): appropriate antibiotic prophylaxis has been ordered; DVT prophylaxis method determined; blood products are confirmed available and appropriate for the estimated blood loss; and the surgical site been marked.

Safety and efficiency in the operating room rely on good communication among the team members. Having all team members introduce themselves facilitates subsequent communication. Prior to commencing the operation several issues should be discussed: confirm correct patient and transplant procedure; the organ allograft is available including donor/recipient ABO compatibility documentation. Surgeon reviews the critical portions of the transplant procedure, its duration and estimated blood loss, including need for central venous access and direct arterial blood pressure monitoring. Surgeon and nursing staff review availability of surgical equipment, retractors, electrocautery devices, lighting, etc. Confirm that appropriate antibiotics and immunosuppression are given.

Exposure

The surgical techniques for pancreas transplantation are diverse (Figure 60.2). The principles are consistent, however, and include providing adequate arterial blood flow to the pancreas and duodenal segment, adequate venous outflow from the pancreas, and secure management of the pancreatic exocrine secretions. The native pancreas is not removed. Adequate exposure is also necessary for intra-abdominal implantation of the deceased donor kidney when an SPK transplant procedure is performed.

Proper exposure is accomplished by either a midline incision or a right lower quadrant oblique incision. Most use a long midline incision, especially if it is an SPK transplant because both organs can be implanted through the single incision. The pancreas is preferentially placed on the right-sided iliac vascular supply. After the midline incision, a modified Cattell maneuver is often used to properly expose the recipient vessels for the pancreas transplant. The right colon is reflected anteriorly and medially by dividing along white line of Toldt lateral to cecum and ascending colon. This will expose the right iliac vessels, aorta, and inferior vena cava. It is not necessary to expose the right kidney and renal vessels or 4th part of duodenum. An alternative method is to retract the small intestines cephalad without mobilizing the right colon and dissecting the iliac vessels and distal vena cava through the retroperitoneal layer. To properly expose the iliac vessels for the intra-abdominal kidney transplant, the descending colon and sigmoid are mobilized medially by dividing along white line of Toldt lateral to descending and sigmoid colon.

If a right lower quadrant incision is used, the retroperitoneal iliac vessels are dissected akin to a kidney transplant. The internal iliac veins are stick tied and divided to achieve liberal mobilization of the vein lateral to the iliac artery. The pancreas is implanted with the head caudal. The peritoneum is opened and the pancreas rotated intra-abdominally, the tail of the pancreas tacked to the anterior abdominal wall and either a duodenocystotomy or duodenoenterotomy performed.

Methods

The surgical techniques for pancreas transplantation are defined according to the methodologies of insulin venous delivery and pancreatic exocrine drainage technique. Also, for the intra-abdominally placed pancreas, it may be oriented with the duodenum cephalad

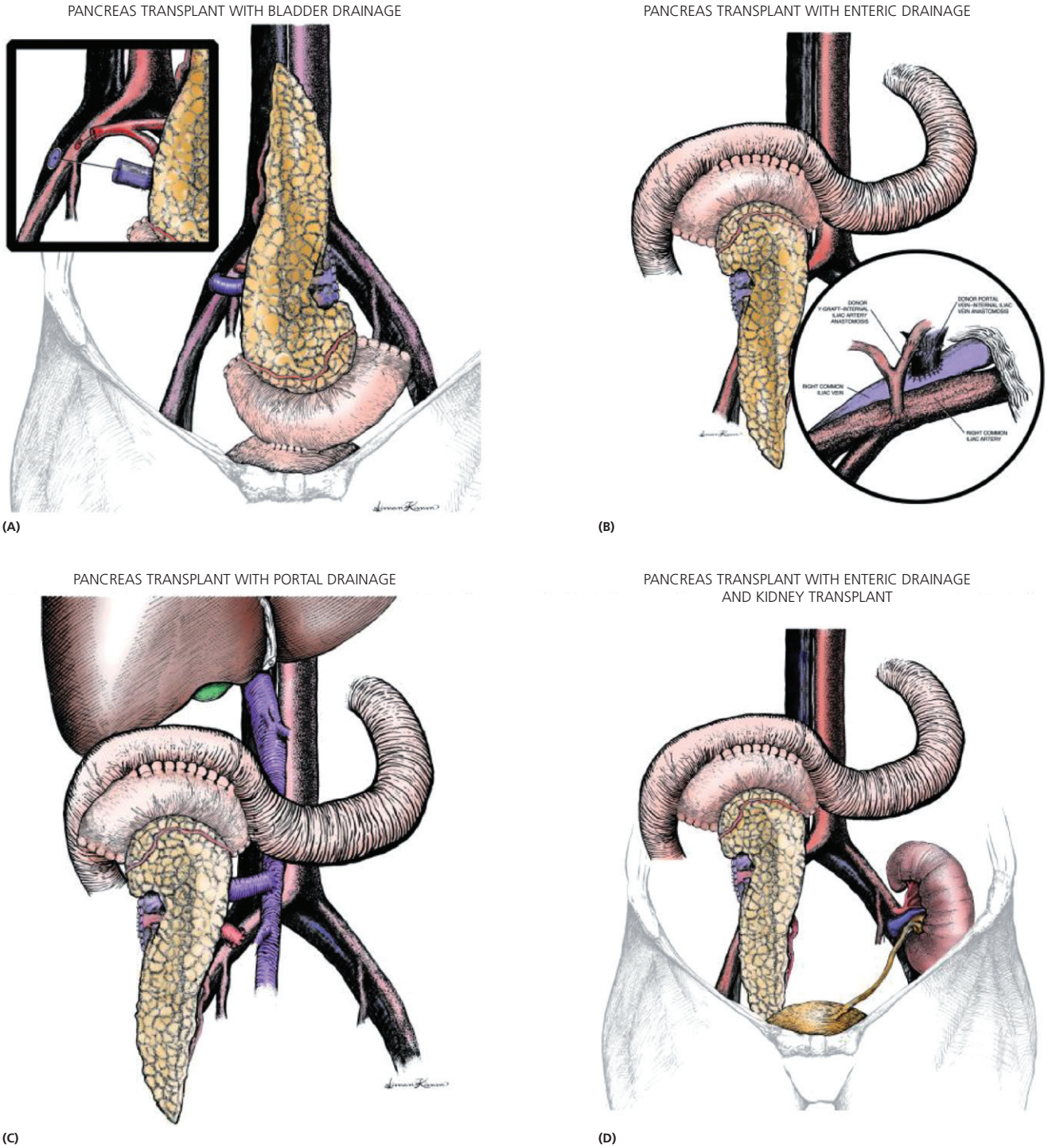

Figure 60.2. (A) Pancreaticoduodenal allograft with systemic venous insulin delivery and bladder exocrine drainage. (B) Pancreaticoduodenal allograft with systemic venous insulin delivery and enteric exocrine drainage. (C) Pancreaticoduodenal allograft with portal venous insulin delivery and enteric exocrine drainage. (D) Pancreaticoduodenal allograft with systemic venous insulin delivery and enteric exocrine drainage with kidney transplant. Reprinted with permission from [20] Stuart FP, Abecassis MM, Kaufman DB. Organ Transplantation, 2nd edition. Georgetown: Landes Bioscience, 2003, p. 168. Copyright © 2003, Landes Bioscience. Illustration by Simon Kimm MD, Department of Urologic Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY.

or caudad. Systemic venous insulin delivery/enteric exocrine drainage is the most commonly applied method, usually with the pancreatic head cephalad. Occasionally, the method of portal venous insulin delivery/enteric exocrine drainage is used, but accounts for approximately 10% of cases. In this case the superior mesenteric vein is used for the venous anastomosis. This vessel is exposed at the root of the mesentery. This technique requires longer arterial length of the common iliac artery Y-graft so it can be dropped through the mesentery to the recipient right common iliac artery. It may be useful for re-transplant procedures in which difficulty of systemic venous drainage is encountered. The other common method is systemic venous/bladder exocrine drainage (portal venous drainage is not possible with exocrine bladder drainage). This technique has waned in popularity coincident with advances in immunosuppression.

Enteric drainage is performed almost uniformly in SPK transplant cases. Bladder drainage is used occasionally with solitary pancreas transplants because of opportunity to utilize urinary amylase monitoring for rejection in this category of cases because of the relatively higher rates of immunological graft losses.

Arterial reconstruction

The pancreas graft arterial revascularization is usually accomplished using the recipient right common or external iliac artery. The Y-graft of the pancreas is anastomosed end-to-side. Positioning of the head of the pancreas graft cephalad or caudad is not relevant with respect to successful arterial revascularization. The arterial anastomosis is typically performed after the venous anastomosis, except when the SMV is used for a portal drained pancreas. In that case, it may be useful to place the arterial graft first. Commonly used arterial clamps are Fogarty Hydragrip vascular clamps with inserts. They are less traumatic on vessels (often diseased) in diabetic patients. The arteriotomy is performed with an 11 blade and the vessel flushed with heparinized saline. An arterial punch can be used to expand the size of the arteriotomy to match the size of the common iliac artery of the Y-graft. The end-to-size anastomosis is performed using 6-0 monofilament suture as a running stitch.

Venous reconstruction

There are two choices for venous revascularization—systemic and portal. Systemic venous revascularization commonly involves the distal vena cava/right common iliac vein, or right external iliac vein. If portal venous drainage is used, it is necessary to dissect the superior mesenteric vein at the root of the mesentery. The pancreas portal vein is anastomosed end-to-side to a branch of the superior mesenteric vein. This anastomosis may influence the methodology of arterial revascularization and necessitates using a long Y-graft placed through a window in the mesentery to reach the right common iliac artery. Portal venous drainage of the pancreas is more physiologic with respect to immediate delivery of insulin to the recipient liver. Portal drainage results in diminished circulating insulin levels relative to those in systemic venous-drained pancreas grafts. The route of venous drainage has no documented clinically relevant differences in glycemic control.

Commonly used vascular clamps for the vein are the single Lambert–Kay or dual atraumatic straight DeBakey clamps. The former may be preferred if partial venous occlusion is sought, often if the venotomy site is the distal vena cava and a previous kidney allograft is in place. The venotomy is performed with an 11 blade or an ellipse cut with the scissors. The vessel is flushed with heparinized saline. The size of the venotomy is fashioned to match the size of the portal vein or the external iliac venous extension graft. The end-to-size anastomosis is performed using 6-0 monofilament suture as a running stitch.

Reperfusion

Following completion of the anastomoses the vascular clamps are carefully removed, venous then arterial. A well-perfused pancreas will have uniform pink color including the duodenum. The duodenum will begin filling with secretions. The revascularized pancreas bleeds more profusely than a kidney allograft. Bleeding vessels are controlled by clamps and suture stick ties. The three most common areas of bleeding are the mesenteric vessels of the pancreatic uncinate, the splenic vessels of the pancreatic tail and arterial vessels of the common bile duct. It is common for systolic pressure of the recipient to transiently decrease due to the blood loss and reperfusion effect. Excellent communication with the anesthesiology team is critical prior to revascularization to ensure adequate central venous pressure, glycemic control, and electrolyte balance of the recipient.

The pancreas is carefully positioned according to the orientation of the pancreatic head. If it is in the cephalad position, the tail is positioned inferiorly into the pouch of Douglas or lateral to the cecum in the right paracolic gutter, depending on how the intestines were previously mobilized. The pancreas is rotated so the splenic artery lies adjacent to the retroperitoneum with the mesenteric vessels oriented superiorly. Visual and manual palpation of the vessels will confirm a strong splenic arterial pulse and soft portal vein without misalignment. The intestines are repositioned around the allograft with head of the pancreas oriented cephalad and superior adjacent to the midjejunum.

Pancreatic duct management

There are several methods of managing the exocrine drainage of the pancreas. Pancreatic exocrine drainage may be handled via anastomosis of the duodenal segment to the bladder or by anastomosis to the small intestine. The bladder-drained pancreas transplant modification was introduced about 1982. This technique significantly improved the procedure's safety by minimizing the occurrence of intra-abdominal abscess from leakage of enteric-drained pancreas grafts. With the successful application of the new maintenance immunosuppressive agents and reduction in the incidence of rejection and leaks, enteric drainage of pancreas transplants has enjoyed a rebirth and largely replaced bladder drainage.

Enteric drainage of the pancreas allograft is physiologic with respect to the delivery of pancreatic enzymes and bicarbonate into the intestines for reabsorption. The enteric drained pancreas can be constructed with or without a Roux-en-Y intestinal limb. The enteric anastomosis can be made side-to-side or end-to-side with the duodenal segment of the pancreas. The anastomosis may be hand sewn or accomplished with the stapler. The former is more common. Typical is a two-layered anastomosis using 3-0 silk suture as seromuscularly placed interrupted stitches as the outer layer and 3-0 absorbable monofilament suture on the through-and-through hemostatic inner layer.

The risk of intra-abdominal abscesses is low with enteric drainage [18], and the avoidance of the bladder-drained pancreas has significant implications with respect to potential complications that include bladder infection, cystitis, urethritis, urethral injury, balanitis, hematuria, metabolic acidosis, and the requirement for enteric conversion. Currently, approximately 75% of pancreas

transplants are performed with enteric drainage, and the others with bladder drainage.

The options of enteric versus bladder drainage depend on the choice of venous drainage and the clinical scenario of the pancreas transplant. For the portal drained pancreas transplant, bladder drainage is not an option. For the recipient of an SPK transplant, enteric drainage is the technique of choice because there is no urinary monitoring benefit and the morbidities as described above are significant. In the cases of PAK and PTA, bladder drainage has two important advantages: (1) urinary monitoring for rejection and (2) placement of the graft, allowing access for percutaneous biopsy for diagnosis of rejection. In the latter situation the advantages of monitoring outweigh the morbidities associated with bladder drainage, at least in the short term, when the risk of immunologic graft loss is significant. However, many programs have had good experience with enteric drainage of the solitary pancreas transplant using other markers for rejections, such as clinical signs and symptoms of pancreas graft pancreatitis and serum amylase or lipase levels coupled with biopsy.

Closure

The fascia is closed with an absorbable or non-absorbable monofilament 0, #1, or #2 size suture. If a peritoneal dialysis catheter is present in a uremic patient, it may be removed prior to fascial closure. Closed suction drains are occasionally placed in the dependent portions of the lower abdomen adjacent to the pancreas into the pouch of Douglas. The subcutaneous adipose is approximated with 3-0 absorbable suture and the skin edges are re-approximated with surgical staples and covered with bacitracin ointment and an occlusive dressing, or as a subcuticular stitch using 4-0 absorbable suture followed by benzoin adhered steri-strips and occlusive dressing.

Conclusion

At the conclusion of the procedure the sponge, needle, and instrument counts are verified as accurate. Appropriate radiological studies are obtained. Postoperative recovery and care plan for the patient is reviewed prior to leaving the OR. Accurate descriptive documentation is completed in operative report shortly after conclusion of the procedure. Communication with patient's family members is immediately conducted in a face-to-face manner.

Summary

A remarkable amount of progress has been made in the field of pancreas transplantation since the first successful procedure in humans in 1966. Pancreas transplantation has evolved from an experimental procedure, with high morbidity and mortality, into an operative procedure with expected excellent outcomes akin to a kidney-alone transplantation. The rise of the field is due to great strides in surgical techniques, immunosuppressive protocols, donor evaluation, preoperative recipient assessment, and postoperative management. The techniques described have been in routine use at our center with excellent results and continue to evolve to make pancreas transplantation a safer and more effective treatment option for patients with diabetes.

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CHAPTER 61

Islet Cell Transplantation Procedure and Surgical Technique

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Introduction

Clinical islet transplantation (CIT) has transitioned rapidly from a rare, experimental curiosity to a reasonably routine clinical procedure in selected centers for patients with unstable forms of type 1 diabetes. Over 850 procedures have been performed worldwide [1], and, at least in expert hands, may now be considered amongst the technically safest of all the transplant procedures when compared to any other solid organ surgery. Given improving short-term outcomes (see Chapter 108), clinical islet transplantation is, in several countries, including Canada, the United Kingdom, Australia, Switzerland, Italy, France, and other parts of Europe, considered and funded as "non-research" standard clinical care. In the United States, the Food and Drug Administration (FDA) is moving rapidly towards a Biological Licensure Application (BLA) for CIT, supported by two parallel trials run through the CIT Consortium. A BLA will facilitate reimbursement through Medicare and Medicaid, and will have major bearing upon future activity in islet transplantation.

This chapter discusses the surgical techniques for pancreas procurement, islet isolation and culture, and minimally invasive techniques for intraportal delivery of islets in the recipient. Strict attention to detail throughout all aspects of the process is required in preparation of clinical Good Manufacturing Practice (cGMP) grade, highly viable islets of sufficient quantity to reverse diabetes in the recipient with minimal risk of complications.

Surgical procurement of the pancreas

The cGMP process used for clinical islet isolation, purification, and culture requires pancreas organs to be procured with utmost precision, with the goal being to ensure pancreatic capsular integrity, to maximize pancreatic oxygenated blood flow prior to aortic cross-clamp, and to minimize handling that could trigger graft pancreatitis [2]. The best islet yields are obtained from donors on minimal inotropic support, and the ideal islet donor overlaps considerably with the ideal donor used for whole pancreas transplantation [3]. The higher body mass index (BMI) donor with a more fatty pancreas tends to digest easier, but donors with underlying type 2 diabetes often have defects in insulin secretion that preclude their suitability in islet transplantation [4–7]. For this reason, donors with a hemoglobin A1C (HbA1c) elevated above 6.5% should not be used routinely for islet isolation. Up till recently, islet yield from the young ideal donor (18–30 years old) tended to be poor, in part due to the increased collagen and more fibrous nature of the younger gland [8–10]. Currently, success rates at several of the CIT Consortium centers have indicated marked improvement in islet yield from the younger pancreas and, combined with excellent clinical outcomes, this may have important bearing on future allocation of pancreas organs for whole organ versus islet utilization [11–15].

The principles behind optimal pancreas procurement are set out in Table 61.1. In general, the techniques used for pancreas procurement for islets are similar to those applied in procurement for whole pancreas transplantation, which are covered in depth in Chapters 22 and 60. The University of Wisconsin (UW) solution (Viaspan, Bristol-Myers-Squibb, New York, USA) was originally designed for optimal preservation of the pancreas [16]. UW "intracellular" solution has been used as the standard pancreas preservation solution but, more recently, perhaps more driven by economic cost saving than direct protective benefit to the pancreas, there has been a shift towards use of other solutions including histidine–tryptophan–ketoglutarate (HTK, Custodiol, Brantford, Ontario, Canada). HTK was originally developed as a cardioplegia solution in the 1970s, and is considered an "extracellular" preservation solution that is low in potassium. The use of HTK has been promoted by the liver transplant community as it avoids the risk of acute cardiac standstill with high potassium bolus previously observed with UW. There are, however, concerns that HTK may lead to inferior outcomes in whole pancreas transplantation, at least in selective centers [17,18]. The Canadian transplant programs switched from use of UW to HTK solution in 2009, and encouragingly this had little impact on the success of islet isolation, and, in fact, with use of the CIT Consortium and modification of their isolation Standard Operating Procedures (SOP), the isolation to transplant success ratio has recently risen from 40% to almost 70% (and an increase in the single donor insulin independence rates), clearly suggesting that HTK is not detrimental to clinical islet isolation success, and indeed may even be beneficial. Caballero-Corbalan et al. compared HTK and UW for human islet isolation and in vitro functionality, and found very similar outcomes provided the pancreas preservation time was kept below 10 hours [19].

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Table 61.1. Surgical principles applied in the procurement of the pancreas for islet isolation

1	Selection of stable donors on minimal inotropic support
2	Absence of underlying diabetes (HbA1c < 6.5%)
3	Minimal handling of the pancreas throughout all stages of procurement
4	Maximize arterialized blood flow before cross-clamp, keeping the superior mesenteric, gastroduodenal, and splenic arteries patent
5	Avoidance of venous congestion of the pancreas during cold perfusion (ideally, complete avoidance of a mesenteric venous cannula, or, if used, maintaining a height differential of >40 cm between arterial and venous cannula)
6	Minimize warm ischemia immediately after cross-clamp by opening lesser sac, packing ice-saline slush behind and in front of the pancreas, and submersion of the pancreas after explant at 4°C in UW or HTK solution
7	Consideration of pancreatic ductal cannulation and low-volume ductal injection with 30 mL of UW, HTK, or ET-Kyoto preservation solution
8	Minimize cold ischemic transport time by use of expedited transportation where available (keep below 6 hours if possible)
9	Use the experienced procurement team from the receiving islet center when possible

HbA1c, hemoglobin A1C; UW, University of Wisconsin solution (Viaspan, Bristol-Myers-Squibb NY USA); HTK, histidine-tryptophan-ketoglutarate solution (Custodiol Inc., Brantford Ontario); ET-Kyoto, ET Kyoto Solution, Otsuka Pharmaceuticals, Tokyo Japan.

Table 61.2. Donor selection criteria[33]

Inclusion criteria	Exclusion criteria
SCD or ECD	
Multiorgan donor	Type 1 or 2 diabetes (HbA1c > 6.5%)
Male or female	Malignancy other than 1° brain tumor
Age 15–75	Untreated septicemia
Warm ischemic time <10 minutes	Viral hepatitis
Cold ischemic time <12 hours	HIV or AIDS
	Syphilis
DCD	
Controlled setting	Viral encephalitis
Warm ischemia <30 minutes	Creutzfeldt–Jacob disease
Cold ischemia <4 hours	Rabies
	Tuberculosis
	Sustained periods of hypotension
	Elevated serum creatinine (>3 × normal)
	Abnormal liver function (>3 × normal)
	Elevated serum amylase or lipase
	High-risk sexual behavior (unless NAT testing available and negative)
	History of recent i.v. drug use

SCD, standard criteria donor; ECD, expanded criteria donor; DCD, deceased circulatory death (non-heart beating); NAT, nucleic acid testing for HIV.
Data from Ponte et al. [33].

The "two-layer technique" has been promoted previously as a more optimal means to increase oxygen delivery to the pancreas during transportation. Originally developed by Kuroda and colleagues in Japan, this technique employs oxygenated perfluorocarbon (PFC) and UW solution, where the pancreas lies at the interface between the two solutions [20]. Subsequent uncontrolled studies suggested improved islet yields when the two-layer method was used [21–27]. However, more recent studies have brought these benefits into question, and, currently, in part due to complexity of widespread application and additional costs, the two-layer method is falling out of favor [28–32].

Appropriate selection of donors for islet isolation remains a critical component of isolation and transplant success [33]. Multivariate analyses have consistently shown that donor age >20 (and possibly <50), high donor BMI (in the absence of type 2 diabetes), donor normoglycemia, absence of sustained hypotension or cardiac arrest, and minimal inotropic requirements are all key variables in islet isolation success (Table 61.2) [14,34–41]. Selection of donors age

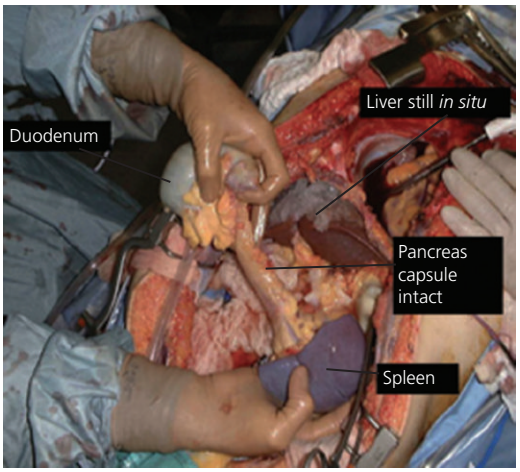

Figure 61.1. Pancreas is removed en bloc together with the first and second portion of the duodenum and spleen, with the pancreatic capsule intact.

<50 with BMI >27 kg/m^2 may have been one of the critical determinants of single donor engraftment success in the University of Minnesota series [42,43].

The surgical steps in multiorgan retrieval follow standard procedure, with minimal handling of the pancreas and minimal vascular dissection before the supraceliac aortic cross-clamp is applied. The lesser sac should be opened by separation of the omentum from the transverse mesocolon, and the anterior surface of the pancreas inspected for signs of trauma, capsular injury, pancreatitis, or mass. The duodenum should be fully mobilized by a Kocher maneuver. The duodenum should be divided distal to the pylorus, and again at the junction of the second and third portion, with two firings of a linear cutting 75 mm stapler. The splenic artery should be exposed at its origin and controlled with a vascular loop. The presence of a replaced right hepatic artery from the superior mesenteric artery is not a contraindication to pancreas retrieval either for whole pancreas or islet isolation, and especially in islet isolation there is no requirement for an SMA vascular pedicle. The gastroduodenal artery should be left patent until after cross-clamp, and may then be ligated and divided. Immediately following supraceliac aortic cross-clamp and chilled UW or HTK vascular flush, the spleen, tail, and body of the pancreas should be dissected and brought to the midline, allowing chilled saline slush solution to be placed both behind and in front of the pancreas, resulting in rapid cooling and avoidance of warm ischemic injury. Ideally, the pancreas should be the first abdominal organ removed after cross-clamping, after division of the common bile duct and portal vein (Figure 61.1). This actually facilitates the completion of the dissection of the hepatic vasculature, and optimizes visualization and protection of a replaced right hepatic artery. The pancreas is removed en bloc with the first and second portion of the duodenum, and is placed in UW or HTK solution at 4°C and triple-bagged for sterile transportation to the islet isolation center. A portion of the spleen is usually removed at this point for HLA-typing.

The Baylor islet isolation team has advocated precannulation of the pancreatic duct via the ampulla of Vater in the operating room, with flushing of the pancreatic duct with 1 mL/g pancreas of ET-Kyoto solution (Otsuka Pharm Factory Inc., Naruto, Japan), and claim that this facilitated consistent high-yield isolations [44–46]. This technique has yet to be broadly adopted, in part as it adds to

the complexity of the postretrieval preparation, and mainly because ET-Kyoto solution is not widely available. Further controlled studies are needed to clarify the utility of this promising approach.

Clinical islet isolation and culture

Restoration of endogenous islet function through intraportal transplantation of large numbers of purified human islets rests on the intricacies of the 6–8 hour islet isolation process, which extracts the 1–2% of islets contained in a 70–100 g pancreas gland that otherwise consists entirely of unwanted exocrine tissue. A series of digestive, rinse, and purification steps reduce the 60 mL digest down to an enriched islet fraction of 5 mL considered safe to infuse into the portal vein. Generally, an islet mass of 5000 IEQ per kg, based on the recipient's weight, is required for a transplant. A single donor transplant is more likely to succeed if the islet transplant mass exceeds 6000–7000 IEQ/kg. An inability to extract such large numbers of high-quality human islets represented a formidable challenge in the 1970s and 1980s and precluded success in early clinical transplant attempts. The introduction of an automated method for islet isolation by Camillo Ricordi in 1986, working in Paul Lacy's laboratory in St. Louis, enabled for the first time an increasingly reliable technique for high-yield human islet isolation, and was the cornerstone of the success of the first pilot clinical trials [47,48]. Although the process has evolved substantially in recent years, the Ricordi automated method remains pivotal, and has been adopted universally by all clinical islet isolation laboratories. More recent refinements have mainly focused on optimized collagenase enzyme blends and delivery, better purification gradients, and stringent control and record of the entire cGMP process (adoption of detailed SOPs, documentation of all steps in the process, and ultra-clean room facilities for appropriate "manufacture" of clinical grade islet product).

Centers working as part of the CIT Consortium have refined detailed SOPs relating to all steps of the clinical isolation and culture process. These protocols remain unpublished, but may be accessed through direct application to the consortium group [49].

Pancreatic ductal perfusion and digestion

The critical steps involved with the digestion and purification of human islets are illustrated in Figure 61.2. The principles involve progressive disassembly of the pancreas down to enriched, intact islets using enzymatic, mechanical, and centrifugal purification. The pancreatic duct is first cannulated (Figure 61.3), and the pancreas is distended with collagenase blend enzymatic solution. The enzyme is first delivered at 4–10 °C for 10 minutes, then warmed for 4 minutes to 37 °C, with the goal being to deliver active collagenase enzyme to the islet–acinar interface [9,50]. The pancreas is then sectioned into several large pieces (usually nine), transferred to the Ricordi chamber containing steel marbles for gentle mechanical agitation against a 500 μm screen. The enzymatic solution recirculates at 37 °C for approximately 15–30 minutes (Figure 61.2A) [51]. This process results in gradual digestion of the pancreas into fragments of decreasing size, until cell clusters containing intact islets are able to escape through the screen. Once islets begin to be released (identified by red color on dithizone staining [52]), the enzymatic digestion is halted by rapid cooling to 4 °C, diluted with multiple wash steps; human albumin solution is added to further quench enzymatic activity. Expert judgment and skill are required to determine when to halt digestion, as early or late dilution results in either inadequate fragment digestion or disintegration of intact islets, resulting in failure to yield sufficient islets for transplantation [8,53].

Collagenase enzymes

Variability in collagenase blend, lot activity, stability, and enzymatic target profile has previously created a substantial challenge to reliable large mass human islet isolation [8]. The introduction of Liberase HI (Roche Diagnostic Pharmaceuticals, Indianapolis USA) was seen as a major advance as this more standardized collagenase enzymatic blend provided more lot-to-lot stability and higher islet yields than previous collagenase types [54]. The Edmonton group found that excess collagenase class II activity was detrimental to islet isolation [55]. It subsequently became apparent that Liberase HI involved exposure to bovine growth factor extract as a feeder layer for *Clostridium histoloyticum* bacteria, with concern that clinical grade Liberase HI could potentially expose patients to a very small risk of Creutzfeld–Jacob related prion disease [56]. While the risk was judged to be exceedingly low (less than one in 100 million), this was felt to be avoidable, and alternative enzyme blends were sought for clinical islet application [56].

Serva Inc. developed a bovine-product-free GMP-grade collagenase and neutral protease designated NB 1 (Serva Electrophoresis GmbH, Heidelberg) with similar high-pressure liquid chromatography (HPLC) profile to Liberase HI [14,57,58]. The Miami group found that the pancreatic digestion times with NB 1 were longer than with Liberase HI, and that beta cell mass and viability was less optimal [59]. The Uppsala group found similar results with reduced islet yield with NB 1, but higher islet purity and glucose stimulation response [58]. The Edmonton group found that separation of the NB 1 and neutral protease components, with ductal delivery of NB 1 and neutral protease addition to the recirculating enzyme, led to less enzymatic degradation and higher and more potent islet yields [60]. In a proportion of cases, additional neutral protease was not required with NB 1 [61]. The University of California San Francisco (UCSF) group conversely found improved digestion profiles in donors age <45, with NB 1 dosing at 16 U/g of pancreas combined with neutral protease at 2 U/g of pancreas and 2.5 mL of DNAse (Pulmozyme, Genentech, San Francisco) [62]. In the UCSF experience, 9 of 12 pancreata were clinically transplanted from young donors, and high clinical functional potency was observed [12,63].

Most recently, a newer version of Liberase Mammalian Tissue Free (MTF) has been developed by Roche Diagnostics Inc. Caballero-Corbalan et al. observed reduced digestion times with reduced degradation of collagenase class I, and suggested that a two-component product with individual adjustment of thermolysin would be beneficial [64]. O'Gorman et al. found excellent digestion characteristics of MTF with similar functionality to NB 1 [14]. An alternative collagenase, called Vitacyte HA (Vitacyte LLC, Indianapolis), has been developed with controlled component activity and reduced class II degradation, and is being further evaluated in clinical islet isolation [65].

Purification

The final step in the process involves purification of the pancreatic digest using density-gradient separation, carried out on a refrigerated COBE2991 cell processor [66,67]. Typically, the purification centrifugation run takes 10 minutes. Originally, discontinuous Ficoll gradients were used, but more recently continuous gradients of iodixanol (Optiprep, Axis-Shield, Oslo), as introduced by Hering et al., have demonstrated increased purification efficiency [42]. The use of iodixanol has been associated with less inflammatory

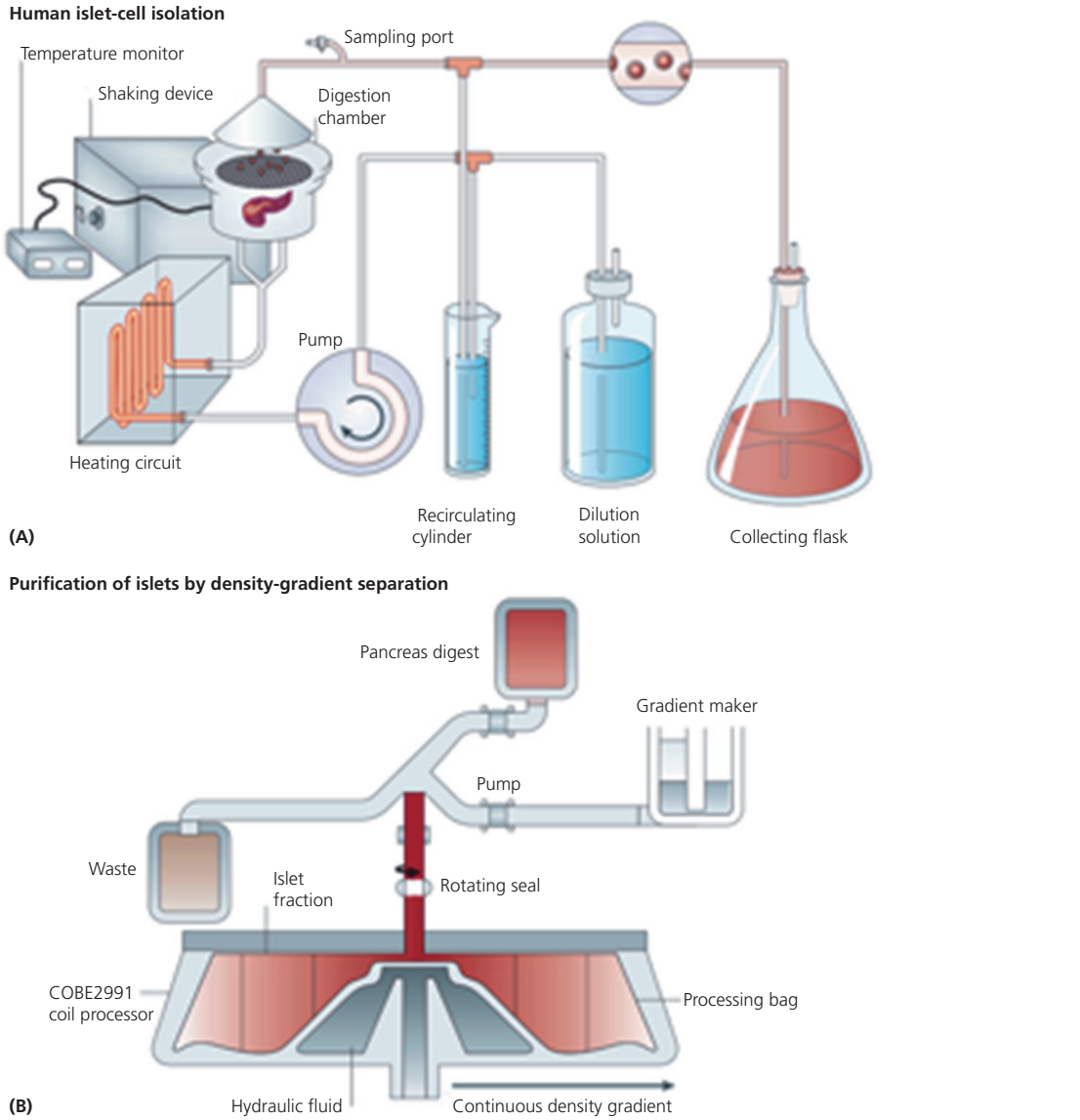

Figure 61.2. (A) Schematic diagram of the Ricordi chamber, 500-μm screen, steel marbles, and recirculating pump system used to digest the pancreas. (B) Diagrammatic representation of the COBE 2991 centrifuge cell processor used for purification of the pancreatic digest using continuous density gradient separation. Reproduced from [51] Ricordi C, Strom TB. Clinical islet transplantation: advances and immunological challenges. Nature Reviews Immunology 2004;4(4):259–268. Copyright ©2004, with permission from Nature Publishing Group.

activation compared with the previous Ficoll gradients [68]. Barbaro et al. demonstrated improved purification efficiency when iodixanol-based gradients are combined with UW solution to increase the differential density of exocrine versus islet tissue on the COBE2991 [69].

The use of rescue gradients to improve islet recovery on purification have been promoted by several groups [70–72]. The Vancouver group found that repurification of the impure tissue fractions after a further 12–36 hours of culture resulted in a 20% increase in islet yield [70]. The Miami group found that addition of a repurification step using discontinuous gradients substantially increased the rate of islet preparations suitable for clinical implantation based on islet mass, and that the additional step had no negative impact on islet functionality [72,73].

Islet culture

After further wash and recombination steps, the final islet preparation is placed in culture media and incubated for 24–72 hours before release and clinical transplantation. A period of islet culture leads to increased purification, and transplantation of islets and impurities in a less inflammatory state. Furthermore, this period facilitates patient management by allowing transportation time to the transplant center, and opportunity to administer T-depletional and other adjunctive therapies to the recipient, while avoiding exposure of newly transplanted islets to a potentially injurious cytokine storm [39,74–79]. Up to 20% of the islet mass may be lost during islet culture, with prolonged cold ischemia, lower islet purity, and higher islet index being important factors on multivariate analysis [80]. Islet culture at 24°C was previously shown by

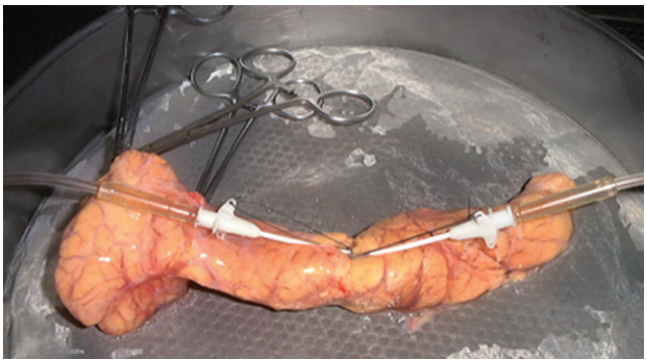

Figure 61.3. The main pancreatic duct is cannulated and a solution containing a blend of collagenase enzymes is recirculated, leading to distension of the pancreas and delivery of the enzyme to the acinar–islet interface.

Table 61.3. Islet product release criteria

Sterility
Gram stain: no bacteria
Endotoxin content: <5 EU/kg based on recipient weight
Culture: no bacteria or fungal elements (available post hoc after 14 days' culture)
Potency
Static insulin release: stimulation index >1.0
Volume
Packed tissue volume ≤5.0 mL or settled tissue volume ≤7.5 mL
Purity
≥30% (based on dithizone staining)
Viability
≥70% (based on membrane integrity staining with FDA/PI or Syto green)
Minimal islet mass (protocol dependent):
≥6000 IEQ/kg for single donor protocols
≥5000 IEQ/kg for routine initial transplants
≥4000 IEQ/kg for retransplant in multiple infusion protocols
Compatibility
ABO blood group compatibility
Negative cytotoxic cross-match (required if PRA > 10%, or depending on protocol)

FDA/PI, fluorescein diacetate/propidium iodide.

Markmann et al. to markedly reduce MHC antigen expression [81]. The addition of insulin, transferrin, zinc, selenium, and pyruvate to CMRL-based culture media as promoted by the Miami group with the addition of nicotinamide [82], and recently modified by the Little group, appears to optimize islet survival in culture [83–85]. A period of islet culture also permits islet preparations to be shipped between cGMP isolation and clinical transplant centers, concentrating skill and expertise locally and minimizing costs associated with islet isolation [73,86,87].

Islet graft assessment and product release testing

The FDA have emphasized the need for predictive islet potency assays to correlate with clinical islet efficacy, as islet transplantation moves forward to BLA. This has been a challenging area as the standard tests of islet viability using dye-exclusion based membrane integrity assays are insensitive [88], and in vitro insulin secretion in response to glucose with either static incubation or islet perifusion often fail to predict functionality in vivo. Several approaches are currently under active investigation to help better refine the sensitivity and positive predictive value of these tests. Cabrera et al. developed a combined high-throughput multiple automated islet perifusion system and kinetic flux imaging system for beta-cell potency, which may fulfill the niche required by the FDA for predictive potency testing [89]. Pappas et al. introduced an oxygen consumption rate analysis system that strongly predicts both graft function of human islets transplanted in to immunodeficient mice as well as clinical outcome after islet transplantation [90]. Novel methods designed to quantify the viability of specific islet cell subsets using flow cytometer is proving to be one promising approach. Ichii et al. have developed a multiparametric analysis of beta-cell content, viability, and cellular composition using laser scanning cytometry and mitochondrial apoptosis, and this is currently undergoing more detailed testing within the CIT centers [91].

Finally, prior to release for clinical transplantation, the cultured islet preparation must meet all product release criteria (Table 61.3). These minimal requirements reflect a need to confirm sterility and compatibility of the final islet product to prevent transmission of infection to the recipient, while providing an adequate, viable islet implant mass of sufficient purity to minimize risk of portal venous thrombosis.

Percutaneous intraportal islet transplantation in the recipient

The fact that the portal vein can be accessed through a non-surgical, minimally invasive approach by percutaneous transhepatic ultrasound and fluoroscopic guidance (Figure 61.4) distinguishes islet transplantation from whole pancreas transplantation, and makes it one of the safest, lowest risk, and attractive of all the different transplant procedures, at least in expert hands. The percutaneous approach was first described by Weimar et al. using a combination of CT and fluoroscopy [92]. The Edmonton group used a combination of ultrasound and fluoroscopic-guided percutaneous portal access for their initial series of seven patients attaining insulin independence [93]. This technique has been further refined by close collaboration between groups, for a technique that now safely allows for full therapeutic anticoagulation with heparin in the immediate peritransplant period together with effective methods to eliminate the risk of bleeding after the procedure. Effective methods that simultaneously prevent risk of portal venous thrombosis and prevent bleeding are essential if this procedure is to be carried out with the lowest morbidity.

Most interventional radiologists have considerable expertise in percutaneous transhepatic access in the setting of transhepatic cholangiography and intrahepatic biliary drainage procedures, and this technique has a proven safety track record.

The use of preliminary color duplex ultrasonography has been shown to reduce the number of liver capsular punctures, avoids inadvertent biliary punctures, and significantly reduces the procedural time [94,95]. A high-resolution fluoroscopic C-arm set up with rotational digitized imaging facilitates portal catheter placement. Access to a second or third-order portal branch, away from the central liver hilum, minimizes risk of hepatic arterial injury, and provides a better parenchymal track for subsequent ablation.

Before proceeding with the percutaneous transhepatic approach, patients must have no evidence of right-sided hepatic hemangioma, and ultrasound screening of the liver to confirm this is important before the patient is listed for the procedure. There should be no underlying coagulopathy or portal hypertension, and the underlying liver parenchyma should be non-cirrhotic. Antiplatelet agents (e.g. clopidogrel, dipyridamole) and direct thrombin or Xa

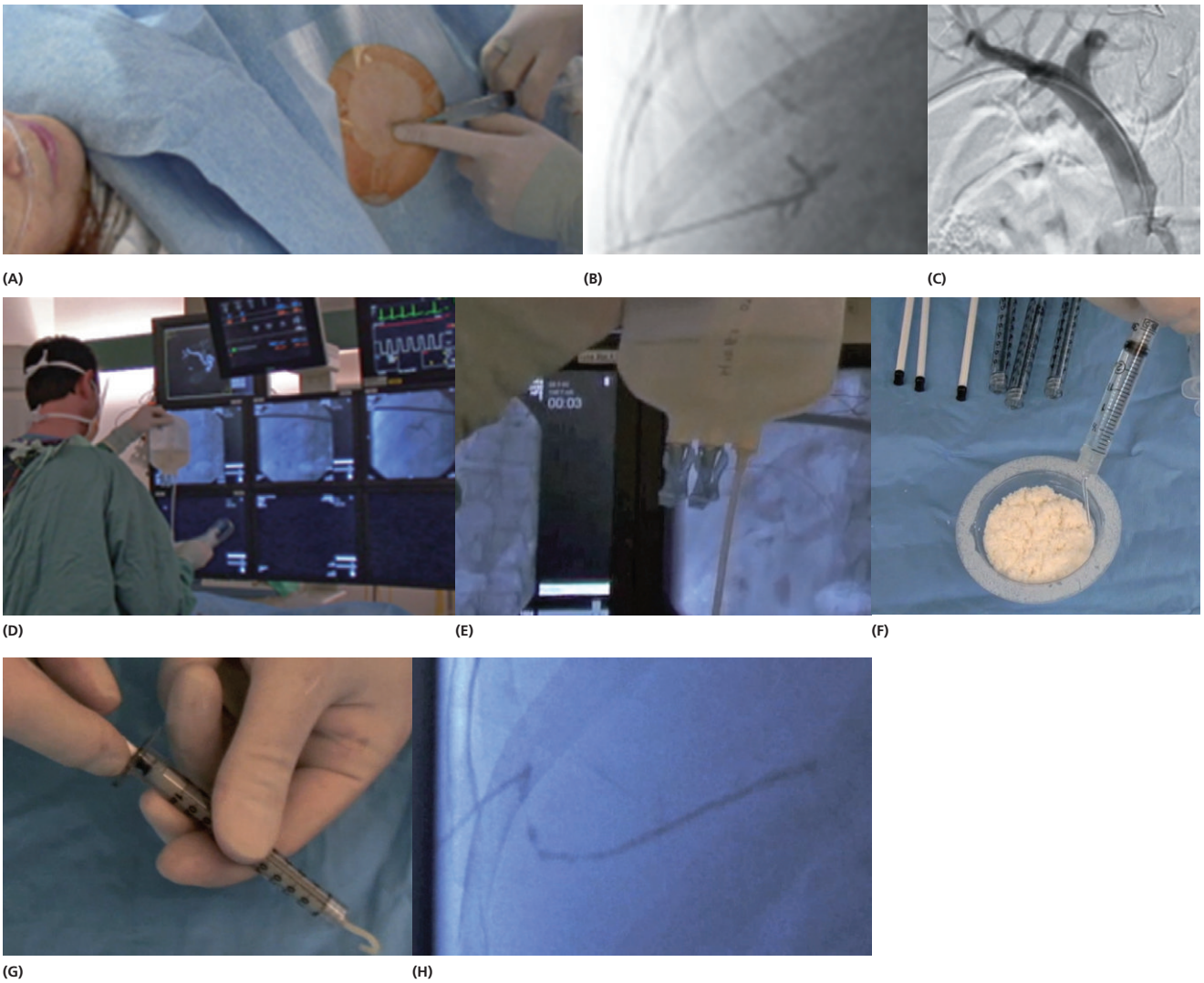

Figure 61.4. Steps involved with percutaneous islet transplantation via an ultrasound-guided fluoroscopic approach: (A) Doppler ultrasound interrogation followed by local anesthesia; (B) direct puncture of a peripheral second or third-order right-sided branch of the portal vein, with recognition of the branching pattern on fluoroscopy; (C) portal angiogram with the tip of a 4 or 5 French catheter at the portomesenteric splenic junction; (D) islet infusion under gravity after addition of 70 U/kg (based on recipient weight) heparin to the islet bag, with intermittent direct monitoring of portal pressure; (E) close-up of the i.v. tubing with transplant tissue visible; (F) making up microfibrillary collagen paste (Avitene) with 1 g powder and 3 mL saline and 3 mL radiological contrast media; (G) loading the paste in a 1-mL syringe; (H) obliteration of the catheter tract for a minimal length of 4 cm, under direct fluoroscopic visualization.

inhibitors (e.g. dabigatran, rivaroxaban) should be discontinued within 7–14 days of the procedure, which for practical purposes means that patients on the top of the transplant list should be discontinued from all such medications if safe to do so, or delisted for the procedure. Patients with a known underlying thrombophilia disorder (protein C, S, antithrombin III, factor V Leiden deficiency) should not undergo percutaneous intraportal islet transplantation [96].

The radiologic techniques used for percutaneous portal access have been described in detail by Owen et al., Gaba et al., Goss et al. and others [97–102]. In brief, after local anesthetic skin infiltration, a 22 gauge Chiba needle is advanced in to a peripheral branch of the right portal system. An 18 gauge guide wire is then advanced through the needle into the main portal vein. The guide wire is then exchanged for a single-lumen 4 or 5 French angio catheter (NEFF, Cook Canada, Stouffville, Ontario) or equivalent, with the tip directed down to just above the portomesenteric–splenic venous confluence. A portal venogram is then obtained to confirm position, and a baseline measurement of the portal venous pressure is obtained.

The final islet preparation, suspended in transplant media in a Ricordi bag, is then loaded with heparin (70 units per kg heparin based on recipient weight), and infused under gravity while the portal pressure is measured intermittently throughout the infusion [103]. If the baseline portal pressure exceeds 20 mmHg, or if the portal pressure rises above 22 mmHg during infusion, no further islets are given until the pressure normalizes, to avoid added risk of portal vein thrombosis.

Upon completion of the islet infusion, the portal catheter is slowly withdrawn through the hepatic parenchyma, while infusing

thrombostatic paste. The Miami group has used D-STAT (Vascular Solutions, Minneapolis, MN) for this purpose with success [104]. The Edmonton group has advocated use of Avitene paste (Medchem Products, Woburn, Massachusetts, USA) made up as 1 g of Avitene powder in 3 mL saline and 3 mL radiological contrast media [105]. Microfibrillary collagen made up in this way forms a thick paste that is radiographically visible by fluoroscopy, and, when deployed over a minimal track distance of at least 4 cm, has eliminated the risk of liver capsular bleeding in Edmonton, now in an accrued experience of over 360 percutaneous access islet procedures [105,106]. Alternative techniques include use of Gelfoam plugs and metal coils, but are less reliable and more easily displaced, at least in our experience.

Once the track is adequately ablated, therapeutic systemic heparinization is continued in the radiology suite at a rate of 3 units per kg per hour, and titrated to maintain a partial thromboplastin time (PTT) of 60–80 seconds once the patient is being monitored on the ward. The heparin infusion is continued for 48 hours, and this may improve single-donor islet engraftment and minimize activation of the instant blood mediated inflammatory reaction (IBMIR) [107,108]. At the time of patient discharge from hospital, low molecular weight heparin is continued (enoxaparin 30 mg s.c. twice daily for 7 days) together with aspirin 81 mg enteric coated for 14 days.

We recommend that in the rare event of inadequate track ablation, or where portal access was traumatic, that postprocedural heparin be withheld temporarily, and a repeat Doppler ultrasound be obtained at 2 hours post procedure. This will facilitate early diagnosis of potential intraperitoneal bleeding, and allow appropriate heparin management. With this combined Avitene and therapeutic heparinization approach, the risk of both bleeding and portal thrombosis has been effectively eliminated [95,105,106]. If bleeding occurs from the capsular surface, it may be dealt with by direct laparoscopic cautery, as in this rare instance of hepatic trauma the precise site is known and easily accessible by this approach [95].

Post transplant, hemoglobin and liver function should be monitored closely, and a routine Doppler ultrasound of the portal system and liver should be obtained at 24 hours and at 7 days. Complete occlusion of the entire portal tree is exceedingly rare after clinical islet transplantation (0% in 364 consecutive islet allograft procedures in Edmonton) but can have serious potential consequences [109]. Partial branch venous occlusion of a peripheral, anterior, or posterior segmental branch may occasionally occur, and while this carries very low risk of further propagation, is best managed by intravenous heparin followed by 3 months of Coumadin therapy with serial Doppler screening for evidence of resolution.

Small arteriovenous fistulas may rarely be observed after percutaneous transhepatic access (risk less than 1%), and can be safely ablated by percutaneous hepatic arterial embolization [100,110].

Open techniques for intraportal access

Where percutaneous access is not possible (e.g. large hepatic hemangioma or lack of local radiological expertise), an open surgical technique has been advocated. Fewer than 1% of procedures require an open approach in the largest clinical centers. In this case, the portal system can be accessed surgically by a minimal approach by dissection around the umbilicus and recanalization of the obliterated left umbilical vein, which provides access to the left portal system [48,111]. Alternatively, a limited laparotomy with delivery of an omental or mesenteric vein into the wound allows direct catheterization. The most effective approach is to pass a dual-lumen 9 French Broviac-type central line until the tip reaches the main portal vein, as this allows for simultaneous monitoring of portal pressure through the smaller lumen while islets are infused through the larger lumen [112].

Summary

The detailed techniques of surgical procurement of the pancreas for islet isolation, and the steps involved in cGMP human islet manufacture for clinical transplantation are reviewed in this chapter. The alternative approaches to safe portal vein access are reviewed, and the critical steps to avoid risk of portal thrombosis and bleeding are discussed in detail. Expertise in all aspects of islet preparation and clinical care are needed to avoid potential complications.

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CHAPTER 62

Intestinal Transplantation Procedure and Surgical Technique

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Introduction

Intestinal transplants may be performed alone or in combination with other organs. When the jejunoileum alone is transplanted, this is referred to as an **isolated intestine allograft** [1]. This allograft is based on the vascular supply from the superior mesenteric artery, with drainage through the superior mesenteric vein. However, intestinal allografts may be modified based on the co-morbidities existent in other abdominal organs of a particular patient. Some patients have undergone prior colon removal (Crohn's colitis) or stomach surgery (gastric bypass); some have developed chronic pancreatitis (associated with parenteral nutrition); while others have suffered renal failure (oxalic acid stones, repeated dehydration) or have experienced a host of other complications of intestinal failure or parenteral nutrition.

The colon may be transplanted along with the small bowel and, less commonly, the foregut may be transplanted, such that a stomach, duodenum, and pancreas are included in the graft [2,3]. Multivisceral allografts [4] including the liver are described separately in Chapter 63. Determination of the type of allograft to use is up to the individual, and anatomical or functional considerations may be addressed with a variety of approaches. Recipient characteristics also determine the most commonly employed specific implantation technique to use [5]. Preoperative considerations are addressed in Chapter 42 and postoperative management and outcomes can be found in Chapters 74 and 109, respectively.

Surgical technique

Isolated intestinal transplantation

Abdominal exposure

The most common clinical indication for isolated intestine transplantation is a patient with short bowel syndrome. Such patients have always undergone prior abdominal surgery, and the abdominal wall may contain areas of scar from enterostomy, enterocutaneous fistula, or prior gastrointestinal tubes or drains. When these complicate the abdominal wall, a midline incision is usually the most versatile for implantation of an isolated intestine allograft. Occasionally, abdominal wall reconstruction with advancement flaps, tissue loss, or skin grafts complicate the midline. In such cases a broad bilateral subcostal incision placed midway between the xiphoid and umbilicus may be preferable if it can be performed above the area of reconstruction. The choice of allograft for such transplants is critical to the outcome. Closure of the abdomen is best achieved utilizing a small graft from a donor one-third to one-half the size or weight of the recipient. Open abdomen with temporary closure is sometimes necessary, but it is associated with significant morbidity and a decreased survival rate. A variety of methods have been used to obtain coverage of the allograft [6–11]. In rare circumstances, a composite allograft of the abdominal wall has been transplanted along with the intestinal allograft.

After the incision is made, adhesiolysis is performed to clear the sidewalls of the abdominal cavity to the retroperitoneal space. The loss of domain inherent with short bowel syndrome usually requires maximizing the abdominal cavity and relaxing incisions on the lateral endoabdominal fascia or component separation to enhance laxity of the overlying abdominal wall. A self-retaining retractor is utilized for exposure. The diseased remaining small intestine or colon is a frequent cause of postoperative morbidity if not removed, so mobilization of this bowel is imperative. In cases such as Crohn's disease, areas of prior strictureplasty, enteroenteric anastomosis, fistula, or stricture are best mobilized from the pelvis and removed with a stapling device. Maintaining at least 3–5 cm of jejunum from the ligament of Treitz is helpful for restoration of proximal intestinal continuity. However, long segments of even healthy bowel may make enteroscopy for later graft surveillance more difficult. We recommend saving no more than 20 cm. Similarly, maintaining some native colon is advantageous for water absorption after closure of the stoma. If healthy distal colon is present, we attempt to salvage the left hemicolon for continuity. The remaining diseased bowel is then removed after high ligation of the mesentery.

Vascular exposure in short bowel patients

In most cases of short bowel syndrome the vascular approach will use the infrarenal abdominal aorta for inflow, with systemic outflow through the infrarenal vena cava [2]. The aortic exposure is best obtained after mobilizing the duodenum gently from the retroperitoneal attachments. Exposure of the crossing left renal vein (LRV) then demonstrates the superior extent of dissection. Ligation of lymphatics helps prevent post-transplant fluid collections or chylous ascites. Care to preserve the inferior mesenteric artery (IMA) is important, as the loss of collateral circulation from the small bowel and prior surgery sometimes leave this as the sole blood supply to the descending colon or sigmoid. A few lumbar vessels sometimes require ligation between the left renal vein and inferior mesenteric artery and then a Satinsky clamp will control the aorta (Figure 62.1A–C). Vena cava exposure is aided in some

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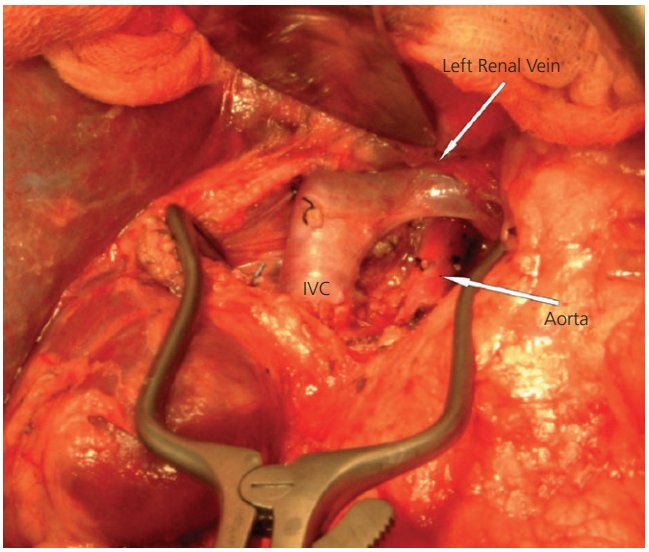
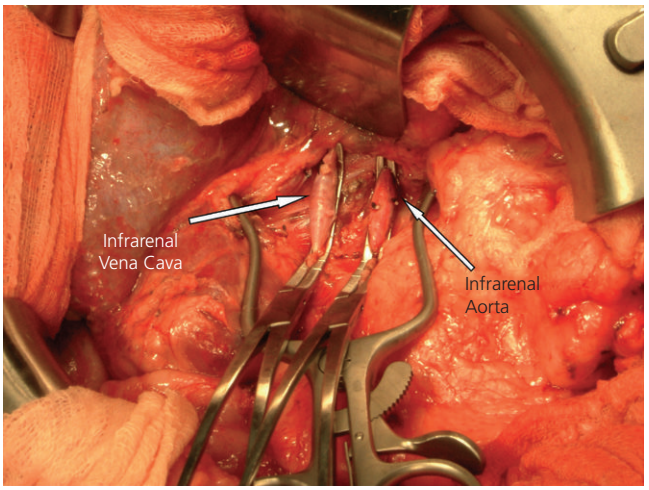
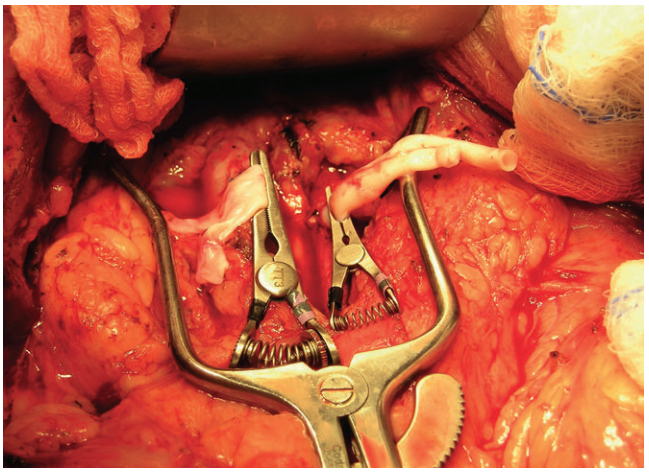


(A)

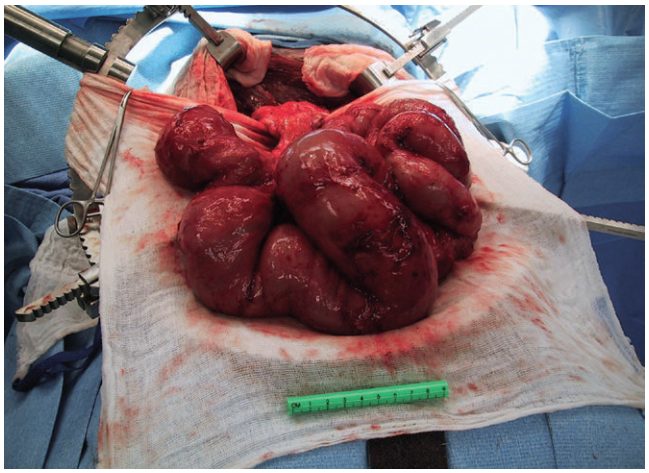


(B)

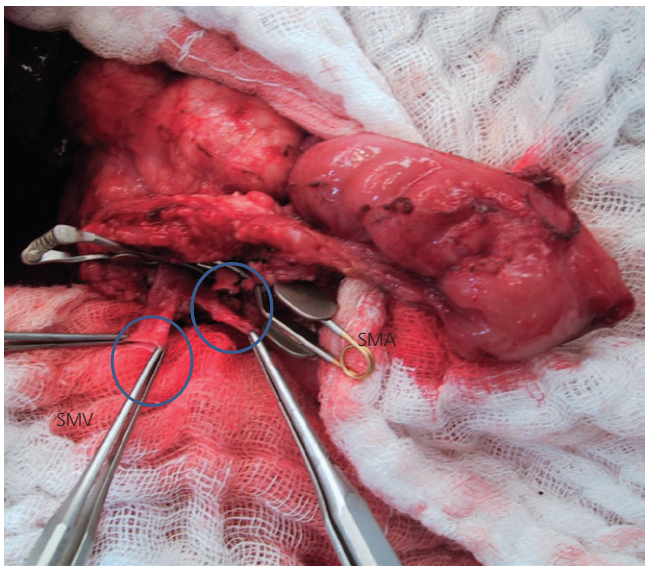


(C)

Figure 62.1. (A,B,C) Exposure and preparation of the infrarenal aorta and vena cava for extension graft placement or direct anastomosis.



(A)



(B)

Figure 62.2. Removal of diseased native bowel (A) with preservation of the mesenteric vessels, transection of proximal jejunum, and preparation for graft implantation with SMV on left and SMA on right (B).

situations by division of the right gonadal vein and, sometimes, by one or two lumbar veins. Preoperative imaging should identify a retroaortic or circumaortic left renal vein if it exists. Care must be taken not to injure either the dependent third portion of the duodenum or the renal veins during this exposure.

Exposure for functional intestinal disorder patients

In cases where the patient suffers from a primary functional disorder, such as an enterocyte or motility disease, the native intestinal tract will most commonly be in place. In such cases, total enterectomy is required. These patients usually have less or no loss of domain and, particularly in dilated motility disorders, a lax abdominal wall is common. Either midline or bilateral subcostal incision is suitable. Enterectomy proceeds along the same vascular lines as for donor enterectomy (Figure 62.2). The ligament of Treitz is mobilized, and the right colon is mobilized along the white line of Toldt with a broad Catell–Brasch maneuver to the base of the

mesenteric vessels. Division of the left transverse colon and dissection of the base of the transverse mesocolon allows the entire small bowel and right colon to be mobilized to the right lower quadrant. This move and division of the proximal jejunum define a plane along the base of the small bowel mesentery. We have found it helpful to place a laparotomy pad behind the mesentery and under the bowel to isolate the base of the mesentery (Figure 62.2A). The mesenteric fat and lymphatics are then dissected and ligated, allowing the superior mesenteric artery (SMA) and vein (SMV) to be skeletonized (Figure 62.2B). Knowledge of the anatomy of the proximal mesentery is critical here. The proximal jejunal arcade should be preserved to the residual native jejunum. The main jejunal vein then usually courses posterior to the superior mesenteric artery in most cases. Isolation of a 2-cm portion of artery and vein just below where they cross over the duodenum allows mesenteric implantation of the allograft with direct anastomosis.

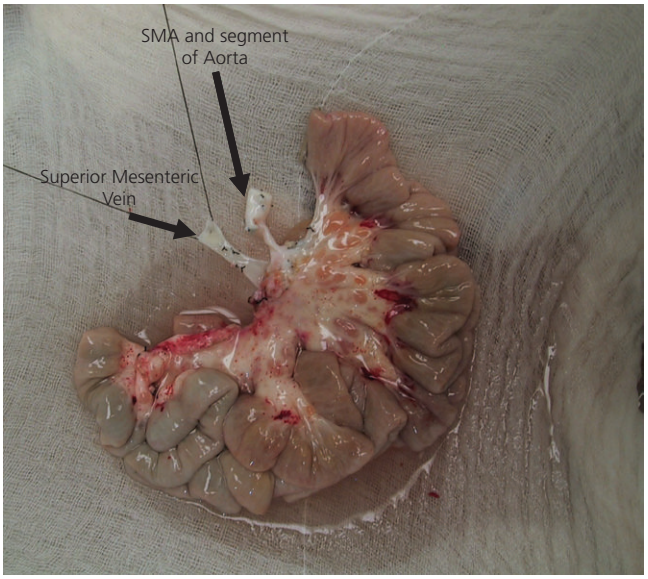
Systemic drainage graft implantation

When the allograft is being implanted, care should be taken to match vessel length to the particular recipient. In the case of systemic implantation, if no pancreas was procured from the cadaveric donor and the full SMA and SMV remain with the allograft, direct anastomosis to the aorta is preferred by some surgeons. Control of the aorta is established with a partially occluding clamp, and an anterior aortotomy is made. However, small bowel loops of the graft sometimes descend in a deep retroperitoneum, as is typical of small children. Gentle flattening of the base of the donor mesentery with upward retraction toward the point of arterial anastomosis by the assistant is critical to facilitate implantation. Here, it is important to be aware that the normal small bowel mesentery sits with an angle to the retroperitoneum, with the jejunum slightly higher in the anterior–posterior dimension, and the ileum more posterior. When the graft is implanted, the mesentery should be positioned to lie flat and parallel to the retroperitoneum. Even in systemically drained cases with a full donor artery, I prefer placing short segment interposition grafts on the aorta and vena cava prior to taking the allograft off the ice [12]. This allows easy positioning of the graft with anastomosis somewhat less recessed in the retroperitoneum.

End-to-end anastomosis of the artery is performed and a growth factor is used to allow dilatation of the artery at reperfusion. The vein length is measured so that no redundancy and kinking occur. The venous anastomosis is performed as in the manner of portal vein anastomosis in liver transplantation, with a growth factor through which preservation fluid may be flushed prior to removal of the vena cava clamp. As in the case of mesocaval shunt construction, the systemically drained allograft is at risk for slight twists and shuttering of the outflow anastomosis. For this reason, a wide ellipse of anterior vein wall is always excised. Another pitfall to be avoided is vascular kinking. When no colon exists, or the size of the donor organ is significantly smaller than the native bowel, the allograft will tend to sink towards the pelvis after reperfusion. Traction on the vessels may result if they are too short, causing arterial narrowing. Thus, the interposition graft or the donor SMA should be left long enough to create an arc similar to that of the native SMA, but not long enough to kink.

Mesenteric drainage graft implantation

When the native mesenteric vessels are used for anastomosis, the proximal vessels are controlled with atraumatic bulldog clamps, and no aortic or vena cava exposure is necessary. Pre- and postimplantation orientation is shown in Figure 62.3 A and B, respectively. The mesenteric vessels should be large enough in caliber to match the size of the donor vessels. While these vessels may be isolated in some short bowel syndrome patients, they are often atretic due to the low flow state and provide insufficient flow. The length of the remaining native vessels should then be evaluated. The donor bowel will lie below the transverse duodenum, over which the native vessels course. Therefore, the weight of the allograft may produce tension on these vessels if they are of insufficient length. In this case, interposition grafts of external iliac artery and vein are often useful. The alignment of the graft usually places the jejunum slightly higher than the ileum in the right lower quadrant, such that the slight angle of the graft from higher on the left side to lower on the right side is reapproximated. The vessels are then reconstructed in the same manner as described above.



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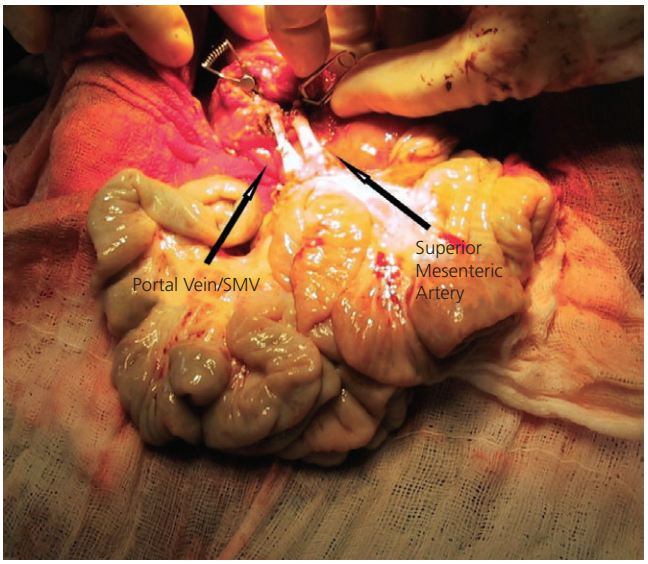


(B)

Figure 62.3. Before (A) and after (B) implantation with mesenteric drainage. The cuff of aorta has been removed and direct end-to-end anastomosis performed between native and graft mesenteric vessels.

Reperfusion

The intestinal allograft is large and capable of causing significant reperfusion-associated hemodynamic instability. For this reason, we always perform a blood flush in which the venous anastomosis is performed with a growth factor. This is left open and the distal venous outflow clamp remains in place during arterial reperfusion. The graft fills with fresh oxygenated blood, and the preservation fluid is flushed through the growth factor prior to removal of the distal clamp. The venous suture is then either tied or, if the growth factor is generous, a gating suture may be used. Volume resuscitation should be used during reperfusion, and agents causing splanchnic vasoconstriction should be avoided. Small bleeders in the hilum of the mesentery should be ligated with fine monofilament suture with care to avoid narrowing a major venous branch.

Mesenteric fixation

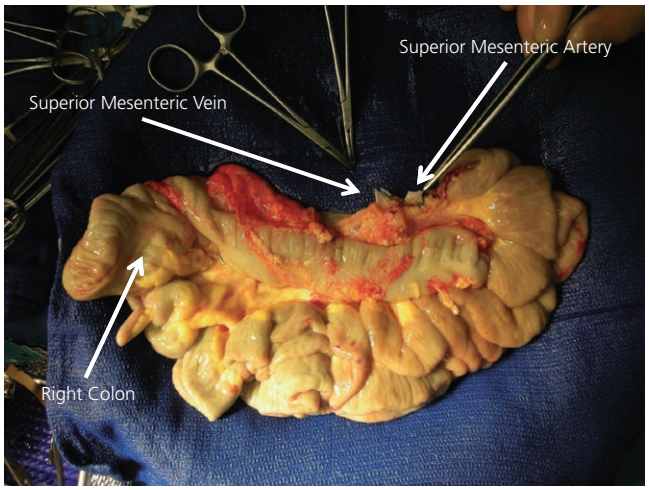
After reperfusion the allograft has a narrow point of fixation at the mesenteric hilum. This places it at risk of volvulus and the bowel rapidly develops a secretory state and becomes laden with mucin and heavy. The base of the mesentery must be fixed to the retroperitoneum with interrupted sutures. Care should be taken to avoid the ileocolic vessels along the cut edge of the allograft, and both sides of the mobilized mesentery should be fixed in place. Another pitfall to be avoided is ureteral injury in the patient who has undergone multiple prior operations. We liberally employ preoperatively placed ureteral stents in such patients.

Restoration of enteral continuity

After fixation of the mesentery, the proximal allograft jejunum may be reconstructed to the native jejunum in the usual hand-sewn manner. A well-preserved allograft usually holds sutures well, and the mucosa of the proximal jejunum provides information about the degree of reperfusion injury to be expected. If a gastrojejunal tube is being used to permit early jejunal feeding, this should be placed through the stomach, threaded around the duodenum, and placed across the anastomosis under direct visualization after the posterior wall has been sewn. The anastomosis is then completed. Distal reconstruction can be accomplished according to the native colon that is retained. If a significant portion of colon is not retained, an end ileostomy may be created. If left colon is retained, either an end-to-side anastomosis can be constructed with a loop ileostomy approximately 20 cm proximal, or a Santulli-type ileostomy can be constructed. It is critical in either case to both fix the base of the ileal mesentery and attempt to close the lateral defect around the ileostomy to the endoabdominal fascia.

Colonic transplantation with the isolated intestine

Patients with a functioning rectum but no appreciable colonic length to allow water reabsorption may be candidates for colonic inclusion in the allograft. In this case, the graft implantation is similar, but there is no true cut edge of the mesentery; therefore fixation of the base of the mesentery relies on the posterior leaflet instead. Usually, the right hemicolon is transplanted, and the distal transverse colon must reach to the pelvis for restoration of enteral continuity [13]. The usual orientation involves mobilization of the transverse colon such that the ascending colon lies somewhat transversely and the transverse reaches the rectum. An example of the preimplantation orientation is shown in Figure 62.4A. A proximal loop ileostomy is created, allowing proximal surveillance of the graft and distal evaluation of the colon (Figure 62.4B). Closure of the defect posterior to the mesentery to prevent herniation is important, if it is possible. Alternatively, the colon may be transplanted and an end colostomy created, for later closure to the rectum. I do not prefer this technique, as endoscopic surveillance through the ileocecal valve is required of the endoscopist to sample the ileum. Additionally, daily monitoring of the mucosal pattern of the exteriorized ileum is required, and ileostomy effluent is not available for early rejection monitoring. The colon is rarely subject to early rejection.



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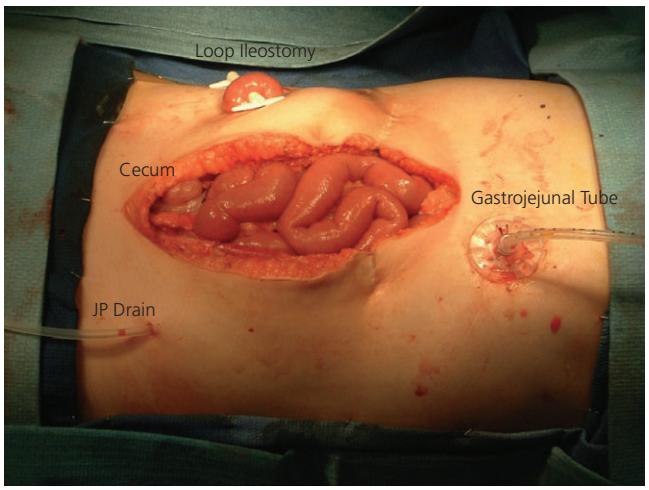
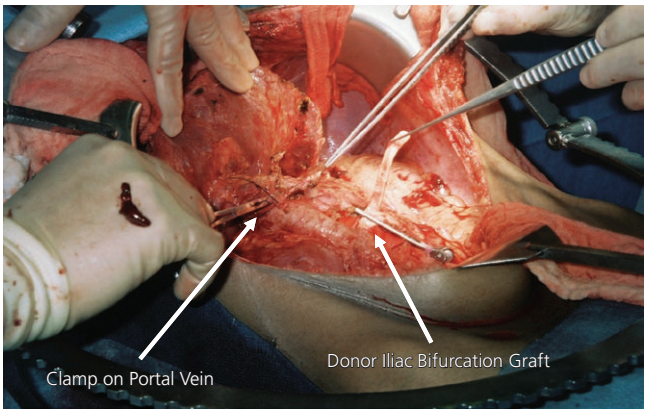


(B)

Figure 62.4. Allograft prior to implantation with right colon in continuity (A). After implantation and anastomosis of right transverse colon to rectum, with creation of loop ileostomy. Cecum is seen to far left in inferior abdomen (B), drain and gastrojejunostomy tube in place. The patient's head is to the right.

Small bowel transplantation with the pancreas and stomach

Some patients who do not require liver transplantation with the small bowel may, nevertheless, be best served by inclusion of the pancreas and stomach with the intestinal allograft. Examples include those who have undergone prior gastrectomy or pancreatectomy, have severe motility disorders that affect the foregut and result in megaduodenum, or those for whom mesenteric tumors may encase the base of the mesenteric vessels and extend into the



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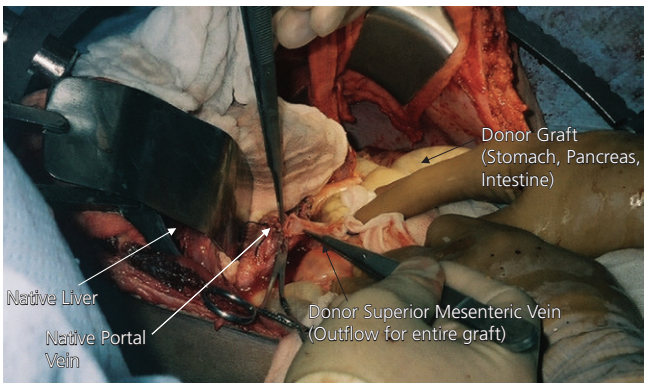

(B)

Figure 62.5. The native liver with arterial inflow preserved, but portal vein clamped (top left) remains, while the foregut has been removed. An arterial bifurcation graft has been placed on the infrarenal aorta (A). The allograft sits underneath the assistant's hand, while the celiac and superior mesenteric arterial anastomoses have been completed, and the portal outflow into the liver is being constructed (B).

pancreas. Foregut exenteration maintaining the arterial supply to the liver is required, and the en bloc stomach, pancreas, and jejunoileal graft is then implanted. This has been referred to, also, as modified multivisceral transplantation [14]. In brief, the portal structures are dissected, the gastroduodenal artery ligated, and the bile duct divided low near the duodenum. A wide mobilization of the duodenum and head of the pancreas is performed to identify the SMA from the right side. The portal vein is skeletonized and the spleen and tail of the pancreas are rotated medially to the base of the SMA. The left gastric artery is ligated, and the splenic artery is identified and ligated, leaving only the celiac supplying the hepatic artery, which is intact and supplying the liver. The proximal stomach can then be divided with a stapling device. If colon is present, the right and left colon are mobilized from the lateral attachments, and rotated medially such that the entire intestinal tract is held only by the SMA. The SMA is ligated and divided and the whole foregut, with the small bowel, is now mobilized and ready for removal. When the portal vein is clamped low near the pancreatic tunnel, it can be divided and the native organs removed. Allograft implantation can then begin (Figure 62.5A,B).

Modified multivisceral implantation

To provide inflow to the allograft, a bifurcating iliac artery graft is placed on either the supraceliac or infrarenal aorta. This is placed in similar fashion to the extension graft utilized for isolated bowel implantation. The two limbs of the bifurcation graft then are reconstructed to the celiac artery (CA) and SMA of the allograft. The portal vein, emanating from above the duodenal allograft, is reconstructed end-to-end to the native portal vein, in the fashion of liver transplantation. In essence, the implantation is akin to upside-down liver transplantation. The portal vein implantation is completed with a growth factor to allow flushing of the preservation solution at reperfusion. After reperfusion of the graft, a choledochocholedochostomy may be constructed. The pylorus is denervated, so a pyloroplasty or pyloromyotomy is performed to facilitate gastric emptying. The stomach is then reconstructed to the esophagogastric junction over a nasogastric tube. A gastrojejunal feeding tube is likewise placed. The stomach is fixed to the abdominal wall and the mesentery is sutured to the retroperitoneum, as with isolated small bowel transplantation, to avoid kinking of the vessels or volvulus of the graft. The spleen is removed or may be removed on the back table, with ligation of the short gastric vessels. Distal reconstruction is performed as detailed above. Variations on this operation have evolved to allow preservation of the spleen, and/or pancreaticoduodenal complex in select circumstances [15,16].

Unusual circumstances

Short bowel syndrome and thrombosis of the infrarenal vena cava due to femoral line placements for parenteral nutrition delivery, or inferior vena cava filter

In this circumstance, we usually mobilize the inferior vena cava superior to the renal veins. Ligation of short hepatic veins from the right lobe of the liver allows a greater length of caval exposure. Sometimes, resection of the right caudate enhances exposure, particularly when mild liver disease has resulted in hepatomegaly. A common iliac vein may be used as an extension graft, and the hypogastric vein ligated. The external iliac vein lies nicely after Kocher mobilization of the duodenum, and aligns next to an infrarenal aortic conduit used for arterial inflow.

Short bowel syndrome due to aortic dissection or other severe vascular occlusive disease

If the infrarenal aorta is not usable, an iliac artery graft may be placed onto either: (1) the supraceliac aorta, (2) an aortobifemoral conduit previously placed, (3) end-to-end to the splenic artery, or (4) the right iliac artery. All have been performed, but aligning venous outflow is individualized and of critical importance.

Combined small bowel and pancreas transplantation

In the case of a type 1 diabetic with intestinal failure, the composite pancreas–intestinal allograft has been employed. The bifurcation iliac artery graft may be placed on the infrarenal aorta, and the inflow celiac and superior mesenteric arteries reconstructed to the bifurcation graft. Venous outflow requires an interposition iliac vein graft placed on the infrarenal vena cava and to the portal vein remnant of the allograft.

Isolated intestinal transplant in patients with poor gastric emptying

The isolated intestine can be transplanted into the patient in whom poor foregut function is documented. Often, gastric emptying in patients with primary motility disorder of the small bowel improves after removal of the native small bowel and transplantation. However, the isolated bowel may be transplanted and proximal

jejunojejunostomy accomplished. A loop gastrojejunostomy approximately 20 cm downstream may then be constructed. Alternatively, with megagastris and severe foregut gastric dysfunction, near-total gastrectomy may be performed. In this circumstance, the proximal graft may be reconstructed to the proximal stomach remnant, and the native jejunum brought into the side of the allograft approximately 40 cm downstream to avoid bile acid reflux. This creates a Roux-en-Y reconstruction that has proved effective in individual cases.

Summary

Intestinal transplantation remains a technically challenging procedure that requires good spatial reasoning, advanced planning, and extemporaneous adaptation to the recipient vascular, and (often) re-operative anatomy. Proper choices for the vascular reconstruction, the specific bowel components included in the reconstruction, and the means of re-establishing continuity have been increasingly standardized over the past two decades. However, numerous technical decisions remain critically important to the success of the procedure, requiring individuals practicing this transplant be experienced in the highly variable potential scenarios that present in this patient population.

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CHAPTER 63

Multivisceral Transplantation Procedure and Surgical Technique

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Introduction

Multivisceral transplantation (MVTx) is the en bloc transplantation of liver pancreaticoduodenal complex, and intestine [1]. This chapter will focus predominantly on the technique of MVTx. The techniques for isolated intestinal transplantation are specifically covered in Chapter 62. We usually do not incorporate stomach as a part of the multivisceral allograft; however, at other centers, stomach and/or colon may form a part of the multivisceral allograft. Patients with history of severe gastric dysmotility, extensive upper abdominal adhesions, and trauma to the stomach are potential candidates for stomach-inclusive MVTx. Not only is such a procedure orthotopic in nature, it provides for a relatively simpler vascular reconstruction as portocaval shunt is not required. Occasionally, colon may also be transplanted as a part of multivisceral allograft in patients who may eventually benefit from a coloanal pull-through, for example patients with aganglionosis of the large intestine. MVTx accounts for a quarter of all intestinal transplant operations in adult patients and nearly 13% of such operations in children [2]. MVTx is the treatment of choice for patients who suffer from irreversible intestinal failure, typically short bowel syndrome combined with intestinal failure associated liver disease (IFALD). Diseases that compromise the entire alimentary tract, such as chronic intestinal pseudo-obstruction, dense adhesions complicated by multiple enterocutaneous fistulas, severe abdominal injuries resulting in gastrointestinal tracts that cannot be otherwise reconstructed, and locally invasive malignancy such as desmoids, are the other main indications for MVTx [3,4].

MVTx can be divided into foregut-sparing and non-foregut-sparing operations, depending upon the extent of recipient organectomy. This is primarily dictated by the underlying cause of the intestinal failure. Patients with involvement of the stomach, as in severe dysmotility or hollow visceral myopathy, usually undergo non-foregut-sparing organectomy. In the foregut-sparing operation, the recipient stomach, pancreas, and duodenum (foregut) are preserved in situ. The non-foregut-sparing procedure includes excision of most of the stomach, duodenum, pancreas, and spleen in the recipient. Removal of the entire small bowel along with colon up to mid-transverse colon is common to both types of procedures. The specific reasons as to why one removes or leaves the recipient's foregut are discussed in Chapter 33. Depending on the surgical team's preference and the circumstances, the stomach and/or colon may be transplanted en bloc as part of the allograft. The basic surgical techniques and principles that apply to liver transplantation (covered in depth in Chapter 56) are generally applicable to MVTx. These include careful attention to dissection during explantation, meticulous hemostasis and vascular reconstruction, and management of coagulopathy. One of the unique aspects of MVTx is the difficulty that can be present in obtaining adequate vascular access for anesthetic management and resuscitation, as discussed in the following section.

Anesthetic considerations

An experienced anesthesiologist is critical to the success of these complex operations. An exhaustive preoperative evaluation of the patient and a thorough discussion with the surgeon about the planned procedure will serve as the foundation for a successful outcome. Besides a systemic approach to history and physical examination, accurate assessment of the patient's vascular access, coagulation status, and aberrations of nutritional, acid–base, fluid, and electrolyte balance are of prime importance.

As mentioned above, many patients who require MVTx present a challenging situation for vascular access. This is secondary to history of multiple central line placements for long-term hyperalimentation and subsequent venous thrombosis due to catheter-related complications. Experienced preparation and care is required in evaluating these patients before they arrive in the operating room so that central venous access can be obtained. Occasionally, assistance of an interventional radiologist may be required preoperatively in highly complex cases to obtain central venous access through the translumbar or the azygos route. Preoperative vein mappings, as well as intraoperative vascular ultrasounds, often times are essential for successful placement of a large-bore peripheral line or central venous catheter. If all else fails, a large-bore high-volume catheter may be inserted by the surgeon directly into the inferior vena cava in the surgical field during the early part of the operation. In addition to the standard anesthetic delivery equipment, hemodynamic monitors for continuous cardiac output and mixed-venous oxygen saturation are generally used, as well as the monitors capable of simultaneously displaying four pressure channels. In adults, we routinely use a Swan–Ganz catheter (Edward Lifesciences Corp., Irvine, CA) to monitor the pulmonary artery pressure, capillary wedge pressure, and central venous pressure. Two arterial lines is standard practice at our institution. Most of the

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recipients of MVTx belong to the pediatric age group in whom it is difficult to place arterial lines secondary to their small size. As these patients need multiple arterial blood gas analyses as well as precise blood pressure monitoring, it is prudent to have good arterial access. The use of intraoperative transesophageal echocardiography is also standard and serves as an excellent monitor for the assessment of preload and ventricular wall motion. It is prudent to have ready access to a venovenous bypass circuit in the event of significant bleeding, hemodynamic instability, and anticipated prolonged implantation time. Close attention to electrolytes, arterial blood gases, coagulation profiles, glucose, and hematocrit are mandatory. It is not uncommon to encounter massive blood loss in these operations and the availability of blood salvage equipment and some type of rapid blood infusion device can be of great utility under such circumstances. Maintenance of a normal core body temperature is essential to prevent worsening of the patient's coagulation status. The use of upper and lower body forced airway devices, heating blankets, and fluid warmers are helpful in minimizing heat loss, and, at times, it may be necessary to raise the ambient temperature of the operating room.

From an anesthesia perspective, reperfusion of the graft can be the most challenging portion of the procedure. This phase can be marked by hypotension, hypocalcemia, hyperkalemia, metabolic acidosis, hypotension, and hypothermia. Aggressive volume infusion and prompt vasopressor support should be instituted as indicated. Additionally, the serum potassium levels should be optimized prior to reperfusion in anticipation of potassium efflux from the reperfused graft. This preunclamping optimization is achieved using the same algorithm as is used for the treatment of hyperkalemia. If hyperkalemia is noted, it should be aggressively corrected with insulin, dextrose, sodium bicarbonate, loop diuretic, and calcium. In the rare event of asystole secondary to hyperkalemia, prompt DC cardioversion should be performed. It is standard practice at our institution to prepare the entire chest in the surgical field and have sterilized shock paddles available on the operative field prior to reperfusion. The value of experience in these operations cannot be overemphasized, especially in pediatric recipients and infants.

Incision

The type of incision made on these patients is variable, based on their size, age, and prior abdominal surgeries. Most of these patients have had prior surgeries, so individualization of the incision is usually required. For many patients, a prior midline or subcostal incision can be re-used for the transplant procedure, sometimes with extensions either side to side or cephalocaudad. On rare occasion, extension of the incision may be required in a perpendicular direction to the previously placed one. However, it is important that any new incision that is made should not compromise the blood supply to the abdominal wall. At the same time, the need for generous exposure cannot be over emphasized.

Removal of abdominal organs (organectomy)

The next stage of the operation involves removal of the recipient's organs. The level of proximal transection depends on whether stomach forms a part of the allograft or not. In cases where stomach forms a part of the allograft, the proximal level of transection is either distal esophagus or proximal most stomach. Usually, a rim of stomach immediately distal to the esophagus is left in situ, as it not only makes the proximal anastomosis (gastrogastrostomy) relatively simpler, it also decreases the chances of reflux and or stricture formation at this anastomosis. In cases where stomach does not form a part of the allograft, the level of proximal transection depends on foregut-sparing versus non-foregut sparing MVTx. First, the recipient liver is mobilized in the fashion typical of a liver transplant operation. This step is common to both foregut-sparing and non-foregut-sparing techniques. Ligamentous attachments are taken down and the hilar structures are identified. Depending on the type of caval anastomosis planned (bicaval versus piggyback), complete or limited mobilization of the vena cava is performed. We usually perform bicaval liver transplant at our center and use the same technique for implantation of the liver from a multivisceral allograft. Subsequent dissection is dictated by proximal level of transection of the intestine, as is detailed below. The distal level of transection is at the level of mid-transverse colon, preserving the left colic artery.

Foregut-sparing multivisceral transplantation

In this approach, the small bowel remains in place but is mobilized cephalad to the level of the left renal vein and superior mesenteric artery. In this circumstance, the splanchnic venous drainage from the stomach, pancreas, spleen, and duodenum needs to be preserved. To this end, an end-to-side portal caval shunt is created. Following completion of the portal caval shunt, clamps are placed on the upper and lower inferior vena cava and the liver is removed.

Non-foregut-sparing multivisceral transplantation

Non-foregut-sparing organectomy for MVTx involves relatively extensive dissection. The first step is usually transection of the stomach. The stomach is completely mobilized by dividing the omentum along the greater curvature up to its mid-portion. The hepatogastric ligament is also divided to free the mid-portion of the stomach circumferentially. This mobilization of the stomach allows for the placement of a stapling device in its mid-portion for transection. Care is taken to preserve the left gastric artery as this is the main blood supply to the proximal gastric remnant. If a total gastrectomy with replacement is anticipated, the stomach and the gastroesophageal (GE) junction are mobilized circumferentially. Because the GE junction receives its blood supply from the lower esophageal branches, the left gastric artery is not preserved in this case. This step exposes the supraceliac aorta and often times exposes the origin of the celiac axis. Next, medial visceral rotation (the Mattox maneuver) is performed to mobilize the spleen and the pancreas towards the midline. The residual small bowel and the colon are mobilized from a caudal to cephalad direction by mobilizing the right colon laterally. The small bowel and the right colon are then dissected off the retroperitoneum to expose the inferior vena cava and the aorta. This dissection is taken up to the level of the left renal vein, which is an excellent landmark for the identification of the superior mesenteric artery. Kocherization of the duodenum is performed to expose the superior mesenteric artery from the right side, along with complete mobilization of the entire base of the mesentery. Next, the left or transverse colon is divided, usually with a stapling device. The left colic and the inferior mesenteric vessels are preserved because they are the only blood supply to the remaining hindgut. This dissection can be difficult and time consuming due to dense adhesions. Significant amount

of blood can be lost if one is not careful, setting up a vicious cycle of coagulopathy and bleeding.

After complete mobilization of these structures, the superior mesenteric artery (SMA) is identified and doubly ligated, typically with silk ties. The common hepatic and the splenic arteries are subsequently identified and ligated. As mentioned above, the left gastric artery is preserved if the stomach is divided at mid-body. In case the stomach is transected at the level of the GE junction, the celiac axis is ligated in a fashion similar to SMA ligation. At this time, the native foregut and midgut are attached only to the liver through the portal vein. At this juncture, a large clamp or stapling device is used to completely transect the hilum of the liver and this composite of midgut and foregut organs are removed from the abdomen. The main reason for leaving the liver in situ on the vena cava is that, despite the lack of inflow, it provides some hemodynamic stability.

There still can be considerable bleeding from the retroperitoneum due to the extensive dissection and this is a good time to obtain meticulous hemostasis. After obtaining adequate hemostasis, the crus of the diaphragm are split to identify the supraceliac aorta to expose the space between the parietal pleura superiorly and the celiac axis inferiorly for placement of the aortic conduit, which will provide the arterial inflow to the allograft.

In the previously described technique for combined liver/small bowel transplant by Grant et al., the portal vein was skeletonized but left intact, leaving it as the only structure bridging the liver and the intestinal portion of the allograft [2]. This was done primarily to avoid inflammation of the donor pancreas or the development of a pancreatic duct fistula [5]. In our initial experience, however, this technique posed several technical problems. Firstly, it made back-table dissection of the allograft technically challenging, especially in pediatric donors, including the risk of injury to the celiac trunk, superior mesenteric artery, and the portal vein. Secondly, we observed significant morbidity associated with bile leaks from the biliary–enteric anastomosis. Lastly, there was a high incidence of torsion of the portal vein during the implantation process [6].

A modified technique to resolve these technical issues was first reported in 1996 [7]. Primarily, the changes include elimination of the hilar dissection, leaving the hepatoduodenal ligament undisturbed (Figure 63.1). This not only made the back-table preparation easy, but it also avoided torsion of the allograft and eliminated the need for a biliary–enteric anastomosis. The subsequent description of the operative technique is based on our modifications as described by Sudan et al. [6].

Next, dissection is performed at the diaphragmatic hiatus and the crural fibers of the diaphragm are divided to expose the aorta in the supra celiac region. After adequate exposure has been obtained for placement of clamps, a portion of the donor aorta is brought to the operative field (the conduit). A curved occluding clamp is placed on the recipient's supraceliac aorta. The donor aorta diameter is used to estimate the size of the aortotomy on the recipient aorta. Using an 11-blade, the recipient's aortic wall is incised, followed by creation of aortotomy using an aortic punch. A 4 to 6-cm portion of the conduit is anastomosed to the recipient's aorta in an end-to-side fashion with a running suture, typically 5-0 or 6-0 polypropylene. After completion of the anastomosis, a bulldog clamp is placed across the conduit and the supraceliac aortic clamp is released to restore perfusion of the abdominal viscera and lower extremities (Figure 63.2). Subsequently, a test occlusion of the suprahepatic inferior vena cava is performed to assess whether the patient can tolerate IVC clamping for the duration of the transplant

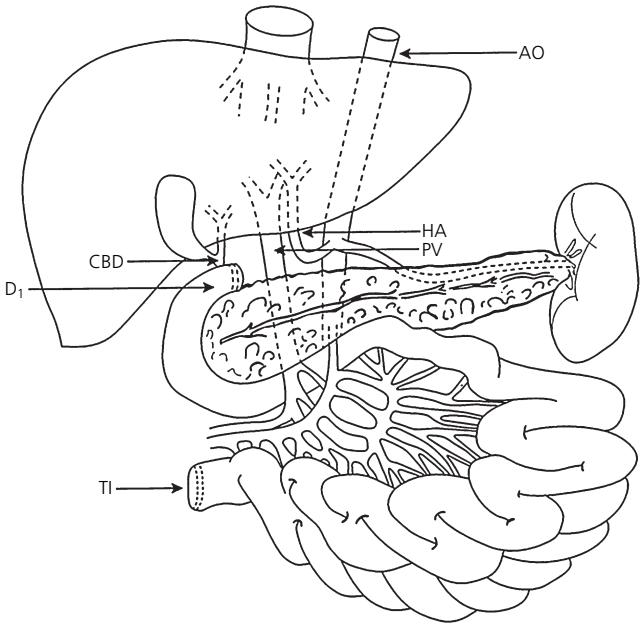

Figure 63.1. Donor operation. Note that the liver, small bowel, pancreas and spleen are procured en bloc. There is no hilar dissection and the celiac trunk and SMA are included on a long segment of aorta. AO, thoracic aorta; HA, hepatic artery; PV, portal vein; CBD, common bile duct; D1, first part of duodenum, stapled; TI, terminal ileum, stapled. Reproduced from [6] Sudan DL, Iyer KR, Deroover A, et al. A new technique for combined liver/small intestinal transplantation. Transplantation 2001;72(11):1846–1848, with permission from Lippincott Williams & Wilkins, Inc.

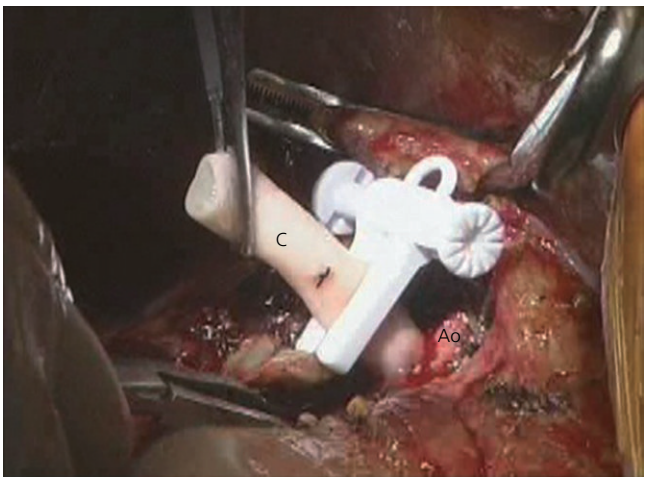

Figure 63.2. Aortic conduit clamped with a bull dog clamp, immediately prior to removal of aortic clamp. C, conduit; Ao, recipient aorta.

procedure. If there is no hemodynamic instability, the infra and suprahepatic IVC (inferior vena cava) are clamped and divided. The liver and the retrohepatic IVC are removed. The patient may be placed on venovenous bypass at this time to prevent congestion of the kidneys and the lower extremities. At our institute, we seldom use venovenous bypass during the anhepatic phase. Over the years, we have gradually moved away from the venovenous bypass technique, which continues to be standard at many institutions.

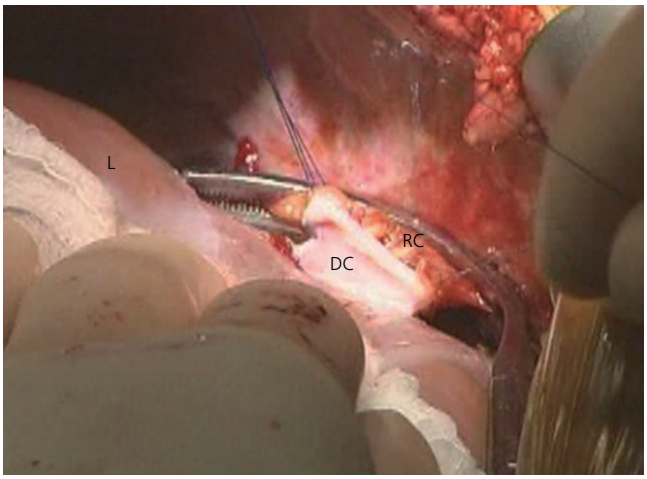

Figure 63.3. Upper caval anastomosis between the suprahepatic IVC of the allograft and the cross clamped recipient IVC. L, liver; DC, donor IVC; RC, recipient IVC.

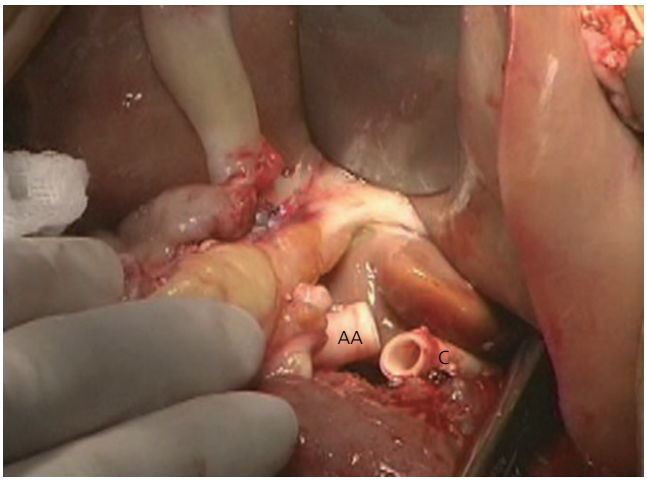

Figure 63.4. Positioning of the allograft aorta next to the aortic conduit prior to anastomosis. AA, allograft aorta; C, conduit.

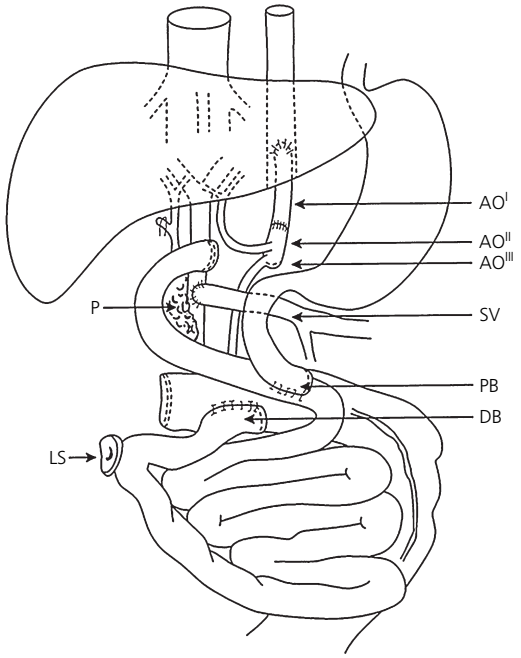

Figure 63.5. Recipient operation. AO^{I}, interposition graft of aorta; AO^{II}, Carrel patch bearing celiac trunk and SMA; AO^{III}, aortic end oversewn, below SMA takeoff; SV, native splenic vein; P, pancreas, with duch and parenchymal edge oversewn; PB, proximal small bowel; DB, distal, ileocolonic anastomosis; LS, diverting loop ileostomy. Reproduced from [6] Sudan DL, Iyer KR, Deroover, A et al. A New technique for combined liver/small intestinal transplantation. Transplantation 2001;72(11):1846–1848 with permission from Lippincott Williams & Wilkins, Inc.

We have not seen any significant difference in overall outcome of patients who are not placed on such bypass compared to those who are.

The multiorgan allograft is then brought to the operative field. The upper caval anastomosis is performed in a fashion identical to that performed in liver transplantation (Figure 63.3). A running 3-0 polypropylene is used in adults and a 4-0 or 6-0 suture is used in children. Subsequently, the infrahepatic cava on the liver allograft is anastomosed to the recipient's infrahepatic IVC using a running 4-0 polypropylene suture. The donor aortic conduit, which had been anastomosed to the supraceliac aorta of the recipient, is anastomosed to the aorta of the composite allograft with a running 6-0 polypropylene suture (Figure 63.4).

At this point, the clamps are removed, typically beginning with the suprahepatic IVC clamp. This allows for venous back flow into the graft and early control of any major bleeding. Subsequently, the infrahepatic clamp is released. This usually helps stabilize the patient's hemodynamics as the venous return from the lower half of the body is restored. Lastly, the large bulldog clamp on the aortic conduit is released to re-establish inflow to the composite allograft. The integrity of all the major anastomosis is ensured. Small bleeding sites on the donor organs are then carefully sutured or cauterized as necessary. Once hemostasis has been obtained, continuity of the gastrointestinal tract is re-established.

If the stomach is not to be transplanted, a Roux-en-Y gastrojejunostomy is constructed between the stomach remnant and the allograft jejunum. This is typically done with a hand sewn, two-layer anastomosis. The jejunojejunostomy for the Roux-en-Y is also hand sewn in two layers. Lastly, the ileocolonic anastomosis is performed in the same two-layered fashion. Alternatively, stapling devices can be used for these gastrointestinal anastomoses, but this can sometimes results in bleeding from the staple line. It should be noted that, more commonly, the entire pancreas is left with the allograft and not resected, as depicted in Figure 63.5. Allograft cholecystectomy is performed at this time. This is followed by construction of a diverting loop ileoostomy.

We prefer to bring this ostomy out in the right lower quadrant whenever possible and mature it in "Brooke" fashion (Figure 63.6). The siting of the stoma is critical and can be very challenging because of previous incisions, prior stoma, prior stoma site hernia, large abdominal wall defect, and the extent to which the allograft mesentery can be stretched (due to donor recipient size mismatch). This may cause complications with the maturation of the ostomy and application of the stoma appliances. A poorly placed stoma

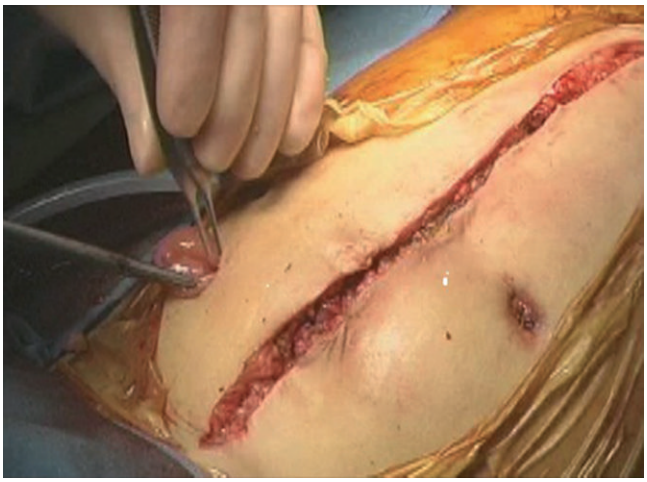

Figure 63.6. Completed multivisceral transplant followed by creation of the Brooke type of ostomy.

not only causes skin and wound complications, it also impacts the psychological and emotional well being of the patient. It is important for the patient to be evaluated preoperatively by a stoma care specialist so that the best possible site for stoma placement can be identified. During this evaluation, attention is paid to abdominal scars/contractures, posture, and mobility of the patient. Key physical considerations include abdominal folds, suture lines, waistline, iliac crest, previous stomas, and presence of hernia. Multiple stoma sites should be marked as an alternative in these complex patients, although it may not be always possible. Ideally, an area below the belt line that is visible to the patient should be chosen. Additionally, it should be placed at least 5 cm from incision lines to aid in proper appliance placement.

One of the most challenging or rather complex parts of the procedure is the closure of the abdominal wall. Primary fascial closure is the goal in all patients. The abdominal fascia is closed with heavy polypropylene suture in a running fashion, and often times the skin is left open. This goal, however, may not be achievable in all patients. It is very important to avoid closure under tension, as (1) the risk of wound dehiscence is significantly increased, (2) the perfusion in the graft may be compromised secondary to intra-abdominal hypertension, (3) the patient's respiratory status may be compromised with resultant difficulty in ventilation secondary to increased airway pressure, and (4) an abdominal compartment syndrome may occur secondary to allograft swelling and third space fluid loss. Several options are available to address this situation, including a skin-only closure, or the use of prosthetic mesh or a biologic mesh. In situations where prosthetic mesh is used, a second-look operation is performed once the swelling in the allograft has gone down (usually 48–72 hours postoperatively) to assess graft viability and attempt the fascial closure. In our experience, the best option in these cases is to achieve temporary closure of the abdominal wall with the use of biologic agents such as acellular human dermis or absorbable mesh. Over the next few days, the patient is brought to the operating room several times for washout and gradual cinching of the mesh so that a fascial closure can be accomplished. In rare circumstances, especially when there is significant amount of tissue defect, abdominal wall transplantation may be the only available option, as described below.

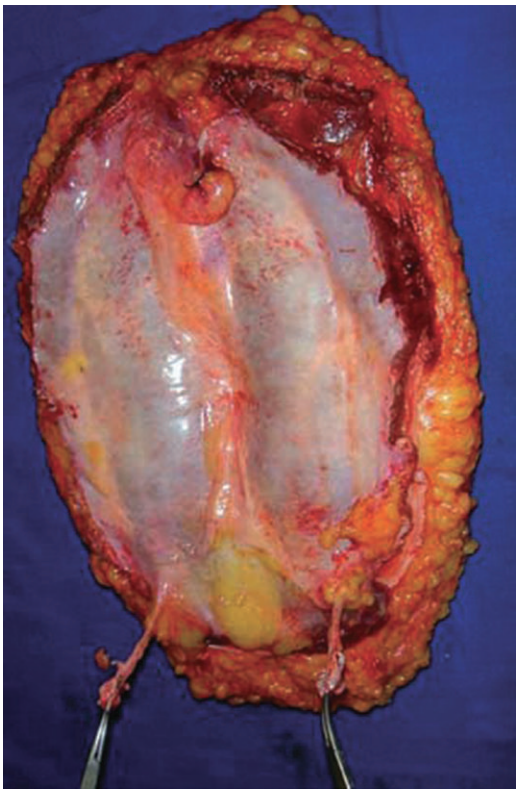

Figure 63.7. The abdominal wall allograft isolated with two epigastric pedicles. Reproduced from [8] Cipriani R, Contedini F, Santoli M, et al. Abdominal wall transplantation with microsurgical technique. American Journal of Transplantation 2007;7:1304–1307, with permission from John Wiley and Sons Ltd.

Transplantation of the abdominal wall

Patients undergoing MVTx or intestinal transplant may encounter difficulties with the closure of the abdominal wall. Due to multiple prior laparotomies, enterocutaneous fistulas, or prior stomas there is limited mobility of the musculoaponeurotic tissues in these patients. Additionally, they may also have fixed abdominal wall defects that preclude re-establishment of peritoneal domain. Also, many of these patients have had the removal of their entire midgut, which can result in an abdominal cavity that is often too small to receive the composite allograft. Additionally, the loss of peritoneal domain may necessitate the use of smaller donor organs and closing the abdominal compartment may be impossible without transplanting abdominal wall. Under these unique and limited circumstances, one has to consider abdominal wall transplantation to restore the peritoneal cavity. This abdominal wall allograft is considered a vascularized composite allotransplant (VCA) which can facilitate reconstruction and closure of the abdominal compartment, especially in patients with prior complex abdominal wall defects.

Procurement of the composite abdominal wall allograft is carried out as part of the multiorgan procurement. The allograft consists of an oval myocutaneous flap comprising of the rectus abdominis muscles, and parts of oblique muscles as well as a layer of parietal peritoneum. The vascular pedicle of this composite allograft is based on the inferior epigastric vessels, which are harvested up to their origin from the external iliac vessels (Figure 63.7) [8].

Inflow to this VCA is achieved by anastomosing either the left or right donor external iliac artery or vein to the recipient iliac artery and vein, respectively. Alternatively, under the right circumstances, a direct end-to-end anastomosis between donor/recipient epigastric vessels can be performed using 10-0 non-absorbable suture using microsurgical techniques. After successful revascularization, bleeding should be noted from the cut edges of the muscle. This myocutaneous flap is then sutured to the recipient abdominal wall musculature in two layers. The successful transplantation of the VCA helps facilitate a tension-free and biologic closure of the abdominal wall.

Reduced-size allografts

Because of the difficulty in obtaining suitably small donor organs, particularly for infants, it is often times necessary to reduce the size of the donor organs prior to transplantation. Typically, a donor that is greater than 25% larger than the intended recipient may require a size reduction of the donor organs. The size reduction is performed on the back table and these surgical techniques follow the same general guidelines outlined in the chapter on live donor liver transplantation (Chapter 57). Typically, the left lateral segment or left lobe will be transplanted. The portion of the small bowel to be removed is variable but is often times limited to one-third of the mid gut. Whether the reduced-size graft is placed in a bicaval position or a piggyback configuration is dependent on the allograft anatomy.

Summary

Multivisceral transplantation is one of the most complex surgical procedures performed in the current era. Even though our experience with MVTx has increased with time, these operations still carry high risk, especially in a patient population that is critically ill. Despite the detailed description of the operative technique, varying degrees of individualization are required depending on patient characteristics, such as age, nature of underlying disease, history of previous operations, and feasibility of vascular access. Meticulous attention to sound surgical technique and hemostasis, an earnest effort to limit allograft ischemia time, and attentive perioperative management by an experienced team are essential for a good patient and graft outcome.

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CHAPTER 64

Vascularized Composite Allograft Transplantation Procedure and Surgical Technique

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Introduction

Each year, millions of individuals sustain injuries, have tumors surgically resected, or are born with congenital defects that require complex reconstructive surgeries to repair the resulting tissue defects. However, limitations of current reconstructive procedures for such major tissue loss include poor functional and aesthetic outcomes, multiple surgical revisions, prolonged rehabilitation, donor site morbidity resulting from use of autologous tissues, and high costs of multiple surgeries and hospitalization. Transplantation of vascularized composite allografts (VCA) offers a new alternative and possibly a superior solution to the requirement for native tissue.

The idea of transplanting body parts from one individual to another dates back to the ancient times [1]; although this vision was partially realized through the first kidney transplants in the 1950s, the potential for VCA was manifest on May 23, 1962, when Ronald Malt performed the world's first arm replantation at the Massachusetts General Hospital. In doing so, he forever changed the way patients suffering from traumatic amputation and limb loss were to be treated to restore form, function, and appearance [2]. This groundbreaking surgery, combined with increasing success in microsurgical techniques, immunosuppressive drug development, and experiences in solid organ transplantation, generated renewed interest in the concept of limb and composite tissue allotransplantation in the 1980s and 1990s. The ultimate result was the first successful hand transplantation in the modern era of immunosuppression on September 23, 1998, performed by a team of surgeons in Lyon, France lead by Dr. Jean-Michel Dubernard [3].

Since then, reconstructive transplantation has become a clinical reality with over 200 procedures of different types of VCAs successfully performed around the world, including hand, forearm, arm, partial facial tissue and full face, abdominal wall, larynx, trachea, vascularized bone and joint, tongue, and even uterus and penis [4,5]. The cumulative world experience substantiates the fact that vascularized composite allotransplantation has become an important treatment option for patients suffering from complex tissue injuries with devastating tissue defects that are not amenable to conventional reconstructive approaches and techniques. However, despite the fact that the surgical procedures and functional outcomes have been largely successful and have shown highly encouraging results, the morbidity associated with chronic immunosuppression limits the broader application of these procedures and prevents them from becoming standard of care [6]. Nevertheless, with thoughtful patient selection, detailed preoperative screening and planning, meticulous surgical technique, and diligent follow-up care, excellent functional overall outcomes can be achieved [7]. This chapter will focus on the general processes of VCA, recognizing that each case is technically unique and worthy of individualized works in their own right. The chapter will complement Chapters 75, 87, and 110, which cover the treatment of VCA rejection, histology of VCA rejection, and long-term VCA outcomes, respectively.

Recipient selection, wait list management, and donor considerations

Candidate selection is critical because VCAs are not life saving, but instead are offered to improve the quality of life, and thus the individual risk–benefit assessment must justify the procedure. Given the requirement for immunosuppressive drug adherence and rigorous rehabilitation, it is arguable that selection of a properly motivated patient is the most important factor for achieving a successful outcome in reconstructive transplantation. Recipient screening and selection, therefore, must include a multistage process that entails an all-encompassing look into the patient's past medical history and pretransplant life [8]. To this end, anatomic, physiologic, psychological, and social factors must be considered and evaluated prior to determining the patient's eligibility for transplantation. Only careful and thorough evaluation of the suitability of a potential candidate will result in an outcome that is satisfactory to both patient and physician. In particular, psychological and social screening becomes critically important in assessing a candidates capacity for compliance with immunosuppressive and rehabilitative treatments. Psychosocial screening is more of an art than a science, but it continues to be refined and emphasized in the transplant literature [9]. From the world's experience with VCA we have learned that only a patient who is physically and mentally healthy, has realistic expectations, is fully capable of understanding and appreciating the complexity of the procedure, and is highly motivated to undergo at least 6 months to 1 year of intensive rehabilitation, should be considered as an appropriate candidate. Indeed, psychological stability and the capacity to comprehend all aspects of the operation and postoperative course need to be confirmed by an independent psychiatrist or psychologist who is unrelated to the

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Table 64.1. Standard screening test panels

Hematologic panel
Complete blood count, differential, reticulocyte count, platelet count
ABO blood typing and Rh-factor, HLA typing
Panel reactive antibodies (PRA)
PT/PTT with INR
Metabolic panel
Serum electrolytes and renal function panel
Urinalysis and creatinine clearance
Liver functions tests
Dexascan (if indicated)
Infectious disease panel
Cytomegalovirus, Epstein–Barr Virus, HSV, toxoplasmosis and VZV (IgG and IgM when indicated), HIV antigen, HTLV I–II antibody, antibodies to HIV 1 and 2, hepatitis C virus, syphilis, hepatitis B core antibody, and hepatitis B surface antigen titers
PPD (purified protein derivative)/Mantoux skin test
Cardiopulmonary panel
Electrocardiogram and multigated acquisition scan (MUGA) or echocardiogram to check the heart
Pulmonary function tests with DLC02
Radiologic panel
Ultrasound of the abdomen (to rule out tumor) and ultrasound imaging of the hand/stump
CT scans (CT angiography and/or conventional angiography)/musculoskeletal MRI of the forearm or hand, fMRI (functional magnetic resonance imaging) studies, and MEG (magnetoencephalography) scans
Sinus X ray (to rule out infection or tumor)
Chest X ray
Plain X rays of bilateral hand/wrist/forearm/elbow/etc. as indicated

Table 64.2. Screening consultations and assessment

Consultations
Dentistry
Ophthalmology
Gastroenterology including esophagogastroduodensocopy (EGD) and colonoscopy
Otoloryngology (ENT)
Male candidates: urology (evaluate for prostate or testicular cancer)
Female candidates: gynecology (evaluate for possible ovarian/endometrial/uterine/cervical cancer)
Transplant psychiatry
Transplant psychology
Transplant social work
Certified hand therapist
Psychosocial screening and functional assessment standardized evaluation tools
SF36 Health Survey: questions on the patient's views about their health
DASH Disabilities of the Arm, Shoulder and Hand Instrument: questions on the patient's current health and ability to perform certain activities
Psychiatric examination together with one of the following questionnaires: Perlin Self-Mastery Scale, Rosenberg Self Esteem Scale, Coping Responses Inventory, Sherwood's Self Concept Inventory, NEO Personality Scale Short Form

transplant team. Only when such psychosocial criteria are met can further patient screening be initiated. Depending on the type of injury and extend of tissue loss, the unique anatomical situation of each candidate, including bone, muscular, vascular, soft tissue, and neural status, is evaluated for surgical planning. This is usually done by physical exam and by using various imaging modalities such as computed tomography (CT) scan (± three-dimensional reconstruction), angiography, or CT angiography and magnetic resonance imaging (MRI). Photographs of the evaluated limb or anatomical region and the contralateral side, even if uninvolved, are compared to assist with the reconstructive procedure and in donor–recipient matching (i.e. size, skin tone). Considerations regarding the potential reconstructive options to be taken in the event of graft loss are also critically important.

To exclude any co-morbidity that would contraindicate chronic immunosuppression (e.g. malignancies or infections) or the transplant procedure itself, potential candidates undergo further transplant-specific screening, including gastroscopy, colonoscopy, and the evaluation of their renal, cardiovascular, pulmonary, hematologic nutritional, and dental status (for a summary of the screening tests see Table 64.1). Transplant-specific immunologic screening includes ABO blood typing, HLA typing, alloantibody screens, and viral serologic screens for hepatitis viruses A, B, and C, cytomegalovirus (CMV), Epstein–Barr virus (EBV), and human immunodeficiency virus (HIV). Any specific medical problem the candidate has or is encountered during screening should trigger a more specific and in-depth evaluation (Table 64.2).

The results of these tests are also provided to the Organ Procurement Organization (OPO) to initiate donor screening. Although no uniformity or standardization has been currently imposed on this process, in general the protocols developed by VCA programs adhere closely to protocols used in solid-organ procurement [10]. Explicit inclusion and exclusion criteria for potential donors are developed jointly by the VCA team and the OPO. In this regard, potential donors for VCA need to be matched not only for the usual parameters such as ABO type, age, and serologies but also for size, gender, and the color, tone, and texture of skin. Blood type must be compatible. To date, HLA matching has not found to be necessary and for practical reasons is disregarded. However, the lymphocytotoxic cross-match needs to be negative for all donor–recipient combinations. CMV matching of donors and recipients to avoid a donor positive /recipient negative combination is desirable, as the risk of CMV infection in VCA is high and complications of CMV reactivation can be significant [11–13]. It is important to note that despite "presumed consent" legislation in some countries, specialized and informed consent for donation of a VCA should be obtained. This should include a detailed explanation of the exact nature/extent of the VCA procurement and how the subsequent donor defect will be restored (e.g. alginate prostheses, facial masks, cosmetic limb prosthetic) for the comfort of the donor's family. Most importantly, coordinators must approach the families of potential VCA donors in such a way that the consent for life-saving solid organs is not derailed [10,14]. At present there are no specific listing criteria for potential VCA recipients or allocation policies and there is also no dedicated nation-wide waiting list for VCA. Thus, each case is considered individually by the transplant center and their local and regional OPO. Up to now, the number of patients awaiting reconstructive transplantation has been small and therefore competition for VCA donors is still negligible. However, as the number of VCAs increases (and the trend observed over the past several years for both face and hand transplantation clearly indicates an upward trend) it will be of utmost importance to implement respective policies and oversight to promote broad sharing of potential VCA donors. On a national level, the most appropriate governing body to oversee VCA donation will be the United Network of Organ Sharing (UNOS), which operates and acts through the Organ Procurement Transplant Network (OPTN) [15–17]. The same oversight mechanisms already established by the OPTN for solid-organ transplantation can be adapted for VCA donation to provide the same level of oversight, standardization, effectiveness, safety, and transparency (see Chapters 128 and 143 for in-depth treatments of governmental oversight in transplantation).

Since there are no standardized protocols, the surgical strategies developed for each VCA recipients have to be adapted and tailored

to that particular patient. For each patient, a precise surgical plan is generated and, in some cases, additional surgical training on a cadaver is completed in order to perfect a new reconstructive technique.

There is emerging evidence that prolonged cold and warm ischemia significantly impairs short- and long-term graft function [18]. Muscle tissue is particularly sensitive to ischemia and damage. Interstitial edema, microvascular constriction, and/or damage of myocyte membranes may result in muscle dysfunction as early as after 2.5 hours of warm ischemia [19]. Thus, allocation requiring long-distance transportation should be avoided and simultaneous preparation and dissection of the donor graft and recipient tissue in adjacent operating theaters is recommended.

Surgical technique: hand, abdominal wall

Donor surgical technique for hand procurement

Once an immunologically appropriate donor has been selected, further "matching" must take place to ensure adequate similarity of skin color, age, gender (although based on recent experiences from several centers world wide, gender mismatch may occur if skin texture, hair patterns, size, etc. are appropriate), length, circumference, and body habitus [10]. Evaluation of possible vascular injury from invasive monitoring (e.g. arterial lines), injury related to the mechanism leading to brain death, and vasopressor effect must also be performed to make sure the limb is suitable for transplantation. Radiographs evaluating injury and disease should be performed. They can also assist with size matching.

After being deemed an acceptable donor, procurement commences in concert with traditional donor organ procurement (covered in depth in Chapters 21 and 53). Given the ability to extend the limbs and isolate them from the operative field, limb procurement does not alter substantially the procurement procedure. Limb allograft retrieval may occur prior to or during procurement of the thoracic and abdominal viscera. In either case, the brachial artery and vein are exposed and placed under tourniquet control prior to aortic cross-clamping. This ensures that the required dissection can be performed in complete isolation from the systemic circulation which reduces risks of hemodynamic destabilization and prevents interference with solid organ procurement. A perfusion catheter is inserted into the brachial artery and instillation of perfusion solution commences. The authors prefer 500–1000 mL of Custodiol (HTK) solution infused with 5000 units of unfractionated heparin. The instillation continues until venous drainage is clear. The remainder of the procurement can either proceed prior to cross clamping, or occur in concert with thoracic and abdominal viscera retrieval.

A circumferential incision is made with rapid transection of flexor/extensor muscle compartments and median, radial, and ulnar nerves. It is helpful to preserve an extra length of the nerves and vessels so that they extend beyond the soft tissue envelope for ease of identification on the back table. Disarticulation through the elbow (for more distal to mid forearm transplants) is performed. Alternatively, for more proximal procurements, a humeral osteotomy is performed. In some instances, maintenance of abundant length of median, ulnar, radial, and musculocutaneous nerve is necessary. In addition, dissecting out an extended length of basilic and/or cephalic vein and extra length of deep veins and brachial artery may be desirable. The superficial veins may be reflected from proximal to distal prior to tourniquet placement. Once safely reflected, a sterile tourniquet may be placed high on the arm to facilitate proximal nerve and vascular dissection. The tourniquet is inflated until all circulation has ceased. The limb, which has been perfused with preservation solution, is then wrapped in moist gauze or towels, and placed in sterile bags on ice for transportation per standard organ preservation protocol.

Surgical technique recipient: hand transplantation

For upper extremity transplantation, the patient is prepared similarly to a liver transplant recipient. Large-bore peripheral and central venous access and arterial lines are placed away from the extremity or extremities to receive the VCA(s). The patient may be given immunologic induction therapy (e.g. thymoglobulin) per the center-specific protocols. Given the length of a VCA procedure, care should be exercised in anticipating thymoglobulin-associated cytokine release and its related hemodynamic instability. Regional block catheters (peripheral nerve catheters) may be placed but should not be dosed with long-acting anesthetic agents. Ultrasonographic evaluation and mapping of the superficial venous system is performed to assist with venous reconstruction during the revascularization period of the operation. Following induction, the patient is maintained under general anesthesia with monitoring similar to that used in liver transplant recipients. Fluid resuscitation should be performed with colloid (packed red blood cells and fresh frozen plasma) instead of crystalloid to minimize peripheral edema.

The back-table donor limb dissections and recipient site dissections are performed simultaneously and have been well described [20,21]. Of note, all donor limb dissection needs to be performed on ice, using sterile ice packs and crushed ice on the field with towels between the ice and tissue to prevent cold injury to the limb. The recipient dissection is performed under sterile tourniquet control. Skin flaps on the recipient are raised in either a volar–dorsal or radial–ulnar manner, depending upon the level of transplantation, with opposite skin flaps elevated on the donor. Extra care should be taken to preserve small veins and their branches, as they will be necessary for venous reconstruction. Extensive dissection of skin flaps and arterial pedicles should be avoided as devascularization and excessive bleeding are the likely acute result and progressive arteriopathy the potential long-term result [22]. Dissection should be limited to what is considered absolutely necessary for adequate exposure. Any scar potentially limiting functional outcome or soft tissue viability should be excised. Individual muscle–tendon units are identified using tags written with permanent ink sutured to the individual structure. The process continues until all named arteries, nerves, and muscle–tendon units have been identified. The forearm bones are exposed and only once the donor limb has been prepared, are calculations for osteotomies performed. For unilateral cases, the contralateral forearm length should be matched. For bilateral cases, both forearms should be of equal length and within normative values for the patient's height while ensuring adequate tendon, nerve, vessel, and soft tissue length. The same process is performed simultaneously on the back table for the donor limb. At this time, the tourniquets are released and compressive wrapping of the forearm performed, followed by careful bipolar and ligature-achieved hemostasis.

Once bone lengths have been determined, the radius osteotomies are performed and plated and the two separate "work stations" (back table and recipient site) are condensed to a single workstation. The donor limb should remain on ice until revascularization. The authors will frequently preplate the donor radius and ulna on the back table. Once the radius osteosynthesis is complete, the ulna cut is made. Using an oblique osteotomy may help maximize bone

contact surface area while allowing a small amount of "wiggle room" to cheat the ulna in position or length. The use of 3.5-mm locking compression plates (LCP), which are low profile, are used for rigid fixation. This enables rapid advancement of load bearing therapy in the mid and proximal forearm regions. For the distal forearm region, the authors prefer volar locking extended distal radius and distal ulna plates. For above-elbow transplants, we use a single 4.5-mm LCP placed through an anterolateral approach.

At this point, the surgical sequence may diverge from that of standard "replantation" techniques [20,21]. If the ischemia time of the limb has been relatively short, surgeons may continue to reconstruct muscle–tendon units and nerves prior to re-vascularization so that these can be performed rapidly and in a bloodless field. However, just what the time limit is for this technique is as yet unknown (see above). The authors routinely complete all extensor tendon work, most flexor tendon work, and some nerve reconstruction prior to revascularization.

Achieving tendon balance in a transplant is substantially different from that of a replant. Relative tendon balance is non-existent in such a scenario. The extensors are repaired first, placing the thumb and digits into full extension with the wrist in mild flexion so that extension may be checked with tenodesis using 20–30 degrees of wrist flexion for full thumb and digit extension. The wrist extensors are repaired next. Rapid repair of large dorsal veins may be performed at this time if possible. However, if the veins are not immediately obvious or do not lend themselves to a quick anastomosis, the authors proceed to the flexor tendon reconstruction. The flexor digitorum profundus and superficialis tendons are repaired as well as the flexor pollicis longus. The wrist flexors can be repaired following revascularization. At this point, the nerves may be repaired in a bloodless field, or revascularization may commence first. Nerve repairs are performed in a grouped fascicular manner with identification and separate repair of the anterior interosseus nerve or group (if possible) as well as motor and sensory tracts if identifiable by surface topography and level of repair to the median and ulnar nerves. The superficial radial nerve, dorsal ulnar cutaneous, and palmar cutaneous nerves should be repaired if available.

Larger veins lend themselves to a blood-free operative field and rapid anastomosis. When possible, the authors prefer to use a venous coupling device, which is reliable and saves time. Smaller veins may require anastomosis after revascularization when they are distended and more easily identified. Arteries are repaired with the least amount of dissection possible. This includes the brachial, radial, ulnar, and/or anterior interosseus arteries. When the recipient's proximal forearm is being preserved, brachial artery repairs should be performed as an end-to-side anastomosis. For transplants at the mid to distal forearm, individual end-to-end anastomoses of the radial and/or ulnar and/or anterior interosseus artery and their venae comitans (whenever possible) are performed. For above elbow transplants not preserving any distal recipient arm, spatulated end-to-end brachial artery anastomoses are performed. Beware of previously placed radial arterial lines in the ICU of the donor, as the radial artery may be thrombosed. This has been reported to propagate and lead to acute failure of the transplant, though the authors have successfully transplanted such a hand using only an ulnar artery anastomosis for arterial inflow [23]. Only at the point that clamps are removed from the arteries and in flow re-established, is the sterile ice removed from the field. Warm saline is used to irrigate and re-warm the limb. Blood is allowed to egress from uncoupled veins for several minutes until the dark coloration begins to lighten. A cell-saver may be used to recycle this intentional blood loss.

After successful reperfusion of the graft, any remaining nerves are repaired and veins are identified and hand-sewn or coupled. This is usually the most time-intensive portion of the operation. Adequate venous outflow is critically important and can be difficult to achieve. The authors prefer at least four veins per hand, but will reconstruct as many as are anatomically possible. The authors use an implantable venous Doppler probe on each transplanted limb applied to what is believed to be the most reliable vein. Once the microsurgical portion of the transplantation is complete, the superficial flexor tendon–motor units are repaired and irrigation and hemostasis are performed. The four skin flaps are interdigitated and closed over drains. Any non-viable-appearing portions of the skin flaps are excised. Skin grafting from the donor may be required if there is a skin shortage. Finally, bulky gauze dressings and a non-compressing splint are applied with the digits completely visible. Pulse-oximetry probes are placed on the thumb and small finger with saturations and waveforms compared to either the contralateral native hand or the contralateral transplanted hand and a separate reference site in the event of a bilateral transplant.

Donor surgical technique for abdominal wall procurement

The abdominal wall allograft (or alloflap) may be safely procured at the same time as the abdominal and thoracic viscerae, with some modification to the abdominal approach. The midline sternotomy incision is stopped just below the xiphoid process and bilateral subcostal incisions are performed, extending down the lateral borders of the rectus sheath to both groins. A transverse suprapubic incision is then performed [24]. The flap is circumferentially incised, leaving the deep inferior epigastric vessels intact, and then turned face down inferiorly to provide wide access to the intra-abdominal organs for procurement. Procurement proceeds per protocol until the abdominal organs have been retrieved. At this point, the vascular pedicle to the abdominal wall is isolated upon the common to external iliac system and infusion of preservation solution commenced. The alloflap is procured with a cuff of distal vena cava and aorta if desired, or may be procured anywhere along the common/external iliac vessels for large vessel anastomoses [24]. Alternatively, the flap can also be based on the deep inferior epigastric vessels taken directly off the external iliac vessels for microanastomoses [25]. The abdominal wall graft is then wrapped in moist gauze, tripled bagged, and kept on ice for transportation per standard transplant protocols.

Surgical technique recipient: abdominal wall transplantation

An abdominal wall transplant is usually indicated to follow a multivisceral abdominal transplantation or transplantation of the small bowel [26]. Following successful completion of these transplants, the recipient vessels are selected and prepared. End-to-side anastomosis of the more proximally procured vessels (distal aorta, proximal common iliac level) may be performed to the recipient common iliac artery/vein or the infrarenal aorta and inferior vena cava. Alternatively, microsurgical technique may be employed to perform end-to-end anastomosis of the more distal and smaller deep inferior epigastric vessels to the proximal take-offs of the same recipient vessels or to the circumflex iliac vessels [24–26]. It is theoretically possible to attempt superficial epigastric vessel anastomosis as well as the deep inferior epigastric system, though this is likely

unnecessary. Motor nerve coaptations have not been necessary in previously reported series [26].

After ensuring adequate arterial inflow and venous outflow into the abdominal wall, fascia-to-fascia repair is preformed and subcutaneous and skin closure is then performed. The abdominal wall graft may be monitored by implantable Doppler probe, external hand held Doppler probe, and by clinical evaluation of skin color, capillary refill, edema, and turgor.

An additional technique incorporates a vascularized abdominal wall/fascial component in continuity with a liver allograft. The posterior abdominal wall fascia connected and vascularized via the falciform ligament has been performed successfully in pediatric liver transplant recipients and provides additional fascial tissue for closure, though the recipient's skin has been closed primarily in each presented case. This composite allograft has been termed the posterior rectus sheath (PORSH)-liver composite vascularized allograft [27,28].

Surgical technique: face

Pretransplant anatomical review

Each face transplant candidate should be assessed in the same orthognathic fashion as other patients undergoing facial skeletal surgery [29]. It is important that the surgical team documents each individual's facial skeletal deficit based on three regions: (1) upper third (scalp/skull), (2) middle third, and (3) lower third. With this in mind, the face transplant team should plan an individualized approach for each candidate [30]. The relevant anatomy and function of each missing facial subunit should be assessed, thereby allowing the team to foresee reconstruction in an ideal setting using customized free-tissue transfer. All pre-existing vascular inflow/outflow aberrancies must be documented during the candidate's pretransplant head/neck surgical screening phase [31]. Detailed preoperative radiological analysis includes three-dimensional CT scan reconstruction and angiography (Figure 64.1). Of note, one needs to be aware of both the donor and recipient surgical/medical history and distinct skeletal anatomy surrounding the facial organ, since both the alloflap recovery and recipient preparation dissections can be equally challenging.

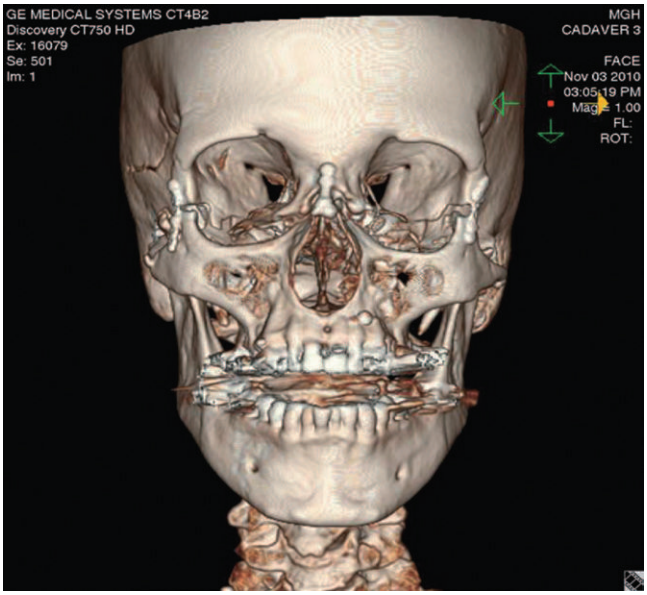

Figure 64.1. Three-dimensional computed tomographic scans (frontal view) depicting an optimized hybrid skeleton in the recipient.

Transplant procedure: timing and logistics

Timing and coordination of both the donor and recipient operation are based on the complexities of the recipient's pretransplant anatomy. In our program at Johns Hopkins, we employ a multidisciplinary approach combining plastic/reconstructive surgery, craniomaxillofacial surgery, microsurgery, and facial plastic surgery/head and neck surgery. For those transplants necessitating complex facial skeletal osteotomies, we prefer to transport the donor to our home institution and use two neighboring operating rooms. This allows us to use and coordinate two teams, one based on craniomaxillofacial expertise and the other based on head/neck surgery–microsurgery expertise. If both rooms are available side-by-side, we aim to start the donor operation (VCA recovery) and recipient operation (preparation) in similar fashion. However, if the recipient contains a large amount of facial scar tissue and complex microanatomy, we choose to delay the donor operation until progress has been made on the recipient procedure [32]. Ideally, the alloflaps are dissected to completion at the same time with all target vessels and nerves labeled. If the recipient's dissection is delayed, the donor's alloflap is fully islandized until ready for transplant so as to minimize alloflap ischemia. However, this may not be possible if the facial alloflap needs to be recovered at an outside institution. In this situation, the alloflaps are perfused, wrapped in moist gauze and kept on ice, according to solid organ transplant standards.

Donor surgical technique for face allograft procurement

A prerecovery, elective tracheostomy (percutaneous or open) should be performed to allow safe access to the face/neck and to assure donor stability. In some instances, the patient may already have a pre-existing tracheostomy in place. Given the central nature of the facial donor site, some special logistical arrangements are required to facilitate facial procurement without impeding the progress of the procurement of the thoracic organs. These are nicely discussed by Brazio et al. [33] and displayed in Figure 64.2. Skin and soft tissue incisions are dissected in the mirror image of the necessary size limits based on the recipient's deficit and with respect to aesthetic unit boundaries. For the purpose of graft monitoring, some groups, including ours, choose to leave a redundant portion of skin at the inferior edge of the facial flap so that protocol skin biopsies can be performed in an inconspicuous region. Others perform a second alloflap (sentinel skin flap) for this purpose. The authors believe that preserving redundant tissue is better than adding a sentinel flap, because facial skin rejects differently in different anatomical locations [34]. The donor facial vessels should be dissected with utmost care from the proximal level extending superiorly. An operating microscope is often necessary for microneurovascular reconstruction. If there is a vasovagal reaction due to carotid bulb stimulation, 1% plain lidocaine should be injected into the carotid bulb. In certain cases, exposure of the facial vessels may require a mandibular osteotomy. One should determine pretransplant which facial vessels are most appropriate given the exact deficit being reconstructed based on angiography. This includes considering superficial temporal vessels for the upper third, transverse facial/superficial temporal vessels for middle third (facial) defects, and ipsilateral neck vasculature for the lower third.

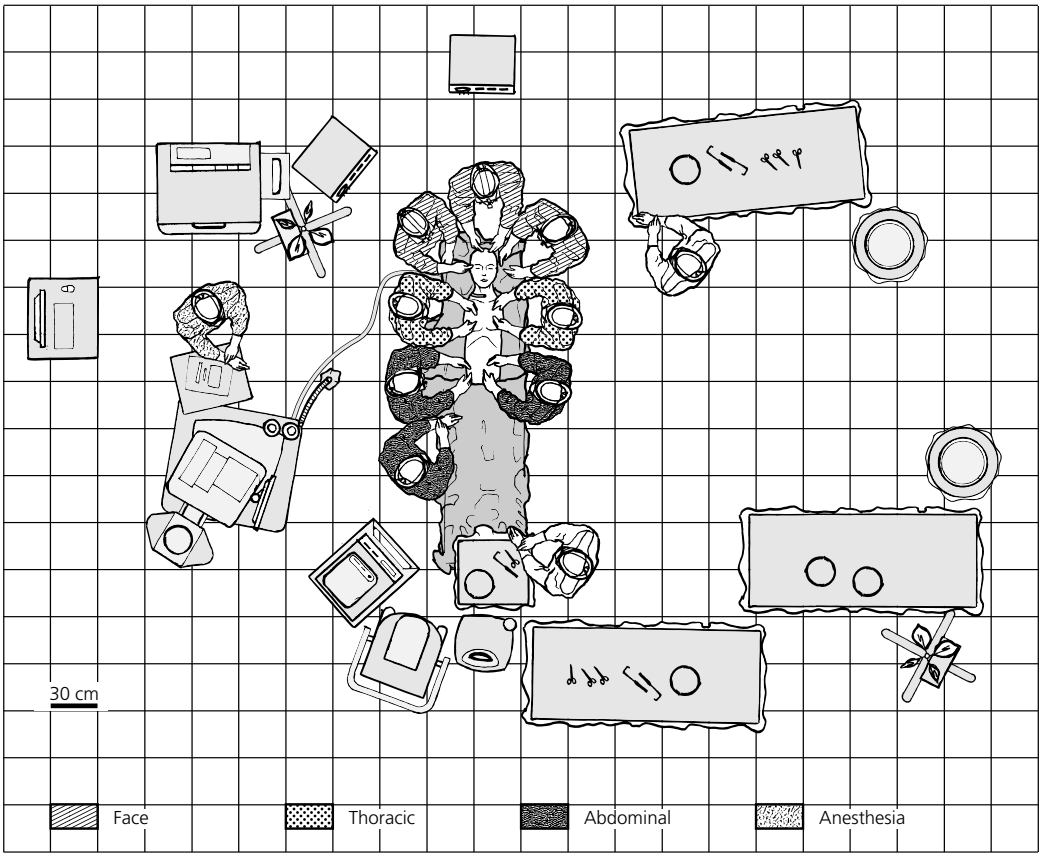

Figure 64.2. Donor operating room set up for multiorgan procurement including a face allograft. The arrangement allows face, thoracic, and abdominal teams to work concurrently with the sole exception of sternotomy. In addition to standard anesthesia and monitoring equipment, there were two OR lights, two monopolar and one bipolar electrocauteries, three suction assemblies, and two head lamp light sources. The operating table was complemented by one back table at each end for face and solid organ teams, respectively, and a back bench for organ preparation. From Brazio et al. 2013 [33], with permission.

When the optimum vessels are not available, one can use alternative vessels, such as adjacent small vessels in the neck branching from the external carotid and/or internal jugular vein systems. Due to the common zone of injury for those with severe facial injuries, having to identify an alternative vessel is a common situation. This may prolong operative time and therefore should be considered in advance by all teams. Pretransplant imaging (e.g. Doppler ultrasound, angiography) provides the microsurgical team with important information about target vessel patency, which saves time and helps prevent technical failures. Again, depending on which facial tissue types (soft tissue with or without skeletal components) are included in the facial alloflap, the vascular supply may vary. The Cleveland experience demonstrated that the majority of facial skin, glandular tissue, muscular, and a modified Le Fort-III maxillofacial structure can all be adequately supplied by the bilateral facial artery system [35]. Studies have also shown that the periosteal blood supply to the facial bones provides significant perfusion [36]. For a flap transplanted with lateral cheek tissues, ear, scalp, and forehead, one should consider including the superficial temporal, internal maxillary, and/or facial branches during recovery. In addition, the craniofacial dissection should strive to preserve all of the overlying periosteum for the sake of viability. Regardless of the vessels chosen, one should dissect to the most proximal level where the caliber is greatest to reduce the risk of technical failure. However, one should avoid recovering the carotid artery proximal to its bifurcation as inhibiting blood flow in the internal carotid artery could result in acute cerebral edema. Following the arterial/venous dissection, the facial nerve branches are defined bilaterally. Most often, the main venous drainage is based on the external jugular venous system. The facial nerve dissection at the area of the stylomastoid foremen allows one to raise the flap at the donor's main trunk level. In contrast, the Boston group recommends an inset with distal neurorraphies so as to prevent synkinesis [37].

If applicable, the Le Fort-based osteotomies are best left to the final stage, prior to final flap recovery, so as to minimize bone bleeding. These bone cuts should be tailored to the recipient's needs and are best performed once the soft tissue envelope has been dissected and labeled. The craniofacial team should request relative hypotensive anesthesia and reverse Trendelenburg positioning during the Le Fort recovery to prevent iatrogenic bleeding. Another surgical consideration in maxillofacial alloflap recovery is the adequate exposure of the lower orbital floors in preparation for a Le Fort-type osteotomy. One should consider using a small vibrating saw (Sonopet and Nakagawa Serrated Knife, Stryker, Inc., Kalamazoo, MI) with constant irrigation to protect the donor globe for possible cornea donation. If the recipient's maxillary defect is primarily anterior, thereby restricting the inset of a full maxilla, it may be reasonable to employ a "veneer approach." This "maxillary onlay" technique entails harvesting the anterior, medial, and lateral maxillary walls, leaving behind the rest of the maxilla so as to not

disrupt the pertinent soft tissue bony attachments and retaining ligaments.

The face transplant team should talk with their solid organ transplant colleagues before commencing surgery to coordinate certain maneuvers such as vessel cannulation and cross-clamping so as to not jeopardize the procurement of life-saving solid organs. It is imperative that all parties be aware of each other's surgical plan and specific techniques, which could be altered or abandoned in the setting of donor instability. We prefer to recover the facial alloflap and place a customized on-lay mask prior to the solid organ team beginning their operation. The goal is to prevent any major disturbance to the solid organ recovery process, while at the same time protecting the identity of the donor when they are transported from the operating room to the morgue. To avoid any donor disfigurement, it is recommended that a facial prosthesis is placed on all donors following facial tissue recovery irrespective of cremation or open-casket burial.

Surgical technique recipient: face transplantation

As the donor team is recovering the face, the recipient team simultaneously dissects and prepares the patient's head/neck in order to minimize total ischemia time. Great care is taken to identify and label viable nerve and vessel endpoints, excising excess scar, and creating skin flaps for closure. All pertinent endpoints are dissected to predetermined lengths and mobilized accordingly. Prior to implantation, all UW fluid should be flushed from the alloflap and replaced with cool lactated ringers (LR) solution.

Similar to an extremity replantation, a face transplant including bone begins with partial osteosynthesis to provide necessary stability for neurovascular reconstruction. Cranial osteosynthesis may involve the maxilla, orbital rim, mandible, zygomatic–maxillary complex, and/or cranial vault, depending on the recipient's extent of alloreconstruction. The hard tissue component of the alloflaps is rigidly fixated with multiple titanium plates and screws. In certain cases, the width of the donor facial bones may be wider or narrower compared to the recipient's, and if this is the case, the donor's bizygomatic distance will be modified using surgical techniques (e.g. greenstick fracturing) to allow adequate apposition with the recipient's zygomatic arch [29,30]. Careful attention is needed to make sure the X-, Y-, and Z-axis is accurately positioned for facial width and projection, in addition to appropriate dental occlusion and orbital volumes. We employ a novel approach using a "HYBRID splint" and pretransplant cephalometrics to predict the ideal Le Fort inset position. Of note, this portion of the transplant can be challenging if the donor and/or recipient has poor dental hygiene and periodontal disease. Before face–jaw transplantation, preoperative dental preparation and intraoperative tooth extraction may be necessary if caries are present. This will diminish the risk of infectious complications after the recipient is placed on lifelong immunotherapy. If dental extraction is necessary for the donor's maxilla or mandible, a modified Gunning splint will allow proper vertical spacing for ideal maxilla–mandibular relation.

One should be also cautious in aligning the frontonasal sinus tracts during Le Fort-III type alloreconstruction because the donor-to-recipient junction can be a potential drainage obstruction, leading to future problems such as infections and/or mucoceles. For this reason, it may be prudent to burr down the donor's frontal sinus mucosa using a high-speed burr, with possible obliteration depending on the team's comfort level. A single nasofrontal osteosynthesis permits the microsurgical team to complete unilateral vascular reconstruction, which allows contralateral perfusion and minimizes ischemia time. Total ischemia time from alloflap division to first artery and vein repair should be considered most critical and be monitored. Once the perfusion of all facial tissues has been completed, the craniofacial team can then complete the remaining osteosynthesis, depending on the recipient's stable skeleton. The goal is to create the most optimized hybrid skeleton using many well-tested maneuvers, as described above (Figures 64.1 and 64.3).

After osteosyntheses and vascular perfusion has been secured, facial nerve and sensory nerve repairs are completed if possible. Donor nerve cable grafts can be utilized for such repairs, including the likes of spinal accessory, sural, vagus, greater auricular, and hypoglossal nerves. The authors prefer to replace motor nerve defects with motor grafts, and sensory nerve defects with sensory grafts. Ideal nerve diameter is accounted for as well. Depending on the patient's mechanism of injury, it may be challenging and/or impossible to reconstruct all sensory branches. However, if possible, all trigeminal nerve branches should be repaired. Also, if the facial nerve trunk is scarred, a mastoidectomy releasing the facial nerve from its canal may also be necessary. The ultimate goal is to perform a tension-free neurorraphy to the donor's facial nerves

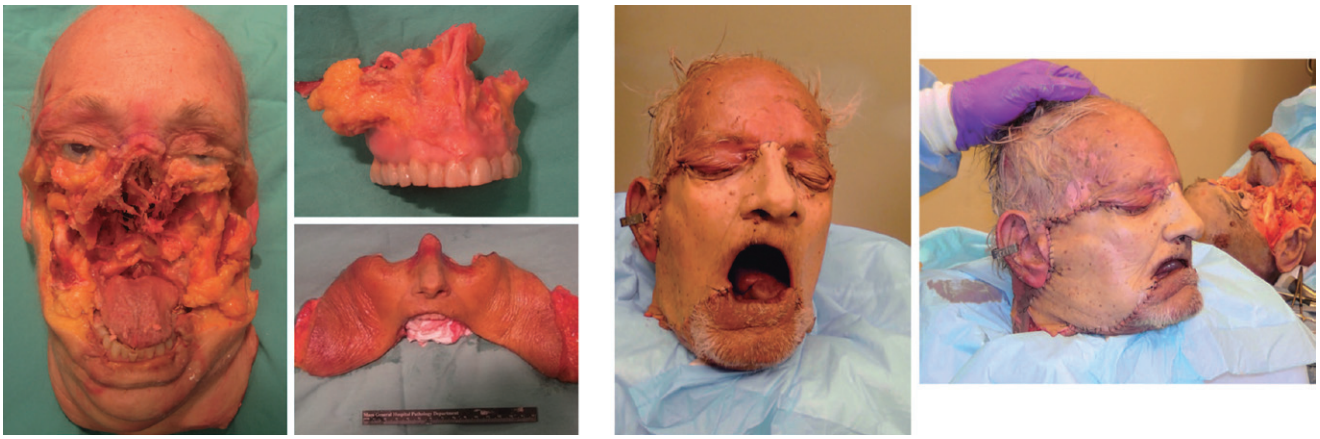

Figure 64.3. Frontal and lateral photographs of cadaver face transplantation (edentulous technique), demonstrating class I skeletal profile.

bilaterally. The authors avoid a donor parotid dissection and leave the overlying facial tissue intact so as to prevent possible alloflaps vascular compromise and/or iatrogenic injury to smaller facial nerve branches. This results in the recipient having excessive lower-face bulkiness for a short time post-transplant. However, redundant tissue provides an added benefit for tissue biopsies. At a later stage, all extraneous tissue can be excised during a soft tissue staged revision for optimal aesthetics.

For those recipients with inadequate sensory nerve length, osteotomies to the orbital floors can be used to increase the length of infraorbital nerves for reconstruction. The need for this procedure only becomes apparent when the recipient's anatomy has been fully characterized. Interestingly, the Cleveland patient gained optimal two-point discrimination even though she was unable to undergo infraorbital nerve reconstruction at the time of transplantation due to her close-range ballistic injury [38].

Finally, the soft tissue should be inset using multilayered closure. Closed suction drains are placed strategically and sewn in place. Skin incisions made at the start of the transplant should be designed to assure a tension-free closure of donor–recipient skin edges. More importantly, they should be made within aesthetic unit junctions and fall within aesthetic lines (e.g. nasolabial folds). If incomplete skin coverage is obtained, donor skin grafts may be needed for closure. The face transplant incisions should be dressed with antibiotic ointment and semipermeable gauze, thereby allowing physiotherapy to commence within 1 week. It is difficult to minimize unwanted motion after this type of surgery. As such, it is beneficial to have a patent airway via tracheostomy and gastrostomy for nutritional feeds.

Particular considerations for craniomaxillofacial transplantation

In a recent cadaver study, Gordon and colleagues [34] evaluated the value of orthognathic planning for face transplants that include underlying skeletal components. Key principles and practice, such as dental splint fabrication and cephalometrics, were shown to improve the final facial skeletal results. This, in turn, (1) optimized "hybrid occlusion" between the donor and recipient, (2) provided the team surgical with guidance for aesthetic facial–skeletal harmony during flap inset/rigid fixation, and (3) enhanced the ability to predict and plan for intraoperative and post-transplant hard tissue discrepancies between donor and recipient. With this approach, all face transplant patients should receive a much-improved hybrid occlusion and facial–skeletal relation.

In a setting where the facial alloflap includes an accompanying jaw (e.g. maxillofacial transplant), all efforts should be made to improve the recipient's hybrid occlusion (the dental relation of the two jaws). To begin, one should place the donor maxilla and recipient mandible into ideal "hybrid" occlusion during the transplant by way of a "hybrid occlusal splint" (donor impression on one side, and recipient impression on the other side). Next, the team should perform rigid fixation of the orbital segments while assessing the recipient's orbital volumes, malar projection, and nasofrontal junction. These findings help to determine how much under- or over-rotation of the recipient mandible is needed to obtain an orthognathic profile (e.g. Class I skeletal relation). For instance, if the recipient has a steep mandibular plane angle and prominent maxilla (e.g. Class II relation), one should rotate the face and jaw alloflap in the counter-clockwise direction (as viewed from the right) to allow for close bone-to-bone distance at the nasofrontal junction. This allows for some dis-impaction from the recipient bone posterolaterally at the zygomaticofrontal regions and improves the sagittal projection of the midface and mandible. With these movements, one can improve the overall facial–skeletal form of the recipient (as compared to pretransplant) and preserve ideal orbital volume without compromising ideal occlusion (Figure 64.4). However, this may come at the cost of creating a few millimeters of posterior vertical maxillary excess, as well as interbony gaps between the upper bony segments. In this instance, posterior vertical maxillary excess may manifest clinically as an anterior open bite. This potential problem can be anticipated preoperatively and dealt with by leaving a small, anterior open bite with the condyles in centric relation, which maintains ideal facial–skeletal projection and optimizes midfacial convexity and orbital volume. An open bite could be corrected in the post-transplant phase without resorting to osteotomies of the alloflap (both challenging techniques in the setting of impaired wound healing, questionable vascularity for maxillary osteotomies, and mandated immunosuppression). There are two potential options for post-transplant open bite correction. For small anterior open bites, correction can be accomplished with non-surgical methods, including occlusal grinding of posterior teeth, molar intrusion, or with the assistance of minianchorage devices. Alternatively, a delayed bilateral sagittal split osteotomy (BSSO) may be used and can be combined with secondary aesthetic procedures at a later date [39]. The second option is less ideal, as it is subject to the same challenges of wound healing and immunosuppression as mentioned previously; however, vascularity of the virgin mandible may not be as concerning. For example, a patient with a Class II skeletal pattern and excessive overjet could be treated at 6–12 months postoperatively, with BSSOs and mandibular advancement/setback, as indicated, in combination with bilateral donor parotidectomies and skin tailoring (i.e. face and neck lifting).

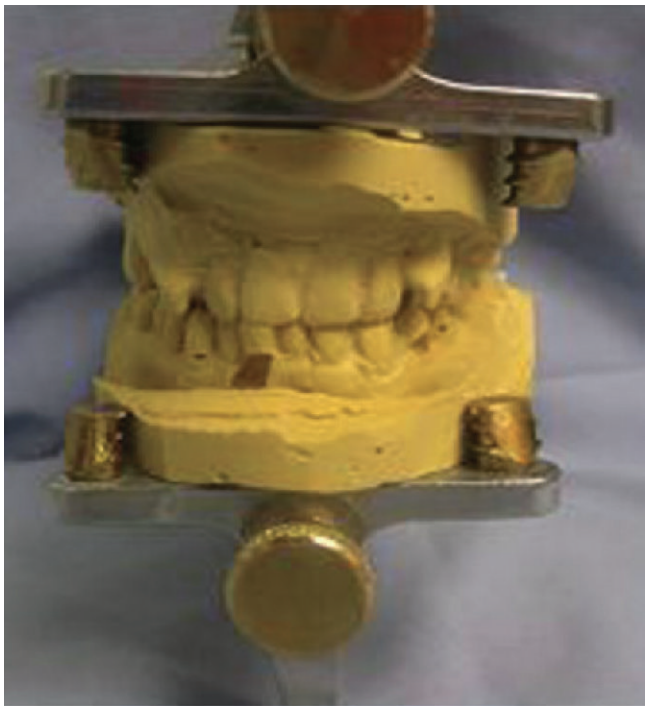

Figure 64.4. A best-fit occlusion with optimal intercuspation is shown for donor maxilla and recipient mandible models.

Surgical strategies in the event of a technical failure

As with any microsurgical procedure, there is always a risk of arterial and/or venous complications in the immediate postoperative period. If this happens, the facial alloflap requires emergent vascular revision in the operating room. As such, the facial alloflap should be monitored closely in an ICU setting for at least 48 hours postoperatively using frequent observation and continuous skin oxygen level monitoring. When the diagnosis of vascular insufficiency is made early, the flap is often salvageable. However, there is always a chance that the transplant or salvage surgery fails, requiring removal of the alloflap. As such, it is imperative that each institution conduct these surgeries under an institutional review board (IRB) protocol. Within the protocol, a full description of techniques for salvage should be provided. In many cases, this will most likely include a combination of skin grafts and/or autologous free tissue transfer. Notably, one should consider a parascapular–scapular free flap due to its large surface area and its usual availability in patients with severe facial deformities for salvage procedures.

Surgical technique: larynx, trachea

Anatomical considerations for laryngeal transplantation

Vascularized laryngeal allotransplantation is one of the less common VCA procedures performed. To date, only two such transplant cases are known to have been performed in the United States and only one, performed by Dr. Marshall Strome at the Cleveland Clinic in 1998, has been published in any detail in the scientific literature [40].

Although the reported outcomes (including a functional voice, protective cough sensation, and swallowing function without significant aspiration) were relatively good, there are only a few programs capable of laryngeal transplantation. Due to the paucity of published information, the surgical details discussed below pertain to this single case. There are of course alternative methods and surgical techniques that could be used and, indeed, are used in animal models of laryngeal allotransplantation. The exact surgical procedure to be used in future larynx allotransplantation has yet to be determined [41].

The surgical details of laryngeal transplantation are dictated by the anatomical intricacies of the larynx itself. The larynx consists of the airway, the vocal cords and their musculature, and a cartilaginous framework to provide support. However, these structures lie in intimate relationship with the epiglottis superiorly, the trachea interiorly, the lobes of the thyroid laterally, and the hypopharynx and esophagus posteriorly. The intimate relationship of these structures with the larynx and their frequent partial or total resection at the time of laryngectomy mean that they must be considered when planning a laryngeal transplant procedure. Additionally, the blood supply of the larynx comes from a combination of the superior and inferior laryngeal arteries, which are branches of the descending superior thyroid artery and inferior thyroid artery, respectively. Additional small arteries penetrate the larynx from the surrounding tissue of the pharynx and esophagus, and its blood supply is intimately connected with that of both thyroid and anterior pharynx and esophagus. These factors make it difficult, though certainly not impossible, to design a vascularized laryngeal graft and must be considered carefully during the planning for the procedure.

Another critical factor to consider when regarding the surgical details of larynx transplantation is the nerve supply to the larynx. All of the necessary functions of the larynx require successful recovery of both sensory and motor component re-innervation of the vocal cords. The superior laryngeal nerve provides the majority of protective sensation that prevents aspiration. The superior laryngeal nerve also supplies the cricothyroid muscle, which, by varying tension on the cords, allows variation in voice pitch. The function of this muscle alone is insufficient, however, to lateralize the cords for adequate respiration. This function relies on the recurrent laryngeal nerve, which supplies the remaining muscles of the larynx. Unfortunately, repair of the recurrent laryngeal nerve, even as an isolated injury, frequently results in synkinesis. The inability to predictably re-innervate the abductors of the vocal cords is one of the reasons that patients treated with this technique have continued to require tracheostomy and why the overall utility of larynx allotransplantation is limited [42].

Donor operation (procurement)

Potential laryngeal donors are identified having proper consent and no history of airway trauma, surgery, or neoplasm. Prior thyroid surgery, laryngeal nerve injury, or neuromuscular disease would be an absolute contraindication to donation. Heavy smoking history must be considered as a relative contraindication. The entire pharyngolaryngeal complex, including the larynx the thyroid/parathyroid glands, the anterior surface of the pharynx, and a portion of the superior trachea, are resected en bloc. This can be done before or after cross-clamping and thoracic organ harvest provided the donor has a tracheostomy below the level of planned tracheal en bloc resection. Standard nerve detection techniques can be utilized, if necessary, to identify the nerves; however, it is important to remember that motor function will degrade rapidly once the graft is no longer perfused. The vascular supply can be harvested with the internal jugular veins. Arterial supply can be harvested at the level of the superior thyroid and inferior thyroid artery or the carotid or thyrocervical trunk can be harvested if additional vascular pedicle is necessary. Following harvest, the donor graft is perfused on the back table and stored in University of Wisconsin solution until revascularization. Ischemia time in the first transplant was approximately 10 hours.

Recipient operation (transplantation)

During the recipient operation, careful planning is required to guarantee the safety of the airway at all times. If not already present, the patient undergoes tracheostomy at the beginning of the surgery. The level of the ostomy should be at the level of planned tracheal repair. Only once the graft is available for transplant, the recipient dissection is carried out. The patient's native remaining laryngeal structures are exposed and dissected free of surrounding tissue and scar. Every attempt is made to identify all four nerves (although in the first case the left recurrent laryngeal nerve could not be identified). Recipient vessels are identified and the graft re-perfused using microsurgical anastomosis with standard techniques. Any available arteries and veins may be utilized. In the case of the first transplant patient, the superior thyroid artery was anastomosed to the recipient superior thyroid artery and the internal jugular vein and anastomosed to the right facial vein. Laryngectomy was then performed leaving the thyroid gland intact and lateralized. The graft was then rotated into position. The contralateral superior thyroid artery was then reconnected and the left middle thyroid vein was anastomosed end-to-side into the recipient's left internal jugular vein. The thyroid

cartilage was secured to the hyoid superiorly. Posteriorly, approximately 75% of the donor's pharynx was utilized. Inferiorly, the trachea was repaired at the level of the tracheostomy [43]. These final details may by modified to fit the particular circumstances of each patient's anatomy but the success of the transplant done in such a way demonstrates that this particular sequence can result in a viable and functional graft.

Anatomical considerations for tracheal transplantation

Surgical reconstruction of the trachea continues to be a daunting surgical challenge and one for which an ideal solution has not yet been found. Despite the wide variety of different techniques for surgical reconstruction of the trachea with autologous, alloplastic, and allogenic materials that have been proposed and performed over the years, no single technique has resulted in consistent and reliable long-term results [44]. This provides the opportunity for reconstructive transplantation to play an important role in advancing the field. However, tracheal reconstruction presents significant challenges. In particular, the arterial and venous supply to the trachea makes it difficult to design a robust vascularized flap that can be transplanted in a single surgical procedure. The majority of the trachea is supplied by branches from the inferior thyroid arteries that course through the gland before giving very small branches to the trachea. The inferior third of the trachea is supplied by arterial branches from the bronchial arteries and the posterior, membranous portion of the trachea is supplied in part by perforating branches from the immediately adjacent esophagus. Several animal models have been designed using various vascular pedicles and containing composite structures, including the thyroid gland and in some cases the esophagus. Although these models do allow transfer or transplant of the trachea as a vascularized graft in a single stage, use of similar techniques in humans has not been published. Several groups have used living tracheal allografts in the human by utilizing revascularization of the isolated tracheal graft in a recipient vascular bed [45,46]. This can be either the sternocleidomastoid muscle, the omentum, or a radial forearm flap. This can be performed immediately and in situ; however, most cases utilize heterotopic placement during the revascularization phase followed by a second-stage relocation into the orthotopic position using free tissue transfer. The most recent and promising studies have come from the Leuven Tracheal Transplant group in Belgium and their technique is outlined below [46,47].

Donor operation (procurement)

Following harvest of the thoracic organs, an 8 cm long tracheal segment is harvested from immediately below the cricoid cartilage to the point of transaction of the trachea for double lung transplant. Care is taken to remove the thyroid gland and esophagus without damaging the adventitial structures of the trachea itself. The graft is stored in University of Wisconsin solution on ice during transport. Cold ischemia time may be up to 10 hours. A full-thickness skin graft is also harvested from the donor and stored in identical manner.

Recipient operation (transplantation)

The recipient bed is prepared for first-stage heterotopic placement by elevation of the skin overlying the vascular territory of the radial forearm flap. The radial artery and underlying fascia serve as a bed for revascularization of the tracheal allograft. The graft is placed longitudinally in this bed with the superior portion facing proximally in the arm for proper orientation of the cilia once the graft is transferred to the neck. During initial surgeries, it was found that the membranous portion the trachea necrosed prior to revascularization. It is therefore removed during implantation in the arm. Full-thickness buccal mucosal grafts may be harvested and inserted in place of the membranous portion. The radial forearm skin is then closed over the trachea and the tracheal ends left open to facilitate drainage and observation. The donor skin graft is sutured behind the patient's ear for immunological monitoring. The graph is maintained heterotopically for 4 months, at which point the immunosuppression may be tapered. Once the skin graft behind the ear has fully rejected and the tracheal graft remains viable, orthotopic transfer may be performed. At this stage the graft is raised with the radial artery and the forearm fascia as a free flap. The skin may be taken with the flap if it is needed for coverage in the neck or for a monitoring sentinel flap. Otherwise skin flaps are elevated prior to raising the flap and the forearm skin left in place on the arm. The flap is then transferred to the orthotopic position after removal of the damaged segment of airway. Oral tracheal incubation is maintained while the graft is positioned and suited in place. The radial vessels are then anastomosed to adequate recipient vessels in the neck in standard microvascular fashion. It is recommended that a small flap of skin or fascia be externalized in order to monitor the microvascular status of the transplant. This may be excised when the patient leaves the hospital or soon thereafter. The patient can be extubated once they no longer require mechanical ventilation and a stent may be placed in the early postoperative period if necessary.

Summary

Despite initial skepticism and debate, reconstructive transplantation is now a clinical reality. As illustrated in this chapter the technical surgical aspects of VCA transplantation are reasonably well established and are no longer the factors that limit the widespread and routine application of this therapy. Nevertheless, microsurgical challenges will remain the Achilles' heel of any type of reconstructive transplantation. Excellent results require meticulous technique, in combination with intelligent immunosuppressive therapy, a high degree of patient compliance, and close follow-up. As our experience increases, the safety, efficacy, and applicability of these life-changing reconstructive modalities will continue to improve dramatically to the benefit of our patients.

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SECTION 6

Post-transplant Management

CHAPTER 65

Induction Immunosuppressive Therapy

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Introduction

The introduction and improvement of immunomodulatory medications have paved the way for organ transplantation to become the treatment of choice for most causes of organ failure. Over the decades, the immunomodulatory regimens used for organ transplantation have evolved into three general classifications: induction, maintenance, or rescue therapy. Induction therapy is intense, prophylactic treatment that is used at the time of transplantation when the recipient's immune system is exposed for the first time to the donor organ. It can include medications that are specific to the induction indication, medications used at high dose during induction that are used chronically at lower dose, and other immunomodulatory maneuvers (e.g. donor-specific transfusions) to facilitate the initial acceptance of an allograft. Its use has emerged from the empiric observation that the early requirements for immunomodulation exceed those late after transplantation. Over time, the biological basis for this observation has become increasingly apparent and related to numerous mechanisms of immune activation.

In general, the intensity of induction regimens is such that prolonged exposure is prohibitively toxic, leading to infectious morbidity and impaired wound healing. Nevertheless, when used appropriately, induction therapy can reduce the incidence of early acute rejection and avoid immune injury at a newly transplanted organ's most vulnerable time: its recovery from the ischemic consequences of the transplant procedure. This avoidance of early rejection simplifies the early immune management of transplant recipients; whether it improves the long-term outcome post transplant remains a matter of continued investigative interest. Regardless, the use of induction therapy is increasingly commonplace, as reflected in the 2010 Scientific Registry of Transplant Recipients. In 2010, more than 80% of kidney transplant patients received some form of induction immunotherapy beyond the ubiquitous bolus methylprednisolone that serves as the base regimen for most induction approaches. More specifically, 58% of kidney transplant patients received a T-cell depleting antibody, 21% received an IL-2RA (interleukin-2 receptor antibody), and 4% received both a T-cell depletional therapy and an IL-2RA (Table 65.1, Figure 65.1).

As the need for immunomodulation decreases, induction therapy is replaced by maintenance therapy—medications of lesser potency and improved tolerability for chronic use. Should acute rejection emerge during maintenance therapy, rescue therapy is deployed. Rescue therapy is similar to induction therapy in that it is intense and used for a short period of time. This chapter will cover the emerging biological basis for induction therapy and review the specialized induction agents currently available for clinical use. The general clinical rationale and composition of maintenance and rescue therapies will be covered in Chapters 66 and 67, as well as in the section Clinical Allograft Rejection Syndromes: Diagnosis and Management, which contains organ-specific chapters on the clinical diagnosis and treatment of acute rejection.

The biologic basis for induction therapy

A recipient of a transplanted organ first encounters that organ handicapped by two non-physiological circumstances that favor a pathological immune response. The first is the recipient's thymic and hematopoietic selection has selected a lymphocyte repertoire that is appropriate for them, in that it has a relatively low precursory frequency of autoreactive cells (cells responding to self-MHC and/or with self-peptides). Those cells that have autoreactive potential are generally of such affinity that their activation is dependent on accessory signals (discussed below) for activation. The second non-physiologic factor driving a graft-specific immune response is related to the ischemia, reperfusion, and related tissue injury that inevitably result from the process of transplantation (Figure 65.2). These factors alter mechanisms of antigen presentation, change the activation threshold of T and B cells, and alter cell trafficking so as to favor an alloimmune response (Table 65.2). Thus, the processes leading to alloimmunity derive from physiological immune processes encountering an organ under non-physiological conditions (injury within a high allospecific precursor frequency). This forms the basis for alloimmunity and as such serves as an appropriate conceptual framework for the initial immunomodulatory approach for avoiding alloimmune rejection. Additional detail regarding these concepts can be found in Chapter 11. In this section, we will relate the immunologic basis that necessitates induction therapy to the therapeutic maneuvers used at the time of transplantation. Current clinically common induction regimens are focused on T-cell-mediated rejection (largely based on the available agents, not to discount the importance of alloantibody development post transplant).

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 65.1. Induction therapy between 1994 and 2009: the percent of patients receiving any form of specialized (not including bolus methylprednisolone) induction therapy is shown for each organ type

Organ	Any specialized induction agent (%)		
	Year		
	1994	2003	2009
Kidney	25	70	82.8
Pancreas	30	79	81.4
Liver	13	20	24.6
Intestine	13	74	56.7
Heart	36	48	49.5
Lung	25	44	59.7

Data from the Scientific Registry of Transplant Recipients (SRTR) and Organ Procurement and Transplantation Network (OPTN). SRTR/OPTN 2010 Annual Data Report. Department of Health and Human Services, Health Resources and Services Administration, Healthcare System Bureau, Division of Transplantation. Am J Transplant 2012; 12 (Suppl. 1).

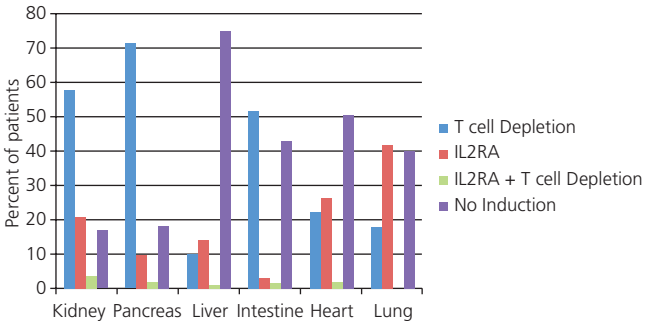

Figure 65.1. The percent of patients receiving a specific induction agent, by agent type and organ transplanted. Data from Scientific Registry of Transplant Recipients (SRTR) and Organ Procurement and Transplantation network (OPTN). SRTR/OPTN 2010 Annual Data Report. Department of Health and Human Services, Health Resources and Services Administration, Healthcare System Bureau, Division of Transplantation. Am J Transplant 2012; 12 (Suppl.1).

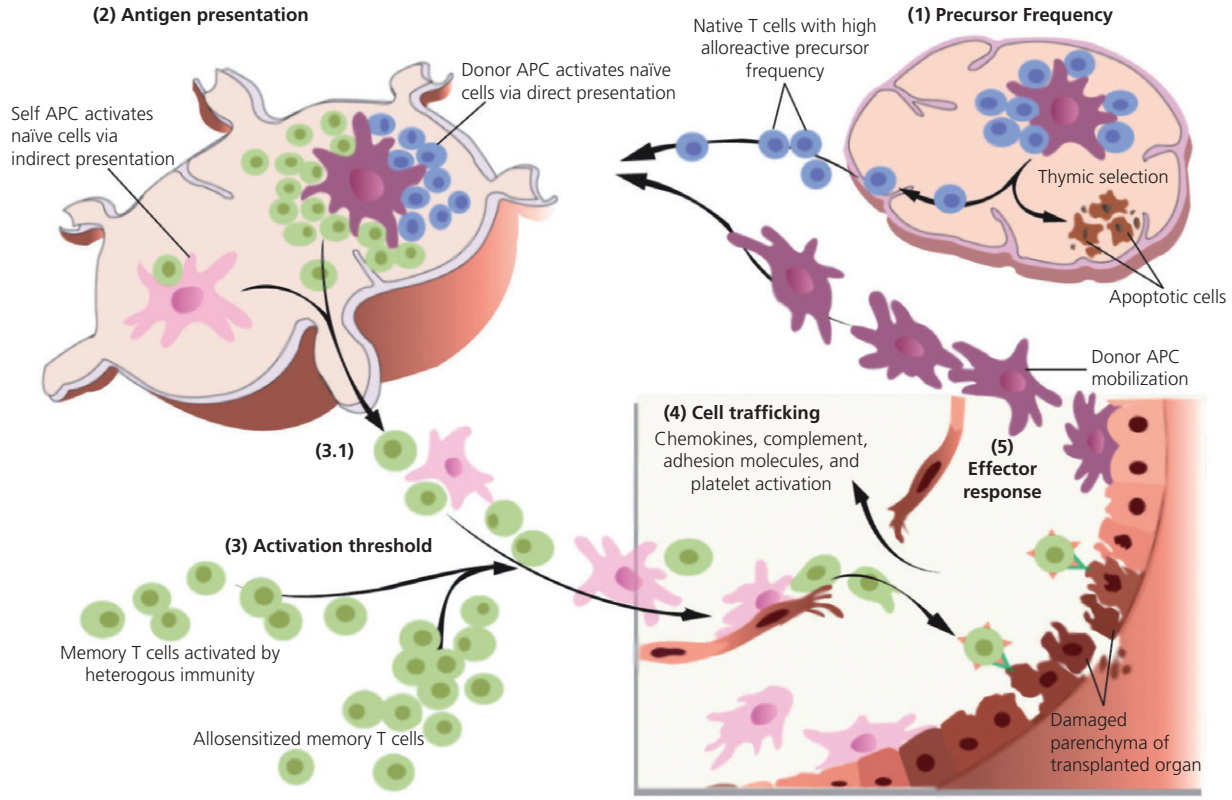

Figure 65.2. Factors necessitating induction therapy.

(1) Precursor frequency. The absence of alloantigens in the native thymus at the time of thymic selection leads to the release of T cells without regard for allo-MHC and, as such, leads to a non-physiologically high precursor frequency in the naïve T-cell pool generated. This is influenced by MHC mismatching and can be manipulated, in theory, by the introduction of donor antigen into the recipient thymus.

(2) Antigen presentation. Allospecific antigen presentation (direct through migration of donor APCs to the nodes/spleen and indirect through autologous APC antigen uptake and presentation) occurs in the secondary lymphoid tissues (e.g. peripheral lymph nodes and the spleen). It can be manipulated by costimulation blockade (e.g. belatacept), T-cell depletion, steroids, and anti-IL-2R antibodies. Strategies that alter T-cell activation threshold (CNI, mTORs) or cell division (antimetabolites) also affect a T cell's receptivity to antigen presentation.

(3) Activation threshold. T cells can be activated in the lymph nodes (3.1) or locally adjacent to the graft. Memory T cells bypass the need for nodal presentation.

(4) Cell trafficking. Recipient APCs and activated T cells are attracted by chemokines and adhesion molecules to the recently injured graft. Reperfusion injury promotes donor APCs migration to the nodes for direct presentation.

(5) Effector response. Reinforced by a milieu enriched in T-cell-derived cytokines (IL-2), donor-specific CTLs damage the transplanted organ through direct effects of the cells and indirect effects of proinflammatory cytokines.

Immunologic concepts relevant to the need for induction therapy

Several basic concepts conspire to determine a recipient's initial capacity for an alloimmune response. As induction immunosuppression is predicated on blocking the initial response, and its risk–benefit assessment is based on using this potent immunosuppressive approach only when required by the conditions of the recipient, it is useful to understand, in general terms, the factors that determine the veracity and effect of that response. These factors can be loosely defined through an understanding of the general factors determining a recipient's T-cell activation threshold, their allospecific precursory frequency, the degree to which the T-cell repertoire is in a memory as opposed to a naïve state, the amount of injury accompanying the antigen exposure, and the overall susceptibility of the organ being transplanted. These factors will be briefly summarized.

Table 65.2. The biologic basis of induction therapy

	Precursor frequency	Antigen presentation	Activation threshold	Cell trafficking
Biologic factors	MHC mismatch	IRI, APC activation	Sensitization, homeostasis	IRI, endothelial injury
Therapeutic interventions	OKT3, ATG, alemtuzumab, DST, mTOR	Steroids, belatacept	mTOR, CNI, MMF, steroids, anti-IL2R, ATG	Steroids

IRI, ischemic reperfusion injury; DST, donor-specific transfusions; mTOR, molecular target of rapamycin; CNI, calcineurin inhibitors; MMF, mycophenolate mofetil; ATG, antithymocyte globulin.

Threshold of activation: the three-signal model of the immune response

The three-signal model of immune activation is conceptually useful for understanding T-cell activation (Figure 65.3). Indeed, most commonly used immunosuppressive agents target specific pathways that fall within this framework.

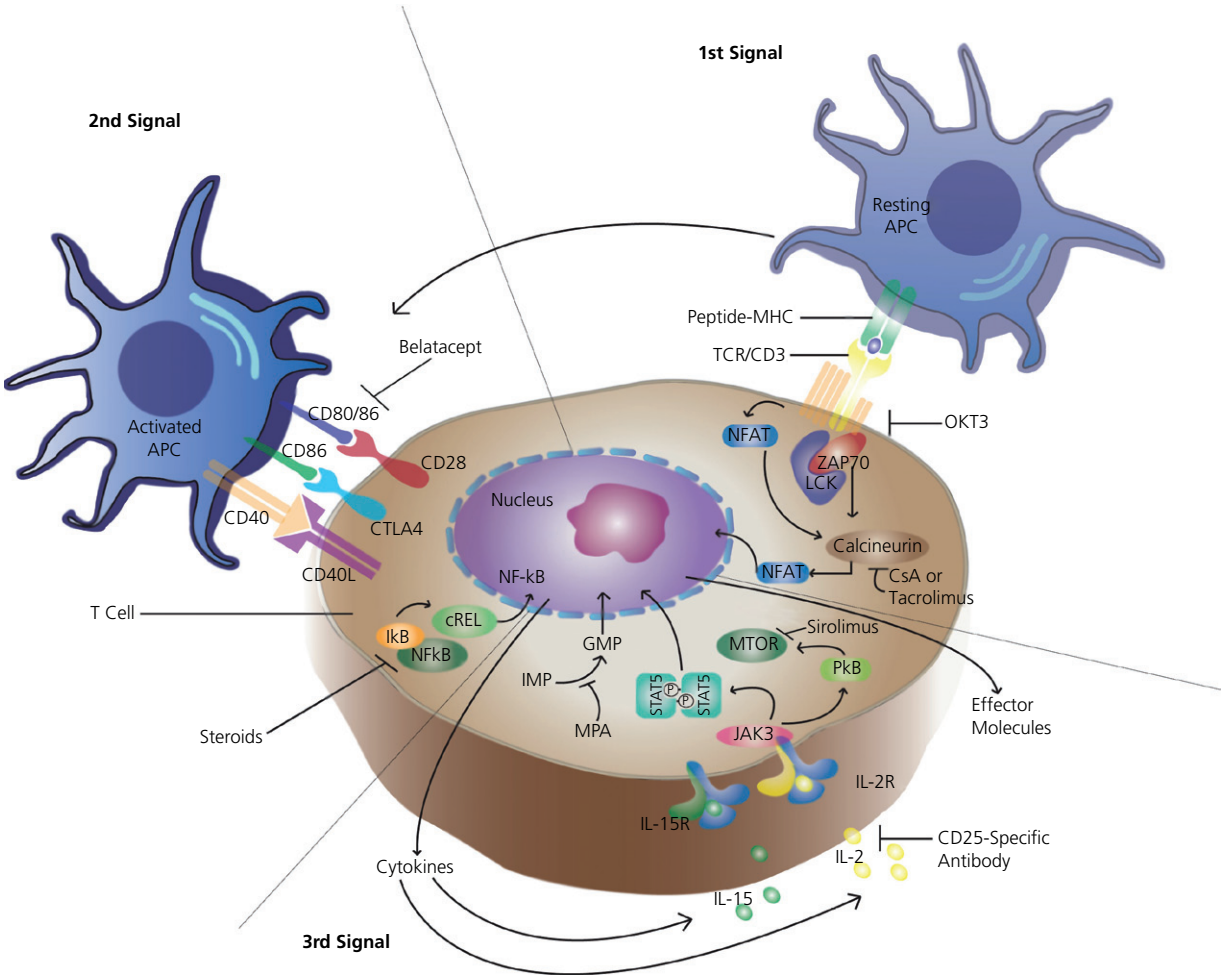

Figure 65.3. The three-signals model for T-cell activation. Signal 1 is the antigen-specific signal conferred by the TCR. Signal 2 is received by costimulatory molecules, and signal 3 is derived from postactivation cytokines. OKT3, CsA, and tacrolimus act on signal 1; belatacept and steroids act on signal 2 (and to some extent on signal 1 by limiting MHC expression); CD25-specific mAbs and sirolimus block signal 3. APC, antigen-presenting cell; CTLA-4, cytotoxic T lymphocyte antigen 4; GMP, guanosine monophosphate; IκB, inhibitory κB; IMP, inosine monophosphate; JAK3, Janus kinase 3; L, ligand; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; PKB, protein kinase B; R, receptor; STAT5, signal transducer and activator of transcription 5; TCR, T-cell receptor; TLR4, Toll-like receptor 4; ZAP70, zeta-chain-associated protein 70.

Alloantigen-stimulated activation is largely a process that occurs in secondary lymphoid tissues. Donor dendritic cells are activated at the time of transplantation and are mobilized to move to T-cell areas of secondary lymphoid organs (Figure 65.1). The chemotactic signals derived from a recently reperfused organ also attract recipient-type dendritic cells and other cells with mobile antigen-presentation capabilities (e.g. monocytes and macrophages) to the graft where donor antigen uptake can occur. These cells also migrate to the secondary lymphoid tissues. The resulting condition is one in which donor antigen is richly represented in the secondary lymphoid tissues and available for presentation to allospecific T cells. In this location, antigen-bearing dendritic cells engage naïve and central memory T cells in non-physiologically high numbers.

"Signal 1" is transmitted when an antigen on the surface of antigen presenting cells (APC) triggers T cells with the appropriate receptors through the CD3 complex. This process is labeled as direct antigen presentation when T cells are responding to donor-derived antigen-presenting cells; it is referred to as indirect antigen presentation when T cells are responding to recipient-derived antigen-presenting cells (Figure 65.4). Furthermore, antigen-presenting cells provide costimulation, also known as "signal 2", when CD28 present on T cells is engaged with CD80 and CD86 on the surface of dendritic cells. There are numerous costimulatory pathways, many of which (e.g. CD152, also known as CTLA-4) provide down-regulatory signals. Thus, the combination of signal 1 and signal 2 can lead either to antigen-specific activation or antigen-specific anergy. The details of this are outlined in Chapter 5. At the time of transplantation, generally CD28-derived activating costimulation signals tend to dominate. Thus, signals 1 and 2 activate three signal transduction pathways in the T cell: the calcium–calmodulin calcineurin pathway, the RAS-MAP kinase pathway, and the nuclear factor kappa-B (NFκB) pathway. These pathways lead to the expression of many new molecules, including IL-2, CD154, and CD25. Ultimately, IL-2, IL-15, and other cytokines activate the target of rapamycin (TOR) pathway via JAK-STAT dependent signaling through the common gamma chain of IL-2 and IL-15 (and others) cytokine receptors leading to "signal 3", the trigger for recruitment of additional cells and enhanced cell proliferation, which leads to a large number of effector T cells [1].

Of note, B cells also are activated when antigen engages their antigen receptors in lymphoid follicles or extrafollicular sites, like the red pulp of the spleen, or even the transplanted organ. Specific therapeutic manipulation of this pathway, though important, is not, in general, clinically available. Consequently, within a short period of time the unrestrained immune response can mobilize effector T cells and alloantibody.

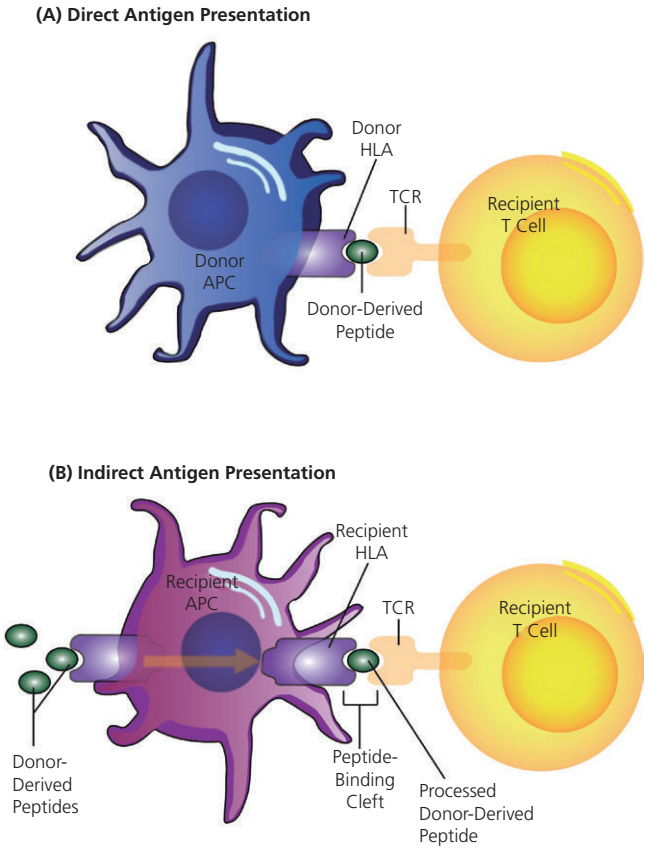

Figure 65.4. (A) Direct allorecognition. T cells responding to direct antigen presentation by donor-derived antigen-presenting cells (APCs) recognize determinants on the allogeneic HLA molecule as well as structures found on the bound peptide. (B) Indirect allorecognition. Indirect recognition requires antigen processing and presentation by recipient-derived APCs. HLA, human leukocyte antigen; TCR, T-cell receptor.

Precursor frequency

The allospecific T-cell precursor frequency is the number of T cells in the host immune repertoire that are able to respond to donor alloantigen, either through direct or indirect means. Unfortunately for transplant clinicians, in most recipients this donor-specific precursor frequency can be many logs-fold higher than the precursor frequency to any nominal antigen presented on a self-MHC. In other words, the number of T cells that are available in a recipient to respond to donor antigen is much higher than the number of T cells that can respond in a physiologic manner to a typical viral infection. Keeping in mind that immune responses are exponential in nature, a slight change in the initial donor-specific precursor frequency can pose a tremendous change in the immune response as a whole, and present rapidly escalating immunity that exceeds the regulatory controls that have evolved for much more conscribed responses. Figure 65.5 portrays the concept of precursor frequency in more detail and illustrates the effect of precursor frequency on clinical immune responses [2].

The effective precursor frequency differs widely among recipients and is mostly affected by the degree of MHC mismatch between donor and recipient. Matching the MHC, in effect, matches the recipient's thymic selection more closely to the MHC of the donor and, in doing so, assures a more appropriate T-cell precursor frequency for the incoming organ. Other factors that influence the recipients' precursor frequency are prior immune history and MHC sensitization (Figure 65.6). Up to 10% of the recipients' immune repertoire can be alloresponsive to a given donor. As such, the processes of activation that occur in the secondary lymphoid tissues are greatly (and non-physiologically) facilitated, making the chance for a productive APC–T cell interaction inevitable and much less likely to succumb to homeostatic regulatory pressure.

Memory T cells

Immunological memory is one of the characteristic features of the mammalian adaptive immune response and, in general, it makes secondary responses much more efficient than primary ones.

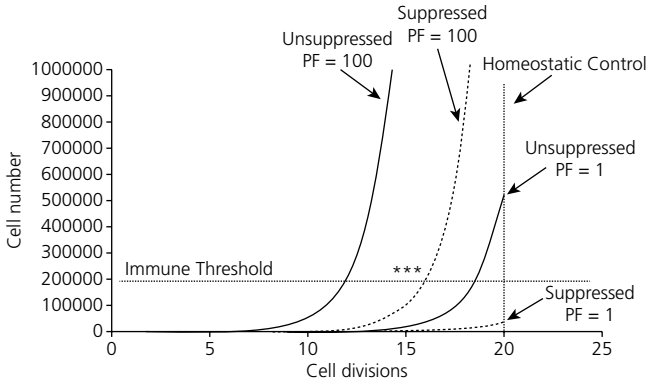

Figure 65.5. The effect of precursor frequency on clinically relevant immune responses. An efficient (or unsuppressed) immune response starting at a low precursor frequency (PF) can be surpassed by an inefficient (suppressed) immune response starting at a high precursor frequency. To mount an effective adaptive immune response, T cells must reach an "immune threshold" (theoretically a certain number or activity level), without exceeding the homeostatic control. During an optimal immune response under normal physiologic conditions (precursor frequency of 1 and no immunosuppression), T cells divide and proliferate (approximately 20 cell divisions; cell doubling at y = 0.5e0.69x) to reach the immune threshold and mount the immune response. The homeostatic control bar marks the end of T cell proliferation. When T cells are deprived of their costimulation (suppressed; and PF = 1; cell doubling at y = 0.03e69x), the homeostatic control point will be reached before the activation threshold, and this immune activity fails to register as an immune phenotype. On the other hand, raising the precursor frequency to 100 (unsuppressed cell doubling at y = 50e0.69x) will tremendously assist the immune activity reach the immune threshold to mount an immune response, way before the homeostatic control point. Even when suppressed, those cells at a precursor frequency of 100 will still reach the threshold of activation more efficiently than unsuppressed cells at a PF of 1.

Memory T cells pose a significant barrier to tolerance induction (Figure 65.7). They are the product of prior antigen exposure delivered in the setting of sufficient costimulation to induce cell division, and persist and survive as a pool of long-lived antigen-specific cells [3]. Memory T cells are characterized, based on surface phenotype, into central memory (Tcm) and effector memory (Tem). Central memory cells (Tcm) migrate to secondary lymphoid organs (lymph nodes, spleen) and, from those locations, generate bursts of new effector T cells upon recall. Effector memory cells (Tem) migrate to peripheral non-lymphoid sites and provide immediate effector function to foreign antigen (for example, alloantigen following organ transplantation) [3]. The common characteristic of both is an ability to reactivate with substantially less need for costimulation and fewer repetitions of MHC-TCR binding. As such, therapies that work well for naïve cells may fall short when directed against memory cells. Of note, memory cells tend to have reduced capacity for proliferation and reduced survival once reactivated. As such, this barrier, though substantial, can be attenuated with brief therapies targeted toward transient elimination, a principle of induction.

The influence on memory is manifest experimentally in considering the effects of costimulation blockade. In rodent models, depriving T cells from costimulation at the time of antigen presentation induces transplantation tolerance successfully [4]. However, this approach is less successful in outbred models (e.g. humans) and memory T cells are thought to be largely to blame. Specifically, memory T cells in young laboratory mice raised in specific pathogen-free conditions represent only 5–10% of the total T-cell compartment. On the other hand, over 50% of the T-cell compartment in humans and non-human primates is composed of memory T cells. This discrepancy can be explained by the difference in the environmental exposure and the resultant immune history. Thus, humans and socially housed non-human primates possess an armamentarium of potent memory T cells that is in a sense on standby to mount a barrier to transplantation tolerance induction [3].

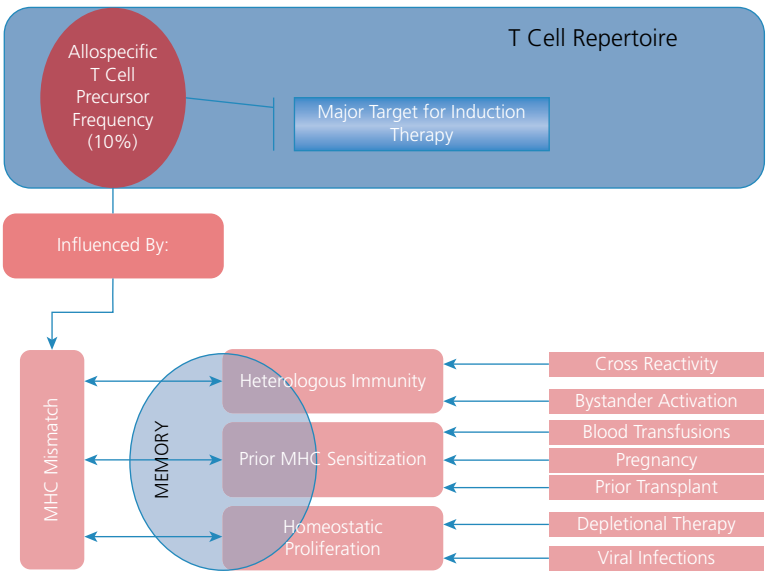

Figure 65.6. Allospecific precursor frequency is a major target for induction immunosuppression.

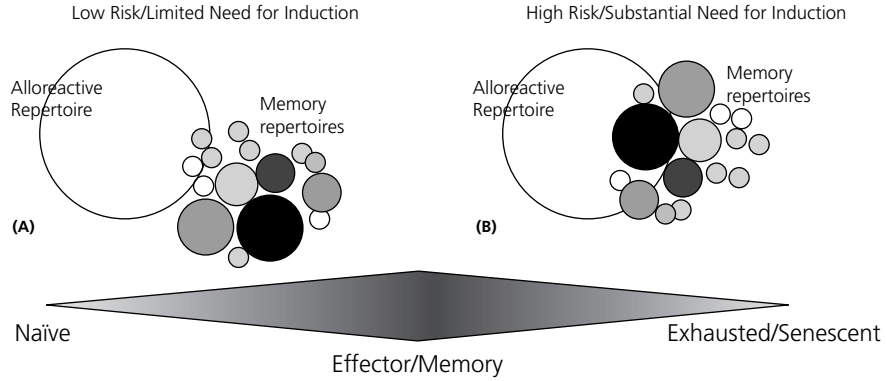

Figure 65.7. Conceptual scheme for induction. The spectrum of naïve T cells through expanded activated effector memory states, moving to senescent phase, is shown at the bottom of the figure. Looking at the allorepertoire and multiple viral reactive repertoires at variable stages of maturation, we would hypothesize that individuals with a very low degree of heterologous cross reactivity (A) will be at low risk for rejection, and individuals who have a high degree of cross reactivity (B) (with large expanded activated populations that are in fact incorporated in the alloimmune repertoire, represented by black circles) would be at a very high risk of rejection. So, if we can distinguish between A individuals and B individuals we might be able to tailor our immune management such that we would avoid complications and unnecessary risks.

In addition to their prevalence in adult humans, memory T cells exhibit an increased resistance to depletional therapy and are also less inhibited by the activity of regulatory T cells than naïve T cells. This large pool of memory T cells in adult humans stochastically contains a high frequency of donor reactive memory T cells, which imposes a major factor in determining the relative success of tolerance induction protocols during transplantation [5].

When developing a tolerance induction strategy for human patients, one must consider both the naïve component of the alloimmune response and the memory counterpart. This is important regardless of the pathway that generated those memory cells. In fact, several mechanisms have been described to explain the generation of donor-reactive memory T cells. These include heterologous immunity, homeostatic proliferation, prior MHC sensitization, and T cells with dual receptors. The relationship between memory and alloimmunity is described briefly below, but covered in depth in Chapter 9.

Heterologous immunity. Previous immunological exposures can influence the course of future responses to completely unrelated stimuli. At least two mechanisms can explain this phenomenon: T-cell cross reactivity and bystander activation [6]. A normal immune response to a pathogen, like a virus, occurs when APCs process the viral proteins and present antigenic peptides complexed to self-MHC molecules in secondary lymphoid organs. Molecular mimicry, leading to T-cell cross-reactivity, occurs when this viral antigen–self-MHC complex imitates a foreign MHC molecule containing self-peptide. Alternatively, a heterologous response could occur through bystander activation, when viral-specific T cells release growth factors, cytokines, and inflammatory mediators, thus activating bystander alloreactive T cells in a non-specific manner [6] (Figure 65.8).

Homeostatic proliferation. T cells can extensively proliferate under conditions of lymphopenia, a process named homeostatic proliferation. This process shows high resistance to costimulation blockade and generates alloreactive memory T cells that show resistance to tolerance induction. Clinically, lymphopenia can be induced by a viral pathogen or by therapeutic depletion of T cells for the treatment of autoimmune disease or organ transplantation. The residual T cells undergo rapid division and acquire a memory phenotype, hence posing a strong barrier to transplantation tolerance [7].

MHC sensitization and other mechanisms. Prior exposure to antigens can occur through pregnancy, blood transfusions, and transplantation. Recently, it has become apparent that other mechanisms exist by which donor reactive memory T cells might be generated. Memory T cells specific for a pathogen-derived epitope have in some cases been shown to express a second T-cell receptor that can be alloreactive [8].

Tissue injury mechanisms necessitating induction therapy

Injury and immunity are intimately associated in that injury leads to exposure to pathogens, and pathogens themselves ultimately cause tissue destruction—a vicious circle. Types of injury include endothelial damage, complement activation, ischemia and reperfusion, surgical wounding in the recipient, donor injury, and brain death, and all impact the intensity of the immune response. These factors propel immunity by increasing the effectiveness of antigen presentation, and the trafficking of cells to the transplanted organ and its draining lymph nodes [9]. Recognition of, and response to, injury is typically viewed as a role of the innate immune system, which is covered in depth in Chapter 7.

Several other aspects of injury conspire to promote alloimmunity. For example, complement deposited within a reperfused transplanted organ can facilitate naïve T-cell activation. Ischemia and reperfusion have been shown to increase costimulatory molecule expression, thus leading to increased efficiency of both resident and migratory APCs, and also improving the response of engaged T cells. Furthermore, the cytokine milieu created right after reperfusion strongly favors activation and differs markedly from that seen months after engraftment [9]. Damaged endothelium and parenchyma secrete chemokines that attract effector cells to the organ; at the same time, reperfusion injury initiates APC mobilization to the lymph nodes and spleen. Both of these events increase the possibility of a specific T cell meeting its suitable APC or target antigen, thus creating a locally enhanced T-cell precursor frequency [9].

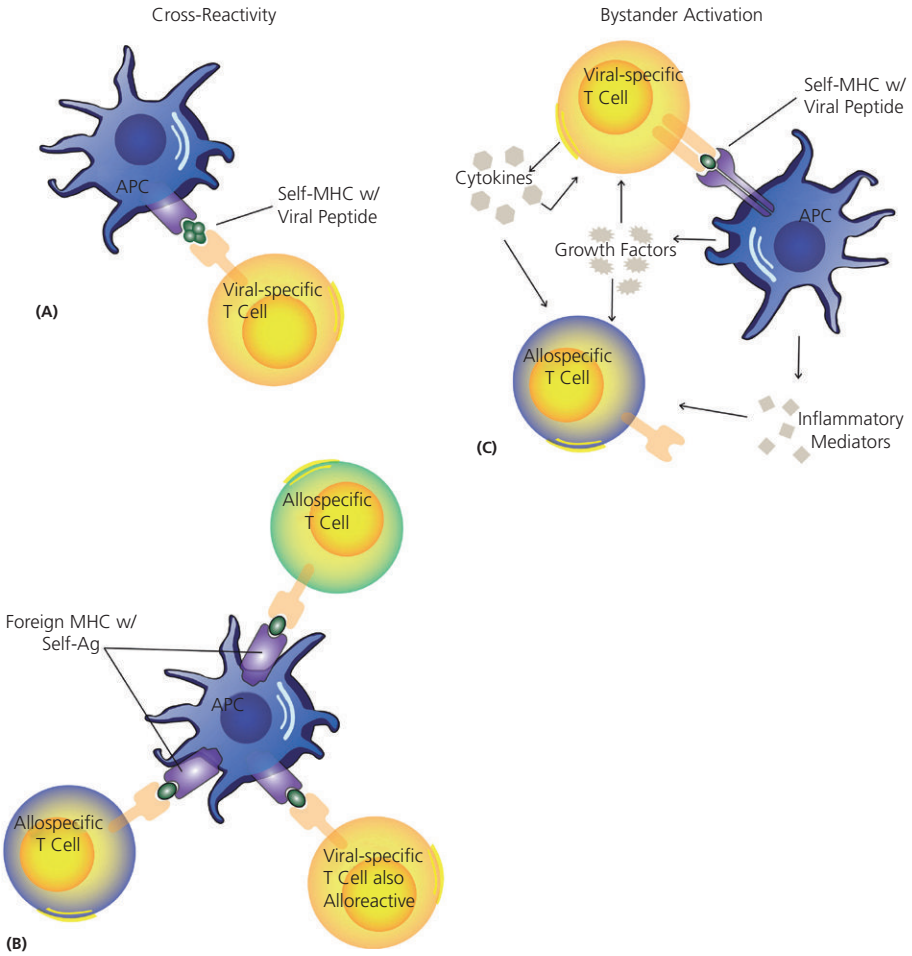

Figure 65.8. Heterologous immune response: (A) normal immune response. APCs process a viral protein and present the antigenic peptides complexed to self MHC; (B) cross-reactivity through "molecular mimicry"; (C) bystander activation.

Organ susceptibility as a variable in the need for induction therapy

It is important to keep in mind that transplanted organs markedly differ with regard to their susceptibility to injury. For example, a heart needs to function immediately and continuously and is dependent on the electrical activity of the conduction system (a small volume of tissue); it also is susceptible to the negative inotropic effect of local cytokines. Contrast this to a kidney, which can fail for several weeks and still regenerate its tubules and function even after a marked injury, or a liver, which can regenerate over half its mass in response to injury. Furthermore, the need to avoid immunity is greater in older organs with pre-existing injury. Thus, the magnitude of an acceptable immune insult varies widely not solely based on the size of the response, but also on the ability of the organ to withstand the response and still recover in sufficient time to be of use. In summary, a survivable alloimmune response varies not only from patient to patient, but also from organ to organ and donor to donor. Consequently, the perioperative immunosuppression required to obtain a good outcome also varies [9].

Linking the above factors to a clinical scenario is complex and has yet to be achieved in a prospective quantitative approach. Still, one can, in a very broad sense, identify that there are various considerations at play. Factors like age, prior pregnancies, EBV status, prior organ transplantation, MHC match, donor clinical condition, and ischemia time are to be taken into consideration. Ideally, induction therapy would be tailored to the specific characteristics of the recipient and donor [9]. In practice, however, a finely tuned means of assessing the myriad circumstances at play in a quantitatively proportional way has not been developed. As such, general approaches are taken to address common themes of early immune activation, and induction agents are used based on broad institutional or demographically based guidelines.

Targeting specific elements of an initial immune response

The term immunosuppression has a very broad meaning and gives no specific information about a patient's risk of rejection. Since each patient receiving an organ has a unique immune history and each graft has its unique vulnerabilities, induction therapies should then vary in their required intensities, duration, and target. The most reasonable approach is to look at those variable factors that warrant induction therapy and choose medications that address specifically those factors (Table 65.3).

Targeting precursor frequency

Depletional induction agents can non-specifically reduce precursor frequency. Depletional agents include polyclonal anti-T lymphocyte/thymocyte preparations (thymoglobulin, ATGAM,

Table 65.3. Immunosuppressive medications that can lower T-cell activation threshold

Immunosuppressive Drug	Mechanism of Action
Calcineurin inhbitors	Decrease signal transduction
Anti-TCR antibodies	Limit efficiency of TCR binding
mTOR inhibitors	Limit the effect of activating cytokines
IL-2R antagonists	Limit the effect of activating cytokines
Glucocorticoids	Change nuclear proteins avaliable to TCR
Costimulation antagonists	Block CD28 engagement

ATG-Fresenius), and monoclonal preparations specific for common lymphocyte determinants like CD3 (OKT3) and CD52 (alemtuzumab). These agents mediate rapid T-cell depletion with varying degrees of durability and tolerability. In doing so, they reduce the number of cells available to harm the graft and, ideally, reduce the allospecific T-cell frequency to more closely approximate that of a physiologic immune response. An important observation is that not all T cells are depleted equally. T cells with a memory phenotype are less susceptible (still susceptible but proportionately less so) to antibody-mediated depletion than are naïve T cells (likely a reason they are tolerated despite markedly reducing the T-cell count). It is also important to note that following partial T-cell depletion, the remaining T cells undergo homeostatic proliferation, thus creating a population of T cells with a memory-like phenotype. This considerably lowers the threshold of activation of each cell, leaving remnant cells that are relatively more active and potent. Thus, following depletion, patients are capable of rejecting with very few T cells. As such, depletion always requires additional immunosuppression as the recipient emerges from the depletional therapy. Clinical trials using aggressive depletion with OKT3, alemtuzumab, or thymoglobulin have all been performed without additional maintenance immunosuppression and shown rejection as soon as T-cell repopulation begins, even with T-cell counts of less than 10 cells/μl.

One theoretical way of avoiding the bulk activating effects of post-depletional homeostatic activation is to target specifically activated T cells. IL-2R (CD25)-specific monoclonal antibodies (daclizumab and basiliximab) were designed with this in mind. Although designed with hopes of selective depletion, their clinical effect has been modest with little or no depletional capacity.

Donor-specific transfusions (blood or marrow) have been shown to facilitate allospecific T-cell depletion when combined with agents that promote activation-induced cell death (AICD). Theoretically, providing donor antigen to activate a cell while at the same time depriving it of signal 3, the cytokines required to promote its division, leads to apoptosis. Among these agents are the mTOR inhibitors (sirolimus and everolimus). The possible mechanisms by which donor-specific transfusion works include clonal deletion, activation of regulatory T cells, anergy, and chimerism. Regardless, the effects of this, though evident in experimental models, have not been shown to be practically operative in general clinical situations.

Targeting antigen presentation

As mentioned earlier in this chapter, two different dynasties of APCs are available after organ transplantation to initiate rejection: those derived from the donor (involved in direct presentation) and those of the recipient (involved in indirect presentation) (Figure 65.4). Both can lead to rejection; however, donor-derived APCs likely influence the initial risk of rejection most as they tap into the bulk of the alloreactive T-cell repertoire.

Numerous steps can be taken to limit APC mobilization, beginning with good preservation technique and expedient transplantation. This limits factors such as oxidative stress that have been shown to influence APC activation and function. From a therapeutic medication standpoint, methylprednisolone is used almost without exception at the time of organ reperfusion, and is known to decrease APC trafficking. While it is common to dismiss bolus steroid use as not "truly" being induction, in fact it is one of the more effective induction strategies, and has been accepted as such to be considered just part of the transplant procedure rather than a specific induction approach.

Polyclonal antibody preparations have antiadhesion effects. Therefore they potentially block APC activation and trafficking, and this might differentiate polyclonal preparations from monoclonal preparations in that the former have a mechanistic reach extending beyond mere depletion. Deoxyspergualin (DSG) has also been found experimentally to block APC activation, and has been used anecdotally on this basis without substantial effect [10]. Costimulatory molecules are crucial in APC function. Most prominent are the B7 molecules, CD80 and CD86. Biologic agents have been specifically designed to block this pathway, the most advanced of which is belatacept [11]. Belatacept has been studied as a maintenance drug, but may have some effect on the initial APC function. Data do not support it as having substantial efficacy over and above general maintenance agents, even when used at the time of transplantation.

Targeting the threshold of activation

Many factors are involved in T-cell activation. T cells reach an activation threshold as a result of marked antigen affinity, excessive antigen presentation, or weak antigen presentation supplemented by optimal costimulation, or even abundance of stimulatory cytokines in the T-cell immediate environment. Given that T cells are crucial for organ rejection, most commercially available immunosuppressive drugs alter the T-cell activation sequence of events (Table 65.3). These effects are covered in depth in Chapter 17. With regard to the use of standard immunosuppressive drugs with induction in mind, most clinicians view achievement of therapeutic drug levels early post transplant to be an important goal. As such, aggressive dosing of calcineurin inhibitors, antimetabolites, and mTOR inhibitors all have been explored. In general, the tolerable doses of these drugs are limited by toxicities and, as such, high "induction dose" use of drugs altering T-cell activation is limited. Alternatively, glucocorticoids can induce a change in the nuclear binding proteins available for a signal transmitted through the T-cell receptor, and likely influence this mechanism, adding to its efficacy as an induction agent.

Targeting cell trafficking

Ischemia and reperfusion promote cell trafficking toward the transplanted organ through a multifactorial process, which includes adhesion receptor up-regulation, endothelial retraction or damage, and chemotactic cytokine release [12]. This phase of the alloimmune response is gaining wide recognition as worthy of specific targeting. In general, glucocorticoids limit adhesion molecules expression and polyclonal antilymphocyte medications also have antiadhesion molecule effects. As such, this mechanism has been cited as being in part responsible for the rationale of methylprednisolone and/or polyclonal antibodies during the induction phase. However, numerous investigational agents are being considered based on the importance of trafficking at the time of reperfusion.

Integrins play a vital role in leukocyte activation and trafficking to sites of inflammation. Found on a variety of immune cells, including T cells, B cells, neutrophils, and macrophages, integrins are heterodimeric cell surface receptors that mediate adhesion between these cells and other cells in their immediate environment. In particular, leukocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4) are the most studied integrins. LFA-1 plays a vital role in the formation of the immune synapse between T cells and antigen-presenting cells, while both LFA-1 and VLA-4 have been shown to play a role in the arrest of rolling lymphocytes at sites of inflammation and the migration of T cells into sites of inflammation. Integrin antagonists are discussed later.

One class of agents specifically targets the chemokine pathways that are essential in cell homing and trafficking. In particular, sphingosine-1-phosphate (S1P) is a critical element in the regulation of chemokine sensitivity and theoretically represents an interesting target for blockade. Fingolimod (FTY720) was designed to bind to S1P and leads to internalization of this molecule, thus inhibiting lymphocyte migration from the secondary lymphoid organs. It has been shown to reduce the rate of relapses in relapsing–remitting multiple sclerosis. Unfortunately, fingolimod has been found to have a first-dose bradycardia effect that has limited its development in transplantation.

Clinical results with specialized induction agents

Many agents are clinically available to target the components of alloimmunity that are amplified shortly after transplantation. Several of these agents have been studied in clinical trials in combination with standard maintenance regimens and shown to be efficacious, but few prospective studies have compared the prominent agents and no agent has distinguished itself as clearly superior in all situations. Most trials have used the surrogate endpoint of early acute rejection rather than a more definitive endpoint of patient or graft survival. Almost all trials have layered "specialized" induction agents on an induction base of bolus methylprednisolone.

When compared to a standard bolus of methylprednisolone induction, specific induction agents considered as a whole have been shown to reduce the incidence of early biopsy-proven acute rejection (BPAR) in kidney recipients, particularly those who are allosensitized and on the historical standard of cyclosporine (CsA), azathioprine (AZA), and prednisone [13,14]. Induction in simultaneous kidney–pancreas transplantation offers a modest trend toward reduction of rejection [15,16], and its efficacy in heart transplantation remains controversial [17]. Induction has not been shown to benefit liver transplantation. Long-term analysis has failed to show a measureable effect in kidney transplantation after 5 years [13], likely because the effects of maintenance therapy and recipient co-morbidities become dominant over time.

As with all forms of aggressive immunosuppression, induction therapies have been associated with increased incidences of infectious and malignant complications. The benefits of induction do not come at the cost of higher technical complications [18]. However, several induction strategies measurably increase the risk of post-transplant lymphoproliferative disease (PTLD) and death from malignancy when combined with conventional maintenance immunosuppression [19,20]. Specifically, the expected PTLD rate is 0.5% in patients who do not receive specialized induction, or who receive CD25-specific therapy. OKT3 induction increases the risk to 0.85% as does polyclonal depletion at 0.81%, particularly in recipients newly exposed to the Epstein–Barr virus at transplantation. Given this trade off, it remains unclear whether long-term outcomes are improved by induction therapies. Regardless, the reduction in early rejection does ease the early management of a transplant recipient, and this has led to a general increase in induction therapy use. Table 65.4 summarizes the monoclonal and polyclonal antibodies used in induction.

Table 65.4. Monoclonal and polyclonal antibodies

Name	Form	Source	Target
AntiThymocyte Globulin (ATGAM)	Polyclonal	Horse	Anti-CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR1
AntiThymocyte Globulin (Thymoglobulin)	Polyclonal	Rabbit	
Muromonab (OKT3)*	Monoclonal	Mouse	Anti-CD3
Basiliximab (Simulect)	Monoclonal	Recombinant (chimeric)	Anti-CD25
Daclizumab (Zenapax)#	Monoclonal	Recombinant (humanized)	Anti-CD25
Alemtuzumab (Campath)	Monoclonal	Recombinant (humanized)	Anti-CD52

*Withdrawn from global market in 2009; #Withdrawn from US in 2009

Muromonab-OKT3

OKT3 (muromonab-CD3; Orthoclone OKT3, Janssen-Cilag) is a mouse IgG2a antihuman CD3 antibody which was the first monoclonal antibody approved for induction therapy. OKT3 binds at the epsilon chain of the CD3 protein, resulting in the transient activation of circulating T cells and their release of cytokines (IL-2, -3, -6, IFN-γ); eventually, opsonized T cells undergo massive lysis and disappear from the circulation [21].

Muromonab was first introduced in the early 1980s and quickly shown to be efficacious in treating acute rejection. Despite its clinical success, its adverse side-effect profile was challenging, most secondary to the immense cytokine release from T cells inducing a clinical cytokine release syndrome which included hypotension, fever, nausea, vomiting, and pulmonary edema. Rarely, OKT3 caused aseptic meningitis or intragraft thrombosis. Furthermore, being a mouse antibody, patients developed antimurine antibodies that limited OKT3's usefulness after the first dose. Other major side effects of OKT3 were PTLD, fungal infections, and CMV infection. As a result, OKT3 was removed from the market and is mentioned here for historical purposes [21]. A number of humanized OKT3 Fc variants have been developed with attempts to achieve the antirejection effects without cytokine release; however, none have been shown to be useful in the setting of transplant induction.

Anti-CD25 antibodies

The side effects of the pandepleting muromonab was substantial incentive for the development of better-tolerated induction immunosuppression. As the cytokine IL-2 was well recognized as a prominent T-cell growth factor, basiliximab and daclizumab, both mABs targeted to the alpha subunit of the IL-2 receptor (CD25), were developed. Both competitively inhibit IL-2 binding and preventing T-cell expansion [21]. Most importantly, the serum sickness and expedited drug clearance associated with horse, mice, and rabbit

protein-derived mABs are absent with basiliximab (chimerized) and daclizumab (humanized). A theoretical advantage lies in the fact that these mABs target a receptor that is uniquely expressed by activated T cells.

Daclizumab and kidney transplantation. Daclizumab is a humanized IgG1 monoclonal antibody, introduced in 1998 after it was proven to be safe and efficacious in kidney transplantation in a prospective placebo-controlled trial [22]. On a background of triple therapy (AZA, CsA, steroids) or double therapy (CsA, steroids), daclizumab has been shown to modestly reduce BPAR at 6 months [23]. In other studies, daclizumab-treated patients, when compared to placebo, had a better graft function, a similar post-transplant lymphoproliferative disease incidence, and decreased infections with CMV [24–26]. Importantly, treatment with daclizumab did not induce a systemic inflammatory response syndrome (SIRS) or its associated symptoms.

Induction with daclizumab in the setting of triple therapy but with a lower dose of CsA was shown to be roughly equivalent to a standard CsA dose; unfortunately, weaning off CsA completely was associated with a significant increase in BPAR [27–29]. A regimen of daclizumab induction on the background of a triple therapy with mycophenolate mofetil (MMF), corticosteroids, and low-dose tacrolimus (TAC) was advantageous in terms of renal function, graft survival, and acute rejection episodes, when compared to regimens containing daclizumab induction with low-dose CsA or low-dose sirolimus, or standard CsA alone [30].

For high-risk patients, defined as patients who had lost a previous graft or had alloantibody (not donor specific), induction with the depletional agent rabbit antithymocyte globulin (RATG) on a background of triple therapy (MMF, TAC, steroids) was significantly more effective than induction with daclizumab in terms of steroid-resistant rejection and BPAR [31].

Daclizumab with other solid organs. Daclizumab has also been studied in liver, heart, and lung transplantation. In liver transplantation, daclizumab in a two-dose regimen had a similar effect to that observed in kidney transplantation [32]. Several studies showed lower acute rejection episodes with daclizumab when added to conventional MMF and TAC regimens and, more recently, several studies revealed that corticosteroids could be safely eliminated from this regimen [33–35].

In heart transplant patients, the standard five-dose regimen of daclizumab reduced the frequency of acute rejection episodes when compared to conventional immunosuppression. Eventually, a two-dose regimen of daclizumab was shown to be as effective as a five-dose regimen in heart transplant patients [36–39]. As for lung transplant patients, daclizumab was slightly superior to RATG in two controlled studies [40,41].

Although daclizumab was on the market for approximately 10 years, its use was discontinued in September 2009. This was largely related to market share competition with its contemporary, basiliximab, rather than for biological differences. The most prominent difference between daclizumab and basiliximab is the number of doses recommended for induction (five versus two, respectively) and this difference has swayed in favor of basiliximab.

Basiliximab in kidney transplantation. In a 1997 randomized, prospective trial of basiliximab induction on a background of CsA and steroids, compared to placebo, patients who received basiliximab had a lower 6-month BPAR, a lower incidence of steroid-resistant rejection, no evidence of the cytokine release syndrome, and a similar rate of infections and PTLD [42]. These results were again verified in three other trials at different institutions; induction with basiliximab in the setting of a triple background therapy (CsA, AZA, and prednisone) also showed a lower 6-month acute rejection rate [43–46]. Trials looking at single-dose basiliximab versus double doses showed that single-dose regimens (40 mg on post operative day 1) had a slightly better BPAR and acute rejection rates [47–49]. Most importantly, several trials have shown that in the setting of induction with basiliximab, calcineurin inhibitor-sparing regimens were well tolerated [50–52].

Furthermore, another study compared RATG plus delayed CsA to basiliximab plus CsA induction on a background of MMF and steroids. Graft survival rate, patient survival, and BPAR rates were comparable in both groups [53].

Basiliximab with other solid organ transplants. A two-dose basiliximab induction in liver transplant patients was well tolerated in clinical trials and effective at reducing BPAR rates (though to a lesser extent in HCV-positive patients) [54–56]. Interestingly, basiliximab allowed for a delayed TAC administration and a better renal function in liver transplant patients [57]. Most importantly, in HCV-positive patients, induction therapy with basiliximab with a steroid-free background showed a higher BPAR rates (39.4% basiliximab minus steroids vs. 24.3% basiliximab plus steroids) but better patient survival (84.3% without steroids vs. 61.0% with steroids). In addition, HCV recurrence was lower in the steroid-free regimen with basiliximab induction [58–60].

The jury is still out on the efficacy of basiliximab in heart transplant patients. In one trial, basiliximab induction was used to facilitate delayed CsA administration, thereby decreasing renal toxicities in post-heart transplant recipients [61]. Even comparative studies with RATG are still controversial [62,63]. A recent meta-analysis of several smaller studies has found little evidence that IL-2R-specific therapy improves outcome [64].

In lung transplantation, a recent analysis of the International Society of Heart and Lung Transplant Registry data have shown IL-2R-specific therapy to be beneficial in single and double lung transplant recipients [65].

Alemtuzumab

CD52 is a membrane glycoprotein with an unknown function. It is present on T cells, B cells, macrophages, monocytes, eosinophils, and cells of the male reproductive system. Alemtuzumab is a humanized IgG2b specific for human CD52, which has been used for induction and rescue therapy [66–69]. Its administration leads to rapid and effective T and B-cell depletion, and some degree of monocyte depletion. It is indicated for the treatment of lymphogenous malignancies, and is being developed as a therapy for multiple sclerosis. Although not specifically indicated for transplant induction, it has long been used off-label for this indication, and is now used in approximately 10% of kidney transplants performed in the USA. See Chapter 101 for an in-depth discussion of off-label drug use.

Alemtuzumab gained the attention of the transplant community following its use to facilitate low-dose CsA monotherapy in kidney transplantation [67]. Subsequent investigations explored its use as a tolerogen [68], clearly demonstrating that despite profound depletion, alemtuzumab did not induce tolerance, but rather facilitated low-dose maintenance therapy. Subsequent studies, combining it with more conventional immunosuppressive approaches, have

guided this agent into reasonably routine use [69–71], showing that it is effective in reducing the rate of BPAR, and provides a window of several months where maintenance immunosuppressive needs can be reduced, although in the absence of sufficient maintenance immunosuppression late BPAR is seen.

Comparative studies. Few well-controlled comparative studies have been performed using alemtuzumab, and this reflects its lack of a specific indication in transplantation and the fiscal implications this has on trial conduct. In an early trial using a historical control group, alemtuzumab was compared to basiliximab on a background of MMF and TAC. In this large single-center study, alemtuzumab-treated patients had a lower rejection rate at 3 months, but similar rejections rates at 1 year [70]. Other small studies followed, suggesting benefits of alemtuzumab induction with short-term follow-up [71,72].

The single most important study investigating alemtuzumab as an induction agent was a multicenter, randomized, controlled trial that stratified patients based on perceived rejection risk (defined by repeat transplant, a peak value of panel-reactive antibodies above 20%, or Black race) compared with low-risk patients randomized between basiliximab and alemtuzumab, and high-risk patients randomized between RATG and alemtuzumab [73]. The rate of BPAR was significantly lower in the alemtuzumab groups at both 6 (3% vs. 15%, $P < 0.001$) and 12 months (5% vs. 17%, $P < 0.001$). At 3 years, the rate of BPAR in low-risk patients was lower with alemtuzumab than with basiliximab (10% vs. 22%, $P = 0.003$), but among high-risk patients, no significant difference was seen between alemtuzumab and RATG (18% vs. 15%, $P = 0.63$). Adverse-event rates were similar among all groups. This study has done the most to establish alemtuzumab as a viable option for induction therapy, but has not convinced its maker, Sanofi, to seek an indication in transplantation.

Alemtuzumab has not been systematically or extensively studied in extrarenal organs. One study compared alemtuzumab and low-dose TAC to conventional TAC and steroids in liver transplant recipients. Alemtuzumab-treated patients needed less steroids for maintenance, had less renal toxicity, and, most importantly, the incidence of acute rejection at 1 year was lower with a longer median time to rejection [74]. Lung transplant patients suffering from refractory acute rejection or bronchiolitis obliterans syndrome received alemtuzumab. The histological rejection scores in rejection did improve, as did freedom from broncholitis at 2-year follow up [75]. These liver and lung studies show that alemtuzumab might have some benefits in organs other than the kidney.

Polyclonal antibodies

Several polyclonal antibody preparations have been explored, beginning in the earliest days of transplantation. Polyclonal antibodies are heterologous antibody preparations that are produced by whole-cell immunization of a variety of animals, with the clinical preparations being derived from either rabbits (RATG) or horses (ATGAM). Rabbit preparations are overall noted to be the most effective in multidrug renal transplant immunosuppression protocols [76,77]. Polyclonal antibodies contain antibodies that exhibit binding specificities to a variety of cellular antigens, including TCR cell-surface markers involved in antigen recognition, T-cell adhesion, and costimulation (e.g. CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45), as well as MHC molecules and non-T-cell molecules.

The affinity spectrums for polyclonal antibody preparations are diverse and variable. Even the clinically approved RATG and ATGAM agents designed to contain only IgG remains diverse, highly immunogenic and consequently associated with a vast array of undesired adverse effects, including pancytopenia [78,79]. Currently, three preparations are used clinically: antithymocyte immunoglobulin-R (ATG-R or RATG), antithymocyte globulin-Frensius (ATG-F), and ATGAM (horse prepared). While all are used to some extent, by far the most commonly used is RATG, the result of comparative trials showing its higher efficacy in kidney transplantation [80]. The dominant mechanism of action of polyclonal preparations is currently unknown but has been inferred to be related to a combination of precursor frequency reduction, inhibition of leukocyte adhesion, and blockade of accessory molecule signals required for lymphocyte activation.

The side-effect profile of polyclonal antibody induction is well documented. Cytokine release syndrome tends to be the most common adverse effect observed with therapy, affecting up to an estimated 20% of all treatment recipients. This physiologic phenomenon is thought to be caused by a similar means to that discussed above for muromonab, but in general is substantially less severe. Additionally, these drugs generally require administration via central venous access to minimize the risk of thrombophlebitis. Other concerning adverse effects relate to their potent immunosuppressive effects and include PTLD, opportunistic infections, and reactivation of latent herpesviruses. Dose-limited side effects include leukopenia and thrombocytopenia, serum sickness, and anaphylaxis.

Polyclonal antibodies in kidney transplantation. Polyclonal antibody preparations are used for induction for approximately 50% of all kidney transplant recipients in the USA. The rates are lower in Europe and elsewhere in the world. To date, most trials have been designed to evaluate polyclonal antibodies added to an otherwise rigorous maintenance regimen, and, as would be anticipated, this intense approach has both reduced acute rejection rates and increased infectious morbidity [81,82]. Given that polyclonal antibodies target many of the mechanisms compelling induction and have lasting effects both in terms of CD4 depletion and cell surface molecule modulation, recent trials have been designed to reduce the need for maintenance therapy. Two pilot studies have demonstrated that RATG facilitates monotherapy maintenance immunosuppression in selected patients with graft and patient survival comparable to the current standard [83,84]. Longer follow-up has suggested that upon lymphocyte repopulation, late rejections can be seen, and, as such, the advisability of this approach remains a matter of debate.

Trials dating back to the pre-CsA era have shown that RATG induction delays early acute rejections [76]. More contemporary trials have shown RATG to be more efficacious and better tolerated in most situations compared to ATGAM [85,86]. As modern concerns have turned toward cost analysis, evidence has suggested that the modest long-term benefits of polyclonal induction may not warrant the additional cost associated with their purchase and administration [87].

The most robust evidence defining the proper use of polyclonal induction has been provided in the form of a randomized, prospective trial comparing RATG to basiliximab in patients deemed at high risk for acute rejection or delayed graft function [88]. In this study, 278 patients were randomized to RTG or basiliximab. The RATG group had less BPAR (15.6% vs. 25.5%, $P = 0.02$) and less

steroid resistant rejection (1.4% vs. 8.0%, $P = 0.005$), but no differences in the incidences of graft loss (9.2% and 10.2%, respectively), delayed graft function (40.4% and 44.5%), and death (4.3% and 4.4%). The incidences of adverse events, serious adverse events, and cancers did not differ but those patients receiving RATG had higher rates of infection (85.8% vs. 75.2%, $P = 0.03$) but, paradoxically, less cytomegalovirus disease (7.8% vs. 17.5%, $P = 0.02$). Thus, polyclonal induction has a role in the management of high-risk patients in that it reduces the BPAR rate, but it does not alter the rate of delayed graft function or improve long-term outcomes.

Extrarenal use of polyclonal antibodies. Both ATG-R and ATG-F have been prospectively demonstrated to be superior to OKT3 in heart transplantation with respect to reducing the rate and consequences of acute rejection [89,90]. When compared to each other, their results have been equivalent in heart transplantation [91]. Induction appears to be most beneficial for patients with the highest risk of rejection (African Americans, allosensitized patients, patients dependent on a ventricular assist device); otherwise, induction therapy with polyclonal antibodies in heart transplant patients is associated with a higher incidence of infectious complications and malignancies [21]. Polyclonal induction agents have not been shown to be of use in liver transplantation [92], although induction with RATG can be employed to delay calcineurin inhibitor introduction in patients with a pre-existing renal failure [93].

In lung transplantation, RATG on a background of triple therapy was found to reduce the rejection episodes with no effect on the incidence of bronchiolitis obliterans syndrome [94]. Analysis of the International Society of Heart and Lung Transplant Registry indicates that a benefit to induction with RATG is evident in recipients of double lung transplants [65].

Investigational agents of note

Antiadhesion drugs. Given the clear role of adhesion molecules in the trafficking of lymphocytes to the graft and the draining secondary lymphoid organs, the blockade of the adhesion has long been conceptually attractive as an induction strategy. Several approaches have been explored and remain of potential importance.

LFA-1 (leukocyte function-associated antigen 1), a B2 integrin, is present mainly on the surface of T cells, and has two distinct functions: (1) cell arrest and migration on surfaces that express an LFA-1 ligand (intracellular adhesion molecule-1, ICAM-1); and (2) contact with APCs and stabilization of an immunological synapse. LFA-1 helps T cells withstand the shearing force of blood flow, and helps T cells create a firm adhesion and transmigration into the site of injury [95].

Efalizumab (Raptiva; Genentech) is a humanized LFA-1 IgG1 antibody that was initially approved for psoriasis treatment in 2003. Vincenti et al. studied efalizumab induction in 38 kidney transplant patients receiving standard triple maintenance immunosuppression [96]. The study suggested efficacy in that the rate of rejection was low, but the immunosuppressive potential added to an already effective regimen was excessive, leading to several cases of PTLD. Subsequent use in islet transplantation demonstrated remarkable tolerability and efficacy in two pilot studies [97,98]. These studies inspired a resurgence of interest, which was unfortunately halted by efalizumab's removal from the psoriasis market in 2009. As the drug's only indication was psoriasis, off-label use in transplantation has stopped.

Alefacept (Amevive; Astellas) is an LFA-3/IgG1 fusion protein that, like efalizumab, was indicated for psoriasis. Preclinical data in primates was promising in showing that alefacept provided a depletion of memory T cells and as such facilitated costimulation-blockade based immunosuppression [99]. While its logical use would be in this setting, the development of alefacept moved to the clinic as an agent added to a TAC-based regimen, and, in this setting, no efficacy was demonstrated. Regardless, like efalizumab, alefacept has been withdrawn from the psoriasis market, making it unavailable for off-label investigation in transplantation.

TNF inhibitors. TNF blockade, with the TNF-specific mAb infliximab, or more recently with the TNF receptor antagonist etanercept, is commonly used in inflammatory bowel disease, where its efficacy has been clearly demonstrated. In transplantation, TNF-specific therapy has been used as an induction agent in islet cell transplantation. Although it has been shown to be effective in rodent models [100], its use in islets has been based on uncontrolled common usage.

Etanercept was used in the induction phase along with antithymocyte globulin and daclizumab in eight human recipients of single-donor marginal-dose islet transplantation. All recipients achieved insulin independence and freedom from hypoglycemia [101]. Etanercept also was studied in patients receiving supplemental islet transplantation (those are patients who received allogeneic islet cell transplantation with progressive graft dysfunction). Despite the small sample size and lack of randomization, the results suggested an improvement in engraftment, long-term survival, and function [102]. More recently, etanercept was used in 17 patients with graft dysfunction receiving supplemental islet infusions. Induction therapy consisting of etanercept and thymoglobulin was used in 13 patients, while four patients received daclizumab induction. Maintenance consisted of sirolimus and TAC or TAC plus MMF. There was no difference in the insulin independence rates or durations between the two induction regimens used [103].

Summary

Induction therapy is now a common practice in most transplant surgery programs in the United States (in all if bolus methylprednisolone is considered). As detailed in this chapter, precursor frequency, innate immunity, tissue injury during transplantation, and ischemia reperfusion injury are the main factors compelling the use of induction therapy. Its benefits are centered on the decrease in the rate of early acute rejection episodes and a general simplification of the early perioperative immune management of patients, compensating for early low drug levels, and reducing the likelihood of rejection when non-rejection related causes of dysfunction are more common (e.g. reperfusion injury, technical issues). Thereafter, the benefits of induction remain less well defined, but may include incremental reductions in rejection balanced by an increased risk of infections (viral and fungal) and malignancies (virally induced cancers, PTLD). As such, the use of induction therapy should be considered based on the individual risks of the patient and their particular organ, and chosen to match the biological factors most likely to influence those risks.

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CHAPTER 66

Maintenance Immunosuppressive Therapy

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Introduction

Advances in immunosuppressive strategies over the past decades have led to significant improvements in the field of solid organ transplantation. Cyclosporine revolutionized transplant practice by lowering acute rejection rates and improving *short-term* graft survival in the 1980s (see Chapter 1). In the 1990s, post-transplant outcomes improved with tacrolimus and mycophenolic acid. Despite these advances, clear evidence for a beneficial effect on *long-term* graft survival is lacking, especially in the field of renal transplantation. Additionally, significant improvements in early graft survival have come at the expense of associated adverse side-effects, including metabolic side-effects that increase the risk of cardiovascular and cerebrovascular disease. The use of newer immunosuppressive agents, including the mammalian target of rapamycin (mTOR) inhibitors and belatacept, are being incorporated into evolving strategies that aspire to minimize lifelong exposure to calcineurin inhibitors and corticosteroids and improve long-term survival rates.

In an effort to improve outcomes, clinicians have used several general maintenance immunosuppression strategies that are applicable regardless of the organ transplanted. One approach involves assessing patient risk factors for rejection. Patients at higher risk for rejection may receive more potent immunosuppression—either higher doses or longer duration of therapy—while patients at low risk for rejection may receive less potent immunosuppression. Immunosuppression may also be adjusted based on the time after transplant. Immediate postoperative needs are different from long-term maintenance therapy needs. Other management strategies involve monitoring viral infections, which appear to be a sign of excessive immunosuppression and may guide immunosuppressive adjustment and reduction. Likewise, maintenance immunosuppression adjustment may be beneficial in patients with malignancy or recurrent disease.

An ideal immunosuppressive regimen limits toxicity and prolongs the functional life of the graft. This chapter will review maintenance immunosuppressive strategies and provide an overview of the pivotal trials in kidney, pancreas, lung, liver, heart, and intestinal transplant.

Trends and types of maintenance therapy

Immunosuppression regimens for transplant recipients change over time and vary by center. Currently, the five drug classes that comprise maintenance regimens include calcineurin inhibitors (cyclosporine and tacrolimus), mTOR inhibitors (sirolimus and everolimus), antiproliferative agents (azathioprine and mycophenolic acid), costimulatory blockers (belatacept), and corticosteroids (Tables 66.1 and 66.2). The mechanisms of these drugs are covered in detail in Chapter 17, and their approved indications are covered in detail in Chapter 98. In the past two decades, tacrolimus has been substituted for cyclosporine, and mycophenolic acid has been substituted for azathioprine as primary therapy in most US transplant centers, making tacrolimus plus mycophenolate mofetil/mycophenolate sodium, with corticosteroids the most common maintenance immunosuppressive regimen (Figure 66.1A–F). When considering data from the US Scientific Registry for Transplant Recipients (SRTR), there are few deviations among the commonly transplanted solid organs (Figure 66.2). Currently, a majority of lung transplant recipients receive tacrolimus and mycophenolate, but 20% of patients are discharged on a combination of tacrolimus and azathioprine [1]. Secondly, most intestine transplant recipients receive tacrolimus without mycophenolic acid, most likely due to the gastrointestinal side-effects of mycophenolate [1]. Lastly, approximately 20% of heart transplant recipients receive cyclosporine, while the majority receive tacrolimus [1].

Maintenance immunosuppressive therapy can be classified as quadruple, triple, dual, or monotherapy. Quadruple therapy consists of an induction agent at the time of transplant, followed by triple maintenance therapy. Triple therapy, a three-drug regimen, is the most commonly used maintenance regimen because it targets various sites of the immune response, and drug synergies allow minimization of drug toxicity. Historically, the main goal of immunosuppressive therapy was to prevent acute rejection and allograft loss. The relative achievement of these goals has shifted the focus to strategies that minimize immunosuppressive therapy and associated infection and toxicity without compromising efficacy. Therefore, some centers use corticosteroid- or calcineurin inhibitor-sparing regimens in the hope of attaining these goals. There are two principal steroid-sparing strategies: (1) steroid withdrawal/ avoidance and (2) steroid free. These regimens consist of corticosteroids for a short period after transplant or no corticosteroids at all, respectively. According to current data, there has been a movement to withdrawal of corticosteroids in renal, liver, and pancreas transplants, while most heart, intestine, and lung transplant recipients still receive corticosteroids (Figure 66.3) [1]. Many of these regimens consist of two maintenance medications or dual maintenance

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Table 66.1. FDA approved immunosuppressive medications used for transplantation

Drug	Dose	Side-effects
Prednisone	Maintenance: 2.5–10 mg/day Rejection: 250–1000 mg/day × 3 days i.v.	Mood disturbances, psychosis, cataracts, hypertension, fluid retention, peptic ulcers, osteoporosis, muscle weakness, impaired wound healing, glucose intolerance, weight gain
Cyclosporine	4–5 mg/kg p.o. twice daily	Neurotoxicity, gingival hyperplasia, hirsutism, hypertension, hyperlipidemia, glucose intolerance, nephrotoxicity, electrolyte disturbances
Tacrolimus	0.05–0.075 mg/kg p.o. twice daily	Neurotoxicity, alopecia, hypertension, hyperlipidemia, glucose intolerance, nephrotoxicity, electrolyte disturbances
Sirolimus	2–10 mg/day p.o. daily	Hypertriglyceridemia, anemia, thrombocytopenia, mouth sores, hypercholesterolemia, gastrointestinal disturbances, bone marrow suppression, poor wound healing, edema, pleural effusions, pericardial effusions
Everolimus	0.75–1.5 mg p.o. twice daily	Hypertriglyceridemia, anemia, thrombocytopenia, mouth sores, hypercholesterolemia, gastrointestinal disturbances, bone marrow suppression, poor wound healing, edema, pleural effusions, pericardial effusions
Azathioprine	1–2.5 mg/kg/day p.o. daily	Leukopenia, thrombocytopenia, gastrointestinal disturbances, pancreatitis, hepatotoxicity
Mycophenolate mofetil	500–1500 mg p.o. twice daily	Leukopenia, thrombocytopenia, gastrointestinal disturbances
Mycophenolate sodium	360–1080 mg p.o. twice daily	Leukopenia, thrombocytopenia, gastrointestinal disturbances
Belatacept	10 mg/kg administered, prior to implantation, on day 5, and at the end of weeks 2, 4, 8, and 12, then 5 mg/kg every 4 weeks (plus or minus 3 days)	Increased acute rejection, post-transplant lymphoproliferative disorder, progressive multifocal leukoencephalopathy, tuberculosis

p.o., by mouth; i.v., intravenously.

Table 66.2. Classification of immunosuppressive agents

Classification	Drug (generic)	Drug (trade)	Generic	Dosage form
Corticosteroids	Methylprednisolone	Solumedrol® (Pfizer, New York, NY)	Yes	Injection, oral
	Prednisone	Deltasone® (Pfizer, New York, NY)	Yes	Oral
	Prednisolone			Oral
Calcineurin inhibitors	Cyclosporine, CsA	Sandimmune® (Novartis, East Hanover, NJ)	Yes	Injection, oral
	Cyclosporine microemulsion	Neoral® (Novartis, East Hanover, NJ) GenGraf® (AbbVie, Chicago, IL)	Yes	Injection, oral
	Tacrolimus, FK506	Prograf® (Astellas, Northbrook, IL)	Yes	Oral
	Astragraf (Astellas)	Hecoria® (Novartis, East Hanover, NJ)		
mTOR inhibitors	Sirolimus, rapamycin	Rapamune® (Pfizer, New York, NY)	No	Oral
	Everolimus	Zortress® (Novartis, East Hanover, NJ) Certican ® (Novartis, East Hanover, NJ)	No	Oral
Antiproliferative	Azathioprine, AZA	Imuran® (GlaxoSmithKline, Mississauga, Ontario, Canada)	Yes	Injection, oral
	Mycophenolate mofetil, MMF	Cellcept® (Genentech, San Francisco, CA)	Yes	Injection, oral
	Mycophenolate sodium, EC-MPS	Myfortic® (Novartis, East Hanover, NJ)	No	Oral
Costimulation blockade	Belatacept	Nulojix® (Bristol-Myers Squibb, New York, NY)	No	Injection

therapy. Calcineurin inhibitor sparing/ avoidance therapy is being investigated, but is not commonly used. The last type of maintenance therapy is monotherapy, which is rarely used outside of clinical trial settings [2,3]. Candidates for monotherapy or dual therapy include patients with transplanted organs that are perceived to have less immunogenic potential and perceived to be at less immunological risk. Both groups have been defined primarily upon epidemiological data and not on clearly defined immunologic data such as human leukocyte antigen (HLA) matching (see Chapter 36), which—although used in kidney transplantation—is uncommonly used or ascertained in other solid organ transplantation [4–7].

Risk factors and maintenance immunosuppression

Patients perceived to be at increased risk for acute rejection may receive more intense maintenance immunosuppressive regimens. These perceived risks have had variable degrees of utility but, in general, no clear metric for one's need for immunosuppressive therapy has been validated. Immunologic, donor, and recipient risk factors are discussed below.

Immunologic risk factors

Multiple tests may be performed prior to transplant to assess a patient's risk for acute rejection, including panel-reactive antibodies, an immunologic cross-match, HLA matching, and blood/ tissue typing. The methods and basis for these tests are covered in depth in Chapter 36. Highly sensitized patients, those with alloantibodies directed against HLA antigens of the donor, are at higher risk for acute rejection. Circulating antibodies to the donor's antigens may be a result of prior transplantation, pregnancy, or multiple blood transfusions. As the panel-reactive antibody level rises, the chance of a positive B or T cell cross-match and an immunologically incompatible transplant is increased. In a study of the effect of sensitization on graft survival in haploidentical living related transplants, patients with greater than 50% panel-reactive antibody titers had a significantly shorter time to graft failure [8]. A positive T-cell cytotoxicity cross-match is a contraindication to kidney transplant without effective "desensitization" because of the associated risk for hyperacute rejection and graft loss. Patients with positive B-cell cross-match are known to be at risk for accelerated acute rejection, and more potent immunosuppressive regimens are used, although this practice has not been well studied. While not a

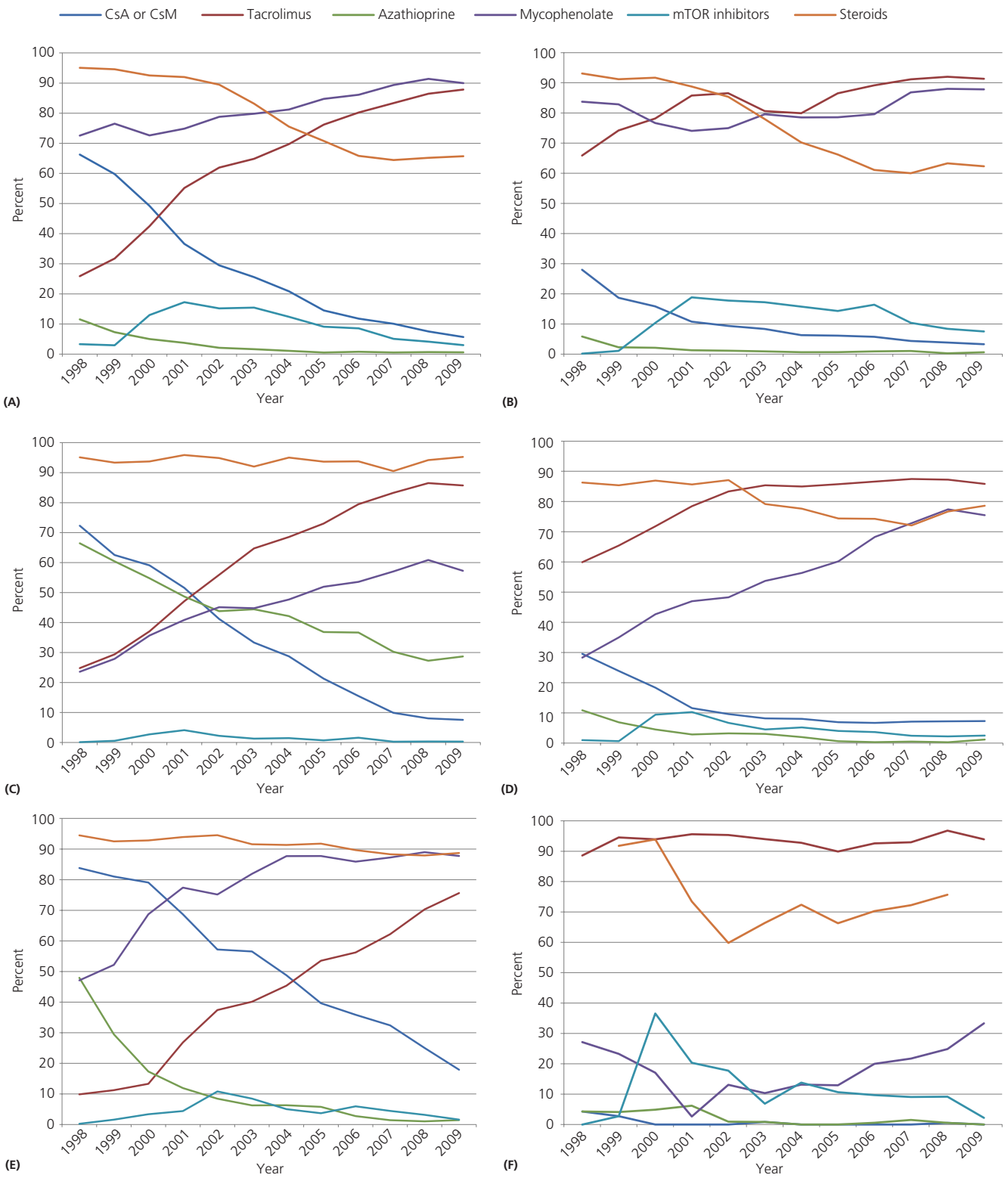

Figure 66.1. Trends over time in transplant immunosuppression: (A) kidney transplant; (B) pancreas transplant; (C) lung transplant; (D) liver transplant; (E) cardiac transplant; (F) intestinal transplant.

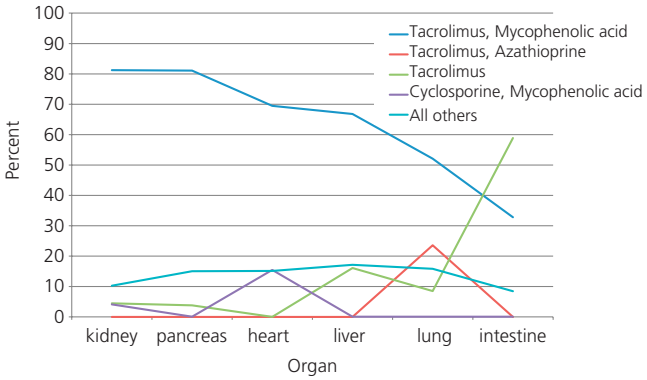

Figure 66.2. Maintenance immunosuppression at the time of transplant.

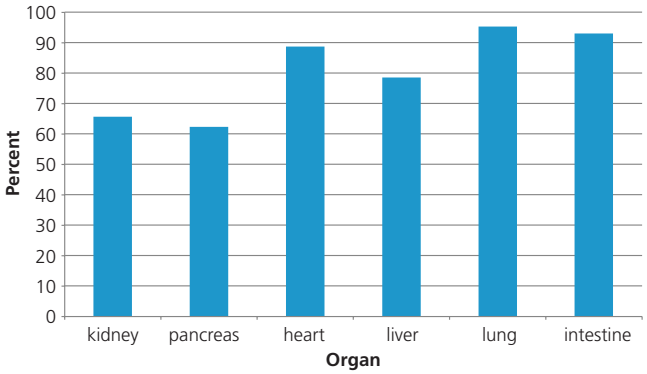

Figure 66.3. Corticosteroid use at transplant discharge by organ type.

contraindication to transplant, a positive flow cross-match is also associated with poorer graft outcomes and may warrant augmented immunosuppression or more intensive post-transplant monitoring [9]. In general, donor specificity is the fundamental characteristic of antibodies conferring high risk, with the risk of non-donor specific alloantibodies less well established.

Human leukocyte antigen matching

In kidney transplant, HLA antigens are important in determining the risk of rejection, especially when potent immunosuppressive agents are not used. The greater the number of antigen mismatches between the donor and recipient, the higher the chance of the recipient having a rejection episode. Numerous studies have shown that better HLA matching leads to better graft survival [10,11]. Despite this, a study of the United States Renal Data System (USRDS) database analyzing 23 443 patients transplanted between 1995 and 2002 revealed a mere 4–8% lower use of immunosuppression with mycophenolate mofetil and tacrolimus at 6 months in the "No HLA ABDR mismatched group" compared to the DR mismatched group. Thus, adjustment of immunosuppression based on HLA matching represents a possible opportunity for "tailoring" of immunosuppression based on immunologic rather than epidemiologic risk of rejection that appears under-utilized.

Studies have suggested that HLA mismatching is associated with rejection and graft outcomes in non-renal transplantation as well. HLA mismatching impacts acute rejection rates in liver transplant but does not impact 1-year graft survival [12]. In lung transplant, HLA mismatching is associated with acute rejection as well the development of bronchiolitis obliterans syndrome (BOS) and decreased patient survival [13,14]. Studies examining the effect of HLA matching in cardiac transplantation have been conflicting, with recent larger single-center studies demonstrating no impact on survival [15]. For non-renal solid organ transplant, considerations such as matching the size of the organ to the size of the recipient and the constraints of cold ischemia time have overshadowed routine use of HLA matching, but with the new calculated panel-reactive antibody (cPRA) using pretransplant single-bead anti-HLA antibody assessment, HLA matching may become useful across all organs to guide maintenance immunosuppressive therapy.

Other immunologic assessment

Additional assays and markers have been investigated to identify preformed T-cell memory, enabling additional assessment of pre-transplant risk or for post-transplant monitoring. These are covered in more detail in Chapters 89, 90, and 91. Ideally, this would provide information enabling individualized treatment, with patients at greater immunologic risk of rejection treated with augmented immunosuppression and those with lower risk considered for drug minimization [16]. The interferon (IFN)-γ ELISpot assay is a technique to detect alloantigen-specific memory T cells. Evidence suggests that high frequencies of donor-reactive memory T cells are associated with poorer graft outcomes [17] and that panel-reactive memory T-cell reactivity is independent of PRA [18]. Markers that reflect general immune activation have also been examined, such as soluble CD30, which is generated by the proteolytic cleavage of membrane-bound CD30 in activated T cells [19]. Although sCD30 levels have been used to predict post-transplant development of donor-specific antibodies [20], high levels do not necessarily correlate with rejection [21]. The ImmuKnow assay (Cylex, Inc., Columbia, MD), which measures ATP released in vitro (iATP) from isolated, polyclonally stimulated $CD4^+$ T cells, has also been examined as a method to discriminate between under- and over-immunosuppression. A meta-analysis suggests that this assay has poor predictive capability for either rejection or infection, and its use to directly manage patients is generally discouraged [22]. Overall, further prospective information regarding the clinical utility of these tests in various patient cohorts and immunosuppression regimens is warranted before any can be recommended for routine clinical use to guide immunosuppressive therapy.

Donor and recipient characteristics

Donor risk factors for acute rejection include the duration of cold ischemia and delayed graft function [23–26]. Cold ischemia time and delayed graft function also influence outcomes. In a multicenter analysis, cold ischemia was reported to be strongly associated with delayed graft function, with a 23% increase in the risk of delayed graft function for every 6 hours of cold ischemia. Acute rejection occurred more frequently in grafts with delayed graft function, which was independently predictive of 5-year graft loss [27]. Delayed graft function has been associated with a significant reduction in renal allograft survival before and after the cyclosporine era [28]. Use of potent immunosuppression in patients with delayed graft function is common in clinical practice, although this practice has not been well studied. Sirolimus, through its inhibiting effect on cell proliferation, may prolong delayed graft function [29].

Recipient characteristics that place a patient at risk for rejection include age, pretransplant dialysis, and re-transplantation. Aging is associated with a reduction in immunoresponsiveness due to the generalized decrease in T-cell production and response [30,31].

However, the Belatacept Evaluation of Nephroprotection and Efficacy as First-Line Immunoprotection Trial (BENEFIT-EXT) showed that rejection was more common in older recipients of expanded criteria donor kidneys [32]. Since an expanded criteria donor has more histologic damage, this may represent "priming" for rejection or reflect that older donor organs have histologic changes that mimic acute rejection. Randomized clinical trials addressing the management of immunosuppression in older patients are needed. Longer waiting time on dialysis and repeat transplant status also have been reported to impact post-transplant patient and graft survival negatively [33]. Historically, re-transplantation was unsuccessful. However, more recently, success has improved with potent immunosuppressive regimens [1]. Although the use of more potent immunosuppressive agents in patients with these risk factors is common, this practice has not been well studied.

Race

Historically, differences in renal transplant outcomes have existed between African-American and Caucasian recipients [34–37]. African Americans are at higher risk for rejection, even among living related two haplotype matched recipients [38], and may benefit from more aggressive immunosuppressive strategies [39]. Despite overall improvements in 1-year graft survival rates in the cyclosporine era, survival rates remained lower in African-American compared to Caucasian patients [40–43] and although cyclosporine-based quadruple immunosuppression has been reported to improve short-term graft survival rates in African-American as well as Caucasian recipients, long-term outcomes in African-American patients have not been affected [44,45]. Outcomes with tacrolimus and mycophenolic acid have shown more promise. Tacrolimus-based immunosuppression with antibody induction has reduced the occurrence and severity of acute rejection at 1 year post-transplant when compared to cyclosporine, especially in African-American recipients [46]. Beneficial results have also been reported with mycophenolate mofetil, although African Americans may require a higher dose than Caucasian kidney transplant recipients. An analysis that compared azathioprine and mycophenolate mofetil reported that acute rejection was reduced in African-American kidney transplant recipients on mycophenolate mofetil when compared to azathioprine, and that acute rejection was similar in African-American recipients given mycophenolate at a dose of 3 g/day and Caucasian recipients given 2 g/day [39]. This study also showed that there was no dose effect in non-African Americans, and that in non-African Americans the combination endpoint of biopsy-proven acute rejection and treatment failure at 6 months was not significantly different between azathioprine and mycophenolate.

Adherence

Non-compliance is associated with rejection, graft failure, and poor outcomes after transplantation, particularly in adolescents [47–50]. This is covered in considerable depth in Chapter 120. Newer immunosuppressive therapy may provide alternative treatments to patients known to be non-adherent. Sirolimus has a long half-life that affords convenient once-daily dosing. Belatacept may prove useful in patients with adherence difficulties due to increased patient compliance with less frequent (monthly) administration as compared to other daily and twice daily oral regimens. Alternatively, it may be perceived as a barrier to patients without social support, those who have difficult intravenous access, or those for whom an infusion center is not readily available.

Pregnancy

Pregnancy may increase the risk of acute rejection and allograft loss. The effects of pregnancy on transplant outcome are covered in Chapter 97. To minimize this risk, careful immunosuppressive selection and drug monitoring is needed. Mycophenolic acid, sirolimus, and everolimus may be harmful to a fetus, and should be avoided. Although listed as category D in pregnancy, azathioprine has been used safely in pregnant transplant patients since the 1950s. Pharmacokinetics, such as volume of distribution and metabolism of immunosuppressives, are also altered with pregnancy and require monitoring and adjustment.

Summary

Given toxicities of immunosuppressive agents, different maintenance regimens are being explored to minimize adverse short- and long-term effects. However, these strategies remain strongly guided by perceived immunologic risk rather than rigorously validated objective metrics. Patients at risk for rejection may require more potent immunosuppressive medication, more medications, and higher doses. Patient at lower risk for rejection may need less potent medications, lower drug concentrations and dosages, or mono- or dual therapy. At present, strong evidence exists only for risk stratification based on HLA mismatching and preformed alloantibody rather than epidemiological risks.

Infection and maintenance immunosuppression

Newer immunosuppressive regimens have revolutionized transplantation, lowering rejection rates and improving graft survival, but at the cost of higher infection risk. Viruses utilize target host cell machinery to replicate, and an effective antiviral immune response depends on both innate (e.g. natural killer cells) and adaptive (e.g. T cells) responses. In suppressing the host immune system to preserve the allograft, the risk of viral infection is increased. The infectious disease risks inherent in transplantation are covered in depth in Chapters 92, 93, and 94. This section will relate viral infection to immunosuppressive management. In general, viral replication is indicative of robust, and perhaps overly heavy, immunosuppression and should prompt consideration for reducing maintenance immunosuppression.

Highly seroprevalent viruses that are relatively benign in the general population can threaten the immunosuppressed transplant recipient, resulting in allograft damage and malignancy. Viruses that pose the greatest clinical threat include human herpesviruses (HHV) 1 and 2, varicella zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), HHV6, 7, and 8, hepatitis C virus (HCV), hepatitis B virus (HBV), BK virus (BKV), human immunodeficiency virus (HIV), human papillomavirus (HPV), and parvovirus. Although many immunosuppressants have demonstrated antiviral effects in vitro, clinical observations may differ (Table 66.3). Strategies for the management of maintenance immunosuppression in the patient at risk or diagnosed with viral infection post-transplant are discussed; with focus on BK, CMV, Hepatitis B and C, and EBV.

BK virus

BK virus is the most common human polyomavirus activated after solid organ transplant. Seroprevalence of BKV is 82% in healthy blood donors; the virus establishes latency in the renal cortex, medulla, and uroepithelial cells. Despite decreased BKV-specific

Table 66.3. Maintenance immunosuppressive effects on viral infections

	BKV	CMV	HCV	HBV	EBV
Cyclosporine	—	↑↑↑	↓↓↓	↓↓	↑↑↑
Tacrolimus	↑↑↑	—	↑	—	—
Azathioprine	↓	↓↓	—	↑↑	↑
Mycophenolic acid	↑	↓↓	↓↓	↓↓	↓
mTORi	↓	↓↓	↓	↓	↓↓

↓ demonstrated antiviral activity; ↑ demonstrated detrimental effect (e.g. increased viral replication); — demonstrated no effect.
BKV, BK virus; CMV, cytomegalovirus; HCV, hepatitis C virus; HBV, hepatitis B virus; EBV, Epstein–Barr virus.

T-cell responses caused by immunosuppression [51], BK nephropathy (BKVN) rarely occurs in the native kidneys of non-renal solid organ transplant recipients as BKVN results primarily from reactivation of latent BK in the transplanted organ [52]. Thus, BKVN is primarily observed in renal transplant recipients (1–10%). Clinical manifestations include asymptomatic viral shedding, cystitis, tubulointerstitial nephritis, nephropathy, ureteral stenosis, and allograft failure.

A correlation exists between BKV infection and the overall degree of immunosuppression. Antirejection treatment and cumulative steroid exposure are risk factors for BKVN. Several studies have demonstrated that tacrolimus-based regimens are associated with higher risk of BKVN [53–55]. However, no specific induction or maintenance immunosuppressive agent or combination is exclusively associated with development of BKVN.

Eighty per cent of patients developing BK viremia do so within the first 3 months post-transplant [56]. Fortunately, BKV can be detected in plasma prior to development of nephropathy [57]. Therefore, consensus recommendations are to screen recipients for viremia every 1–3 months initially post-transplant [58]. As viremia represents imbalance between BKV reactivation and BKV-specific cellular immunity and effective antiviral agents are lacking, immunosuppression reduction remains the cornerstone of therapy to allow for development of BKV-specific immunity. Immunosuppression reduction in those with viremia (>10 000 copies/mL) can avoid progression to histological and functional renal impairment. Outcomes with screening and preemptive strategies are likely superior to treatment of established nephropathy, although comparative trials have not been performed.

Two approaches to immunosuppression reduction after detection of viremia have been described. In the first approach, the calcineurin inhibitor is decreased by 25–50% followed by reduction in antiproliferative agent [59,60]. The alternative approach is first to decrease or eliminate the antimetabolite followed by subsequent calcineurin inhibitor reduction, if necessary to achieve clearance of viremia [54]. Direct comparison of these two strategies has not been done. One single-center study in which BK viremia was treated with cessation of the antimetabolite followed by calcineurin inhibitor reduction reported long-term outcomes. In 200 patients, viruria and viremia occurred in 35% and 12%, respectively by 1 year. Resolution of viremia was observed in 95% of patients after immunosuppression reduction. Overall 5-year patient survival was 91% and graft survival was 84%. Development of BK viremia did not influence patient or graft survival [61].

In patients with allograft dysfunction, allograft biopsy should be performed to direct therapy. As BKVN is associated with inflammation on biopsy, BK nephropathy may masquerade as rejection or occur concurrently with rejection. The presence of endarteritis, fibrinoid vascular wall necrosis, glomerulitis, or peritubular capillary C4d positivity support diagnosis of concurrent rejection [62]. Staining for SV40 can confirm the presence of BKV nephropathy. Treatment of concurrent BKVN and rejection is controversial; one strategy is the institution of antirejection therapy followed by decreased maintenance therapy. As intravenous immune globulin (IVIG) has also been used for the treatment of rejection, the use of IVIG may be particularly beneficial in patients in whom BK nephropathy and rejection are difficult to distinguish. In patients with acute rejection occurring following clearance of BK viremia by immunosuppression reduction, conventional antirejection therapy should be administered with careful monitoring for recurrent BK viremia every two weeks following treatment. In diagnosed BKVN, alternative maintenance immunosuppressive strategies have been described; for example the substitution of cyclosporine for tacrolimus, which has the additional effect of lowering mycophenolic acid levels [57]. The conversion of a calcineurin inhibitor to an mTOR inhibitor can be considered [63], although this strategy would be useful only in those with preserved renal function lacking proteinuria. Although in vitro and initial small studies suggested that leflunomide, a malononitrile amide, inhibits BKV replication [64,65], a systematic review failed to identify any benefit of the addition of leflunomide on graft survival following development of BKVN [66]. Additionally, a study in kidney transplant recipients with BKVN treated with FK778, a leflunomide derivative, failed to demonstrate a benefit [67].

Re-transplantation can be successfully achieved in transplant candidates with prior graft loss due to BKVN [68]. General consensus is to delay re-transplantation until clearance of viremia is achieved, although there are case reports of transplantation with active viremia. In an Organ Procurement and Transplantation Network (OPTN) database analysis, 126 kidney transplant patients with prior graft loss due to BKVN who were re-transplanted were identified. Induction was utilized in 80% of re-transplants and tacrolimus plus mycophenolate was the most common maintenance regimen (57%). Of these patients, treatment for BKV was reported in 17.5% although only one graft loss due to BKVN occurred and 1-year graft survival rate was 98.5% [68].

Cytomegalovirus

As opposed to BKV, CMV can cause significant morbidity and mortality in all solid organ transplant recipients [69]. Manifestations of CMV can range from asymptomatic viremia to infection (fever, arthralgias, leukopenia, thrombocytopenia) to organ-invasive disease (pneumonitis, gastroenteritis, colitis, hepatitis, encephalitis). Seroprevalence is approximately 60% in those above age 20, and after infection the virus establishes life-long latency. Therefore, CMV infection or disease after transplant can represent reactivation of host-latent virus or primary infection from the donor organ. In addition to donor and recipient CMV serostatus, innate host immunity and net state of immunosuppression are major risk factors for infection; thus risk of disease is greatest after immunosuppression for the prevention and treatment of acute rejection. As CMV infection occurs in over 50% of solid organ transplant recipients without prevention, strategies have been employed which have significantly lowered the incidence of CMV disease. Prophylaxis, or the administration of antiviral agents to recipients during greatest perceived risk, and pre-emptive therapy or initiation of therapy after detection of CMV antigenemia both have been successfully utilized [69,70].

While no single immunosuppressive agent is consistently associated with CMV infection, the use of lymphocyte-depleting agents [71,72] and calcineurin inhibitors (particularly cyclosporine) [73,74] appear to correlate with increased risk, while the use of the mTOR inhibitors sirolimus and everolimus may be protective [75–78]. Clinical studies regarding incidence of CMV with mycophenolic acid versus azathioprine have been conflicting [79], with some but not all demonstrating higher incidence and severity of disease with mycophenolate mofetil. Differences between these studies may reflect variable mycophenolate mofetil and azathioprine dosing used. Additionally, many earlier studies were performed before routine use of ganciclovir prophylaxis. Overall, given the efficacy of antiviral agents for the prevention and treatment of CMV, there is insufficient evidence at present to support the use of any individual maintenance immunosuppressive agent or combination for CMV prevention. Conversion to a mTOR inhibitor may be considered in patients with recurrent or intractable CMV disease [80].

Reduction in the overall degree of immunosuppression should be considered as adjunctive therapy to the use of antiviral agents in the treatment of CMV infection and disease particularly with documented organ involvement. Commonly this is achieved through reduction or discontinuation of the antimetabolite (mycophenolate or azathioprine) although reduction of the calcineurin inhibitor may additionally be required in severe cases [81]. In a trial comparing valganciclovir and intravenous ganciclovir for the treatment of CMV, greater eradication of CMV viremia at 21 days was observed in patients on dual versus triple therapy maintenance regimens and was also observed in patients with low versus high calcineurin inhibitor levels. However, the rates of long-term eradication and risk of recurrent disease was comparable between groups [82]. It remains unclear whether the antimetabolite should be restarted after successful CMV treatment and may be best guided by the perceived immunologic risk.

Hepatitis C

Prevalence of hepatitis C varies considerably between solid organ transplant groups, with a higher prevalence among liver and kidney transplant populations. HCV-induced cirrhosis remains one of the principal indications for liver transplantation [83]. Additionally, the prevalence of HCV among kidney transplant recipients is 5–46% with geographic variances. Viral recurrence following transplantation is nearly universal, leading to accelerated hepatic fibrosis, and decreased graft and patient survival primarily due to cirrhosis and hepatic cancer [84].

Accelerated development of fibrosis, increased incidence of cirrhosis, and fibrosing cholestatic hepatitis occurring in immunosuppressed patients establishes that immunosuppression contributes to disease progression [85]. Extensive in vitro evidence has suggested that cyclosporine possesses anti-HCV activity [86,87], with non-immunosuppressive cyclosporine analogs under investigation as therapeutic agents for HCV [88]. However, clinical studies have not demonstrated a clear benefit of cyclosporine compared with tacrolimus in recurrence rates, or response to antiviral therapy. A meta-analysis examining mortality, graft survival, and incidence of fibrosing cholestatic hepatitis after liver transplantation demonstrated no significant difference comparing cyclosporine and tacrolimus-based regimens [89]. Similarly, a randomized controlled trial demonstrated no difference in survival and severe HCV recurrence in liver transplant recipients randomized to cyclosporine or tacrolimus regimens [90]. However, the mean time to histologic recurrence was longer in the cyclosporine arm. Retrospective studies have suggested that cyclosporine may enhance efficacy of antiviral therapy and improve sustained virologic response rates [85]; confirmatory prospective data is needed.

The effect of corticosteroid use on HCV recurrence and response to antiviral therapy is also controversial. Steroid boluses are generally perceived as deleterious. Steroid withdrawal and steroid-free protocols have not been associated with improved survival or slower fibrosis progression to date [91,92]. Both the antimetabolites azathioprine and mycophenolate have anti-HCV effects in vitro [93]. Clinical studies have, overall, failed to demonstrate a consistent benefit using antimetabolite-based regimens in terms of HCV recurrence, although a systematic review in liver transplant recipients suggests reduced severity of HCV recurrence with azathioprine compared to mycophenolate [94]. Data on the utility of mTOR inhibitors in HCV-positive liver transplant recipients has been limited and conflicting. In one study, time to and severity of recurrence was similar in those patients receiving or not receiving sirolimus [95], while in another study, the use of sirolimus was an independent predictor of minimal fibrosis at 1 and 2 years [96].

Hepatitis B

HBV replication may occur after solid organ transplant due to immunosuppressive therapy. Reactivation of replication is more likely in recipients who were HBeAg positive or had detectable HBV DNA prior to transplant, with minimal reactivation observed in HBcAb-positive, HBsAg-negative patients [97]. Similar to CMV prevention, the two approaches to prevent post-transplant HBV reactivation are prophylaxis (administration of antivirals during period of greatest reactivation risk) and a pre-emptive strategy involving post-transplant monitoring for viremia. HBV reactivation following liver transplant was nearly universal until the development of antiviral therapies, including lamivudine and hepatitis B immune globulin [98].

Although immunosuppression may contribute to HBV replication, a paucity of information exists to guide maintenance immunosuppressive management in the present era with the availability and effectiveness of antiviral agents. Present recommendations support minimization of the immunosuppressive regimen based on individual immunologic risk assessment. For example, in the HBsAg-positive liver transplant recipient steroid withdrawal may be considered as corticosteroid use has been shown to increase HBV DNA synthesis in vitro [99]. Although in vitro studies suggested an inhibitory effect of mycophenolate on HBV replication, the use of mycophenolate did not improve outcomes after reactivation of lamivudine-resistant HBV in a small study of liver transplant recipients [100]. Chronic hepatitis may increase the risk of hepatotoxicity of azathioprine or cyclosporine.

Epstein–Barr virus

Clinically, the most important manifestation of EBV infection after solid organ transplant is the uncontrolled proliferation of EBV-infected B cells contributing to the spectrum of post-transplant lymphoproliferative disease (PTLD). The incidence of PTLD is bimodal. Cases occurring within the first year of transplantation often are in EBV-seronegative recipients receiving an organ from an EBV-seropositive donor, with the vast majority represented by B-cell lymphoma expressing CD20. The risk of PTLD depends on the organ transplanted, with heart–lung recipients at greatest risk (Figure 66.4) [101], and differences between organ type may reflect intensity of immunosuppression used, degree of organ antigenicity, and amount of lymphoid tissue within the graft [102]. The use of

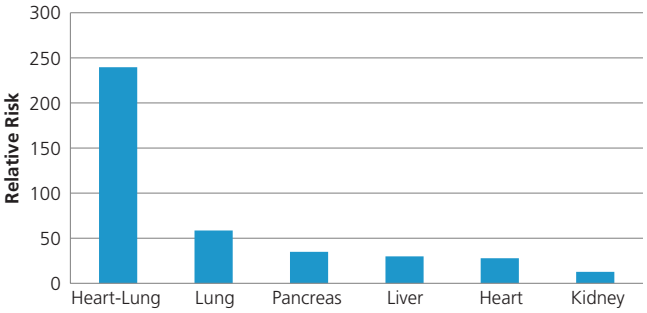

Figure 66.4. Relative risk of post-transplant lymphoproliferative disease (PTLD) by organ type by 5 years post-transplant.

depleting antibody therapy for induction and/or treatment of rejection is clearly associated with increased risk of PTLD development, particularly early post transplant [101,103]. However, use of maintenance immunosuppressive agents and overall degree of immunosuppression also increases PTLD risk by inhibiting EBV-specific T-cell responses. The risk of lymphoma returns to pretransplant levels upon weaning immunosuppression after transplant failure [104]. Patients on quadruple versus dual or triple immunosuppressive agents are at highest risk [103].

Registry analyses have demonstrated increased risk of PTLD in patients on calcineurin-based regimens, particularly in those on tacrolimus [101,103]. However, this increased risk has been observed primarily in patients not receiving induction therapy [103,105], suggesting that the strong immunosuppressive effect of induction initially surpasses any difference in calcineurin inhibitor effect. Rather, the use of a calcineurin inhibitor may impact incidence of late PTLD [104]. Any difference in risk with tacrolimus may be due to the higher level of immunosuppression the drug affords relative to cyclosporine. In contrast to the calcineurin inhibitors, the use of the antimetabolites has not been associated with PTLD, with some studies suggesting slightly decreased risk. Mycophenolic acid inhibits human B-cell lymphoma proliferation in vitro [106]. In registry analyses [104], case–control [107], and prospective cohort studies [108], mycophenolate mofetil was not associated with increased PTLD risk with a trend towards lower risk of malignancy and increased time to malignancy. In a large registry analysis, there was no difference in PTLD comparing mycophenolate and azathioprine; however, the percentage of patients on azathioprine was low [105].

The effect of the mTOR inhibitors sirolimus and everolimus on PTLD is unclear. Use of mTOR inhibitors has been associated with decreased incidence of overall de novo malignancy post-transplant [109]. These agents have been found to inhibit the growth of EBV-positive B-lymphoma cells [110]. Analysis of over 25 000 Medicare transplant recipients did not show an association between PTLD development and sirolimus use [103]; however, only 665 patients of this group were treated with sirolimus. In the ELITE-Symphony trial in renal transplant recipients, which was a randomized controlled trial comparing low-dose tacrolimus, low-dose cyclosporine, standard-dose cyclosporine, and sirolimus-based maintenance immunosuppression, all the cases of PTLD and non-Hodgkin's lymphoma occurred in the sirolimus arm. The rates of acute rejection, however, were also greatest in the sirolimus group; thus the use of T-cell depleting agents may have contributed to higher lymphoma rates [74].

The novel agent, belatacept, a fusion protein that selectively blocks T-cell costimulation, was associated with greater risk of PTLD compared with a standard cyclosporine regimen in phase III studies, especially among those recipients who were EBV-seronegative recipients of an EBV-positive donor [111,112]. Interestingly, rates of rejection were also greater in the belatacept arm, challenging the concept that higher degree of immunosuppression is the sole contributor to increased PTLD risk using maintenance agents. Restricting use of belatacept to EBV-seropositive patients is currently recommended.

Treatments for PTLD may include immunosuppression reduction, antiviral therapy (EBV-associated), chemotherapy, radiation, and local excision. Immunosuppression reduction alone, by 25–50% of baseline level, may be effective in treating limited, polymorphic PTLD. Extensive cases may warrant more aggressive immunosuppression reduction; however, the risk of rejection and allograft loss has to be carefully considered [113]. One analysis pooled 19 renal transplant patients from European centers converted to sirolimus or everolimus with minimization or discontinuation of the calcineurin inhibitor following the development of PTLD [114]. Fifteen patients had remission of PTLD; however, six patients were concomitantly treated with rituximab and another six patients received CHOP (cyclophosphamide, doxorubicin, vincristin, and prednisone), making the contribution of the mTOR inhibitor difficult to discern. Recently, there is increasing interest in strategies to reconstitute EBV-specific immunity through infusion of autologous cytotoxic T lymphocytes [115].

Cancer and maintenance immunosuppression

The use of immunosuppressive agents after transplantation increases the long-term risk of malignancy after solid organ transplant. This risk is covered in depth in Chapter 95. In a large cohort study using linked data from the US Scientific Registry of Transplant Recipients and 13 state and local registries, the frequency of malignancy in over 175 000 patients transplanted between 1987 and 2008 (kidney, liver, heart, and lung) was determined. Over the study period, 10 656 cases of malignancy were reported, correlating to a standardized incidence ratio of 2.1 compared with the general population. Cancers with an increased risk of fivefold or greater compared to the general population were Kaposi's sarcoma, non-Hodgkin lymphoma, skin, lip, vulvar, anal, and liver cancer, illustrating the oncogenic potential of particular viral infections. Cancers commonly screened for in the general population, including cervical, breast, and prostate, were not significantly increased in transplant recipients [116].

Depleting antibody therapy has clearly been associated with increasing the risk of PTLD, commonly associated with EBV infection (discussed above). Beyond this, there is no conclusive evidence that a particular immunosuppressive agent is associated with development of a specific malignancy. Rather, the overall degree of immunosuppression correlates with risk. Several observations support this. The risk of malignancy differs among solid organ transplant recipients, with those receiving more intensive immunosuppressive therapy (e.g. heart–lung) at greatest risk for cancer [101]. Additionally, the risk of certain cancers (e.g. PTLD) is greatest during the first year post-transplant during heightened immunosuppression [101]. Episodes of rejection increase risk of malignancy, perhaps due to treatment with depleting antibodies and/or increased maintenance immunosuppression [117]. The number of immunosuppressive agents correlates with cancer risk

[103]. Certain malignancies (e.g. Kaposi's sarcoma, polymorphic PTLD) may regress completely with decreased immunosuppression [113]. Finally, risk of malignancy dramatically falls after graft failure and weaning of immunosuppression [104]. Mechanisms by which immunosuppression may contribute to malignancy development include impairment of immune activity against oncogenic viruses, decrease in neoplastic cell immunosurveillance, direct DNA damage and disruption of DNA repair mechanisms, and up-regulation of cytokines that promote tumor formation [118].

Malignancy potential for individual immunosuppressive agents, independent of their immunosuppressive effect, is controversial. Studies to date are limited by insufficient power, particularly given the low incidence of some malignancies. Additionally, data on immunosuppressant use may reflect agents used at time of transplant and not capture changes in long-term management. Furthermore, the degree of immunosuppression may vary despite use of similar regimens depending on drug levels achieved [119]. However, certain experimental and clinical findings warrant further discussion. Cyclosporine and tacrolimus may promote cancer progression via the production of transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6) [120,121]. Lower calcineurin inhibitor levels correlate with lower risk of cancer [122]. Among kidney transplant patients, increased de novo tumors, including PTLD, were observed in patients on tacrolimus versus cyclosporine; however, this observation was limited to those who did not receive induction therapy [105]. Azathioprine has been associated with increased risk of squamous cell carcinoma of the skin [123] and lip [124], perhaps via photosensitization to ultraviolet A radiation [125]. Conversely, mycophenolate does not appear to be associated with increased malignancy risk [108,123]. Evidence for an antitumor effect of sirolimus in animal models is increasing; mechanisms by which sirolimus may suppress tumor growth include inhibition of p70 S6 kinase (inhibit cell proliferation), IL-10 (Jak/STATs activity), cyclins (block cell cycle activity), and VEGF signaling (impede lymphangiogenesis) [120,126]. In the Rapamune Maintenance Regimen Study, renal transplant patients randomized to maintenance prednisone plus cyclosporine plus sirolimus versus sirolimus alone, the risk of malignancy was nearly twofold lower in the cyclosporine withdrawal group [127]. In the CONVERT trial, patients converted to sirolimus therapy had lower rates of non-melanoma skin carcinomas compared to patients in a calcineurin inhibitor continuation group [128].

Although reduction of immunosuppression may be useful in treatment of diagnosed malignancy, this approach is useful primarily for renal transplant recipients as graft loss due to rejection is non-fatal. Immunosuppression reduction may be particularly effective in treatment of lymphoma, skin cancer, and Kaposi's sarcoma [129]. Substitution of sirolimus for cyclosporine in renal transplant patients has been associated with complete regression of Kaposi's sarcoma [130,131]. In patients with malignancy, either in remission or under treatment, conversion to a sirolimus-based regimen warrants consideration. However, use of sirolimus in patients with or without a history of malignancy is associated with a more than twofold increased risk of all cause mortality [132]. In patients undergoing chemotherapy, minimization or discontinuation of maintenance agents may be indicated until completion of therapy; azathioprine in particular can increase severity of myelosuppression. Cyclosporine is a potent inhibitor of the efflux p-glycoprotein encoded by the ATP-binding cassette subfamily B member 1 gene (*ABCB1*), also known as multidrug resistance gene 1 (*MDR1*), and may affect or be affected by various chemotherapeutic agents that interact with p-glycoprotein [133].

Maintenance immunosuppression by time after transplant

Immediate postoperative period

Optimizing immunosuppressive management in the early post-transplant period is important for attaining long-term graft function and survival. The practice of using induction agents and/or higher doses of maintenance agents in the early post-transplant period evolved after the early transplant experiences illustrated the heightened risk for acute rejection during the first post-transplant months [134]. Specific induction therapies are covered in depth in Chapter 65. The introduction of cyclosporine revolutionized transplantation, with dramatic improvements in the acute rejection and 1-year graft survival rates. Cyclosporine was initially envisioned as an agent that would prevent rejection and allow for steroid avoidance. However, monotherapy with cyclosporine proved problematic, as the high doses needed for rejection prophylaxis caused significant toxicity, particularly nephrotoxicity [135]. Triple therapy, or the combined use of a calcineurin inhibitor, prednisone, and azathioprine, thus emerged as a strategy to maximize immunosuppressive effect while minimizing toxicity. Although tacrolimus and mycophenolic acid have largely replaced cyclosporine and azathioprine as initial maintenance agents, triple therapy is still the predominant approach after solid organ transplant [1]. Since the risk for acute rejection is highest in the first 3 months after transplantation, higher doses are used during this period, and then reduced thereafter in stable patients to minimize toxicity.

There remains interest in minimization of immunosuppression even in the early post-transplant period. Since the introduction of the calcineurin inhibitors, target levels have been lowered, which has decreased nephrotoxic effects while maintaining acceptable acute rejection rates. Calcineurin-free regimens are uncommonly used given high acute rejection rates, particularly in more immunogenic solid organ transplants. Interest remains in steroid minimization and avoidance. However, the higher rejection rates observed with these protocols [136] despite concurrent use of calcineurin inhibitors have prevented widespread adoption of these strategies.

Particular issues with regards to the use of maintenance agents in the immediate postoperative period warrant further review. In kidney transplant, the mTOR inhibitors sirolimus and everolimus are associated with delayed graft function; thus the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guidelines recommend that if an mTOR inhibitor is used, it should not be started until graft function is established and surgical wounds are healed [58]. Although sirolimus has a relatively low risk of traditional afferent arteriolar vasoconstrictive nephrotoxicity, concomitant use of angiotensin converting enzyme inhibitors (ACEI) may cause acute renal failure [137] or anaphylaxis [138]. Sirolimus is uncommonly used early after lung transplant due to associated risk of anastomotic bronchial dehiscence [139,140]. It has also been associated with hepatic artery thrombosis following liver transplant [141].

Medium to long-term management

Medium to long-term management of transplant focuses on preventing chronic rejection and minimizing drug toxicity. A summary of drug toxicities and management options are listed in Table 66.4.

Table 66.4. Tailoring immunosuppressive regimens on adverse events

Condition	Immunosuppressive cause	Immunosuppressive change
New-onset diabetes after transplantation (NODAT)	Corticosteroid, tacrolimus, cyclosporine, mTORS	Avoidance, dose reduction,
Dyslipidemia	Corticosteroids, cyclosporine sirolimus, everolimus	Avoidance, dose reduction, tacrolimus
Hypertension	Corticosteroid, cyclosporine, tacrolimus, mTORS	Avoidance, dose reduction
Osteoporosis	Corticosteroids	Avoidance, dose reduction, vitamin D
Bone marrow suppression	Mycophenolic acid, azathioprine, sirolimus, everolimus, tacrolimus	Dose reduction
Delayed wound healing	Sirolimus, everolimus	Avoidance
Gastrointestinal side-effects	Mycophenolate mofetil, tacrolimus, sirolimus	Enteric-coated mycophenolic sodium, dose reduction, azathioprine
Proteinuria	Sirolimus, everolimus	Avoidance
Nephrotoxicity	Cyclosporine, tacrolimus, sirolimus	Avoidance, dose reduction, belatacept

Based on data from [58].

Calcineurin inhibitors are associated with numerous toxicities that are often dose dependent. Hirsutism, gingival hypertrophy, hypertension, and hyperlipidemia are more commonly encountered with cyclosporine treatment than with tacrolimus, whereas neurotoxicity, alopecia, and potentially post-transplant diabetes are more commonly encountered with tacrolimus treatment than with cyclosporine.

One concern with tacrolimus is the possible relative increase in the incidence of diabetes, which has been noted in recipients of kidney allografts alone [142]. Several studies have not reported an increase in diabetes with tacrolimus in pancreas transplant recipients [143–146]. Low-dose tacrolimus minimizes the risk of new-onset diabetes after transplantation (NODAT) compared to higher doses of tacrolimus [147]. Calcineurin inhibitors may also cause nephrotoxicity. Calcineurin sparing/ avoidance protocols are discussed in the organ sections below. However, even patients who have never been treated with a calcineurin inhibitor may have histological changes on kidney biopsy consistent with calcineurin inhibitor toxicity [148].

Corticosteroids are associated with side-effects including hypertension, dyslipidemia, glucose intolerance, and osteoporosis. Many new protocols have attempted to withdraw corticosteroids in order to circumvent these adverse effects. These protocols are discussed in corticosteroid withdrawal by organ type in the sections below.

Antimetabolite agents are associated with bone marrow suppression and gastrointestinal side-effects. Close monitoring of complete blood counts is necessary post-transplant. Gastrointestinal side-effects are discussed in antimetabolite section relating to kidney transplant below.

Sirolimus and everolimus may be associated with a number of adverse effects, including leukopenia, thrombocytopenia, anemia, mucositis, hypercholesterolemia, hypertriglyceridemia, pleural effusions, pericardial effusion, ovarian cysts, and edema. De novo use of sirolimus has been associated with delayed wound healing, lymphocele formation, and prolonged delayed graft function.

Failing allograft

The appropriate immunosuppression for the failing allograft is unknown. This is complicated in solid organ transplant recipients where there is no available supportive therapy and re-transplantation is the only option. However, increasing use of, and efficacy of, left ventricular assist devices makes re-transplantation for heart transplant recipients a potential viable option. In kidney transplantation, many physicians will lower the calcineurin inhibitor or replace it with an mTOR inhibitor. The CONVERT study, however, showed that it is unsafe to replace a calcineurin inhibitor with sirolimus when the estimated glomerular filtration rate is less than 40 mL/min, and ineffective and dangerous when the estimated glomerular filtration rate is greater than 40 mL/min when there is more than 100 mg/day of urinary protein, limiting its usefulness in this setting [149]. The appropriate immunosuppression after a patient has returned to dialysis is also unknown. After a patient returns to dialysis, an elevation of the serum creatinine is no longer useful as a marker to identify rejection and calcineurin inhibitors reduce the common signs and symptoms of acute rejection. Some centers withdraw the antimetabolite to reduce the risk of infection, and reduce the calcineurin inhibitor and the prednisone to 5 mg/day as long as the patient is making more than 500 milliliters of urine daily [150]. When the patient becomes anuric, the risk of continued immunosuppression is unwarranted and the calcineurin inhibitor is withdrawn and prednisone tapered slowly. If the patient develops signs and symptoms consistent with acute rejection, treatment may include steroids and possible transplant nephrectomy. An exception to the practice of weaning immunosuppression after graft failure is when a subsequent transplant (i.e. a living donor kidney transplant) is imminent. In this case, maintenance immunosuppression may be beneficial to avoid increased alloantibody levels. Relatively non-toxic immunosuppressive strategies to avoid allosensitization after graft loss are clearly needed given the increasing number of patients returning to dialysis and seeking a new transplant.

Recurrent disease

Disease recurrence is one factor known to adversely affect allograft survival. However, the impact of various immunosuppressive strategies on the occurrence, severity, and progression of disease recurrence is often poorly understood. In general, patients with immune-mediated primary diseases (i.e. autoimmune hepatitis and lupus nephritis) are treated with higher doses of immunosuppression, including corticosteroids, while patients with infection issues (such as HCV) might benefit from less aggressive immunosuppressive regimens. This will be discussed in detail by organ type elsewhere in this textbook (see Chapters 77–80).

Generic immunosuppressants

Cost may be an important factor when choosing maintenance immunosuppression. Cyclosporine, tacrolimus, prednisone, mycophenolate mofetil, mycophenolate sodium, and azathioprine are available as generic products. In order for the FDA to approve a generic product it must contain the same active ingredient, be an identical strength, dosage form and route of administration, have the same use indications, be bioequivalent, and meet the same batch requirements for identity, strength purity, and quality as the brand drug. Generic medications do not need to be proven efficacious and safe in the treated populations, for example transplant recipients. In general, generic drugs are being used with increasing

frequency in all sectors of transplantation, and it is likely that they will become the predominantly used form of maintenance immunosuppression in the coming decade. Minor variations in drug performance surely exist, but are unlikely to be systematically defined.

Maintenance therapy for kidney transplant recipients

In the current era of kidney transplantation, graft survival rates that exceed 90% at 1 year [1] have been achieved with calcineurin inhibitors, antimetabolites, and corticosteroids. Additional discussions on drug mechanism and usage are available in Chapters 17 and 98, respectively.

Calcineurin inhibitors

Over the last two decades, calcineurin inhibitors have been extensively used in post-transplant immunosuppressive regimens and have secured a vital place in today's solid organ post-transplant care for prevention of acute rejection and improved graft survival. Several landmark trials have compared the available calcineurin inhibitors, cyclosporine, and tacrolimus. The first two multicenter studies that compared microemulsion cyclosporine to tacrolimus using the combination of calcineurin inhibitors, azathioprine, and corticosteroids demonstrated a significant decrease in acute rejection with tacrolimus, but there was no difference in patient or graft survival post transplantation [151,152]. A subsequent study randomized first deceased donor recipients to one of three immunosuppressive regimens (all included corticosteroids): (1) tacrolimus with azathioprine; (2) tacrolimus with mycophenolate mofetil; and (3) microemulsion cyclosporine and mycophenolate mofetil [153]. Acute rejection rates were similar in each group (<20%) but the incidence of corticosteroid-resistant rejection was lower in the tacrolimus arms. Three-year follow-up found no statistically significant difference in renal function, patient or overall graft survival, but improved graft survival in recipients with delayed graft function in the tacrolimus arms [153]. In agreement with these data, a meta-analysis reported that for every 100 patients treated with tacrolimus rather than cyclosporine for the first year, 12 would be prevented from having acute rejection, two would be prevented from having graft failure, but five would develop new-onset diabetes after transplantation [154]. More recently, the ELITE Symphony trial demonstrated that a low-dose cyclosporine regimen was not as effective as a low-dose tacrolimus regimen [74]. As a result of these trials, the KDIGO Clinical Practice Guidelines suggest that tacrolimus should be the first-line calcineurin inhibitor for renal transplant recipients [58].

Corticosteroids

Corticosteroids are a key component of most immunosuppressive protocols. However, they are associated with side-effects including hyperlipidemia, glucose intolerance, and osteoporosis. Trials investigating the avoidance or withdrawal of corticosteroids with the hopes of minimizing adverse events and improving graft survival have shown variable results. In the first double-blinded, randomized, placebo-controlled, multicenter, 5-year trial comparing early steroid withdrawal (7 days post-transplant) with steroid maintenance therapy, investigators reported higher rates of acute rejection in the corticosteroid-free arm. Patients with immediate graft function were included in the trial and immunosuppression consisted of induction per the local transplant center, tacrolimus and mycophenolate mofetil. Most patients received antilymphocyte induction with rabbit antithymocyte globulin. At the end of the trial, patients with rapid corticosteroid discontinuation had lower serum triglycerides, less weight gain, and a lower incidence of new-onset diabetes after transplantation requiring use of insulin. There were no differences observed with respect to high-density lipoprotein, low-density lipoprotein, blood pressure, or diabetes [136]. The rate of biopsy-proven acute rejection and chronic allograft nephropathy was twice as high in the rapid corticosteroid discontinuation arm. In contrast, a meta-analysis did show a significant reduction in these cardiovascular risk factors in patients in whom corticosteroids are avoided or withdrawn [155]. Three pooled studies of corticosteroid withdrawal have shown that, despite the increased incidence of acute rejection in the withdrawal arms, short-term results demonstrated comparable patient and graft survival [156–158].

Low-immunologic-risk patients who are at risk for corticosteroid complications may be the best candidates for corticosteroid withdrawal if they receive antilymphocyte induction therapy. High-risk recipients, like African-Americans, may not be the best candidates for corticosteroid withdrawal, although this is controversial [159,160]. Another factor to consider is the timing of corticosteroid withdrawal. Chronic corticosteroid use can lead to dependence and therefore late withdrawal may be associated with an increased risk of rejection. If prednisone is being used beyond the first week after transplantation, it should be continued rather than withdrawn. Given the lack of long-term data defining the optimal timing of corticosteroid withdrawal, the appropriate maintenance agents to continue, and the patient population in which this may be an acceptable practice, the ideal use of long-term corticosteroids remains to be determined.

Antimetabolite agents

Antimetabolite agents are usually considered the "third agent" in triple immunosuppressive regimens, providing additive effects, but less essential than the calcineurin inhibitor or the corticosteroid component. Azathioprine and mycophenolic acid are the commonly used agents in this category. Currently, there are two forms of mycophenolic acid available on the market, mycophenolate mofetil and enteric-coated mycophenolic sodium. The efficacy of mycophenolate mofetil in renal transplantation has been reported in several well-designed trials [161–163]. Mycophenolate mofetil treatment groups demonstrated a reduced incidence and severity of early rejection episodes as compared to low-dose azathioprine-treated patients in treatment regimens consisting of tacrolimus plus corticosteroid as well as cyclosporine plus corticosteroid [161]. Three-year follow-up of these studies found that the decreased incidence of early rejection in the mycophenolate mofetil arm had not translated into a significant improvement in graft function or survival [162,163]. An Italian trial of deceased donor renal transplant recipients using cyclosporine microemulsion and corticosteroids also demonstrated no benefit of mycophenolate mofetil compared to azathioprine [164,165]. Although it may be controversial, as a result of the summative evidence from these trials, the KDIGO Clinical Practice Guidelines suggest that mycophenolate be the first-line antiproliferative agent [58].

Mycophenolate mofetil is often associated with upper and lower gastrointestinal side-effects that are dose related. Enteric-coated mycophenolate sodium has been developed to help circumvent the upper gastrointestinal side-effects by facilitating release in the small

intestine [166]. Two major clinical trials demonstrated that enteric-coated mycophenolate sodium is therapeutically equivalent to mycophenolic mofetil, and that both drugs have a similar incidence and severity of side-effects [167,168]. These trials did not demonstrate a statistically significant difference in overall gastrointestinal symptoms when patients were given equivalent doses of mycophenolate mofetil or enteric-coated mycophenolate sodium. Postmarketing studies have attempted to explore the gastrointestinal profiles of the two formulations of mycophenolic acid [169–181]. Many trials have proven a beneficial effect of enteric-coated mycophenolate sodium [169–177] while others have not reported a difference in gastrointestinal-related adverse effects [178–181]. In the myTIME, Progris, and myGAIN [171,180,181] studies, patients reported improvement in their perception of change in gastrointestinal symptom burden after conversion to enteric-coated mycophenolate sodium using the self-administered Gastrointestinal Symptom Rating Scale questionnaire, Overall Treatment Effect (OTE) scale for gastrointestinal symptoms, and OTE scale for health-related quality of life questionnaires.

It is possible that gastrointestinal events are multifactorial (infectious etiology, related to gastroparesis or other concomitant medications) and enteric-coated mycophenolate sodium may offer benefit to specific populations. If a patient fails mycophenolate mofetil because of the gastrointestinal side-effects, then the patient may benefit if switched to enteric-coated mycophenolate sodium. Also, if the patient is predisposed to gastrointestinal disorders, then enteric-coated mycophenolate sodium may be a better initial choice for the patient. However, enteric coating is unlikely to influence the systemic effects of the drug including effects on enterocyte proliferation and viral infection that may be responsible for gastrointestinal side-effects.

Drug interactions may also occur with the concurrent use of proton pump inhibitors and mycophenolate mofetil. Studies suggest that the combination of mycophenolate mofetil with pantoprazole or omeprazole results in reduced dissolution of mycophenolate mofetil in the stomach, leading to reduced bioavailability and lower systemic mycophenolic acid exposure. In contrast, the enteric-coated formulation of mycophenolic acid passes the acidic environment of the stomach and dissolves after reaching the neutral pH environment of the small intestine [182].

mTOR inhibitors

Excellent rejection rates have been achieved through a regimen consisting of a calcineurin inhibitor, corticosteroids, and an antimetabolite. Although calcineurin inhibitors have significantly lowered acute rejection rates, they are direct nephrotoxins and cause several other side-effects. Historically, preventing acute rejection was the main goal of transplantation. Currently, chronic allograft nephropathy is the most common reason for graft loss and this has become a major concern for clinicians. Calcineurin sparing regimens are an attractive immunosuppressive option to potentially minimize the risk of long-term graft loss while maintaining low rates of acute rejection. An alternative to the calcineurin inhibitor-based regimens use mTOR inhibitors. Two agents, sirolimus and everolimus, have been developed and FDA-approved in the hope of achieving this goal.

Sirolimus may have a favorable role in calcineurin inhibitor-free maintenance therapy [183,184], but caution is warranted in calcineurin inhibitor sparing regimens, as nephrotoxicity and rejection are still concerns. In the Spare-the-Nephron trial, a calcineurin-free regimen of sirolimus and mycophenolate mofetil was compared to cyclosporine and mycophenolate mofetil. At 2 years of follow-up, renal function was not different [185]. ORION (Optimizing Renal Transplant Immunosuppression to Overcome Nephrotoxicity), another calcineurin-sparing trial, was recently halted because of high acute rejection rates in the tacrolimus elimination arm. The trial [186] was a three-arm study of 450 de novo patients evaluating a sirolimus/ mycophenolate mofetil/ corticosteroids combination, sirolimus/ tacrolimus-elimination at 12 weeks/ corticosteroid versus a standard regimen consisting of tacrolimus/ mycophenolate/ corticosteroids. All patients in this study received daclizumab induction therapy. At 2 years, patient and graft survival and glomerular filtration rate were not different between groups and the urinary proteinuria to creatinine ratio was significantly higher in both sirolimus-containing arms when compared with the tacrolimus group. Everolimus has also been studied in calcineurin-sparing regimens.

Everolimus 1.5 versus 3 mg/day with corticosteroids and low-exposure cyclosporine without induction (n = 237) or with induction (basiliximab, n = 256) has been studied in a calcineurin-sparing protocol [187]. In this study, the use of an induction agent eliminated the need for high-dose everolimus. Six-month biopsy-proven acute rejection occurred in 25.0% and 15.2% of patients in the 1.5 and 3 mg/day groups without induction, and 13.7% and 15.1% in the study groups with induction. Calculated glomerular filtration rates (62–67 mL/min) and adverse events were similar in all arms.

Conversion of a calcineurin inhibitor to sirolimus has met with limited success. The CONVERT trial studied 830 renal allograft recipients who were receiving cyclosporine or tacrolimus from 6 to 120 months post-transplant. The participants were randomly assigned to continue calcineurin inhibitor (n = 275) or convert from calcineurin inhibitor to sirolimus (n = 555) [149]. Success with sirolimus was only observed in a subgroup of patients with a baseline glomerular filtration rate more than 40 mL/min and urine protein to urine creatinine ratio less than or equal to 0.11. Early elimination of calcineurin inhibitor by use of everolimus-based immunosuppression may improve renal function while maintaining efficacy and safety outcomes in selected patients. In a later study, everolimus replaced calcineurin inhibitors at 4–5 months after transplantation in patients with mean glomerular filtration rates above 60 mL/min [188]. In this multicenter, European, open-label study (ZEUS), 300 low- to moderate-risk renal transplant patients initially received basiliximab induction, cyclosporine, enteric-coated mycophenolate sodium, and corticosteroids. At 12 months, the everolimus regimen was associated with a significant improvement in glomerular filtration rate in comparison to the cyclosporine regimen (mean difference +9.8 mL/min). Rates of biopsy-proven acute rejection were statistically higher in the everolimus group than in the cyclosporine group after randomization (10% vs. 3%), but similar at the end of the study period (15% vs. 15%). Compared with the cyclosporine regimen there were higher mean lipid concentrations, slightly increased urinary protein excretion, and lower hemoglobin concentrations noted with the everolimus regimen; thrombocytopenia, aphthous stomatitis, and diarrhea also occurred more often in the everolimus group.

Everolimus has also been used as a substitute for mycophenolate mofetil in combination with cyclosporine and corticosteroids in a recent trial (n = 583) [189]. Everolimus (1.5 or 3 mg/day) was as efficacious as mycophenolate mofetil, although the side-effect profile featured increased adverse events. Discontinuation of therapy due to adverse events (hemolytic uremic syndrome,

lymphoproliferative disease, and proteinuria, and higher serum creatinine) was more frequent in the everolimus arm compared to the mycophenolate mofetil arm.

In summary, the de novo use of sirolimus has been proven to be comparable to a calcineurin inhibitor, while it has been associated with early post-transplant adverse events including lymphoceles, prolonged delayed graft function, and poor wound healing [183,184]. Likewise de novo use of everolimus in combination with induction has produced acceptable rates of acute rejection, although adverse events were common [187,189]. It appears that sirolimus conversion is only successful in a subgroup of patients with a baseline glomerular filtration rate more than 40 mL/min and urine protein to urine creatinine ratio less than or equal to 0.11 [149]. Likewise, the ZEUS study demonstrated the everolimus conversion is possible in low- to moderate-risk patients with normal renal function, although this may come at the expense of a higher acute rejection rate. The best evidence for calcineurin withdrawal with mTOR inhibitors is in selected patients. Close monitoring of drug concentration levels and adverse events is warranted. Whether or not calcineurin inhibitor-free/ sparing regimens using mTOR inhibitor maintenance therapy is efficacious in the long term remains unknown. Therefore, at this time, the KDIGO Clinical Practice Guidelines suggest that calcineurin inhibitors be continued rather than withdrawn [58].

Belatacept

Belatacept is the first immunosuppressive to demonstrate a renal benefit over a calcineurin inhibitor-based regimen. It is a second-generation costimulation blocker that received FDA approval for use in kidney transplantation in June of 2011. In the first partially blinded, parallel group, phase 2 study, more intensive belatacept, less intensive belatacept, and cyclosporine administrations were compared [190]. All patients received basiliximab, mycophenolate mofetil, and corticosteroids (n = 218). Similar rates of acute rejection and graft loss occurred in each arm at 6 months, while the glomerular filtration was statistically higher in each of the belatacept arms. The belatacept groups had less chronic allograft nephropathy, diabetes, hypertension, and hyperlipidemia.

Subsequently, two 3-year, phase 3, randomized, multicenter studies—Belatacept Evaluation of Nephroprotection and Efficacy as First-Line Immunoprotection Trial (BENEFIT) and BENEFIT Extended Criteria Donor (BENEFIT-EXT)—tested the efficacy and safety of belatacept in adult de novo kidney transplant patients [32,190,191]. In both trials, patients were randomized into three groups: more intensive belatacept, less intensive belatacept, and cyclosporine in conjunction with basiliximab, mycophenolate, and corticosteroids. BENEFIT-EXT was designed similarly to the BENEFIT trial with the inclusion of expanded-criteria donors. In the BENEFIT trial, despite the higher incidence of acute rejection in the belatacept arm, at the end of the first year renal function was statistically superior in the belatacept arms (more intensive 65 mL/min, less intensive 63 mL/min, and cyclosporine 50 mL/min). In contrast, in the BENEFIT-EXT trial, acute rejection rates were similar and renal function was statistically superior in the more intensive belatacept group, but not the less intensive group (more intensive 18%, less intensive 18%, and cyclosporine 14%) [191]. Three-year follow-up of these trials demonstrated persistent improvement in renal function (mean change +21 mL/min in the BENEFIT and +10 mL/min in the BENEFIT-EXT) [112]. A major concern that arose from these trials was the high incidence of post-transplant lymphoproliferative disease in the belatacept-treated Epstein–Barr virus seronegative recipient arms. Therefore the drug is contraindicated in patients who are Epstein–Barr virus seronegative.

One limitation of the BENEFIT and BENEFIT-EXT trials was that cyclosporine, a less contemporary immunosuppressive, was utilized and at high doses and concentrations. More recently, a trial was reported that incorporated a more contemporary immunosuppressive regimen. In a phase 2, 1-year randomized study, belatacept/ mycophenolate mofetil, belatacept/ sirolimus and tacrolimus/ mycophenolate mofetil, in combination with rabbit antithymocyte globulin and without corticosteroids were compared (n = 89) [192]. Acute rejection was highest in the belatacept/ mycophenolate mofetil arm, graft loss was lowest in the tacrolimus/ mycophenolate arm, and renal function was better in the belatacept arms.

A conversion trial was recently conducted to test the hypothesis that belatacept-based regimens may provide a treatment option for calcineurin-based maintenance immunosuppression. Patients who were more than 6 months but less than 36 months after transplantation with stable graft function (calculated glomerular filtration rate $\geq$35 mL/min and $\leq$75 mL/min) were randomized to switch to belatacept (n = 84) or continue calcineurin inhibitor treatment (n = 89) [193]. At month 12, the mean change in calculated glomerular filtration rate from baseline was higher in the belatacept group versus the calcineurin inhibitor group. Six patients in the belatacept group had acute rejection episodes, all of them within the first 6 months after conversion; all cases were resolved with no allograft loss. At month 24, mean calculated glomerular filtration rate was 62.0 mL/min in the belatacept arm versus 55.4 mL/min in the calcineurin inhibitor arm [194]. The mean change in calculated glomerular filtration rate from baseline was +8.8 mL/min in the belatacept arm and +0.3 mL/min in the calcineurin inhibitor arm. The relative renal benefit of belatacept was observed in patients switched from either cyclosporine (+7.8 mL/min) or tacrolimus (+8.9 mL/min), and was observed regardless of baseline renal function. Patient survival, graft survival, and the overall safety profile were similar between groups.

Belatacept's demonstration of an improvement in glomerular filtration rate over a calcineurin inhibitor-based regimen is the first by an immunosuppressive agent. The chronic intravenous administration and drug cost may influence prescribing patterns and patient compliance. Another special consideration for belatacept is that it has a relatively long half-life and cannot be discontinued in cases of severe infection. Further trials are needed to explore the long-term outcomes, the impact of Epstein–Barr virus on post-transplant lymphoproliferative disease, and development of chronic allograft nephropathy with belatacept use. These trials should include more current immunosuppressive regimens.

Summary

The KDIGO Clinical Practice Guidelines recommend using a combination of immunosuppressive medications as maintenance therapy, including a calcineurin inhibitor and an antiproliferative agent, with or without corticosteroids [58]. They suggest that tacrolimus be the first-line calcineurin inhibitor used and that mycophenolate be the first-line antiproliferative agent. In patients who are at low immunological risk and who receive induction therapy, corticosteroids could be discontinued during the first week after transplantation.

Maintenance therapy for pancreas transplant recipients

In clinical practice, a maintenance immunosuppressive regimen containing a calcineurin inhibitor, mycophenolic acid, and corticosteroids is the preferred regimen for pancreas transplant recipients. Evidence-based medicine has supported the transition from cyclosporine to tacrolimus and azathioprine to mycophenolic acid over the past 15–20 years. A multicenter, randomized trial (n = 205) has proven that tacrolimus reduces the incidence of acute rejection (moderate to severe; 3% tacrolimus vs. 28% cyclosporine) and results in superior pancreas allograft survival (89.2% vs. 72.4%) [145,195]. Likewise, mycophenolate mofetil has also been shown to improve early outcomes after pancreas transplant. In an open-label, randomized, multicenter study, patients received mycophenolate mofetil 1.5 g twice daily (n = 74) or azathioprine 1–3 mg/kg daily (n = 76) for 1 year after transplantation [196]. At 6 months, there was no difference between survival or rejection rates, but the time to rejection or treatment failure was significantly longer with mycophenolate mofetil compared with azathioprine. One-year acute rejection rates for mycophenolate mofetil versus azathioprine patients were similar (35% vs. 47%, respectively).

There is less evidence supporting the use of corticosteroid withdrawal and mTOR inhibitors in pancreas transplantation. According to SRTR data, 38% of pancreas transplant recipients undergo corticosteroid withdrawal [1]. Several non-randomized studies [197–205] and one randomized, single-center study [206] have investigated corticosteroid withdrawal in pancreas transplant recipients. In the randomized study, 50 simultaneous kidney–pancreas recipients received either no corticosteroids or corticosteroid withdrawal at 3 months in conjunction with rabbit antithymocyte globulin, cyclosporine, and mycophenolate mofetil. Acute rejection (4% in both groups), patient survival, and graft survival rates were similar, but the serum creatinine levels were significantly higher after 1 year of follow-up in the complete corticosteroid avoidance group (1.50 vs. 1.30 mg/dL). In summary, early steroid withdrawal with a potent induction agent, a calcineurin inhibitor, and mycophenolic acid may be safe in pancreas transplant recipients, but long-term follow-up is needed. The metabolic benefits of corticosteroid withdrawal may be limited to simultaneous kidney–pancreas patients as pancreas after kidney recipients did not have improved lipid levels in one study [207]. Little is known about the use of mTOR inhibitors in pancreas transplant recipients. Sirolimus appears to prevent rejection in pancreas transplant recipients, but only a few small, non-randomized studies have been published [156,157,208–212].

Maintenance therapy for lung transplant recipients

For the past two to three decades, the most commonly used regimen in lung transplant recipients has consisted of a calcineurin inhibitor plus an antimetabolite, and corticosteroids [1], specifically cyclosporine, azathioprine, and prednisone. There is less evidence supporting the use of tacrolimus in lung transplantation and therefore the switch from cyclosporine to tacrolimus has been delayed when compared to other transplanted organs (Figure 66.3). Currently based on SRTR statistics, 86% of new lung transplants are prescribed tacrolimus at the time of discharge [1]. Many of the trials of tacrolimus in lung transplantation are limited by single-center, non-randomized designs. Most of the studies that compared tacrolimus to cyclosporine revealed a lower incidence of acute rejection episodes/100 patient days in patients treated with tacrolimus [213–216], while one trial did not demonstrate a difference in the incidence of acute rejection [217]. Similarly, many lung transplant centers have not switched from azathioprine to mycophenolic acid. Based on current SRTR data, 55% of lung transplant recipients are given mycophenolic acid at the time of transplant and 30% of lung transplant recipients are given azathioprine [1], perhaps because the published literature suggests an increased incidence of infection and gastrointestinal toxicity with mycophenolate mofetil [217–220]. Two non-randomized studies that compared azathioprine versus mycophenolate mofetil in combination with cyclosporine and prednisone have demonstrated a reduced incidence of rejection in mycophenolate mofetil treated patients [218,221], and less decline in FEV_1 at 1 year of follow-up [218], while randomized trials with similar immunosuppressive regimens have shown no difference in acute rejection rates [219,220]. Additionally, in one study there was no difference in the number of patients who were bronchiolitis obliterans-free at 3 years (75% vs. 73%) [220]. Lastly, nearly all lung transplant recipients are maintained on corticosteroid therapy [1]. One small, non-randomized trial that used potent induction agents has shown promise for corticosteroid avoidance in lung transplant recipients [222].

Sirolimus in not commonly used or FDA approved for use in lung transplantation because it has been associated with several fatal cases of anastomotic bronchial dehiscence when used in the early post-transplantation period [139,140] as well as interstitial pneumonitis [223,224]. In contrast, everolimus has been studied in lung transplantation with more success. In a randomized double-blind trial, 213 lung transplantation recipients were assigned to receive everolimus or azathioprine in combination with cyclosporine and corticosteroids [225]. While everolimus treatment resulted in fewer episodes of acute rejection and less deterioration of FEV_1 in the first year, only the incidence of acute rejection remained significantly less after 2 years. Unfortunately, everolimus treatment resulted in more serious adverse events and elevated serum creatinine levels. Converting lung transplant recipients (greater than 1 year after transplant) to everolimus with low-exposure calcineurin inhibitors has resulted in a renal benefit (+3.2 vs. −2.4 mL/min) that has been sustained at 2 years post conversion [226].

Maintenance therapy for liver transplant recipients

According to the United Network for Organ Sharing (UNOS) registry data, current 1- and 5-year patient survival rates following orthotopic liver transplantation are 88% and 74%, respectively, with graft survival rates of 83% and 67% [1]. These results are due in part to recent advances in immunosuppressive agents. For liver transplant recipients, the regimen that is most commonly used at the time of transplant is tacrolimus plus mycophenolic acid, and corticosteroids, although corticosteroid withdrawal is becoming more common [1]. Although many patients receive triple immunosuppressive therapy at the time of transplant, agents are eventually weaned and dual and single therapy is commonplace after the initial post-transplant period.

Tacrolimus therapy has been associated with less rejection than cyclosporine in two landmark, randomized, multicenter trials (US trial n = 529 and European trial n = 545) of patients receiving a first liver transplant [227]. Tacrolimus was associated

with significantly fewer episodes of acute or refractory rejection, but substantially more adverse events requiring discontinuation of the drug. These studies used Sandimmune®, which requires bile for absorption, and many programs historically used a bile drainage tube at the time of liver transplant. However, similar results have also been reported with the microemulsion formulation of cyclosporine (cyclosporine USP modified) whose absorption is not as bile- dependent. In a randomized trial of 606 liver transplant recipients in the United Kingdom and Ireland, the primary outcome (combined frequency of death, re-transplantation, or treatment failure for immunological reasons) was statistically lower in tacrolimus-treated patients (21% tacrolimus vs. 32% cyclosporine microemulsion) [228]. Three-year follow up of the composite primary endpoint was similar; however, freedom from death or re-transplantation no longer achieved statistical significance [229]. A recent meta-analysis has confirmed these findings [230]. It concluded that treating 100 recipients with tacrolimus instead of cyclosporine would avoid rejection and corticosteroid-resistant rejection in nine and seven patients respectively, graft loss and death in five and two patients respectively, but four additional patients would develop diabetes after liver transplantation [230].

Although mycophenolic acid acts similarly to azathioprine, it is a more effective immunosuppressant with fewer side-effects, including less hepatotoxicity, translating to sparse use of azathioprine in liver transplantation. Mycophenolate mofetil has also been shown to lower the incidence of acute rejection in liver transplant recipients [231]. In a randomized, double-blind comparative study, liver transplant recipients were treated with either mycophenolate mofetil or azathioprine, both in combination with cyclosporine and corticosteroids [232]. The 1-year incidence of acute rejection (68% mycophenolate mofetil vs. 76% azathioprine) and steroid resistant rejection (19% mycophenolate mofetil vs. 36% azathioprine) was statistically lower at 6 months while patient and graft survival rates were similar.

The use of corticosteroids varies between liver transplant programs. Corticosteroid withdrawal or avoidance has been attempted in liver transplant recipients in an effort to reduce adverse events and minimize the consequences of HCV recurrence [233,234]. Three meta-analyses have concluded that when corticosteroids were replaced by other agents, the incidence of acute rejection was reduced [92,235,236] and that corticosteroid-free regimens are beneficial in lowering cholesterol [92,235], cytomegalovirus infection [92,235], hypertension [92], and new-onset diabetes mellitus [209,235].

Side-effects of sirolimus in the postoperative period include hepatic artery thrombosis, delayed wound healing, and incisional hernias, while chronic use has been associated with hyperlipidemia, bone marrow suppression, mouth ulcers, skin rashes, albuminuria, and pneumonia. In 2002, the FDA alerted the transplant community to two studies of sirolimus in liver transplant recipients [237]. In one study of de novo liver transplant patients, sirolimus was associated with excess mortality and graft loss (22% in combination vs. 9% on tacrolimus alone). In this study and another study, de novo sirolimus was associated with an increase in hepatic artery thrombosis (7% in combination vs. 2% in the control arm); most cases of hepatic artery thrombosis occurred within 30 days post-transplantation and led to graft loss or death. In June 2009, the United States Food and Drug Administration issued another alert based on an unpublished study that found increased mortality in stable liver transplant patients after conversion from a calcineurin inhibitor-based immunosuppressive regimen to sirolimus [237]. In this study of stable liver transplant patients at least 6 months post-liver transplantation, an increased number of deaths was observed in the group converted to a sirolimus-based regimen compared to the group who was continued on a calcineurin inhibitor-based regimen, although the difference was not statistically significant (3.8% vs. 1.4%). These warnings have led to sparse use of sirolimus in liver transplant recipients although several studies have explored its use. A systematic published review that included 11 studies found a small non-significant increase in glomerular filtration rate (3.4 mL/min), after 1 year of sirolimus use in liver transplant patients who received sirolimus as primary immunosuppression due to renal insufficiency or who were switched to sirolimus from another regimen due to nephrotoxicity [238]. However, sirolimus use was associated with higher rates of infection, rash, ulcers, and discontinuation of therapy.

Everolimus has also been studied in liver transplant recipients. In a few small, non-randomized studies of liver transplant patients converted to everolimus for renal dysfunction, the creatinine level and glomerular filtration rate improved significantly [239]. A retrospective 15-month study that followed 240 patients who were converted to everolimus for maintenance therapy following liver transplantation found low rates of rejection among patients on everolimus [239]. Because of adverse events, everolimus was discontinued in 47 patients (20%) and the dose was decreased in 59 (25%).

Maintenance therapy for heart transplant recipients

Since the first heart transplant was performed in 1967, immunosuppression has changed dramatically. Since 2000 there has been a steady increase in tacrolimus use [1]. Currently, tacrolimus is the most widely used calcineurin inhibitor (75%), mycophenolic is the predominant antimetabolite agent (88% of patients), and the use of sirolimus and everolimus remains low. Most patients (89%) remain on low-dose glucocorticoids at 1 year post-transplantation.

Multiple single-center and multicenter randomized comparisons between de novo use of tacrolimus and cyclosporine after heart transplantation have been reported [240,241]. These trials have shown similar patient survival and a more favorable side-effect profile with tacrolimus. In the largest trial (n = 314), de novo heart transplant recipients were randomized to either tacrolimus or microemulsion-based cyclosporine in combination with azathioprine, glucocorticoids, and induction therapy [240]. At 6 months, there was a lower incidence of moderate to severe acute cellular rejection (28% tacrolimus vs. 42% cyclosporine, $P = 0.013$), less hypertension (66% vs. 78%), and dyslipidemia (29% vs. 40%), yet more new-onset diabetes mellitus (20% vs. 11%) with tacrolimus.

Substitution of mycophenolate mofetil for azathioprine may reduce mortality and rejection in the first year after cardiac transplantation. In a double-blind, active-controlled trial, 28 centers randomized 650 patients undergoing their first heart transplant to receive mycophenolate mofetil (3000 mg/day) or azathioprine (1.5–3 mg/kg/day), in addition to cyclosporine and corticosteroids. Although survival and rejection were similar in enrolled patients, treated patients had a significant reduction in mortality at 1 year (6.2% vs. 11.4%) and a significant reduction in the requirement for rejection treatment (65.7% vs. 73.7%). Opportunistic infections, mostly herpes simplex, were more common in the mycophenolic

mofetil group (53.3% vs. 43.6%). Enteric-coated mycophenolic sodium and mycophenolate mofetil appear to be similar except that significantly fewer enteric-coated mycophenolic sodium patients required dose reductions during treatment [175,242].

The mTOR inhibitors are typically used in patients with cardiac allograft vasculopathy (CAV) or renal insufficiency because of their inhibitory effects on smooth muscle proliferation and absence of intrinsic nephrotoxicity [226,243]. The high incidence of adverse effects, including lower extremity edema and poor wound healing, may limit the universal use of these agents.

Of the two available mTOR inhibitors, everolimus has the most evidence supporting use in cardiac transplant. In a randomized, double-blind trial of 634 de novo heart transplant recipients, the use of two concentrations of everolimus (1.5 mg/day and 3.0 mg/day) was compared to azathioprine. All patients received cyclosporine and corticosteroids. At 6 months post-transplantation, significantly fewer patients in the 1.5 mg everolimus group (36% vs. 47%) and 3.0 mg everolimus group (27% vs. 47%, $P < 0.001$) reached the primary efficacy composite endpoint of death, graft loss or retransplantation, loss to follow-up, biopsy-proven moderate to severe acute cellular rejection, or rejection with hemodynamic compromise, compared to the azathioprine group. The difference in efficacy between the groups was almost entirely due to a decreased incidence of acute cellular rejection in the everolimus groups. In this study, everolimus therapy was associated with a significant increase in serum creatinine concentration compared to azathioprine, an effect that was likely due to the potentiation of the nephrotoxic effects of cyclosporine by everolimus [244]. The effects of everolimus on rejection rates, allograft vasculopathy, and renal function were sustained at 24 months [245].

In the Nordic Certican Trial in Heart and Lung Transplantation substudy, 111 maintenance heart transplant recipients were randomized to everolimus plus reduced calcineurin inhibitor or standard calcineurin inhibitor [246]. No significant difference in CAV progression was evident between the treatment groups. However, other immunosuppressive therapy is important as azathioprine plus everolimus patients demonstrated attenuated CAV progression and a decline in inflammatory markers, whereas the opposite pattern was seen with everolimus plus mycophenolate mofetil.

Corticosteroids are used in most heart transplant recipients at relatively high doses in the early postoperative period then tapered to low doses or discontinued altogether in the first transplant year [247,248]. Low-risk patients may tolerate earlier (within 1–2 months post-transplantation) corticosteroid withdrawal without long-term adverse consequences [249,250]. An Australian study randomized 112 patients to induction with rabbit antithymocyte globulin, cyclosporine, and azathioprine with or without corticosteroids [251]. The 3-month rejection rate and the number of patients experiencing corticosteroid-resistant rejection were higher in the corticosteroid-withdrawal group. The number of antihypertensive agents and cholesterol levels at 3 years was significantly higher in the maintenance corticosteroid group. Of note, 47% of patients in the steroid withdrawal group required the addition of maintenance corticosteroids during follow-up, largely owing to the increased incidence of rejection.

Maintenance therapy for intestine transplant recipients

Intestinal transplant recipients have the highest rejection rates and the lowest graft survival rates due to the high immunogenicity of the bowel. Newer immunosuppressive drugs have played a significant role in the success with the procedure since the mid-1990s. Currently, most intestinal transplant recipients receive tacrolimus and corticosteroids as maintenance immunosuppression. Fewer than 200 intestine transplants are performed yearly and therefore the clinical trials of immunosuppression are few in number [252].

Summary

In the past 10–20 years tacrolimus and mycophenolic acid have become the cornerstones of immunosuppressive regimens. While awaiting further advances in the immunosuppressive armamentarium, we should be able to improve the functional life of most allografts by tailoring available agents for induction and maintenance therapy. Maintaining the effectiveness of immunosuppressive therapy requires shifting our therapeutic approach from "one-size-fits-all" to a tailored or individualized strategy. Tailored immunosuppression should prevent both under-immunosuppression (rejection) and over-immunosuppression (associated infections and cancer). As such, ongoing vigilant monitoring of transplant patients should be combined with a willingness to respond to rejection or infection with alterations in maintenance immunosuppressive therapy. The information gained through further study in these complex regimens should provide innovative strategies and new immunosuppressive agents that will serve to extend the functional life of allografts without toxicity or infection.

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CHAPTER 67

Rescue Immunosuppressive Therapy

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Introduction

"Rescue therapy" is defined here as any therapeutic intervention designed to address a specific alloimmune-mediated post-transplant process that causes acute or chronic allograft injury. It is of sufficient potency to reverse an emerging acute process, but is prohibitively immunosuppressive to be tolerated for a prolonged period of time. The classic rescue therapy is the treatment of clinically significant acute cellular rejection (ACR). Historically, the management of early ACR was one of the central problems of transplant therapeutics. The development of more effective immunosuppressive regimens has largely solved this once insurmountable problem; however, its management is still important and we discuss this below.

Over the past decade, the role of acute antibody-mediated rejection (AMR) in acute allograft injury has become increasingly appreciated. Mediated by donor-specific alloantibody (DSA), AMR can occur in combination with ACR or can occur in a more pure form—especially in sensitized recipients. Management approaches to these clinical situations are discussed in detail.

With improvements in early post-transplant management, long-term allograft loss has emerged as the major problem facing allograft recipients. In fact, the rate of graft loss beyond 1 year after kidney transplantation has remained relatively constant for decades [1]. We have advocated that the path forward to improving long-term allograft survival is that of detailed patient follow-up to identify specific causes of late renal allograft loss and the development of new therapies designed to address these problems before allograft damage becomes irreversible [2]. We will discuss clinical situations in which chronic allograft damage is encountered and address the connection between subclinical inflammation and chronic injury—specifically within the context of rescue therapy.

Organ-specific guidelines on the clinical diagnosis and treatment of rejection also are presented in Chapters 69–76, and the histopathological criteria defining the various forms of rejection by organ are presented in detail in Chapters 81–87. This chapter will provide an overview of common principles shaping organ-specific therapies, and will serve the reader as a suitable introduction to the treatment of rejection in general.

Before embarking on a discussion of these topics, it is important to note that much of the data does not reach Level I evidence of efficacy, with most widely used practices based on only Level III evidence [3]. This is especially true regarding chronic injury in which there is still controversy on the predominant mechanisms (of which there are likely many) and no consensus exists regarding what therapy may be indicated, if any.

Acute renal allograft dysfunction

While the definition of acute allograft dysfunction is not standardized, a good working definition might be one similar to that used for other forms of acute renal injury: an abrupt (within 48 h) reduction in kidney function (currently defined as an absolute increase in serum creatinine of $\geq$0.3 mg/dL [$\geq$26.4 mmol/L]), a $\geq$50% increase in serum creatinine from baseline, or a reduction in urine output (documented oliguria of less than 0.5 mL/kg/h for more than 6 h) [4]. The differential diagnosis of acute renal allograft dysfunction early after transplantation is broad and, similar to acute kidney injury of native kidneys, includes prerenal, intrinsic renal, and postrenal etiologies (Table 67.1). The majority of this chapter will focus on immunologic causes of graft dysfunction; however, transplant providers also should be familiar with and be able to recognize the non-immunologic causes.

The evaluation of a transplant recipient with acute allograft dysfunction should include the following: a detailed history and physical examination to assess blood pressure and volume status; a urinalysis (paying particular attention to the presence of proteinuria or a nephritic sediment) and urine culture if pyuria is detected; measurement of plasma calcineurin inhibitor (CNI) concentrations (for patients receiving a CNI); measurement of plasma polyoma virus copy number; an allograft ultrasound to detect renal artery stenosis, urine leak, lymphocele, or urinary tract obstruction; measurement of serum alloantibody levels by single antigen bead assay; and placement of a bladder catheter if bladder dysfunction or outlet obstruction are suspected. If no definitive cause of allograft dysfunction is identified from the above interventions, a renal allograft biopsy should be performed. This biopsy should be subjected to routine light microscopic investigations as well as immunofluorescence for IgG, IgA, IgM, C3, albumin, and fibrin. Immunoperoxidase studies should be performed if polyoma virus nephropathy or post-transplant lymphoproliferative disease is suspected. Electron microscopy should be added if de novo or recurrent glomerular disease is suspected. Renal allograft biopsies currently are graded and scored according to the Banff '07 Classification of Renal Allograft Pathology, a consensus-based diagnostic system that is discussed in detail in Chapter 81 [5].

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Table 67.1. Differential diagnosis of acute and chronic kidney allograft dysfunction/ injury

Acute renal allograft dysfunction/ injury
Prerenal
Allograft thrombosis
Atheroemboli
Intravascular volume depletion
Hypotension
Intrinsic renal
Acute cellular rejection
Acute antibody mediated (humoral) rejection
Recurrence of primary renal disease
Polyoma virus nephropathy
Acute calcineurin inhibitor toxicity
Allograft pyelonephritis
Post-transplant lymphoproliferative disease
Postrenal
Ureteral obstruction
Urinary bladder dysfunction
Bladder outlet obstruction
Chronic renal allograft dysfunction/ injury
Chronic antibody mediated rejection
Chronic cellular inflammation
Recurrence of primary renal disease
Chronic drug toxicity (e.g. calcineurin inhibitors)
Polyoma virus nephropathy

Acute cellular rejection

Acute cellular rejection episodes can be described as either clinical or subclinical depending on whether or not allograft dysfunction accompanies the episode. This section will focus on the evidence supporting the treatment of clinically evident acute cellular rejection and subclinical inflammation will be discussed later in this chapter.

In the first three decades of clinical kidney transplantation, ACR was seen in as many as 80% of recipients and was a frequent cause of early allograft dysfunction [6]. Furthermore, a single episode of ACR was found to be predictive of long-term allograft survival [7–10]. More recently, the incidence of ACR within the first 6 months after transplant has decreased significantly from approximately 40% in 1995 to less than 15%, but this decrease has not resulted in a significant increase in long-term graft survival, suggesting that the link between early ACR and long-term outcome may not be as strong as previously suggested [1,11]. Similar to previous reports [12,13], these analyses of registry data show that if complete functional recovery is achieved after treatment of ACR, there appears to be no deleterious effect on long-term outcome. However, those recipients whose graft function (estimated by Cockroft-Gault) did not return to within 75% of baseline after treatment of ACR had a fivefold increased risk of graft loss at 6 years compared to those whose function returned to baseline [10]. Although ACR occurs less frequency than in prior eras and is less consequential, this does not imply that ACR is benign, but rather that its recognition and prompt treatment has become routine practice in transplant centers. Indeed, the progress realized in mollifying the effects of ACR underscores the importance of prompt and properly treating ACR when it is diagnosed. The following sections will discuss the rationale and evidence for the two most commonly used therapeutic agents for the treatment of ACR—steroids and antibodies directed against lymphocytes. The discussion will proceed from a kidney-centric point of view as historically, the management of ACR was initially established in kidney transplantation and largely adapted to other organs as they became more commonly transplanted. Organ-specific considerations follow later in this chapter.

Steroid therapy

Since the early years of clinical transplantation, corticosteroids have been used both as maintenance therapy and as adjuncts to treat ACR [14,15]. Due in part to the rapidly evolving nature of the field of kidney transplantation during these years, as well as to the severe repercussions of not treating established ACR, a randomized trial comparing the use of steroids to treat ACR versus not treating ACR has not been performed. As a result, the consensus regarding the efficacy of these agents in treating ACR has been generated solely from Level III data. Nonetheless, this "experience-based" approach did result in the widespread use of steroids to treat ACR, a practice that continues to the present day. Most current protocols for the treatment of clinically evident, biopsy-proven ACR calls for the use of bolus methylprednisolone therapy for recipients with borderline changes, Banff 1A or Banff 1B scores on biopsy (Figure 67.1). It should be noted that glucocorticosteroids and not mineralocorticosteroids are indicated for the treatment of rejection as they exert a substantial and broad immunosuppressive effect. An ideal dose of methylprednisolone has not been established and varies considerably by transplant center from 100 mg to 1000 mg per dose (or up to 15 mg/kg) for durations that vary similarly by center from 3 days to several weeks, with or without tapering doses. The duration and intensity is often varied by the histopathological grade, although there are no data supporting specific variation.

Steroid-resistant rejection is defined by a failure to clinically respond to steroid therapy, but its precise definition has varied considerably. Most trials have required three full days of bolus methylprednisolone without clinical improvement to meet the definition.

Antilymphocyte therapies

In contrast to the use of corticosteroids to treat ACR, randomized clinical trials (Level I evidence) have been performed using monoclonal and polyclonal antibody preparations directed against lymphocytes. These trials examined the effects of several different antibody preparations [16–36], and reported the effects of a variety of antilymphocyte preparations generated in mice, rabbits, or horses on either the first episode of ACR or in the treatment of steroid-resistant ACR. The nature, results, and limitations of these 21 trials (involving 1394 participants) have been described at length in a Cochrane review [37]. The sum of the data would suggest that antibody therapy is better than steroid therapy at reversing a first episode of rejection and no one antibody preparation is clearly statistically more effective at reversing steroid-resistant rejection.

While it is encouraging to see that these trials were performed, many of them contain limitations that must give the reader pause when interpreting the results. These deficiencies are nicely outlined in the aforementioned Cochrane review [37], but relate mostly to the criteria used to diagnose rejection and define "reversal" of the rejection episode. In the majority of these studies, rejection was diagnosed based upon clinical, laboratory, and imaging criteria without the requirement for an allograft biopsy. In none of the studies was a repeat biopsy performed to verify resolution of the inflammatory process and definitively show reversal of rejection. Lastly, none of the studies examined the effects of these agents in a cohort of patients receiving more contemporary immunosuppressive therapy (e.g. tacrolimus and mycophenolate mofetil ± steroids ± induction therapy). As a result, the true efficacy of these agents

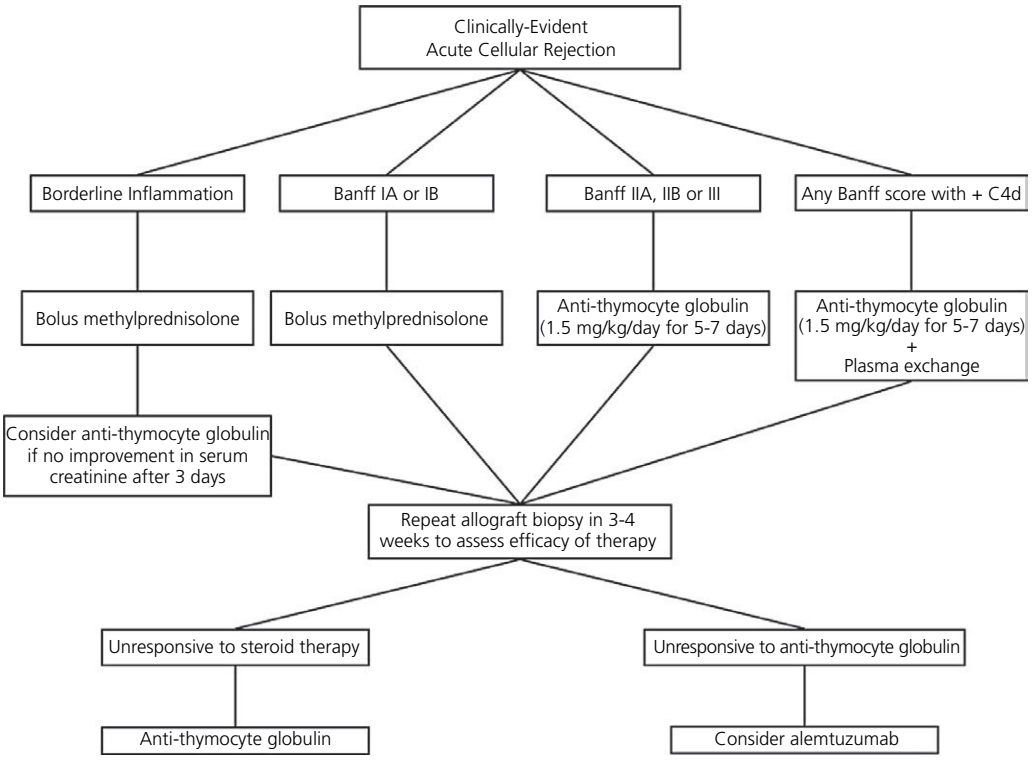

Figure 67.1. Algorithm for the treatment of clinically evident acute cellular-mediated kidney allograft rejection.

in treating ACR in the current immunosuppressive era can be extrapolated from prior results but is, at the current time, unknown. These limitations speak to the need for well-designed clinical trials that include strict inclusion criteria, carefully defined endpoints, and comprehensive follow-up (including repeat allograft biopsies) if the true effects of immunosuppressive agents on rejection episodes and long-term outcome are to be ascertained. Given these caveats, however, our approach is to use antilymphocyte globulin (specifically, antithymocyte globulin [Thymoglobulin®, Genzyme, Cambridge, MA]) for the treatment of a Banff IIA, Banff IIB, or Banff III clinically evident acute rejection episode or in conjunction with plasma exchange for those recipients with "mixed" acute cellular and acute antibody-mediated rejection (Figure 67.1). As with the use of steroids, the use of antibody preparations remains largely center-specific with regard to deployment as a first-line therapy, use in the setting of steroid resistance, and duration of therapy. These policies are driven both by efficacy and cost considerations, noting that antibody preparations are typically costly.

Acute antibody-mediated rejection

Over the past decade, the role of alloantibody in acute renal allograft injury has become increasingly appreciated. The diagnostic criteria for acute AMR according to the Banff classification are: (1) DSA detected in serum; (2) C4d staining in peritubular capillaries; and (3) morphologic evidence of acute tissue injury, including glomerular microthrombi, mesangiolysis, and glomerulitis and/or capillaritis involving neutrophils, macrophages and lymphocytes [38]. Again, acute graft dysfunction differentiates the clinical from the subclinical form. The presence of duplication of the glomerular basement membrane (transplant glomerulopathy) suggests more chronic changes (see below). Early AMR may be seen in association with features of acute cellular rejection (combined ACR and AMR) or may occur alone ("pure" AMR). While the de novo development of alloantibody is likely T-cell dependent and thus implies T-cell activation at some level, but this is not always manifest in the allograft for reasons that remain unclear. The role of T cells in antibody responses in sensitized patients also is unclear.

Pure antibody-mediated rejection

In kidney transplant recipients with high levels of DSA prior to transplantation that are sufficient to cause a positive cross-match (+XMKTx; see Chapter 36 for a complete discussion of DSA detection), the reported incidence of clinical AMR in the first 3 months is high—ranging from 10 to 43% [39]. In many such cases, AMR is a "pure" form and does not involve the histologic features of acute cellular rejection. The diagnosis of AMR in this setting is relatively easy given the presence of DSA, the typical biopsy findings, and the commonly severe graft dysfunction. In our experience at Mayo Clinic, Rochester, we use plasma exchange (PE) and low-dose (100 mg/kg) intravenous immunoglobulin (IVIG) dosed after PE for desensitization; the mean time to acute AMR after +XMKTx was 10 days and all episodes occurred within 3 months of transplantation [40]. This study suggested that the incidence of early AMR correlated best with the level of DSA present at the time of AMR rather than the baseline DSA level. Thus, patients who develop high levels of DSA (either persistent DSA or a "memory" response) develop AMR and those in whom DSA remains low after transplantation do not experience AMR. The onset of AMR in this setting is quite rapid and can lead to graft loss if not aggressively treated. A complete discussion of desensitization protocols is found in Chapter 68.

Table 67.2. Treatment options for acute antibody mediated rejection*

Agents that impact antibody levels
Plasma exchange
High-dose IVIG
Bortezomib
Rituximab
Agents that ameliorate graft damage
Eculizumab (terminal complement blockade)
Corticosteroids
Splenectomy for refractory or severe AMR

*The current Mayo Clinic protocol for acute antibody-mediated rejection (AMR) involves eculizumab and corticosteroids to ameliorate ongoing damage plus plasma exchange and low-dose IVIG to reduce donor specific alloantibody levels. Bortezomib (1.5 mg/kg × 4 doses) is added when DSA levels are very high (e.g. B flow cytometric cross-match channel shift >400 or mean fluorescence index on single antigen beads >10 000).

Surprisingly, acute AMR is usually amenable to therapy and few grafts are lost during the actual AMR episode using current therapy. Because most clinically available immunosuppressive drugs target T cells, they are ineffective in the treatment of AMR. Currently, the treatment of acute AMR may include methods thought to impact antibody levels (PE, IVIG, bortezomib, and rituximab) and/or agents that ameliorate allograft damage via blockade of terminal complement activation (eculizumab or corticosteroids). We discuss these various treatments below with the caveat that this area of rescue therapy is likely to continue to evolve quickly. In addition, the reversal of AMR as an endpoint of a study is sometimes difficult to assess. The studies discussed below fall outside the realm of FDA-approved drug indications and have been termed "off-label" uses. Their use is supported by emerging data that are limited by small numbers of subjects, non-randomized design, and the use of multiple treatment modalities in combination making it difficult to assess the impact of any one agent. An in-depth discussion of off-label drug use in transplantation can be found in Chapter 101.

The mainstay of treatment of AMR is either multiple PE treatments in combination with low-dose IVIG or high-dose (1–2 g/kg) IVIG alone (Table 67.2). In more severe cases such as those with increasing DSA and profound graft dysfunction despite frequent plasma exchanges, splenectomy has been used [41]. Rituximab has been suggested in some reports [42]. The Cedars-Sinai group has reported a two-level protocol using high-dose IVIG as primary therapy and reserving PE for resistant episodes [43]. Both PE and high-dose IVIG based protocols appear to result in the resolution of AMR in >90% of cases [40]. A recent retrospective analysis suggested that treatment of acute AMR with a combination of PE, low-dose IVIG, and rituximab was superior to high-dose IVIG in that DSA levels were lower at 3 months and graft survival at 36 months was higher (91.7% versus 50%, respectively, $P = 0.02$) [44].

Terminal complement inhibition with eculizumab has been shown to ameliorate antibody-mediated damage even when DSA levels are very high after transplantation [45]. Eculizumab also has been used for the treatment of AMR [46]. The advantage of using eculizumab during an early severe AMR episode is that it will significantly block ongoing complement activation and likely ameliorate further damage. The disadvantage is that since it is a humanized monoclonal antibody, it will be removed during PE along with alloantibody and needs to be replaced after each PE treatment. Acute AMR can occur despite treatment with eculizumab, suggesting the existence of C5-independent mechanisms of AMR, but these responded well to PE [45]. Our current protocol for the treatment of acute AMR involves eculizumab (600 mg/dose) and bolus corticosteroids in combination with daily PE and low-dose IVIG. While no controlled studies exist regarding this treatment, this regimen does offer a combination of graft protection while treatment is initiated to reduce DSA levels.

The proteasome inhibitor, bortezomib, also has been used for the treatment of acute AMR—mostly in combination with PE [47–50]. The rationale for bortezomib use is its possible ability to deplete antibody-secreting cells from the bone marrow in vitro [47] and in vivo [50]. It may have additional effects on newly activated lymphoid cells. One of the possible drawbacks of bortezomib is its low bioavailability that likely necessitates multiple doses for efficacy. Studies of bortezomib monotherapy in acute AMR are rare and its contribution to the treatment of AMR remains unclear. Walsh et al. reported that 87% patients with early AMR responded to a combination of bortezomib, rituximab and PE [51].

Combined acute cellular rejection and antibody-mediated rejection

In conventional kidney transplants with a negative cross-match at the time of transplantation, the most common form of AMR is that which occurs in combination with ACR [48]. In contrast to acute pure AMR seen in +XMKTx, this combined AMR and ACR tends to occur months to years after transplantation and has been associated with non-adherence to medical therapy.

Given its late presentation, this form of ACR and AMR is commonly seen in grafts that already have chronic injury. Treatment should address both the cellular and humoral components of the immunologic processes. Thus, bolus corticosteroids and/or anti-T-cell antibodies should be given to treat the T-cell component. PE and/or bortezomib may be considered in order to treat the humoral component. The duration of PE and/or bortezomib has not been clearly defined, but published reports have used one four-dose bortezomib "cycle" [48]. Regardless of the treatment deployed, the long-term prognosis for combined ACR and AMR occurring late after transplantation appears to be quite poor with more than half of the grafts developing chronic injury or graft loss within 4 years of treatment.

Subclinical inflammation and chronic injury

The above discussions have focused on the clinical scenario of early graft dysfunction that is found in association with a biopsy showing features of cellular and/or humoral alloreactivity. Data regarding the management of these entities is now emerging, and outcomes can be followed at least in part by acute improvements in graft function. Subclinical inflammation, that occurring on biopsy but not obviously manifest by graft dysfunction, remains a vexing scenario.

However, over the past decade, it has become clear that the major unsolved problem in transplantation is that of chronic allograft loss and the question arises whether or not rescue therapy might be applied to prevent chronic injury. Recommendations specific to this question require the introduction of two important issues: (1) how chronic injury is defined or diagnosed; and (2) to what degree chronic inflammation (usually present early in a well-functioning organ, i.e. subclinical inflammation) is etiologically linked to chronic injury and graft deterioration. While a detailed discussion of the pathogenesis of chronic injury is beyond the scope of this chapter, a brief summary is warranted in order to understand the theoretical basis for possible future rescue therapy in this clinical scenario.

Chronic injury is usually defined either functionally or histologically. A functional definition usually involves declaring a certain creatinine or glomerular filtration rate (GFR) as a chronically injured allograft. Since allografts tend to have functions across a wide spectrum, it would seem that a decline in renal function in a previously well-functioning allograft also might be a useful metric. Interestingly, very few studies have used declining function to study chronic injury. Defining chronic injury histologically is by far the most common approach. The presence of interstitial fibrosis, usually in combination with poor function, is an accepted definition of chronic injury [52]. Chronic antibody-mediated injury is commonly defined by the presence of transplant glomerulopathy [53].

The association between ACR and interstitial fibrosis was noted in the early years of transplantation. However, Rush et al. definitively showed the connection between subclinical rejection and subsequent interstitial fibrosis [52]. In a randomized trial, they showed that the treatment of subclinical rejection with corticosteroids increased graft survival and likely led to less fibrosis. This early study was from the cyclosporine–azathioprine era of transplantation and subclinical ACR rates were 30%. Tacrolimus-based regimens appear to have much lower rates of subclinical ACR and no randomized trial of treatment in the current era of immunosuppression has been performed. Several transplant programs, including our own, routinely perform surveillance biopsies in well-functioning renal allografts. We continue to find an association between subclinical inflammation at 1 year and lower subsequent graft function and survival (Figure 67.2) [53]. Most of the inflammation found on protocol biopsies is "borderline" in that it is insufficient to meet criteria for ACR. The significance of borderline changes is unclear and has recently been challenged [54]. We and others performing surveillance biopsies tend to treat subclinical inflammation meeting Banff criteria of ACR with bolus corticosteroids. However, the impact of treatment on subsequent outcome in well-functioning grafts is unclear, and the potential infectious risks of additional immunosuppression need to be considered in addition to changes in graft function.

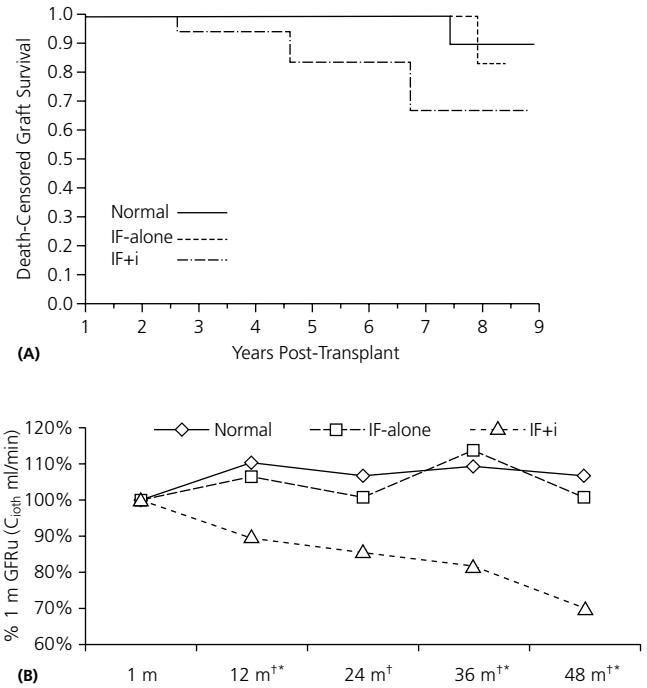

Figure 67.2. (A) Decreased death censored graft survival in patients whose 1-year protocol biopsy showed interstitial fibrosis plus subclinical inflammation (IF+i) compared to normal biopsies and biopsies with fibrosis alone (IF alone). (B) Decreased glomerular filtration (GFR) over time in patients with IF+i (†$P \leq 0.05$ for normal versus IF+I; *$P \leq 0.05$ for IF alone versus IF+i.) Reprinted with permission from the American Society of Nephrology, from [53] Park WD, Griffin MD, Cornell LD, Cosio FG, Stegall MD. Fibrosis with inflammation at one year predicts transplant functional decline. J Am Soc Nephrol 2010; 21: 1987–1997.

Approach to the graft with declining function

A common clinical scenario is that of an allograft with gradually declining renal function—for example the glomerular filtration rate has declined from 60 mL/min at 1 year post-transplant to 40 mL/min at 5 years after transplantation. The rate of decline in GFR may be so slow that the clinician may not consider the event an acute change. The role of therapeutic interventions in these patients is unclear, but a general clinical approach can be outlined.

First, to rule out non-immunologic causes (e.g. partial ureteral obstruction, polyoma virus nephropathy, etc.), the same detailed evaluation should be performed on these failing allografts as those performed on allografts with acute dysfunction (Table 67.1). In addition, assessment for DSA using solid-phase single-antigen beads would be indicated. An allograft biopsy then would be the next logical step in management. Possible findings of the biopsy might include recurrence of the primary renal disease and chronic histologic damage with or without evidence of active inflammation.

However, as described above, a biopsy in this setting also might show cellular infiltration of the interstitium with lymphocytes meeting the Banff criteria for acute cellular rejection. While there is an emerging consensus that subclinical rejection is associated with chronic injury, there is no consensus regarding its treatment. Our treatment protocol involves bolus methylprednisolone similar to the treatment of clinical acute cellular rejection; however, there is no evidence that this or any other treatment prevents the progression of chronic injury. In some instances, when the findings of subclinical rejection accompany recent decreases in immunosuppression (whether prescribed or otherwise), it is likely prudent to attempt to resume the prior drug regimen and levels. Similar to the situation with ACR, there is even less consensus regarding what is an appropriate endpoint to establish the success of treatment of subclinical rejection. Our approach is to repeat the kidney biopsy 1 month after treatment to demonstrate improvement in the severity of the infiltrate.

A second common histologic finding is that suggestive of chronic antibody-mediated injury. The histologic lesion most closely associated with chronic antibody mediated injury is that of transplant glomerulopathy (TG). However, TG also may represent recurrent native renal disease [55]. Chronic active antibody-mediated injury also is suggested by glomerulitis and/or peritubular capillaritis [56,57]. The cellular infiltrate is different from that of cellular rejection, not only in location but also in the fact that macrophages and neutrophils are much more common. In contrast to acute antibody-mediated rejection, the peritubular capillaries commonly do not stain positive for C4d in chronic AMR. The presence of new DSA is further circumstantial evidence that the glomerular lesion is likely due to chronic AMR.

The prognosis of TG appears to be poor, with more than 50% of allografts failing within 5 years of diagnosis [58,59]. Unfortunately,

there are no proven therapies for chronic AMR and few anecdotal reports. We have used multiple PEs to reduce DSA levels, but DSA returns soon after the treatment finishes. High-dose IVIG, rituximab, and/or bortezomib also may be considered, but none have been shown to decrease the progression of TG. Given that chronic AMR may account for more than half of late graft losses [60], more controlled trials are needed to develop effective rescue therapies for this clinical entity.

Calcineurin inhibitor avoidance or cessation as rescue therapy

The past few years have seen increased interest in the avoidance of CNIs using newer agents [61–66]. The major goal of these regimens appears to be avoidance of long-term nephrotoxicity. However, the bulk of the data developed over the past decade have not demonstrated chronic CNI toxicity as a major cause of late renal allograft loss [55]. A major endpoint of CNI-free protocols has been an improvement in renal function at 1 or 2 years after transplantation. However, data demonstrating that improving renal function in the overall renal transplant population will prevent graft loss in the subset of patients destined to fail are lacking. Even the surrogate endpoint of early, improved renal function with CNI-free protocols has been difficult to demonstrate, especially when compared to tacrolimus-based immunosuppression [61]. A recent 10-year renal biopsy study similarly showed the difficulties of using histology to attribute late injury to CNIs [67,68].

When a graft has developed chronic injury, conversion from CNIs to a CNI-free regimen may improve the GFR, but it is unclear if this actually increases graft survival [69–71]. Simply improving the serum creatinine may prolong graft survival marginally, but given the underlying pathology is most likely not due to CNIs, the fate of the allograft may be unaltered.

The end-stage kidney allograft

The role of rescue therapy in the setting of very poor allograft function (e.g. GFR <25 mL/min) and severe, irreversible histologic changes is likely marginal. Most clinicians attempt to maximize medical management by controlling blood pressure and optimizing intravascular volume status. Changes in immunosuppressive regimens also have been suggested, including decreasing the target level of CNIs or discontinuing CNIs with conversion to sirolimus. If there is active inflammation meeting the criteria of ACR, the decision to treat with corticosteroids may be considered; however, the presence of severe (Banff ci3) fibrosis suggests that no treatment will affect the outcome of this transplant. Patients with advanced graft injury should be assessed for retransplantation.

Novel methods of assessing allografts

While renal function and histology have been the cornerstones of allograft assessment for decades, novel methods are being tried to provide a less invasive and/or more detailed profile of intragraft events. Assays for urinary cytokines and other inflammatory markers are being tested for the early detection of ACR [72–74]. These assays are less invasive than biopsies and can be performed more often. In addition, the genomic profile of either the peripheral blood or an allograft biopsy is beginning to help to differentiate different types of inflammation [53,75–79]. In the future, gene expression profiles might be used to decide which grafts need treatment and also could be used as a surrogate marker for the efficacy of treatment. Thus, in the future, "rescue therapy" might be based on the results of urinary assays and/or gene expression profiles.

Rescue therapy for other solid organ allografts

In general, the treatment of ACR and AMR in non-renal solid organ transplantation has evolved along the same lines as in kidney transplantation and, similar to kidney transplantation, the majority of treatment regimens have been adopted without Level I evidence to support their use. However, the implications and treatment of acute and chronic allograft injury in both heart and liver transplantation are unique. Given the life-supporting nature of these organs, the management of alloimmune-mediated allograft injury in these types of transplants deserves attention.

Liver transplantation

The incidence of ACR in liver transplantation varies from approximately 24 to 80% [80,81]. Treatment of ACR with bolus corticosteroid therapy and augmentation of baseline immunosuppression intensity (particularly tacrolimus) typically results in resolution (at least biochemically) of the allograft injury. For those cases that are steroid-resistant, monoclonal or, more commonly in this era, polyclonal antilymphocyte preparations are administered [82,83]. The resilience of the liver greatly exceeds that of the kidney in that livers have a substantial regenerative capacity, making rapid reversal of ACR less pressing. However, a particularly difficult situation arises in the management of ACR in recipients infected with hepatitis C virus (HCV). While a mild episode of ACR does not seem to be associated with an increased risk of graft loss or mortality in non-HCV infected recipients, treatment of ACR in HCV-infected recipients does lead to increased risks of HCV recurrence, graft loss, and mortality [84–89]. At the current time, no consensus exists regarding the most appropriate course of treatment for ACR in the setting of HCV, suggesting the need for prospective trials in this area [90]. As a result, the most appropriate "rescue therapy" for HCV-infected liver transplant recipients may be little or no therapy at all.

Heart transplantation

As with liver transplantation, the implications of graft failure due to allograft injury in heart transplantation are profound. The intensity of treatment of either ACR or AMR generally is determined by the hemodynamic status of the recipient and can vary from increased doses of maintenance immunosuppression or bolus corticosteroid therapy for asymptomatic (subclinical) ACR to plasma exchange $\pm$ bortezomib combined with hemodynamic support for severe episodes of AMR causing cardiogenic shock [91,92].

Similar to kidney transplantation, there are increasing data regarding the role of alloantibody in both acute and chronic heart allograft injury. More specifically, there is emerging evidence supporting the linkage between early allograft injury and the development of cardiac allograft vasculopathy (CAV), a lesion of the epicardial coronary vessels characterized by infiltration of the endothelium, proliferation of vascular smooth muscle cells and subsequent luminal narrowing [93]. Specific management of CAV is discussed in detail in Chapter 79. Patients experiencing either ACR or AMR of the cardiac allograft are more likely to develop CAV [94–96]. Until recently, while percutaneous stenting

and optimizing medical therapy could temporize the condition, the only definitive treatment of severe CAV was retransplantation [93].

The poor prognosis and survival implications of CAV have led to investigations into both preventive and rescue strategies. The most promising immunosuppressive agents in this area appear to be the proliferation inhibitors, everolimus and sirolimus. While the exact mechanisms of action are not completely understood, conversion to or de novo treatment with a proliferation inhibitor has been shown to decrease the incidence and severity of allograft vasculopathy [97–100]. The use of such regimens may have a significant impact on improving the long-term survival related to chronic cardiac allograft damage.

The future role of "rescue therapy" in improving long-term allograft survival

Our ability to manage acute immunologic complications such as ACR and even AMR has made kidney transplantation an almost routine procedure. However, it is has become clear that while early graft loss has almost disappeared, late renal allograft survival has improved little over the past two decades.

We contend that the path toward improved long-term allograft survival will involve detailed patient follow-up to identify specific causes of late renal allograft loss and the development of new therapy designed to address these problems before allograft damage becomes irreversible. Unfortunately, there is a paucity of data regarding the causes of late renal allograft loss and this hampers our ability to devise rational approaches to improve outcomes. Our most recent protocol biopsy study suggests that renal allografts from the era of 1998–2004 demonstrate fewer and less progressive histologic changes in the first 5 years after transplantation than reported in earlier eras [101,102]. For example, the prevalence of moderate to severe fibrosis was only 17% (60/343) at 5 years after transplantation and when mild fibrosis was present on a 1-year biopsy, it progressed to more severe forms at 5 years only 23% of the time. Indeed, two-thirds of all allografts at 5 years showed Banff scores of 0 (none) or 1 (mild) for chronic histologic changes (cg, ci, ct, cv, and ah).

Based upon these data, we have suggested a new path forward to improving long-term graft survival [2]. We recommend that protocols for follow-up be optimized and recent studies into the causes of late graft loss need to be validated. A combination of serum tests (DSA levels and polyoma virus testing) and protocol biopsies might be the best approach. The intensive follow-up aims to identify patients with specific problems at a time point when the graft is still functioning well. Biopsies for cause are likely too late to allow successful intervention.

Thus, we believe that rescue therapy will play an important role in improving long-term graft survival if it is able to move from a reaction to clinical changes to pre-emptive treatment. For example, surveillance for the appearance of de novo DSA might enable "rescue therapy" to be instituted prior to the development of chronic antibody-mediated injury. Similarly, surveillance renal allograft biopsies using either conventional light microscopy or gene expression analyses could be used to identify allografts at risk for chronic injury.

Summary

The concept of rescue therapy is deeply ingrained in transplantation. Therapies for ACR are well established. Treatment protocols for acute AMR, either alone or in combination with ACR, are likely to continue to evolve as new therapies are tried. The role of rescue therapy for subclinical processes remains unclear, but we contend that these will become the most important new areas for intervention as we begin to understand the pathogenesis of chronic allograft injury.

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CHAPTER 68

Desensitization Protocols for Organ Transplantation

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Introduction

Desensitization can be defined as preconditioning treatment aimed at reducing alloantibodies in an allosensitized patient before transplantation. Allosensitization refers to the presence of alloantibody directed toward another genetically distinct individual. Although patients can be B-cell or T-cell allosensitized, in general, the term is typically interpreted as referring to B-cell allosensitization and inferring the presence of alloantibodies against a given donor. The goal of a desensitization procedure is to eliminate donor-directed alloantibody as defined by attainment of a negative or sufficiently low cross-match and, in doing so, allow transplantation to proceed between a previously incompatible donor–recipient pair. Specifics regarding interpretation of a positive cross-match can be found in Chapter 36. At present, the vast majority of data regarding desensitization arise from the kidney transplant literature. As such, this chapter will be solely focused on desensitization prior to kidney transplantation.

Desensitization in the current context of kidney transplantation

The success of desensitization protocols is closely related to the global success of kidney transplantation as a therapy for end-stage renal disease (ESRD). Transplantation provides mechanisms to expand access and reduce cost for successful treatment of ESRD and surpasses dialysis treatments for quality and quantity of life and for cost effectiveness [1–3]. The current major restriction on kidney transplantation rates is the shortage of donated organs. The crisis in organ availability is related to the fact that patients who require a kidney transplant exceed the organs available, with a ratio between donors and potential recipients lower than 1:3 in most high-income countries [4,5]. Worldwide the number of potential candidates for renal replacement therapy is growing rapidly and, in almost all countries, transplantation meets only 10% of global needs [6].

Recent developments have led to an increase in the pool of donors based on excellent results from ABO-blood-group-incompatible transplants and progress made in transplantation of sensitized patients. High titers of donor-specific HLA antibodies also have limited individuals' prospects for transplantation, although better desensitization protocols and paired kidney exchange programs now afford real opportunities for those previously deemed unsuitable for a transplant.

Desensitization protocols for renal transplantation across HLA antibody barriers

Intentionally crossing the HLA barrier is a relatively recent phenomenon related to the development of methods for detecting and characterizing anti-HLA antibodies and to the availability of potentially effective therapies. The field of desensitization has made great gains over the last decade. Although the expanded practice of desensitization has fostered concerns about feasibility, clinical efficacy, durability of allograft function, and patient safety associated with their use [7–12], nevertheless, significant survival benefit for transplant patients after desensitization compared with waiting for a compatible organ has been suggested by recent studies [12]. As such, these therapies are increasingly being considered as valuable strategies to increase the access to transplant for many allosensitized patients.

Desensitization strategy in response to sensitization to HLA: a serious public health problem

Patients who are HLA sensitized are disadvantaged by prolonged wait times or otherwise limited access to transplantation. This is especially true for patients for whom matching is difficult and/or those considered to be highly HLA sensitized [4,5,13]. Thus, these patients with insufficient flow of potential matched donors are destined to remain on the waiting list for extended periods of time while undergoing dialysis, an additional risk factor for death and graft loss [8,12].

HLA-specific antibodies arise through previous blood transfusion, pregnancy, and previous transplants; the last is currently considered the leading cause of sensitization for renal transplant candidates. The growing utilization of solid-phase tests to detect anti-HLA antibodies using both single HLA phenotypes and single HLA antigens has made possible the establishment of calculated panel-reactive antibody (CPRA) as a new method for assessing sensitization to HLA antigens. The details of this procedure are found in Chapter 36. The CPRA is defined as the percentage of potential donors expected to have one or more of the unacceptable HLA antigens indicated on the waiting list for the candidate. CPRA represents a fundamental change in allocation policies, replacing PRA among French transplant candidates in July 2009 and US transplant candidates in October 2009 [14–16]. It provides a more consistent and accountable measure of sensitization among kidney transplant candidates. Prior to the implementation of

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Box 68.1. Key points: sensitization and kidney transplantation

- The introduction of HLA antibody characterization based on solid-phase assays has improved detection and quantification of donor-specific antibodies (DSA).
- The new measure of sensitization for transplant candidates is the calculated panel-reactive antibody (CPRA), which is based upon unacceptable HLA antigens listed on the waiting list.
- Over the last decade, the percentage of very broadly sensitized patients increased (>80% CPRA) while their rate of transplantation fell.
- Depletion of donor-specific anti-HLA antibodies and pretransplant conditioning improve rates of transplantation.
- Transplantation after desensitization provided a significant survival benefit for patients with HLA sensitization as compared to waiting for a compatible organ.

CPRA, transplant centers and laboratories could report a PRA value based on any of a variety of tests with widely differing sensitivities and specificities. Today, the CPRA policy requires laboratories to enter the specific acceptable HLA identified for their transplant candidates on the national computer system.

The introduction of CPRA has increased the efficiency of organ allocation in sensitized patients [15]. However, the CPRA does not solve all issues of transplantation of highly sensitized patients. Furthermore, it has led to new issues, such as the increasing number of hypersensitized patients on waiting lists and their increased difficulty in gaining access to transplants. Currently, between 35% and 50% of the registrants on our active waiting list have evidence of sensitization [15,16]. About one-quarter of transplant candidates are broadly sensitized: 15.8% of registrants on the Organ Procurement and Transplantation Network (OPTN) and 23% on the French National List had >80% PRA/CPRA and this percentage could be increased by the widespread use of single antigen assays. Nearly all of that increase was among registrants with >95% PRA/CPRA. The percentage of registrants who had >95% of PRA/CPRA doubled between 2009 (5.3%) and 2010 (10.2%) . Therefore, we might see a decline as the patients with very high CPRA values accumulate and do not receive any offers. The rate of transplantation in >95% PRA/CPRA fell from 97 to 69 transplants per 1000 patient years [15]. Few options for transplantation currently exist for these patients who are broadly sensitized to HLA.

The access of sensitized patients to transplants can be increased by specific strategies to reduce HLA-specific antibody levels and improve transplantation rates. Desensitization protocols increase the percentage of patients getting a transplant to 80% (16 of 20 patients who underwent desensitization with intravenous immunoglobulin (IVIG) and rituximab received a renal transplant within a mean of 12 months) [11] or higher (98% in a cohort of live-donor kidney transplantation after desensitization) [12]. Thus, desensitization regimens have the potential to greatly increase access to transplant for this disproportionately disadvantaged population of transplant candidates (Box 68.1).

Treatment options for desensitization programs

High-dose intravenous immunoglobulin is the backbone of desensitization therapies

Intravenous immunoglobulin (IVIG) products are known to have powerful immunomodulatory effects [17]. They are, with or without association with plasmapheresis, the backbone of desensitization regimens. IVIG refers to commercial preparations of normal, polyclonal, polyspecific Ig, consisting mainly of IgG isotype antibodies made from plasma pools derived from thousands of healthy donors. IVIG is composed of more than 97% IgG molecules, with a ratio of the IgG1, IgG2, IgG3, and IgG4 isotypes comparable to human sera. The preparations contain antibodies to foreign (non-self) antigens, to self-antigens (natural autoantibodies), and to other antibodies (idiotypic antibodies).

Several non-mutually exclusive mechanisms have been proposed to explain the beneficial effects of IVIG in patients. None has been definitively shown to be operative in all settings, and it is likely that all play some role in IVIGs salutary effects.

1 Provision of anti-idiotypic antibodies [17,18]. Anti-idiotypic antibodies are antibodies with specificity for the antigen binding portion of another antibody. When bound, these antibodies inhibit the effectiveness of their targeted antibody.
2 Impact of IVIG on the cell function. Some of the beneficial effects of administered IgG extend beyond its half-life, suggesting that these effects are not due merely to passive clearance or competition with pathogenic antibodies. These observations raise the possibility that IVIG therapy results in significant alterations in the cellular compartment of the immune system [17,19,20]. Several recent observations have emphasized the effects of IVIG therapy on dendritic cells, the monocyte/ macrophage system, granulocytes, natural killer cells, and various subsets of T cells, in particular the regulatory T-cell (Treg) subset and B cells.
3 Scavenging of complement fragments. Binding of IgG molecules to potentially harmful complement fragments (C3b, C4b, C2a, and C5a) blocks deposition of these fragments onto their targets and prevents subsequent immune damage [21–24].
4 Immunomodulation by sialylated IgG. One such process, proposed by Ravetch et al. [25], is that the beneficial effect of IVIG is mediated mainly by a fraction of antibodies with terminal sialic acid at the glycan linked to asparagine at position 297 (Asn 297) of the constant Fc chain of IgG. The fraction of IVIG rich in these sialic acid-containing antibodies showed an anti-inflammatory effect by enhancing the expression of the inhibitory IgG Fc receptor IIB, and the enzymatic removal of the sialic acid residues abrogated this anti-inflammatory effect. Recent data demonstrate that the anti-inflammatory properties of IVIG can be recapitulated with a fully recombinant preparation of appropriately sialylated IgG Fc fragments [26]. These and other similar mechanisms may be involved in the means by which general immunoglobulin homeostasis is maintained.

These findings demonstrate the considerable progress that has been made in understanding the mechanisms of action of IVIGs and may influence future perspectives in the field of Ig therapy in organ transplantation.

Plasmapheresis and immunoadsorption

Plasmapheresis (PP) and immunoadsorption (IA) are techniques used to remove plasma proteins and specific proteins like alloantibodies, respectively. The end point of these therapies is the elimination of HLA donor-specific antibody either before or after the transplant. PP is not specific for Ig removal and results in lowering of all plasma proteins, including clotting factors, and requires replacement with fresh frozen plasma and albumin. IA includes a sepharose-bound staphylococcal protein A column with a high affinity for binding all IgGs, not just alloantibodies. Anti-HLA antibody titers rebound and return to baseline levels after the completion of PP or IA [27]. This relates to the general homeostatic mechanisms used to regulate physiologic Ig levels such that

physiologically low Ig levels may actually promote antibody production. Thus, these techniques do not result in a durable reduction in HLA antibodies, unless the patient undergoes transplantation within several days after the last treatment.

Some studies showed that immediate pretransplant IA might convert a positive to negative complement-dependent cytotoxic cross-match in sensitized deceased-donor kidney allograft recipients and thus allow transplantation. However, this experience still remains very limited. The first study using IA immediately before transplantation was presented in 1996 by Higgins et al. on 12 cytotoxic or flow cytometry cross-match-positive patients. The rate of rejection was high (13 rejection episodes in nine patients) as was the rate of graft loss (53.8% at 26 months) [28]. Better results were reported more recently by Lorenz et al. on the outcome of 40 deceased-donor kidney transplant recipients, including nine patients with positive cross-matches rendered negative by a single pretransplant IA. Three-year graft survival (78% vs. 71%), acute rejection (11% vs. 20%), and C4d-positive graft dysfunction (33% vs. 32%) were comparable in cross-match-positive and cross-match-negative patients [29].

The rituximab debate

The renewed interest in B cells and antibodies in organ transplantation has stimulated the search for new drugs. Rituximab is a chimeric murine/ human monoclonal antibody that reacts with the CD20 antigen and selectively depletes B cells [30]. The surface protein CD20 is expressed on naïve and memory B cells, is rapidly down-regulated on activated B cells, and is absent on pro-B cells and plasma cells [31,32]. As such, there is no direct influence of rituximab on antibody-secreting cells.

The drug was originally approved by the FDA for the treatment of refractory or relapsed B-cell lymphomas, and has since become widely used in solid organ transplantation for ABO-incompatible transplant [33,34], treatment of rejection [35,36], rejection prevention [37], and desensitization protocols [10,38–40]. Unfortunately, studies describing rituximab use in transplant are small, and generally uncontrolled. This is a critical point and the value of adding rituximab in desensitization regimens is still questioned.

There are two potential means by which rituximab might function in desensitization regimens [41]:

1 Rituximab might deplete specific antidonor antibody, although the mechanism by which this would occur is unknown. The data for this effect is sparse. In a small phase 1 study of nine sensitized dialysis patients treated with single doses of rituximab, only one patient converted a living kidney donor-specific cross-match to negative and underwent a successful kidney transplantation [39]. There were no significant reductions in panel-reactive antibody levels or antibody specificity in most of the patients. In a more detailed analysis using single-antigen beads, Pescovitz found that specificities with lower titers were more likely to fall after rituximab treatment [38]. Other investigations have demonstrated that rituximab treatment for antibody-mediated rejection (ABMR) in combination with multiple other therapies results in the reduction or elimination of donor-specific antibody [36,42]. Use of rituximab has been anecdotally reported to lead to interference with both flow-cytometric cross-match and complement-dependent cytotoxic cross-match for B cells [11].
2 Rituximab may act by eliminating B cells and in doing so interrupt non-antibody secreting functions of B cells such as antigen presentation. Supporting the direct role of B cells in rejection is the conclusion of Sarwal et al., who showed that CD20 gene expression, and $CD20^+$ infiltrates were associated with poor prognosis [43]. Subjects with CD20-positive rejection had worse long-term graft survival [44]. Treatment of these patients with rituximab was successful [45].

There are problems with both of these concepts. First, anti-CD20 activity has no effect on long-lived $CD138^+$ plasma cells, which are the primary source of acute antibody production [46,47]. Second, rituximab has no immediate or powerful effect on circulating antibody levels. These problems might explain the difficulty of assessing the efficacy of rituximab when used as sole treatment. However, the combination with other treatments including IVIG and/or polyclonal rabbit antithymocyte globulin might constitute an improved approach for the management of allosensitization. These combination protocols can reduce both antibody secretion by, and the number of, long-lived plasma cells. Rituximab is likely to reduce or eliminate the silent cohort of resting naïve and memory alloreactive B cells, as well as the short-lived $CD20^+$ activated plasmoblasts of an early anamnestic response.

The new agents

Two new drugs are under consideration for pretransplant conditioning in highly sensitized patients. Both are used off-label (see Chapter 101), and thus neither has been shown to be efficacious in properly powered randomized trials.

Bortezomib is a proteasome inhibitor that targets all cells with high-level protein turn over, including plasma cells. The drug is FDA approved for use in multiple myeloma patients and anecdotal evidence suggests efficacy in transplant patients [48] through many mechanisms. The immune-modulating effects of bortezomib include activity against normal plasma cells, as shown in animal models [49–51], and induction of apoptosis of normal human plasma cells preventing alloantibody production [52]. In addition, proteasome inhibitors have been shown to suppress T-cell functions [53] and to inhibit function of antigen-presenting cells [54,55].

In the setting of transplantation, bortezomib has been used as primary treatment [56] or as a rescue strategy for acute ABMR [48]. Bortezomib has also been used to abrogate HLA-specific antibodies outside of acute rejection in an attempt to improve long-term allograft survival [57]. All these clinical experiences highlight bortezomib's capacity to promptly and rapidly reduce antibody levels as measured by Luminex assays. In these proteasome inhibitor-based therapies, bortezomib is associated with PP. Inclusion of PP provided the advantage of removing previously screened antibody, thereby causing circulating antibody levels to be a more accurate reflection of current production rates by the residual plasma cells population, rather than a reflection of pre-bortezomib antibody production rates [56]. These considerations have led to proposals for prospective clinical trials to evaluate the efficacy of bortezomib in desensitization programs, as proposed by Woodle and colleagues (University of Cincinnati, OH). The frequency of serious side-effects of bortezomib, most notably neurological, also has to be explored in a meaningful number of pretransplant patients.

Eculizumab, a humanized monoclonal antibody directed against the complement protein C5, is another drug under investigation. By targeting terminal components of the complement cascade, eculizumab works on secondary effects of deposited antibody, and thus is not dependent on antibody clearance, per se. In a presensitized murine model of acute ABMR, C5 blockade in combination with conventional immunosuppression was shown to prevent antibody-mediated allograft injury, resulting in prolonged graft survival [58]. As presented by the Mayo Clinic team, the terminal complement

activation is critical for the development of ABMR. Eculizumab decreases the risk of early acute ABMR in sensitized transplant recipients. The incidence of acute ABMR fell from 41.2% in the control group of 51 sensitized patients treated with plasma exchange-based protocol without eculizumab to 7.7% in the 26 patients of the eculizumab group [59].

Inclusion criteria for and success of desensitization protocols

The inclusion criteria for desensitization protocols, as well as the criteria for accepting a donor after completion of the desensitization protocols, are heterogeneous. This reflects the development of the concept of appraisal of the immunologic risk prior to transplant. Today, the techniques that are used to define this risk vary from the classic complement-dependent cytotoxic cross-match and T-cell antiglobulin enhanced complement-dependent cytotoxicity, to more sensitive techniques of solid-phase assays, of which the Luminex-based assays are the most frequently used (discussed in detail in Chapter 36). While the more advanced of these assays have the potential to be quantitative, at present they are approved only as qualitative assays, and this substantially limits the ability of clinicians to make objective determination of a patient's risk for ABMR.

In the first experiences of desensitization protocols in deceased donors, the inclusion and success criteria were exclusively based on cytotoxic tests: cytotoxic PRA >50% or 80% as inclusion criteria and a negative cross-match was required for patients to be eligible to receive a kidney transplant [7,8]. For living donors, the goal of desensitization treatment was the conversion to a negative cross-match before transplantation [9].

In recent years, the policies of graft acceptance have progressed and the strength of cross-matching for donor HLA-specific antibody plateaued at lower levels of reactivity: titer <1:8 on the complement cytotoxicity assay [12] or a T-cell donor-specific flow cytometric cross-match <250 mean flow-channel shifts [11]. This concept is clearly revealed by Johns Hopkins' experience on live-donor renal transplantation. In this study, the definition of HLA incompatibility included three non-overlapping categories of antibody strength: positive complement-dependent cytotoxic or flow-cytometric cross-matching or only detectable donor-specific anti-HLA antibody on Luminex assays. A survival benefit was associated with desensitization in all three categories of HLA incompatibility. This study also suggested that an increase in the donor-specific level was a predictor of reduced graft survival: patients with positive results on the bead assay had the shortest follow-up, with rate survival of 90.8% at 48 months; rates of survival were 92.2% at 1 year, 85.5% at 3 years, and 79.8% at 8 years in desensitized patients with a positive flow-cytometric cross-match; and, respectively, 87.7%, 82%, and 78% in desensitized patients who had positive cross-match on the complement-dependent cytotoxicity assay [12]. Other studies showed a strong correlation between the risk of acute ABMR and the levels of donor-specific antibodies postdesensitization, as defined by mean flow-channel shift on flow-cytometric cross-matching and mean fluorescence intensity on Luminex assays [60,61].

Specific desensitization protocols

There are currently three desensitization protocols for which clinical efficacy has been demonstrated: (1) high-dose IVIG; (2) rituximab and high-dose IVIG (R/IVIG), and (3) plasmapheresis (or immunoadsorption) with low-dose IVIG (PP/IVIG). As of today, there have been no randomized prospective studies comparing their clinical efficacy. Moreover, data from various studies to date are difficult to interpret or compare because of heterogeneity among inclusion and success criteria of desensitization protocols, histocompatibility testing techniques, donor-specific anti-HLA antibody levels, and demographic and clinical characteristic of donor and recipient populations (Table 68.1).

The choice of desensitization regimen relies, for largely logistical reasons, on the origin of the donor, that is living or deceased. In living donor situations, where transplantation can be performed as soon as the projected reduction in antibody level has been achieved, the combination of PP/IVIG is the treatment of choice, with or without the addition of rituximab. When one has to wait for an unknown period of time for a deceased donor, use of high-dose of IVIG, alone or associated with rituximab, is preferred because the decrease in antibody level is far more prolonged, and therefore compatible with the wait for the organ.

High-dose IVIG regimen

High-dose IVIG regimen is a protocol used for both live and deceased donor transplants, but the major advantage of this approach is its applicability in deceased donor kidney transplant candidates. It consists of monthly courses of 2 g/kg body weight of IVIG until either the cross-match is deemed safe or totally negative [7,8,18].

The efficacy of high-dose IVIG protocol was demonstrated in a randomized, multicenter, placebo-controlled trial conducted by the NIH (NIH IG02 study) from 1997 to 2000 [8]. This study, which is the only controlled clinical trial of a desensitization therapy, was designed to determine whether IVIG could reduce PRA levels and improve rates of transplantation without concomitantly increasing the risk for graft loss in this difficult-to-transplant group. IVIG was superior to placebo in reducing HLA-specific antibody levels and improving rates of transplantation (Figure 68.1). The 3-year follow-up showed that the predicted mean time to transplantation was 4.8 years in the IVIG group versus 10.3 years in the placebo group ($P = 0.02$). With a median follow-up of 2 years after transplantation, the viable transplants functioned with a mean serum creatinine of 1.68 ± 0.28 (IVIG) versus 1.28 ± 0.13 mg/dL for placebo ($P = 0.29$). Allograft survival also was superior in the IVIG group at 3 years. From this multicenter, double-blinded, placebo-controlled trial, we concluded that IVIG was superior to placebo in reducing HLA-specific antibody levels and improving transplantation rates in highly sensitized patients with end-stage renal disease.

The rate of acute rejection in desensitized patients was still high (35% to 50%). Although more acute ABMR episodes were seen (20% to 38%), the 3-year allograft survival was satisfactory: 78% in the French experience and 80% in the NIH IG02 study [7,8]. The authors did not report the specificity and the strength of donor-specific HLA-specific antibodies.

Induction treatment in high-dose IVIG protocols was complemented by antithymocyte globulin [7] or daclizumab [18], with similar results. The impact of induction treatment was nonsignificant as shown by Cedar-Sinai Center experience, in which the induction treatment was switched from daclizumab to antithymocyte globulin [62]. The 2-year graft survival was 84% in the daclizumab group (58 patients) and 90% in the antithymocyte group (39 patients), whereas the acute rejection rate was 36% (22% ABMR) and 31% (21% ABMR), respectively.

Table 68.1. Summary of reported desensitization protocols and their clinical outcomes

Author/ year	Number of patients	Donor type	CXM technique	Induction	Mean follow-up (months)	AR/ ABMR (%)	Patient survival (%)	Graft survival (%)
Peritransplant IA								
Higgins 1996 [28]	13	Deceased	CDC; FCXM	Thymo	26	69/–	92	54
Lorenz 2005 [29]	40	Deceased	CDC	Thymo	CXM positive (n = 9) 32	44/33	–	78
					CXM negative (n = 31) 34	52/32	–	71
PP/Low-dose IVIG								
Schweitzer 2000 [63]	11	Living	CDC	OKT3	13	36/27	100	100
Montgomery 2000 [68]	4	Living	CDC	Daclizumab	14	100/100	100	100
Gloor 2003 [64]	14	Living	CDC	Thymo/rituximab/SPL	15	43/43	86	78
Magee 2008 [65]	28	Living	CDC	Thymo/basiliximab/rituximab	22	71/39	93	89
Thielke 2009 [66]	51	Living	FCXM	Thymo/rituximab	23	33/24	95	93
Haririan 2009 [67]	41	Living	FCXM	OKT3 or thymo	47	24/12	78	66
Montgomery 2011 [12]	211	Living	CDC;FCXM	Daclizumab or thymo	96	–	81	–
High-dose IVIG								
Glotz 2002 [7]	13	Deceased	CDC	Thymo	12	8/8	85	85
Jordan 2003 [18]	42	Deceased, living	CDC	Daclizumab	24	31/31	98	89
Jordan 2004 [8]	17	Deceased, living	CDC		24	53/–	76	80
Vo 2006 [62]	97	Deceased, living	CDC	Daclizumab (n = 58)	24	36/22	96	84
				Thymo (n = 39)	24	31/21	100	90
High-dose IVIG/rituximab								
Vo 2008 [11]	16	Deceased, living	CDC;FCXM	Alemtuzumab	12	50/31	100	94
Vo 2010 [95]	76	Deceased, living	CDC;FCXM	Alemtuzumab	24	37/29	95	84
High-dose IVIG vs. PP/ low-dose IVIG/rituximab								
Stegall 2006 [10]	59	Living	CDC	Thymo	12			82

CXM, cytotoxic cross-match; FCXM, flow cytometric cross-match; CDC, complement-dependent cytotoxicity; AR, acute rejection; ABMR, antibody-mediated rejection; IA, immunoadsorption; IVIG, intravenous immunoglobulin; PP, plasmapheresis; SPL, splenectomy; Thymo, antithymocyte globulin.

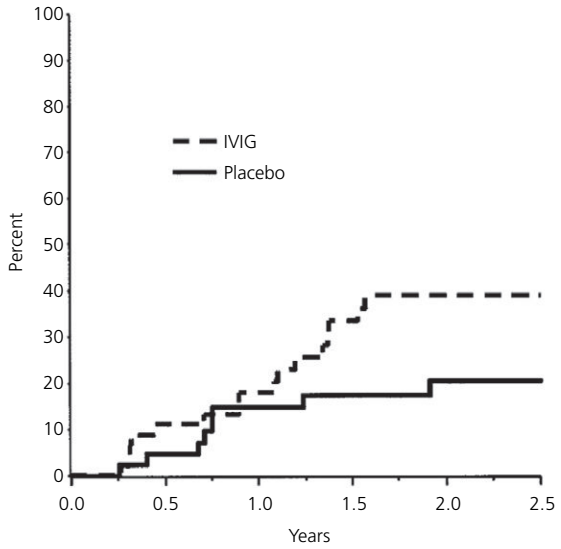


Figure 68.1. The cumulative time to transplantation. Intravenous immunoglobulin (IVIG) significantly ($P < 0.05$) decreased the time to transplantation compared with placebo. The rates of transplantation in the IVIG group were double those in the placebo group and were greater for retransplant patients. Source: Jordan et al. 2004 [8]. Reproduced with permission of Massachusetts Medical Society.

Rituximab and high-dose IVIG regimen

The Cedars-Sinai Medical Center group added rituximab given twice (1 g on days 7 and 22) to a monthly IVIG regimen (2 g/kg body weight on day 0 and 30) and achieved 80% transplant rate among 20 patients with 100% and 94% 1-year patients and graft survival rate [11]. However, the acute rejection rate was very high (50%), and 31% of these episodes were ABMR.

The reduction in PRA was similar to the NIH IG02 study and it remains unclear whether rituximab added any efficacy to the IVIG regimen (Figure 68.2).

Plasmapheresis and low-dose IVIG regimen

This protocol produces a rapid reduction in anti-HLA titers that allows for transplantation after three to five PP treatments, depending of the strength of HLA-specific antibodies before transplantation. The addition of low-dose IVIG adds an immunomodulatory effector mechanism that might be beneficial in keeping the antibody titers low.

The PP/low-dose IVIG protocol was first utilized in 1998 at Johns Hopkins Hospital in cross-match incompatible living-donor kidney transplant candidates [9]. Patients received PP and IVIG at 100 mg/kg after each PP along with tacrolimus and mycophenolate mofetil treatment starting 2 to 3 weeks before transplantation. The treatment plan is individualized on the basis of an assessment of the patient's risk of ABMR [9]. Montgomery et al. reported their 11-year experience in a large cohort of 211 live-donor recipients. They clearly demonstrated that live-donor transplantation after desensitization provided a significant survival benefit for patients with HLA sensitization, as compared with waiting for a compatible

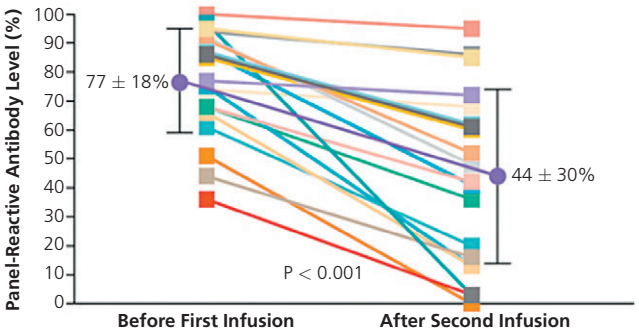
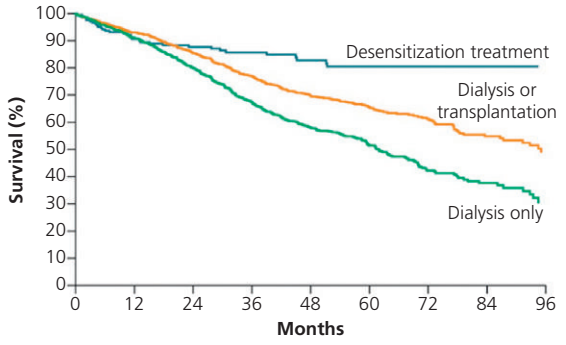


Figure 68.2. Panel-reactive antibody levels. Individual data are shown for patients before the first infusion of intravenous immune globulin and after the second infusion. The pretreatment and post-treatment means are also shown, as determined with the T-cell complement-dependent cytotoxicity panel-reactive antibody assay. The means were significantly different ($P < 0.001$). Bars denote standard deviations. Source: Vo et al. 2008 [11]. Reproduced with permission of the Massachusetts Medical Society.



No. at Risk									
Desensitization treatment	210	170	143	110	75	58	42	28	14
Dual therapy	1027	854	688	497	321	230	157	96	41
Dialysis only	1012	822	626	419	250	159	93	54	17

Figure 68.3. Survival in HLA-incompatible kidney recipients. Kaplan–Meier estimates of patient survival are shown for patients who underwent desensitization treatment before kidney transplantation (treatment group), as compared with two matched control groups of patients on a kidney waiting list who continued to receive dialysis (dialysis-only group) or who either continued to undergo dialysis or underwent HLA-compatible transplantation (dialysis or transplantation, or dual therapy, group). Source: Montgomery et al. 2011 [12]. Reproduced with permission of Massachusetts Medical Society.

organ. By 8 years, the survival advantage more than doubled (80.6% vs. 30.5% for patients in the dialysis-only group) [12] (Figure 68.3).

Similar PP/low-dose IVIG-based desensitization regimens were used by several other teams [63–67]. In these studies the induction therapies were very heterogeneous, including daclizumab [68], OKT3 [63], or antithymocyte globulin/ basiliximab and rituximab [65,66], or antithymocyte associated with rituximab and splenectomy [64]. Despite these modifications, the rates of rejection remained high (between 33% and 100%) with good short-term graft survivals, ranging from 78% to 100% in the first 2 years post-transplantation. The University of Maryland reported its long-term experience in 41 flow cytometry cross-match-positive patients using PP/low-dose IVIG with OKT3 or antithymocyte globulin induction treatment. Patients had lower acute ABMR (12%) compared with the studies presented above, but patient survival and graft survival at 4 years were only 78% and 66% respectively [67]. See Box 68.2 for key points.

Box 68.2. Key points: desensitization protocols

- DSA removal through immunoadsorption or plasma exchange, and DSA inactivation using high-dose intravenous immunoglobulins are the backbone desensitization therapies with good short- to intermediate-term outcomes.
- The value of adding rituximab is still debated.
- New interventions aimed at the prevention of antibody-mediated allograft injury using complement blockade, or the inhibition of DSA synthesis using proteasome inhibitor-mediated plasma cell depletion look promising. Proteasome inhibition with bortizomib is emerging as a potential new strategy.

Safety of desensitization protocols

Specific side-effects of IVIG

There are many IVIG products with product-specific side-effects which depend on the individual preparations' osmolarity, pH, and sodium or sugar content. As shown by the Cedar-Sinai group, who reviewed the side-effects of IVIG in 279 desensitized patients [69], most adverse reactions to IVIG are mild and include headache, nausea, myalgia, back pain, and increased blood pressure, all of which typically respond to slowing the infusion rate or premedication with antihistamine drugs. Rare serious side-effects of IVIG were reported and included: antibody-mediated (Coombs-positive) hemolysis [70]; acute aseptic meningitis [71]; and acute renal failure (ARF). The predominant, known mechanism of ARF after IVIG infusion is the osmotic injury to proximal tubular epithelium associated with sucrose-containing formulations. Thrombotic events, such as acute myocardial infarction, deep venous thrombosis, and stroke, are related to high osmolarity and can be prevented by prophylactic anticoagulation. Very rare, serious anaphylactic reactions may occur in patients with IgA deficiency.

Adverse events related to plasmapheresis

The Johns Hopkins group evaluated the following PP-related adverse events in the largest cohort of live-donor transplant patients desensitized by PP/IVIG regimens (215 patients) [12]: minor reactions (10.9%); major events, including anaphylaxis with hypotension and airway edema (1.4%); and surgical site bleeding complications or bleeding after biopsies, some of which may have been associated because PP depletes coagulation factors.

Risk of infection and malignancy

Desensitized patients receive more immunosuppression that non-sensitized patients, and this confounds the direct association of any of the components of a desensitization protocol with infectious risk. Regardless, the overall impact of a desensitization approach is one of substantial immunosuppression, and this must be balanced against the risks of dialysis and failure to proceed with transplantation.

The development of progressive multifocal leukoencephalopathy (PML) is caused by the reactivation of latent JC polyomavirus. Safety alerts were issued by the FDA and WHO describing cases of PML in patients with systemic lupus erythematosus treated with

rituximab. No cases have been reported as attributed to rituximab in renal transplant patients [72].

Controlled clinical trials in rheumatoid arthritis and lymphoma treatment have demonstrated the safety of rituximab with no significant increase on infection rates [73,74]. Also, the prevalence on infection reported in three studies regarding desensitized patients was not higher than in average transplant patients [11,66,75]. Kamar et al. [76] report on a disturbing association between rituximab use and a significant risk of infection-related deaths post-transplant (9.1% in rituximab-treated group vs. 1.6% in the retrospective controls). However, the dose of rituximab given in this report was four times higher than used in desensitization protocols.

In summary, serious infectious complications can occur after desensitization protocols. Consideration should be given to viral polymerase chain reaction (PCR) monitoring, especially in those patients who receive agents that alter T-cell function in addition to rituximab [77]. As with other immunosuppressive agents, finding the most effective and safe dosing is mandatory.

Desensitization protocols for renal transplantation across ABO blood group antibody barrier

The use of ABO-blood-group-incompatible living donors in kidney transplantation is hardly a new phenomenon [78], having been pioneered by Guy Alexander and advanced subsequently in Japan, with the latter driven by the lack of a definition of brain death and the resulting ABO-compatible donor shortage. In general, the barrier presented by ABO-specific antibodies seems qualitatively less vigorous than that presented by HLA-specific alloantibody. Between 1989 and 2005, more than 850 ABO-incompatible kidney transplants from living donors were performed in Japan [79], giving excellent short and long-term results, with 1-year graft survival currently exceeding 95%.

The desensitization regimens in use differ little from those used for HLA incompatible transplants, and are based on antibody removal, using PP or IA [34], and prevention of antibody resynthesis, initially achieved by splenectomy and now replaced by the use of rituximab, together with conventional immunosuppression. Over the years, the immunosuppressive regimens have changed, imposing a limitation on the numbers of pretransplant PP sessions, replacing splenectomy by rituximab [80], and ceasing post-transplant PP, thus limiting the total amount of immunosuppression without any increase in rejection rates.

The rate of acute ABMR following ABO-incompatible transplantation varies from 15% to 33% [81,82], with a negative impact on graft survival. All protocols are based on pretransplant antibody removal through PP or, more recently, IA [34]. However, very high titers of isohemagglutinins may necessitate a great number of PP sessions, are associated with a high frequency of humoral rejections [83], and might represent a contraindication [84].

In a survey of North American centers, 80% used the combination of plasmapheresis and IVIG [85]. Two seminal observations have been drawn from these transplantations: (1) the pretransplant titer of isohemagglutinins, that is antidonor antibodies, does not need to be reduced to zero, demonstrating that successful transplantation can be performed despite existing low level of antidonor antibodies; moreover, the risk of rejection as well as graft survival are closely correlated with the pretransplant level of isoagglutinins [81,86], a finding also present in HLA incompatible transplants; and (2) long-term excellent function of the graft, without histological damage, is achieved despite persistence of antidonor antibodies, a phenomenon called accommodation [87], yet to be seen in HLA-incompatible transplantation.

Summary: outstanding issues in desensitization protocols

Numerous issues remain regarding the appropriate deployment of desensitization protocols. These have been summarized in the proceedings of a recent FDA-sponsored workshop on ABMR [88].

Who should benefit from desensitization protocols?

All published protocols have selected their patients on the basis of cellular assays, that is positive cross-match assays (either cytotoxic or by flow cytometry) for living donor transplantation, or high cytotoxic panel-reactive antibody for cadaveric donor transplantation. However, access to transplantation for patients on the deceased donor waiting list is now dependent in many countries, such as the USA or France, on CPRA, based on the definition of forbidden HLA antigens by Luminex assay [15,16]. Unfortunately, a growing number of patients are immunized solely by new highly sensitive techniques such as the Luminex assay, with little if any cytotoxic PRA. Those patients may have a high CPRA, barring them from transplantation, but cannot be enrolled in the canonical desensitization protocols based on cytotoxic tests. Thus, we need to reappraise inclusion criteria of desensitization protocols, also based on the current techniques of measure of sensitization and the sensitive techniques for the detection of HLA-specific antibodies.

Pretransplant immunologic risk assessment: how much is significant and what does this mean in term of unacceptable antigen?

There is no consensus on the definition of a forbidden antigen as defined by solid-phase antibody testing methods such as Luminex technology. As for clinical relevance, we have learned that the mere existence of a given HLA-specific antibody directed against the donor (donor-specific antibody or DSA) solely detected by Luminex Single Antigen is no longer a contraindication to transplantation, as excellent graft survival rates have been shown despite the existence of those antibodies [88–90]. Thus, a quantitative approach is mandated but, at the moment, each laboratory has defined its own threshold of mean fluorescence intensity (MFI) to define a forbidden antigen, which may vary from 500 to 3000. At our institution, we retrospectively studied 402 patients who underwent cadaveric transplantation after a negative T-cell cytotoxic cross-match [91]. In 83 of those patients, a DSA was detected using Luminex Single Antigen assay. Although the prevalence of acute humoral rejection rose significantly with increasing MFIs of DSAs, a threshold MFI value of 3000 was associated with increased graft loss. Our main challenge for the years to come is to precisely grade the immunologic risk of rejection and graft loss, allowing clinically relevant definitions of acceptable and forbidden antigens, leading to the precise estimation of the access to transplantation and, finally, to the best possible definition of the population of patients really needing desensitization protocols.

Need for active policy of management of the waiting list

Patients are shifted to desensitization protocols after wait times that are exceedingly long (5 to 10 years). From our standpoint this is an

unacceptable approach. We need to predict probability of transplantation for highly sensitized patients for much earlier identification of those who could benefit from desensitization protocols [16]. Simulation programs should allow us to check whether the national allocation system offers a realistic possibility of transplantation for these hypersensitized patients. We need to identify early—at the time of inscription on the waiting list—which patients have an insufficient pool of donors and will not benefit from the national prioritization programs, in order to allocate them to specific protocols of desensitization.

Integrating paired donation into desensitization protocols

Very broadly sensitized patients with high HLA reactivity who are both difficult to match and difficult to desensitize can be transplanted by combining kidney-paired donation (KPD) and desensitization, with the goal of finding a lower-immunologic-risk donor in the KPD pool, and thus increasing the practicality and success of desensitization. Decision about the best transplant option for a particular donor/ recipient phenotype can then be made rationally using KPD and desensitization as complementary modalities.

The challenge of long-term graft survival in desensitized patients

Preconditioning regimens currently in use for desensitization are administered with an immediate goal of preventing acute antibody-mediated injury. However, it is now clearly recognized that patients with preformed DSA are at risk not only for acute or accelerated ABMR but also for delayed graft function and impaired long-term graft survival event in the absence of clinical ABMR [91]. The studies have led to the discovery of the "underwater" part of the iceberg, representing progression of lesions leading to chronic humoral rejection and late graft loss [92–94]. The main question is, how can we prevent or stop this progression of antibody-mediated injury? Transplant recipients following desensitization protocols therefore require both pretransplant and post-transplant interventions that have separate but complementary aims.

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CHAPTER 69

Clinical Allograft Rejection Syndromes in Kidney Transplantation

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Introduction

Transplant injury from the immune response

Transplantation from a genetically non-identical donor initiates a recipient immune response directed toward donor antigens which is expressed as allograft rejection. Pre-existing immune memory represents residual heterologous immunity from cross-reactive past infections or previous immune stimuli, and correlates with rejection and graft loss [1]. Memory T cells can also become peripherally activated in tissues or in tertiary transplant lymphoid structures [2–4]. Rejection invokes the adaptive immune system of specific T lymphocytes, B cells, and plasma cells generating antibody, but also involves the innate system consisting of complement, NK cells, and macrophages. The alloimmune response is the cardinal threat to the transplanted kidney: uncontrolled rejection destroys the allograft [5].

The clinical syndrome of acute rejection has changed with the introduction of powerful calcineurin inhibitors (CNI) and better immunological matching. In the early azathioprine/corticosteroid era, acute rejection was common, approaching 70–80%, and presented with fever, graft tenderness, oliguria, and a rising serum creatinine. Rejection rates are now below 15% by 1 year, but those remaining episodes are clinically more severe. Disappointingly, as described in depth in Chapter 103, long-term attrition rates remain largely unaltered.

The impact of immune-mediated graft injury varies by the severity, timing, persistence, and histological type of rejection: which in turn determines the level of functional impairment and therapeutic response. Acute interstitial T-cell mediated rejection when treated promptly with pulse corticosteroids usually resolves without sequel. In contrast, vascular, steroid-resistant, or antibody-mediated acute rejection are more resistant to therapy, and—along with recurrent rejection, untreated subclinical rejection, chronic rejection, or late acute rejection from non-adherence—can irreversibly damage the transplant and require robust targeted therapy. Several divergent immune-mediated mechanisms and patterns of rejection can co-exist in a single episode, especially in progressive or late acute transplant dysfunction. Rejection-mediated injury stimulates a healing response within the kidney transplant, modified by immunosuppression [6–9].

Rejection can be described by timing into hyperacute (immediate–minutes), acute (early–days), late-acute or chronic (months to years post transplant); by transplant histopathology (interstitial/cellular, vascular and antibody/endothelial rejection); by histological severity (determined by the extent of inflammation and injury, scored and graded by the Banff schema [10]); by treatment response (steroid-resistant rejection or not); by level of renal dysfunction (acute versus subclinical rejection); and immunobiology (T-cell mediated and antibody of the adaptive immune system versus complement, macrophages, and innate mechanisms). Diagnosed by needle core biopsy, rejection is classified by pathophysiological subtype, which influences prognosis and dictates treatment options [11,12].

This chapter is organized into four clinical scenarios of transplant dysfunction, with their corresponding rejection patterns and non-immune differential diagnoses, occurring in the immediate, early, and late periods after transplantation. It focuses on practical management, with detail of immunological mechanisms, histopathological findings, treatments, and specific differential diagnoses found in Chapters 5–9, 67, and 81.

Primary non-function

Clinical scenario. The transplanted kidney initially fails to function after implantation.

Immediate graft function (IGF) ideally is the norm and occurs after vascular reperfusion, where the kidney transplant passes urine and functions with sequential reductions of serum creatinine. Primary non-function is characterized by post-operative oliguria that is unresponsive to fluid challenge or furosemide, and increasing serum creatinine levels. The most common cause is ischemia–reperfusion injury; however, ureteric obstruction, vascular impairment, and hyperacute rejection are important differential diagnoses.

Hyperacute rejection

Hyperacute rejection is mediated by preformed cytotoxic donor-specific antibodies (DSA), often directed against HLA class I antigens expressed on donor renal allograft endothelial cells

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

[13–15]. Most first transplant recipients do not have pre-existing antibodies to anti-HLA molecules, unless exposed to foreign human cells expressing HLA through pregnancy, blood transfusion, or previous transplantation—which are risk factors for sensitization. See Chapters 36 and 89 for detailed descriptions of HLA antibodies and their clinical detection, respectively.

Hyperacute rejection presents almost immediately after release of the vascular cross-clamps where the kidney becomes flaccid with variable surface mottling, instead of "pinking up" with normal reperfusion. Progressive microvascular DSA deposition is followed by rapid activation of classical complement cascade, innate immune, and coagulation pathways. Acute inflammatory leukocytes then adhere to endothelial cells of glomeruli ("glomerulitis") and peritubular capillaries (PTC). Once established, it is usually impossible to reverse. Endothelial necrosis, platelet, and fibrin deposition and local coagulation then progresses to patchy cortical necrosis and severe acute tubular necrosis [13]. The transplant remains anuric, progressively blackens with large-vessel thrombosis, associated with a consumptive thrombocytopenia and anemia. The operation usually ends with transplant nephrectomy.

Hyperacute rejection was initially described from kidneys transplanted against a positive direct complement-dependent lymphocytotoxicity (CDC) cross-match [16], which later became an absolute contraindication. More sensitive and specific cross-matching techniques (including antihuman globulin augmented CDC cross-match, flow cytometric or solid-phase serum screening; Chapter 89), which identify pretransplant DSA, has largely eliminated this problem [15]. Rarely, undetected non-HLA DSA (such as antiendothelial cell antibodies) can mediate early hyperacute rejection [17].

ABO incompatible kidney transplantation

Hyperacute rejection also occurs with ABO-incompatible transplantation. Anti-A and anti-B antibodies (isohemagglutinins) are isoantibodies directed against blood group antigens expressed on endothelial and red blood cells, and pose a barrier to transplantation. The lesson of ABO incompatibility, discovered a century ago with blood transfusion (massive hemolysis, acholuric jaundice, hemoglobinuria and renal failure), was again relearned in the 1950s with ABO-incompatible kidney transplantation, which rapidly failed with coagulative necrosis from hyperacute rejection. Transplants were then routinely assigned to ABO blood group compatible recipients.

However, increasingly, ABO-incompatible kidneys have been transplanted without hyperacute rejection using perioperative antibody removal in experimental protocols [18]. Pre- and postoperative plasma exchange or immunoadsorption columns (fixed A and/or B antigen remove plasma antibody) reduce antibody titers to low levels, which are combined with i.v. immunoglobulin immunomodulation, splenectomy or rituximab, and antiproliferative agents to prevent antibody rebound [19]. Circulating antibody titers recover after transplantation, adhere to the endothelium, and activate complement (with C4d positivity), but generally fail to cause capillaritis or overt injury, attributed to the phenomenon of "accommodation" of the microcirculation [20], probably mediated by up-regulation of protective endothelial genes. If it occurs, acute antibody-mediated rejection from isohemagglutinin also includes features of early segmental thrombosis of glomerular loops, mesangiolysis, and polymorphonuclear cell recruitment. Long-term results are similar to blood group matched recipients [21], and contrasted with anti-HLA antibody where accommodation is uncommon and poorer graft survival is associated with persistent antibody-mediated rejection. Additional detail on desensitization protocols can be found in Chapter 68.

Differential diagnosis of initial non-function

Ischemia reperfusion injury

Delayed graft function (DGF) is where the implanted kidney passes urine but fails to initially function due ischemia–reperfusion injury related to donor factors, procurement techniques, and prolonged ischemic times. It is defined for registry purposes by dialysis need within 7 days post-transplantation, but other functional definitions (slower fall in serum creatinine, e.g. by 10% daily over 3 days) also correlate with graft failure (HR = 1.47; 95% CI 1.06–2.03) [22]. The DGF incidence has increased from 14.7 to 21–23% over two decades [23], with kidneys from older deceased donors with vascular co-morbidity (expanded criteria donors, ECD), or following circulatory death (DCD or non-heart beating donors), being used because of organ shortages. These marginal kidneys yield inferior initial function rates, and patient and allograft survivals relative to standard criteria donors, but still improve overall recipient survival compared with remaining on dialysis [24]. DGF increases graft loss by 41% (RR 1.41; 95% CI 1.27–1.56) by systematic meta-analysis [25]. Other factors that increase DGF include donor age, organ size and quality (older donor, hypotension, diabetes, renal impairment, and hypertension), and transport or "shipping" issues prolonging ischemia times [26,27]. The turbulent peritransplant stressor impacting deceased donor organs can be mitigated by optimal ICU management, pulsatile ex vivo machine perfusion [28,29], rapid transfer, and prompt implantation [30]. Donor organ quality influences its response to stressors and ultimate longevity.

Metabolically active renal tubular epithelial cells are vulnerable to ischemia, which depletes oxygen, limits cellular metabolism, and Na/K ATPase exchanger function. Reperfusion injury with oxygenated blood generates reactive oxygen species including superoxide, nitric oxide, and peroxynitrates, leading to DNA breakdown, lipid peroxidation, apoptosis, and cellular necrosis. Vascular endothelial cell ischemic injury causes phospholipolysis, thrombin-mediated fibrin deposition, surface signal up-regulation of P-selectin receptors, β-integrins, and chemokines, and leukocyte adherence [23].

Donor brain death or perioperative ischemia–reperfusion injury also stimulates a cascade of chemokines, proinflammatory cytokines (IL-6, TNF-α, IFN-γ), and adhesion molecules (CD54 [ICAM-1], VCAM-1, CD18/11a [LFA-1]), which up-regulate MHC antigen expression within the kidney transplant. Injured tissues express innate danger receptors or Toll receptor system ligands (normal protecting against infectious pathogens) [31], which promote immune cell maturation, activation, and rejection [32]. This increased immunogenicity promotes greater cellular infiltration and rejection rates [32,33].

An on-table postperfusion or early postoperative biopsy of an oliguric kidney often confirms acute tubular necrosis and excludes unsuspected rejection (in rare cases, humoral rejection with DSA only displays acute tubular necrosis and C4d). Commonly, minor non-specific tubular changes from ischemia by light microscopy are contrasted to profound intracellular transcriptome abnormalities. DGF increases clinical rejection (pooled incidence 49% vs. 35%, RR 1.38; 95% CI 1.29–1.47), requiring careful monitoring and immunosuppression (CNI doses are sometimes reduced to promote graft function) [25]. Regular protocol biopsy surveillance of non-functioning kidneys (every 5 to 7 days) screen for undiagnosed

rejection during supportive dialysis (a period ranging from days to weeks), when interval serum creatinine monitoring is unreliable. Functional renal transplant recovery is heralded by an increasing urine output (1–1.5 L/day needed for dialysis suspension), falling predialysis serum creatinine that subsequently plateau, and then progressively decline off dialysis. Up to 3% of implanted kidneys never function [6].

Arterial or venous vascular compromise

Surgical vascular compromise (with patchy perfusion from a suboptimal arterial anastomosis or kinked renal vessels) or overt thrombosis (clinically apparent immediately or soon afterwards with complete anuria) are uncommon differential diagnosis, and are evaluated by urgent Doppler ultrasound. Clinical evaluation of fluid status, blood pressure, and cardiovascular output are also important. It is important to note that urine output is unreliable as a sign of vascular compromise in recipients with residual native kidney urine output, and in these cases a high index of suspicion prompting early urgent ultrasound or re-exploration is required to detect vascular compromise in a clinically meaningful time frame.

Transplant ureteric obstruction

Ureteric obstruction is diagnosed with transplant hydronephrosis by ultrasound imaging. Use of a ureteral stent reduces the potential for as an early complication.

Early graft dysfunction

Clinical scenario. Initial graft function is followed by dysfunction and a rising creatinine within the early transplant period.

Early transplant functional monitoring

Assessment of transplant function is the primary monitoring tool to screen for graft pathology. Routine serum creatinine monitoring begins immediately after transplantation (daily to screen for ischemic DGF and early rejection), reducing to monthly intervals by 1 year in stable patients [34]. The constant daily creatinine generation rate means that serum level is sensitive to relative changes in individual allograft function. Serum creatinine is cheap, convenient, and simple to measure, with a rapid laboratory turn-around time, excellent measurement precision (intralaboratory variation below 3%), and accuracy (about 10–20 μmol/L or 0.10–0.20 mg/dL). For detection of acute rejection, serum creatinine yields adequate sensitivity ("subclinical" rejection is missed), modest biological variation (intrapatient CV is 7%), but is relatively non-specific, because physiological factors such as dehydration and blood pressure also affect glomerular filtration rate (GFR).

Serum creatinine generally falls within the first 2 weeks post-transplant to a baseline level determined by early ischemic injury, donor quality and donor–recipient size mismatch, calcineurin inhibitor levels, and the recipient's muscle creatine generation. Early acute increases in creatinine (e.g. 25% above baseline), or even a failure to decrease, should initially prompt repeat testing with adequate hydration, and exclusion of reversible causes (by Doppler ultrasound for vascular supply and hydronephrosis). A diagnostic transplant biopsy may show rejection, acute tubular necrosis, or be near-normal (acute CNI nephrotoxicity is then inferred, especially if isometric tubular vacuolization and high blood CNI concentrations are present) (Table 69.1).

Table 69.1. Clinical investigation of transplant dysfunction

1. Clinical examination of blood pressure, hydration status, palpation of kidney transplant for tenderness, and auscultation for bruit
2. Check medications for compliance, nephrotoxins, and drugs that alter calcineurin inhibitor concentrations
3. Repeat serum creatinine with adequate hydration and elimination of any reversible factors
4. Check blood calcineurin inhibitor levels for toxicity (higher levels suggest nephrotoxicity versus low or variable levels indicate inadequate dosing or non-compliance with risk of rejection)
5. Renal transplant Doppler ultrasound to evaluate arterial blood flow and exclude ureteric obstruction; ^{99m}Tc mercaptoacetyl-triglycine (MAG3) scan when ultrasound is inconclusive
6. BK evaluation by blood nucleic acid testing, with quantification if positive
7. Urinalysis for proteinuria and hematuria (suggestive of glomerulonephritis or transplant glomerulopathy)
8. Transplant biopsy for specific diagnosis
9. Donor-specific antibodies (if antibody-mediated rejection suspected)

Early acute antibody-mediated rejection

Antibody-mediated rejection (AMR) is mediated by pathogenic antibodies directed against specific donor molecules. Although most DSA are HLA-specific, some are non-HLA directed, which encompasses antibody against ABO blood group antigens and other polymorphic antigens. Antibodies against the polymorphic MICA (MHC class I-related chain A expressed on endothelial cells) [35,36], the AT_1 receptor, or antiendothelial cell antibodies have been rarely implicated in hyperacute, acute, and chronic organ allograft rejection. IgG antibodies targeting the vascular AT_1 receptor have been reported to present with refractory vascular rejection (histologically as endarteritis and/or fibrinoid necrosis) and malignant hypertension, and to respond to adjunctive angiotensin receptor blocker (ARB) therapy (e.g. losartan) [37].

DSA produced by pre-existing plasma cells, or memory B cells converted to new plasma cells, mediates AMR. This anamnestic or "recall" antibody response from previous antigen exposure can rapidly generate high-titer complement-fixing HLA antibody [15], targeted to MHC antigens expressed on donor endothelium (Figure 69.1). Injured endothelial cells release von Willebrand factor and P-selectin (promoting platelet aggregation), inflammatory molecules (such as IL-1α, IL-8, and CCL-2), and chemoattractants (C3a and C5a), causing leukocyte adherence within glomeruli ("glomerulitis") [38] or dilated peritubular capillaries (colloquially called "PTCitis") [13]. Assembly of C5b-9 membrane attack complex leads to localized endothelial necrosis and apoptosis, detachment of cells from their basement membrane, and deposition of capillary fibrin microthrombi. Arterial wall necrosis with hemorrhage or local intravascular thrombosis with distal infarction occurs in severe cases [13].

Acute AMR presents early with inflammatory graft dysfunction (within days after transplantation, but delayed by antilymphocyte induction), acute oliguric graft failure, and sometimes with a low-grade fever and allograft tenderness. Doppler imaging demonstrates reduced diastolic blood flow, allograft swelling, and high resistive indices. Urgent histology shows inflammatory microvascular injury accompanied with polymorphonuclear or mononuclear cells adhering to endothelium of peritubular and glomerular capillaries [38], with C4d deposition and tubular injury [14].

The pathological triad for the diagnosis of acute AMR [9,13] with allograft dysfunction is:

1. Morphological evidence of acute tissue injury (glomerular or peritubular capillary leukocyte endothelialitis, arterial inflammation or acute tubular injury). Leukocyte margination of cortical

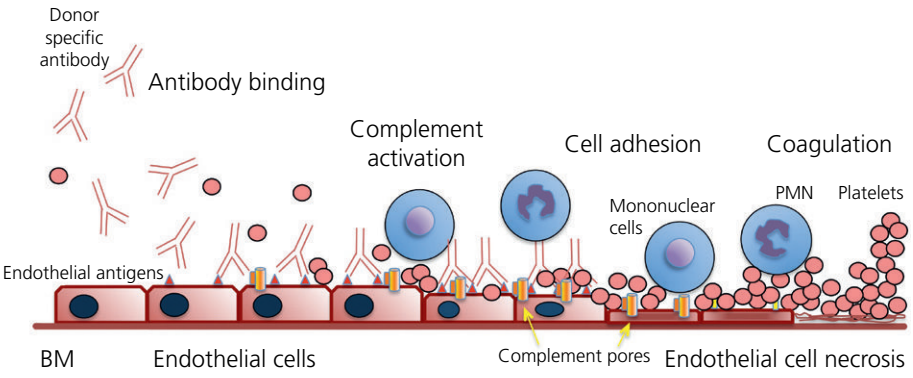

Figure 69.1. Pathophysiology of acute antibody-mediated rejection. Donor-specific antibody binds to antigen expressed on endothelial cells with complement activation, cell adhesion, and endothelial damage followed by platelets deposition and coagulation. BM, basement membrane; PMN, polymorphonuclear leukocyte.

peritubular and glomerular capillaries (outlined by PAS stain) is the most important diagnostic feature, allowing initiation of antirejection treatment with pulse steroids.

2 Immunopathological evidence of antibody activity (C4d staining, classically defined as "intense and diffuse and involving >50% of PTC" but lesser amounts >10% are clinically relevant) [39].

3 Circulating DSA directed towards Classes I and/or II HLA antigens or (rarely) other defined polymorphic specificities.

C4d, a diagnostic marker of classic complement activation, is frequently present in peritubular capillaries but variably insensitive [40], C4d can be occasionally absent in very early biopsies with humoral rejection manifest only by endothelialitis (later becoming clearly positive) [13]. C4d forms a stable and long-lasting covalent bond to endothelium, remaining positive for days to weeks after successful AHR treatment (residual empty dilated PTC may be seen). A new subcategory of "C4d deposition without morphological evidence of active rejection" has been added to the Banff system. Discrepancies in C4d sensitivity (ranging from 23 to 95%) against of a marker of acute AHR (such as DSA), reflect different techniques (less sensitive immunohistochemistry when using paraffin sections but allowing glomerular C4d detection and clear structural visualization versus more sensitive indirect immunofluorescence using impermanent frozen samples), antibody used (monoclonal or polyclonal antibodies), differing cut-off threshold levels (10% versus 50% of peritubular capillaries), and biopsy timing [41]. The focal C4d category (10–50% of PTC with strong linear staining) is associated with capillaritis, glomerulitis, and an intermediate prognosis is abnormal [39,42]. A positive C4d yields an excellent specificity (93–96%) for humoral rejection, especially if supported by DSA, endothelial inflammation, and appropriate clinical context (e.g. regraft, highly sensitized recipient, known pre-transplant DSA) [40,43–46].

Early diagnosis and active therapy are essential to salvage these kidneys. Treatments include pulse corticosteroids, removal of circulating antibody by plasmapheresis (which also removes clotting factors, therefore the first postbiopsy exchange is against fresh frozen plasma to avoid bleeding, and later albumin), low-dose intravenous immunoglobulin (e.g. 100 mg/kg postexchange to a cumulative total of 1–2 g/kg), and antiproliferative agents (i.e. mycophenolate) to suppress B- and T-cell expansion, and limit recovery of antibody synthesis. Monitoring is by renal function, serial DSA levels, and end-treatment repeat biopsy.

Supplementary therapies include rituximab, a chimeric monoclonal antibody against CD20-bearing B lymphocytes, which rapidly depletes B cells in a way that is sustained for up to 1 year (following a single 375 mg/m^2 i.v. dose). Rituximab operates by antibody-dependant cell-mediated cytotoxicity, complement-mediated cytotoxicity, and apoptosis of B cells (but not plasma cells). It is generally well tolerated with occasional acute infusion reactions, and marginal risk of bacterial infections and latent viral reactivation (hypogammaglobulinemia is mild) [47]. In a randomized study of pediatric acute rejection with B-cell infiltrates, rituximab improved graft function, subsequent protocol biopsy rejection scores, and abolished C4d reappearance, independent of unchanged DSA levels [48]. Its role as adjunctive therapy for acute AHR remains unclear because of confounding treatments in observational studies. Antilymphocyte antibody (with some anti-CD20 specificity) can also be used with co-existent T-cell rejection ("mixed" rejection) [11].

Other investigational agents include eculizumab (a humanized C5 cleavage inhibitor of terminal complement activation), which quickly prevents complement pore formation and shows promise in AMR [49,50]. Bortezomib (a proteasome inhibitor directed against plasma cells) targets the source of production and slowly reduces DSA levels (the IgG half life is 7 to 21 days, according to subclass). When given as three to five infusions of 1.3 mg/m^2 (one myeloma cycle dose) with plasmapheresis as rescue therapy, bortezomib has been associated with successfully reduced DSA and reversal of AMR. Side-effects include peripheral neuropathy (about 30%), myelosuppression, and herpes reactivation [50]. No randomized data regarding the use of bortezomib or eculizumab exist.

Previous HLA mismatched transplantation is a powerful sensitizer, commonly generating higher-titer class II HLA antibodies in patients listed for re-transplant [51]. One-third will have antibodies of sufficient titer to reduce transplant offers and increase dialysis waiting times [52]. Transplantation of highly presensitized candidates with DSA is frequently complicated by acute and/or chronic antibody-mediated rejection, with reduced graft function and survival. Many units avoid such donor–recipient pairs, and seek an alternative donor. However, transplantation with prior antibody removal and augmented immunosuppression can succeed, albeit at a cost of greater infection rates and potential later chronic antibody mediated rejection (CAMR) [53]. Living donor exchange programs circumvent the problem of living kidney donors excluded by recipient ABO incompatibility or donor-specific anti-HLA antibodies

[54]. Comprehensive approaches to the sensitized individual are presented in Chapter 68.

Early acute T-cell mediated rejection

Acute cellular rejection is mediated primarily by T lymphocytes invading the interstitial space and renal tubular epithelium; it is clinically signaled by increased serum creatinine levels and confirmed by biopsy [5]. Naïve recipient T cells, activated by direct presentation of donor antigen within lymphoid organs by dendritic cells, differentiate and clonally expand into T helper subsets and return as destructive effector lymphocytes [55]. Circulating lymphocytes tether to endothelium, transmigrate across peritubular capillaries [33], and accumulate in the interstitial compartment in acute cellular rejection, producing inflammatory cytokines and chemokines (such as TNF-α, TNF-β, CCL5/RANTES, CCL3/MIP-1α, and IFN-γ) and inducing IFN-γ genes within the graft (Figure 69.2) [56,57]. CD4 and CD8 T cells may be accompanied by B cells (detected by gene expression signatures and CD20 histochemistry) [58] or eosinophils; both B cells and eosinophils are associated with severe steroid-resistant cellular rejection. Activated macrophages secrete proinflammatory cytokines (such as IL-1, IL-12, and IL-18, TNF-α, and IFN-γ), and are associated with functional impairment and residual T-cell rejection [59].

The invading allospecific CD8 T lymphocytes enter renal tubules ("tubulitis") attracted by secreted chemokines (CCL2, CCL5, and CX3CL1) [60], mediating local tubular injury by direct contact (cell-mediated cytotoxicity), locally released cytokines, or indirect inflammatory cell activation. CD8 T cells release perforin (allowing entry of granzymes A and B through membranes pores), and interact with Fas death receptor by Fas ligand—inducing caspase-mediated apoptosis (Figure 69.2) [61]. Effector CD4 T cells can also mediate cytotoxicity to minor antigens, via TNF [62]. Injured tubular cells can transition into more primitive phenotype of mesenchymal myofibroblasts, or progress to epithelial cell necrosis with basement membrane rupture with urinary leakage, graft dysfunction, and tubular atrophy with severe injury [63].

Clinical immune risk factors for acute rejection include HLA mismatches, living unrelated donor status, pre-existing alloantibodies, particularly when donor specific, regraft, and donor ischemia (e.g. particularly when prolonged or associated with extended criteria donor status), which increases transplant immunogenicity via respondent expression of injury molecules and their recognition by the innate immune system [64]. Tacrolimus (vs. cyclosporine), mycophenolate (vs. azathioprine), and induction therapy have all reduced acute rejection rates in randomized controlled trials. One-year rejection rates have fallen to 10–15%, although remaining episodes are more severe and treatment-resistant than in prior eras. Better HLA-matching and DSA detection technologies (reducing rejection risk) and effective CMV prophylaxis and anti-infective treatments (allowing safe immunosuppressive dose escalations) have also helped. About 4% of kidneys are lost from early rejection [64].

The key diagnostic components of acute T-cell mediated rejection pathology are mononuclear leukocyte cell inflammation of the interstitial space and renal tubules, where their relative proportions and extent determine the grade of severity. The Banff acute T-cell-mediated rejection (TCMR) [9] grades are as follows (also see Chapter 81):

Borderline "suspicious" for rejection. Foci of tubulitis (t1, t2, or t3) are present with minor (i0, or i1 interstitial infiltration score as 10–25% cortex), or moderate interstitial infiltration (i2, i3) with mild (t1) tubulitis (without any intimal arteritis).

Grade IA rejection. Significant interstitial infiltration (>25% of parenchyma affected, i2 or i3 score) and moderate tubulitis (t2).

Grade IB rejection. Significant interstitial infiltration (>25% of parenchyma) and severe tubulitis (t3).

The rejection severity by the Banff schema critically hinges on the tubulitis score, which determines final rejection grade and

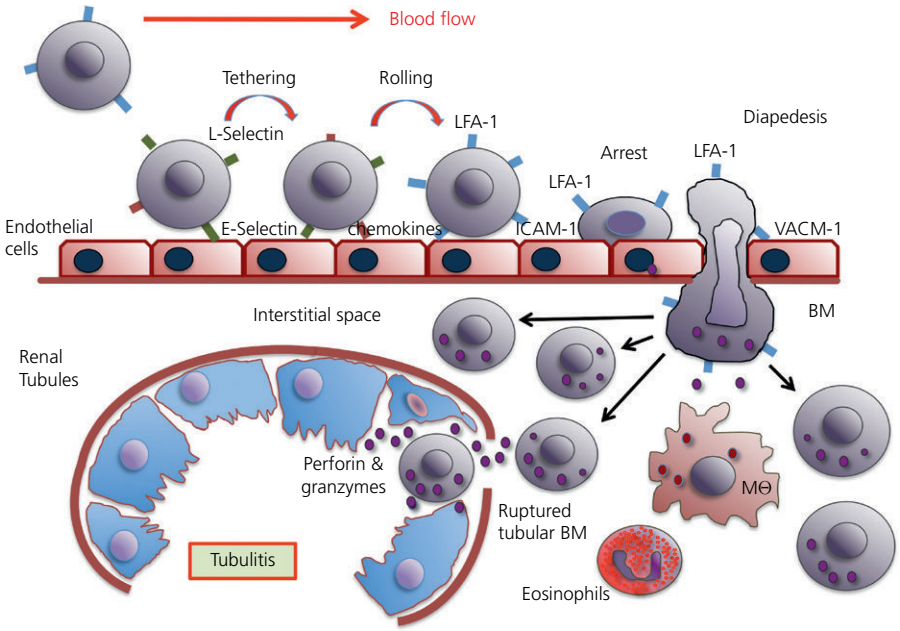

Figure 69.2. Mechanism of T-cell-mediated rejection with trafficking of cells into the kidney transplant. Following initial tethering, rolling, and arrest of effector lymphocytes binding selectins and integrins expressed on endothelial cells, lymphocytes and other immune cells enter the interstitial compartment and invade tubules, mediating local tissue destruction. BM, basement membrane; MO, macrophage.

defaults to the most severe lesion. Localized severe tubulitis can (inappropriately) advance grading if present in a single isolated tubule. Tubulitis can be difficult to appreciate within areas of inflammatory tubular destruction without PAS staining. The extent of interstitial inflammation (Banff i score) is clinically useful for diagnosis and predicts subsequent tubulointerstitial damage if persistent [65]. The older CCTT system used at least 5% cortex lymphocytic inflammation and two injury markers (edema, tubular degeneration/injury or reactive lymphoblasts), with tubulitis (minimum of three tubules affected within the maximal infiltrate) to define rejection. Any patient with an increasing creatinine and an interstitial lymphocytic inflammation exceeding 10% of the cortical area with tubulitis should be clinically treated as rejection until proven otherwise.

Biopsy confirms the diagnosis of rejection, but also categorizes its histological type and severity, which critically determines therapy. Initial treatment for simple cellular rejection is pulse corticosteroids (e.g. 500 mg i.v. methylprednisolone for 3 days) or short-term oral high-dose therapy (e.g. 100 mg daily then tapered), with the serum creatinine expected return to baseline. Successfully treated acute cellular rejection leaves neither histological consequences nor excess graft loss by registry studies. However, partial resolution of renal dysfunction may follow recurrent or serious episodes of acute rejection [64]. Severe, relapsing, multiple or steroid-resistant cellular rejection should be managed by re-biopsy with C4d staining, DSA testing, compliance checks, and strengthened baseline immunosuppression, with consideration for antithymocyte treatment.

Vascular rejection

Vascular rejection (termed "arteritis" or "endarteritis") is a severe, steroid-unresponsive type of rejection characterized by vascular mononuclear infiltration, ingress of intimal myofibroblasts secreting matrix proteins and collagens, and endothelial cell apoptosis. CD4 and CD8 T cells and macrophages invade the subendothelium and intima of small muscular arteries, helped by adhesion molecules (ICAM-1 or VCAM) expressed on activated endothelium, and chemokine (CCL4, CCL5, and CXCL8) gradients [66]. Anti-MHC antibody, T cell–mediated immunity to minor antigens, NK cells pathways and IFN-γ contribute to pathophysiology in experimental studies.

Vascular rejection was originally classified within the Banff T-cell mediated rejection umbrella, and rejection grades are as follows (also see Chapter 81):

Grade IIA rejection. Mild to moderate intimal arteritis (v1 arteritis score).

Grade IIB rejection. Severe intimal arteritis comprising (>25% of luminal area narrowed, v2).

Grade III rejection. Transmural arteritis and possible fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation of the artery (v3).

Vascular rejection clinically presents with progressive graft dysfunction, which fails or partially responds to pulse corticosteroids, with a characteristic arteritis on biopsy. Vascular rejection causes irreversible tubular loss with chronic interstitial fibrosis, frequent incomplete functional recovery, and a greater risk of subsequent graft loss (HR 2.07; 95% CI 1.60–2.68) [64]. Occasionally presenting as isolated arteritis, vascular rejection is generally accompanied by interstitial cellular infiltration, and potent immunosuppression such as antithymocyte globulin with pulse corticosteroids is recommended. Arteritis is often mediated by humoral mechanisms, with poor allograft outcomes, associated with severe arteritis, high DSA levels and associated interstitial inflammation [67]. Robust treatment against pathogenic DSA including PE, IVIG and rituximab, are warrented.

Subclinical rejection

Subclinical cellular rejection

Subclinical rejection (SCR) occurs when acute T-cell mediated rejection (acute or borderline) is present on protocol biopsy-derived histology without concurrent functional deterioration [68]. Allografts with SCR develop more damage on subsequent histology with reduced graft survival, and some will progress to chronic rejection. A causal role for SCR contributing to tubulointerstitial damage is supported by co-localization with prior interstitial inflammation, temporal sequence of SCR before damage, SCR intensity correlating with severity of chronic damage ("dose–response"), biological plausibility, and confirmation in several transplant populations.

Treatment of SCR by pulse corticosteroids decreased acute rejection episodes, chronic 6-month tubulointerstitial scores, and serum creatinine at 2 years, in a randomized trial using cyclosporine-based immunosuppression (background prevalence 30%), with a trend to better survival [69]. Another trial (cyclosporine and some tacrolimus with 28% SCR prevalence) of SCR treatment showed better eGFR at 6 and 12 months [70], but no benefit occurred in a third trial using tacrolimus in low-immune-risk patients where SCR prevalence was only 4.7% [71].

Subclinical antibody-mediated rejection

Subclinical antibody-mediated rejection (SAMR) can be present in protocol biopsies showing a C4d-positive capillaritis associated with DSA. Peritubular leukocyte margination is a sensitive and useful marker of DSA, but is not specific being present in T-cell mediated rejection. Relatively common in "positive cross-match" or sensitized recipients, or following treatment of late antibody-associated rejection, early SAMR is also reported in 10% of standard-risk individuals [7,72,73]. Persistent sublethal microcirculation injury from circulating DSA can activate glomerular endothelial cells and widen the subendothelial space with glomerular basement membrane (GBM) reduplication, which evolves into chronic transplant glomerulopathy, or be associated with PTC basement membrane multilamination, tubulointerstitial damage, renal impairment, proteinuria, and reduced graft survival [72,74–77].

Diagnostic kidney transplant biopsy

Diagnostic needle core biopsy should be considered after exclusion of reversible causes of transplant dysfunction (Figure 69.3). Histology remains the key investigation for the detection of rejection, although transcriptome microarray data for disease categorization and prognostication may supplement histology in the future [58,78]. Most biopsies are indication-driven ("for cause"), but "protocol" biopsies can be undertaken at prespecified times in patients with stable function for surveillance of rejection in several high-risk clinical situations, including delayed graft function, high DSA risk, or ABO incompatibility, or for other situations requiring surveillance of subclinical rejection, particularly clinical trial participation [68].

Biopsies are performed under local anesthetic using an automated spring-loaded needle core deployment device. The risk of major complications (e.g. macroscopic hematuria with ureteric obstruction) is 1%, and graft loss is 0.03% [68]. Minor complications (resolving without intervention) include gross hematuria

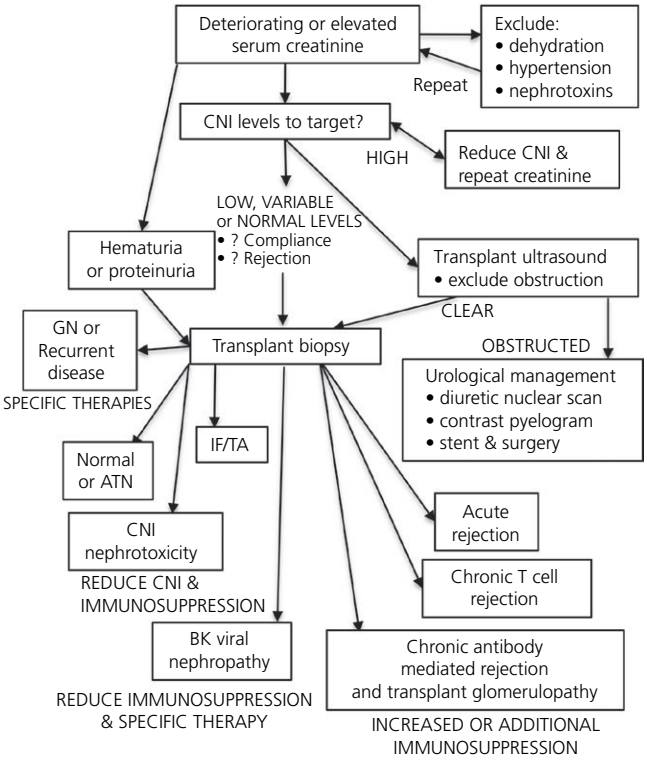

Figure 69.3. Diagnostic evaluation of transplant dysfunction. CNI, calcineurin inhibitor; IF/TA, interstitial fibrosis/tubular atrophy; GN, glomerulonephritis; ATN, acute tubular necrosis.

in 3.5%, perirenal hematomas 2.5%, asymptomatic arteriovenous fistulas 7.3%, and vasovagal reactions 0.5% [10]. Risks are greater with indication (compared with protocol) biopsy, extraperitoneal or transperitoneal adult kidneys in infants, or with larger needles (16 g or greater) [11]. Safety should be maximized by a skilled operator using ultrasound guidance [10]. Particular care is required in patients with uncontrolled hypertension, those on anticoagulation therapy, and those with platelet dysfunction caused by advanced uremia.

While pathology offers diagnostic confirmation of rejection (often clinically obvious), an important additional role is histological categorization and grading of severity, which determines second-line therapy use and graft outcome. Rejection can be patchy, so a minimum of two biopsy cores with two muscular arteries is needed. Assessment of peritubular and glomerular capillaries, small arteries, and C4d staining are important to exclude antibody or vascular rejection subtypes.

Differential diagnosis of early graft dysfunction

Ischemia reperfusion injury with delayed graft function

Ischemia–reperfusion injury can cause early graft dysfunction, with risk factors including donor (extended cold and warm ischemic times, brain death with inflammatory signaling) and recipient (reperfusion injury, cardiovascular compromise) factors. Acute tubular necrosis is confirmed and acute rejection excluded by diagnostic biopsy.

Acute allograft thrombosis

This early technical complication occurs from transplant renal artery injury (usually at the anastomosis), or renal vein obstruction from kinking or external compression, and contributes to 2–7% of early adult allograft losses, and 12–35% of losses in children and infants. It usually occurs within the first week, with a peak incidence at day 2 [79]. Contributing risk factors are the "Virchow's triad" of: (1) vessel wall factors (small, female, deceased donors, vessel wall atheroma, pediatric or diabetic recipient, multiple or reconstructed vessels, small accessory arteries, or vascular clamp or perfusion cannula injury); (2) transplant vascular blood flow (hypotension, cardiac failure, under-filling); and (3) blood coagulation disorders (pre-existing prothrombotic states, factor V Leiden mutation, lupus anticoagulant, antiphospholipid syndrome, high hematocrit). Clinical clues are abrupt catheter-proven *anuria* (when there is no native kidney function, most DGF kidneys are oliguric) and acute graft tenderness with a consumptive thrombocytopenia, confirmed by immediate Doppler ultrasound and/or urgent surgical or radiological intervention for thrombectomy or thrombolysis. Salvaged kidneys experience severe acute tubular necrosis from prolonged warm ischemic injury, poor residual graft function and develops tubular atrophy with interstitial fibrosis.

Ureteric obstruction

The transplant ultrasound typically shows hydronephrosis. However, partial obstruction such as that seen in cases on modest lymphocele-related compression can be less obvious.

Urinary leak

The transplant pelvicalyceal system or ureter can leak (1) externally (diagnosed by elevated creatinine surgical drain fluid concentrations compared with serum) or (2) internally with reabsorption across the peritoneal membrane (suspected by unexplained serum creatinine elevation despite normal graft histology), and diagnosed by nuclear renal transplant scan with delayed abdominal images.

Acute interstitial nephritis

Rarely, routine trimethoprim–sulfamethoxazole prophylaxis causes an acute interstitial nephritis, presenting with subacute graft dysfunction, eosinophilia, and eosinophilic tubulointerstitial nephritis on biopsy. It is easily treated by pulse steroids and co-trimoxazole cessation. Viral acute interstitial nephritis (e.g. from BK, CMV or adenovirus pyelonephritis) differs by graft pathology.

Early recurrent renal diseases

Early acute renal failure can rarely be due to recurrent hemolytic–uremic syndrome (HUS) or primary hyperoxaluria. Recurrent focal segmental glomerulosclerosis (FSGS) initially presents with isolated proteinuria, with progressive graft dysfunction developing much later (see below).

Hemolytic–uremic syndrome

Classical HUS comprises the diagnostic triad of microangiopathic hemolytic anemia, thrombocytopenia, and renal impairment, causing renal failure associated with diarrhea (usually enterotoxic *Escherichia coli* 0157:H7) generating Shiga toxin promoting endothelial injury. Atypical HUS from genetic dysregulation of the alternative complement pathway (occasionally from autoantibodies) causes complement over-activity and chronic renal failure. It is often undiagnosed pretransplant, but can present abruptly with post-transplant HUS and graft dysfunction, especially when combined CFH and CFI (versus lower risk MCP) mutations are present [80]. Treatment is plasma exchange against fresh frozen plasma, eculizumab (an anti-C5 inhibitor developed for paroxysmal

nocturnal hemoglobinuria but effective for both atypical and Shiga-toxin HUS), and CNI avoidance. Combined liver–kidney transplantation corrects the genetic complement pathway defect but with greater patient risks [80].

Primary hyperoxaluria

Primary hyperoxaluria (type 1 is most common) is an autosomal recessive defect of hepatic alanine-glyoxylate aminotransferase, causing systemic overproduction and urinary excretion of oxalate (its metabolic end-product), insoluble calcium oxalate renal accumulation, nephrolithiasis, and progressive renal failure (of "unknown cause"). It manifests as subacute transplant renal failure as circulating systemic oxalate deposit in renal tubules, seen as striking birefringent calcium oxalate crystals by polarized microscopy. Treatment involves hydration, and sodium citrate and/or phosphate administration, hemodiafiltration and pyridoxine in sensitive cases to minimize systemic oxalosis and limit further deposition, but often with disappointing results [81]. High-dose vitamin C causes secondary oxalosis in dialysis patients and should be avoided.

Recurrent focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis is a podocyte (glomerular visceral epithelial cells) disease caused either by: (1) genetic mutations in critical podocyte proteins (such as podocin, identified by DNA analysis) presenting as familial or sporadic nephrotic syndrome; or (2) from a putative 30–50 kDa circulating protein(s) ("FSGS factor or "toxic permeability factors"), which injures the podocyte, increasing glomerular permeability. Idiopathic focal segmental glomerulosclerosis recurs in 20–50%, with half failing by 5 years [82]. Risk factors for recurrence include childhood onset (age <15 years), diffuse mesangial hypercellularity, or collapsing glomeruli, and rapid progression to dialysis in native kidney disease, or fulminant recurrence in previous allografts [83].

Recurrence is suspected by increasing weekly spot urine protein/creatinine ratios (residual native kidney proteinuria gradually abates after transplantation), and is diagnostically confirmed by podocyte foot process effacement on electron microscopy, which occurs well before segmental glomerulosclerosis appears on light microscopy.

A regimen of plasma exchange to remove circulating protein (initially three sessions/week reducing to monthly by 6 months), high-dose cyclosporine (initial 2-hour cyclosporine target 1200–1400 ng/mL, which stabilizes the podocyte actin cytoskeleton), corticosteroids (starting at 1 mg/kg/day and tapering to 10 mg/day), and ACE inhibitors (or ARB) as antiproteinuric agents, can achieve complete or partial remission in 80 to 90%, with occasional late relapses [84]. mTOR inhibitors (sirolimus and everolimus), which affect the podocyte and cause proteinuria, should be avoided.

De novo antiglomerular basement membrane disease in Alport's recipients

Rarely, patients with Alport's disease (from *COL4A5* gene mutations of GBM collagen in X-linked male form) develop anti-GBM antibody disease to the "novel" alpha5 chains (or to alpha3 in recessive disease) of the kidney transplant, appearing "foreign" to the recipient's immune system [85]. Presenting early (few weeks or months), with acute dysfunction, necrotizing and crescentic glomerulonephritis, and linear IgG immunofluorescence resembling Goodpastures's disease can be seen on biopsy. The treatment involves plasma exchange and cyclophosphamide.

Chronic slow graft deterioration

Clinical scenario. Gradual deterioration with slow but inexorably increasing serum creatinine over months to years.

Long-term transplant monitoring and progressive graft dysfunction

Interval serum creatinine is main monitoring tool of transplant health, as GFR correlates with tubulointerstitial injury [86]. Relative changes in serum creatinine level are meaningful, allowing for biological variation, and progressive transplant dysfunction can be the harbinger of graft failure.

Estimated GFR (eGFR) from predictive equations (e.g. "abbreviated" MDRD, Nankivell or CKD-EPI formulae) are more accurate than unmodified serum creatinine measurements; however, they perform indifferently against reference methods because of differences of muscle mass, creatinine generation, tubular creatinine secretion, and assay calibration factors [87,88]. Trimethoprim, routinely used with sulfamethoxazole for prophylaxis against urinary tract and pneumocystis infection, inhibits tubular creatinine secretion and results in a harmless elevation of serum creatinine. Serum cystatin reflects GFR independently of muscle mass, but is more expensive, requiring an optimized formula, and is not clearly superior to creatinine-based eGFR. Plasma clearance of ("cold") iohexol or isotopic-labeled Tc^{99m}DTPA, Cr^{51}EDTA or I^{125}iothalamate are accurate reference methods (replacing inulin clearance), but is used sparingly because of cost and patient inconvenience.

Serum creatinine appreciably rises only after substantial transplant damage has occurred because of its log-linear relationship with GFR, and hyperfiltration from residual nephrons, moderating the increase. The plot of transplant GFR against time is the combination of baseline level ("intercept" from donor quality and early events) and rate of loss ("slope" from continuing injury) (Figure 69.4). Declining reciprocal creatinine (30% reduction RR = 2.56) or increasing creatinine (OR = 2.2/1 mg/dL = 88.4 μmol/L) both

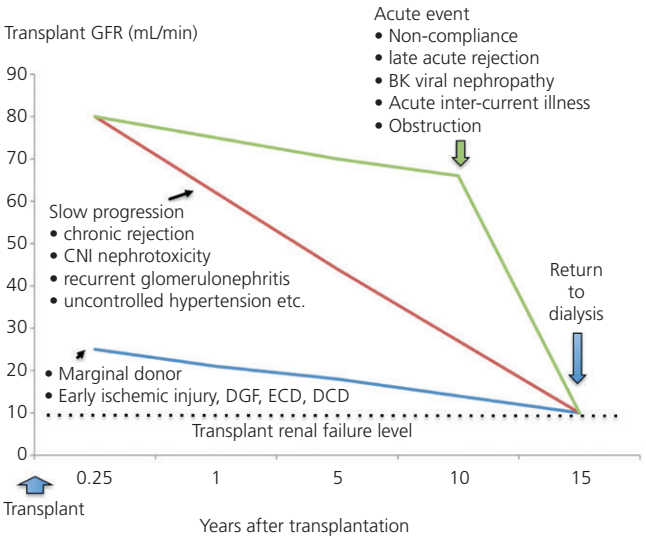

Figure 69.4. Transplant glomerular filtration rate and time to graft failure according to differing pathophysiological events. CNI, calcineurin inhibitor; GFR, glomerular filtration rate; DGF, delayed graft function; ECD, expanded criteria donor; DCD, donation after circulatory death.

correlate with time to graft failure [13]; however, they poorly predict late graft failure when prospectively tested [89,90]. Many individual GFR time curves are non-linear, with a breakpoint and abrupt decline when new pathologies develop (e.g. glomerulonephritis, acute rejection, or BK nephropathy). Alternatively, a transplant with early severe ischemic damage can remain functionally stable for years. Hence, while graft function statistically correlates with graft loss in cohorts, its individual predictive capacity is weak.

Urine testing and transplant imaging

Dipstick urinalysis can be used to screen for transplant glomerular diseases. Proteinuria quantified by spot urine protein/creatinine ratio or 24-hour urinary excretion can also be used. Proteinuria is a powerful independent risk factor for graft loss and patient survival, even at modest levels of 0.5 g/day [91]. Hematuria and proteinuria suggests recurrent or de novo glomerulonephritis.

Initial ultrasonography excludes ureteric obstruction or vascular arterial stenosis as causes of graft dysfunction. Acute and complete ureteric obstruction is rare, presenting with transplant dysfunction, oligoanuria, and hydronephrosis. The obstruction is confirmed and localized by antegrade or retrograde pyelography, decompressed by ureteric stenting, and definitively corrected surgically. Chronic or partial ureteric obstruction is a diagnostic challenge because mild transplant hydronephrosis is common. Diuretic isotopic renography using ^{99m}Tc mercaptoacetyl-triglycine (MAG3), secreted by tubules despite poor graft function, has excellent sensitivity and specificity [92]. Whether untreated chronic transplant vesicoureteric reflux produces irreversible parenchymal scarring is unproven [93–95].

The Doppler "resistive index" correlates with intrarenal compliance, interstitial fibrosis, and subsequent graft loss ($0.80\geq$ values ninefold risk), and recipient mortality (reflecting recipient's noncompliant systemic vasculature) [96,97]. Renal vascular resistance, magnetic resonance, or gadolinium-enhanced dynamic turbo-FLASH are relatively insensitive imaging methods, becoming abnormal only with significant parenchymal damage [98].

Diagnostic algorithm for a chronically failing graft

A gradually deteriorating or persistently elevated serum creatinine should be evaluated. Reversible causes such as dehydration (fluid state examination, diarrhea, diuretic use), acute calcineurin inhibitor nephrotoxicity (by drug levels), nephrotoxins (ACE inhibitors, and angiotensin receptor II blockers, NSAID, or COXII inhibitors) that reduce GFR, early recurrent glomerular disease (by urinalysis), and ureteric obstruction or vascular impairment (by imaging), should be excluded (Table 69.1).

Transplant biopsy should be obtained without undue delay after exclusion of reversible causes (Figure 69.3), as severely damaged grafts lose their diagnostic specificity and poorly respond to therapy. An adequate tissue sample can be processed for immunofluorescence (for glomerulonephritis), light and electron microscopy, and C4d staining. Careful histological and clinical evaluation should establish a cause(s) of transplant dysfunction. A collaborative diagnosis involving clinician (evaluating immune risk, antibody status, previous rejections, current treatment and compliance, donor quality, and renal function) and pathologist (see Chapter 81), optimally interprets findings. A specific etiologic diagnosis is required for rational treatment directed towards underlying pathophysiological cause(s) of transplant dysfunction (Tables 69.2 and 69.3) [86,99].

Chronic cellular interstitial rejection

Chronic active T-cell-mediated rejection involves persistent immune transplant injury from failure of maintenance immunosuppression to control residual alloimmune activity. Progressive renal functional decline is associated with interstitial T-lymphocyte infiltration, often accompanied by B cells and macrophages, and tubulitis. Occasionally, fibrointimal hyperplasia of small muscular arteries (from smooth muscle proliferation and hyperplasia forming a neointima with focal internal elastic lamina destruction) is seen, which can progresses to vascular occlusion [10].

Treatment options involve strengthened immunosuppression, such as conversion from cyclosporine to tacrolimus (initial target levels approaching 10 ng/mL) [100], azathioprine to mycophenolate mofetil [101], and addition or augmentation of corticosteroids. Compliance checks, review of appropriate target blood drug levels, and exclusion of interfering agents (e.g. St John's wort or phenytoin inducing metabolizing enzymes and reducing CNI levels) are recommended.

Chronic antibody-mediated rejection

Chronic antibody-mediated rejection (CAMR) occurs from unrecognized pre-existing low titer DSA (missed by transplant CDC cross-match) or de novo antibodies developing after transplantation [50,102]. Late anti-HLA antibodies correlate with chronic humoral rejection, transplant glomerulopathy, and allograft failure [45,102,103]. Late de novo DSA appearing years after transplantation correlates with early cellular or mixed rejection episodes (often with PTC margination) and HLA mismatch, non-adherence with medications, and indolently progressive transplant dysfunction [77].

CAMR clinically presents with progressive functional decline, hypertension, and worsening proteinuria. Chronic persistent DSA affects the transplant's microcirculation, especially glomerular and peritubular capillaries, producing characteristic activated phenotype with altered production of underlying basement membrane material. Glomerular endothelial cells become hypertrophied and activated by sublethal DSA interacting with capillary endothelium, widening the subendothelial space from fibrillary material, and/or GBM duplication. Peritubular capillary cells produce multilayering of basement membranes (variably defined threshold as five layers for diagnosis, but normal is actually only one layer) [9]. Glomerular capillaries become thickened and, along with mesangial cell interposition, create "double contours" recognized by methenamine silver staining, the hallmarks of transplant glomerulopathy. Differential diagnoses are thrombotic microangiopathy (excluded by blood film, haptoglobin, and lactate dehydrogenase levels) and hepatitis C mesangiocapillary glomerulonephritis (by serology, blood viral nucleic acid testing, and GBM deposits) [104]. Transplant glomerulopathy, the glomerular expression of CAMR, is reported in 5 to 15% of failing and failed grafts [6,45,50,105] and up to 20% of protocol biopsies, where many are subclinical [50,106].

The current diagnostic triad of CAMR includes: (1) circulating donor-specific antibody (often class II anti-HLA antibodies); (2) capillary C4d deposition, the "footprint" of classical complement activation by antibody in peritubular capillaries (but relatively insensitive and often negative); and (3) chronic transplant glomerulopathy, by light microscopy, in the context of organ dysfunction [9,13,45,73,107].

Electron microscopy demonstrating glomerular endothelial swelling, subendothelial widening and GBM duplication, and/or

multilamination of PTC basement membranes confirms the diagnosis. Peritubular leukocyte inflammation and glomerulitis support DSA-mediated microcirculation injury. Progressive fibrointimal arterial hyperplasia from CAMR is relatively rare. Commonly, CAMR is associated with progressive transplant dysfunction, persistent interstitial cellular rejection, and tubulointerstitial damage (interstitial fibrosis and tubular atrophy, IF/TA), indicative of an uncontrolled broad alloimmune response [77].

Evidence for treatment is limited to uncontrolled cohort studies, adapted from acute AMR, involving strengthened baseline immunosuppression, increased tacrolimus and mycophenolate dosages (suppressing B and T-cell expansion), and corticosteroids (re-added if steroid free), and ACEI or ARB to limit proteinuria [13,50]. Aggressive treatments such as plasmapheresis to remove circulating DSA with intravenous immunoglobulin, and rituximab produce variable results. The roles of bortezomib (a proteasome inhibitor of plasma cells) and eculizumab (an anti-C5 antibody inhibitor) in CAMR are unclear, needing further clinical evaluation [13,50].

Differential diagnosis of chronic graft deterioration

Interstitial fibrosis and tubular atrophy

The most common pathology of slowly progressive graft failure is chronic IF/TA, accompanied by vascular changes and glomerulosclerosis [12]. IF/TA occurs in 27–45% of late graft losses [6,7], and is present in most late indication- or protocol-driven biopsies [7,12,108–110]. Formally designated as sclerosing "chronic allograft nephropathy" (CAN) [111], it represents the final common pathway of nephron injury with its fibrotic healing response, rather than a specific diagnostic entity [112]. While multiple potential etiologies and pathogenic pathways can lead to IF/TA, the alloimmune response is the most common and important cause [12,65].

Early tubular damage begins with ischemia–reperfusion injury, and subsequent acute or subclinical rejection episodes superimposed upon pre-existing donor disease. IF/TA associated with inflammation portends a worse prognosis [65,108,113]. A nonspecific pattern in a late biopsy of unknown cause is classified as "IF/TA not otherwise specified" [112]. Later microvascular abnormalities and glomerulosclerosis in transplanted kidneys slowly develop over years from calcineurin inhibitor nephrotoxicity, hypertension, diabetes, and hyperlipidemia, possibly admixed with specific diagnostic entities, such as recurrent glomerulonephritis or late alloimmune injury [12].

One treatment strategy for the IF/TA-CAN pattern is CNI minimization (assuming causal CNI nephrotoxicity as the primary pathology). CNI sparing regimens supplemented by mycophenolate increase transplant GFR (by 4.4 mL/min; 95% CI 2.9–5.9) in a meta-analysis of randomized controlled trials, and tended to improve graft survival (OR = 0.72; 95% CI 0.52–1.01). Acute rejection episodes increased only in trials of mandated CNI elimination, but not with avoidance or dysfunction [114]. The large randomized CONVERT study of transplant dysfunction (CAN proven in 91.3%) switched patients from CNI to sirolimus ("substitution") but failed its primary eGFR difference endpoint versus continued CNI. Sirolimus substitution was futile with severe transplant dysfunction (eGFR <40 mL/min) or proteinuria above 0.5 g/day [110]. CNI elimination (with sirolimus substitution or initial avoidance) reduced vascular and tubulointerstitial damage in other controlled trials [109,115].

The clinical approach to a failing graft with IF/TA begins by considering potential immune and non-immune-mediated etiologies with corroboration of clinical, biological, and histological information [86]. IF/TA should be pathophysiologically classified as primarily attributable to (1) early non-immune causes such as donor disease or ischemia–reperfusion tubular damage; (2) immune injury, including acute, severe, persistent, or late rejection; and/or (3) other specific diagnostic entities, including recurrent disease, BK virus, hypertension, or CNI toxicity. Several causes of injury may coexist at one time, or be differentially expressed over the graft's lifetime (Table 69.2).

Table 69.2. Differential diagnosis of chronic renal allograft dysfunction

Structural/infective
Ureteric obstruction or lower urinary tract obstruction
Recurrent allograft pyelonephritis and/or vesicoureteric reflux
Polyoma (BK) virus nephropathy
Renal arterial stenosis
Alloimmune injury
Late acute rejection (iatrogenic or patient non-compliance)
Chronic T-cell-mediated rejection
Chronic antibody-mediated rejection
Mixed acute or chronic rejection
Other causes
Non-specific sclerosing tubulointerstitial damage (formally designated as chronic allograft nephropathy)
Chronic calcineurin inhibitor nephrotoxicity
Recurrent thrombotic microangiopathy
Acute interstitial nephritis
Recurrent or de novo glomerulonephritis
Recurrent diseases (e.g. diabetic nephropathy)
Poorly controlled hypertension and hypertensive nephrosclerosis
Metabolic disturbances (e.g. uncontrolled hypercalcemia or hyperglycemia)
Concomitant medications: nephrotoxins (e.g. NSAID or COX-II inhibitors, contrast), drugs affecting glomerular hemodynamics and GFR (e.g. ACE-I, ARB, calcium channel blockers) or affect metabolism of CNI (e.g. phenytoin, diltiazem, verapamil, azole antifungal agents such as ketoconazole, fluconazole, etc.)
Acute kidney injury associated with major medical illness

Pointers for treatment assignment include the recipient's prior rejection and non-compliance history, sensitization, HLA mismatch, and circulating DSA status, all of which help quantify immune risks when considering any CNI minimization. Recent graft pathology is important to exclude ongoing alloimmune activity (histological clues include fibrointimal hyperplasia of small arteries, interstitial lymphocytic infiltration, lymphocytes in peritubular capillaries, transplant glomerulopathy, or positive C4d staining). Subclinical interstitial rejection leads to progressive IF/TA assessed by sequential biopsy pairs [65] where immunosuppression could be strengthened.

BK virus nephropathy

BK virus is an endemic polyoma virus causing a mild primary infection in children with a seroprevalence rate of 60–80%. Residual virus can be reactivated by powerful immunosuppression, usually within the first year with asymptomatic viremia. Severe BK virus nephropathy can develop with tubulointerstitial nephritis, leading to progressive renal dysfunction and graft loss (incidence 5% where 46% fail) [116]. Transplant biopsy demonstrates diagnostic intranuclear inclusions with enlarged nuclear chromatin from replicating virus within renal tubular cells, confirmed by SV40T viral immunostaining. Allograft BK infection can be accompanied

by tubular cell degeneration, apoptosis and detachment (forming diagnostic urinary "decoy" cells), and mononuclear interstitial inflammation [117].

Current antiviral agents are ineffective against BK. Prevention is the best strategy by avoidance of excessive immunosuppression, coupled with regular viral surveillance (by blood nucleic acid or PCR testing). Early detection of viremia (with viral load quantification) before graft dysfunction, and cautious reduction of therapy (usually 50% CNI dose reduction and weakening or elimination of antimetabolite), typically reduces or eliminates circulating virus, preventing destructive parenchymal infection.

Established BK viral allograft nephropathy is serious threat and difficult to eradicate [116,117]. Ciprofloxacin (for 1 month), low-dose cidofovir (0.5 mg/kg i.v. every second week with saline hydration), elimination or substitution of mycophenolate with leflunomide (all weak antiviral agents) or azathioprine, conversion from tacrolimus to low-dose cyclosporine (target C2 500 ng/mL), or CNI dose reduction, or regular intravenous immunoglobulin therapy (a non-depleting immunomodulatory agent for BK infection coexisting with "rejection"), had limited benefit in small cohort studies [34,118].

Calcineurin inhibitor nephrotoxicity

The introduction to transplantation of cyclosporine and later tacrolimus substantially reduced early acute rejection episodes, producing excellent 1-year graft survival rates and underpinning modern immunosuppression. But CNIs are nephrotoxic and this represents the primary drawback to this class of drug [119]. Evidence that CNI nephrotoxicity contributes to late graft injury is inferred from: (1) unchanged long-term graft loss rates despite suppression of acute rejection; (2) characteristic toxicity lesions in longitudinal histopathology studies [120]; (3) clinical trials of CNI avoidance, early withdrawal (to prevent toxicity), and dose reduction or late withdrawal (for established CNI damage), demonstrating functional or structural improvement [109,115,121,122]; and (4) by classical CNI histopathology and renal failure occurring in native kidneys of non-renal solid organ transplant recipients or autoimmune disease patients treated with CNI [123].

Progressive arteriolar hyalinosis is the most reliable diagnostic marker of chronic CNI nephrotoxicity, associated with downstream glomerulosclerosis and a common secondary diagnosis in 30% of "troubled transplants" [7]. Arteriolar hyalinosis becomes increasingly prevalent from sustained chronic exposure and increasing transplant age, and so by one decade post-transplant it is present in the vast majority, but with variable severity (suggesting individual susceptibility) [12,120]. Arteriolar hyalinosis can also originate from the donor kidney [124] (older donor age, vascular disease, or smoker, where baseline implantation biopsy helps later histological interpretation), or from subsequent development of other conditions such as diabetes mellitus (as diabetic glomerulopathy with GBM thickening on biopsy and proteinuria), other than CNI nephrotoxicity.

Isolated CNI nephrotoxicity (either acute cyclosporine-mediated glomerular vasoconstriction or chronic changes without rejection), has a relatively good outcome if recognized early, and is easily treated with CNI-sparing regimens [7]. CNI withdrawal from failing transplants reverses the functional decline and improves eGFR in cohort and uncontrolled studies [115]. Randomized trials of CNI reduction or withdrawal improved renal function in 90% of patients with a small risk of rejection and graft loss [121]. Patients interconverted from cyclosporine to low-dose tacrolimus showed improved renal function, lipids, hypertension, and cardiovascular markers, but 5-year graft survival was unaltered [125].

Table 69.3. Management of chronic allograft injury

Prevention and screening
Minimize ischemia–reperfusion damage (shortest ischemic times, optimal procurement, preservation and transport measures)
Minimize donor–recipient histoincompatibility
Rapid histological diagnosis and effective treatment of acute rejection
Early optimal immunosuppression (including IL2R blocker or antithymocyte induction with medium to high immune risk recipients, as appropriate)
Control of early subclinical rejection by adequate immunosuppression (only available as protocol biopsy diagnosis)
Early prophylaxis for CMV with (val)ganciclovir or valaciclovir
Early BK screening (especially if using high-dose immunosuppression)
Regular monitoring of renal function by serum creatinine
Interval dipstick urinalysis and imaging (for ureteric obstruction)
Regular compliance review and (re-)education if required
Control of progression factors
Control hypertension (calcium channel blockers ideally but there are theoretical advantages for ACEI or ARB therapy), diuretics may be needed
Avoid added salt, stop smoking, control lipids, limit weight gain, lifestyle modifications (risk of metabolic syndrome)
Control diabetes and urinary tract infections if these occur
Consider minimization of long-term CNI by dose reduction in low to medium immune risk recipients, or, if chronic allograft injury or CNI nephrotoxicity develops, by elimination or substitution
Avoid under-immunosuppression (risks of subclinical rejection or acute late rejection)
Match immunosuppression with individual immunological risk of rejection (e.g. HLA-mismatch, previous rejection history, re-transplantation, and DSA)
Manage acute transient recipient events (such as acute tubular necrosis from sepsis) with restitution of appropriate immunosuppression after stability of acute insult

When deteriorating function from CNI nephrotoxicity occurs in low immune-risk patients (and subclinical rejection is excluded by biopsy), CNI withdrawal and maintenance with concentration-controlled mycophenolate mofetil and corticosteroids are recommended [126]. For CNI nephrotoxicity in recipients with higher immune-risk, retransplants or previous rejection(s), then CNI can be either minimized or substituted (with sirolimus or everolimus), and carefully monitored by serum creatinine (the rejection risk for withdrawal approximates 10%) [34]. mTOR inhibitors are weaker immunosuppressive agents and poorly tolerated at high doses, and side-effects of mouth ulcers, peripheral edema, proteinuria, anemia, or thrombocytopenia result in discontinuation in 30–45% of patients [127,128]. When active rejection and chronic CNI nephrotoxicity occur together, treatment is prioritized to controlling the alloimmune response, as the greater immediate threat to the transplanted kidney (Table 69.3).

Newer non-nephrotoxic immunosuppressive agents also offer some promise, although clinical data are incomplete. Belatacept (targeting the CD28-CD80/86 pathway and T-cell costimulation) and tofacitinib (a Janus kinase 1/3 inhibitor) when combined with mycophenolate have been shown to decrease graft failure (OR 0.61; 95% CI 0.39–0.96) in a meta-analysis of CNI-sparing trials [129]. Voclosporine (a cyclosporine analog that is immunologically non-inferior to tacrolimus) has claimed reduced toxicity to the kidney. The CD2-specific fusion protein alefacept (targeting LFA3-CD2 pathway and selectively eliminating memory T cells, and effective in psoriasis) and sotrastaurin (AEB071, a protein kinase C inhibitor reducing T-lymphocyte activation and cytokine release) are largely preclinical at present.

Late-occurring glomerular diseases

Recurrent or de novo glomerulonephritis fail up to 8.4% of grafts by 10 years [130–132]. Clinical clues are hematuria or proteinuria, and diagnosis is made by transplant pathology. Despite fewer early rejection losses with modern immunosuppression producing more "at risk" recipients, late graft failures from recurrent glomerulonephritis are not increasing [133]. Transplant immunosuppression usually controls most immune-mediated renal diseases, including ANCA vasculitis, lupus nephritis, membranous glomerulonephritis, and Goodpasture's disease. IgA nephropathy commonly recurs but generally with a relatively milder clinical impact [130]. The use of corticosteroids is associated with reduced recurrent IgA by registry analysis (subhazard ratio 0.50; 95% CI 0.30–0.84) [133]. Mycophenolate (showing benefit in native IgA disease) also is suggested to be of benefit in avoiding consequential recurrence.

Light chain deposition disease mostly recurs, and dense deposit disease recurs in 50–90% with graft failure (there are case reports of benefit with eculizumab). Fabry disease recurs late and requires specific agalsidase therapy. Transplant diabetic nephropathy also reappears late with proteinuria and graft dysfunction and typical pathology. Blood pressure control and renin angiotensin system blockade appear beneficial in cohort studies, and are suggested.

Transplant hypertension and dyslipidemia

Hypertension occurs in 70–90% of recipients, correlating with graft loss and death. The high prevalence is usually related to pretransplant hypertension and recipient vascular disease, glucocorticoid and cyclosporine therapy, transplant dysfunction, or rarely from transplant renal artery stenosis [134]. Calcium channel blockers (antagonizing cyclosporine-mediated vasoconstriction) are well tolerated and effective, reducing graft loss (RR 0.75; 95% CI 0.57–0.99) and improving GFR (by 4.5 mL/min compared with placebo/nil treatment) in meta-analysis [135]. While renin–angiotensin system blockade has theoretical benefits, reducing allograft fibrosis, proteinuria, and graft loss [136], there is no proven benefit for graft outcomes. ACE inhibitors reduce GFR (difference −8.1 mL/min), proteinuria (by 0.28 g/day), and hemoglobin (by 0.28 g/day), and worsens hyperkalemia [135]. Loop diuretics are useful for symptomatic edema or when hypertension originates from the failing transplant's inability to clear salt and water. A target blood pressure of 130/80 mmHg is suggested [34].

HMG CoA reductase inhibitors ("statins") are well tolerated, significantly reduce hyperlipidemia, and tend to reduce cardiovascular events by randomized controlled trial [137]. Recipient cardiovascular event rates approximate 3.5–5% per year, and so treatment of dyslipidemia, a healthy diet and lifestyle, and appropriate management of diabetes mellitus are all suggested [34].

Late ureteric stenosis with obstruction

See Section Urine testing and transplant imaging.

Late acute graft failure

Clinical scenario. Transplant recipient presents (sometimes after prolonged absence) with severe transplant renal failure and a markedly elevated serum creatinine, compared to their previous baseline.

Late acute rejection

Acute rejection arising beyond 3 months ("late") after transplantation, from non-compliance or iatrogenic under-immunosuppression, presents with subacute or acute renal dysfunction. It can develop in sensitized patients with increased immunoreactivity against their kidneys, but usually occurs from patient medication non-compliance (see below) or iatrogenic under-immunosuppression (e.g. reductions following cancer diagnosis or severe infection) [77]. Clinician-directed mycophenolate dose reductions for diarrhea have been associated with acute rejection [138].

Acute rejection occurring late is often severe, relatively steroid-resistant, difficult to reverse, and accompanied by widespread tubulointerstitial damage [46]. Complete or partial cessation of maintenance immunosuppression results in (uncontrolled) allograft rejection—involving the adaptive and innate immune systems, antibody production, and mixed infiltration T and B cells, eosinophils, plasma cells, and macrophages. Initiation of chronic transplant rejection can also occur with persistent cellular interstitial infiltration and/or de novo DSA production (often Class II antibodies) [77]. Diagnostic biopsy usually shows well-established acute rejection with extensive interstitial lymphocytic infiltration and edema, associated with chronic interstitial fibrosis, tubular destruction, and glomerulosclerosis. Antibody-mediated rejection (circulating DSA, endothelialitis, and C4d positive) and vascular rejection are common [6,7,77].

Initial treatment by pulse corticosteroids and subsequent strengthening of baseline immunosuppression has limited success, with ongoing functional decline frequently progressing to end-stage failure. Transplants are often irreversibly damaged by late rejection episodes, displaying severe IF/TA (grade 2 involving 25–50% or more cortex) and limited potential for functional recovery. An important therapeutic question, the use of potent second-line therapies, requires the clinician to consider each individual's risk–benefit ratio carefully. The decision to use antithymocyte globulin and/or plasma exchange with i.v. immunoglobulin or rituximab depends on: (1) the severity of irreversible underlying nephron loss (best assessed by IF/TA biopsy scores, duration and pattern of renal functional decline); (2) the expected treatment response (antibody, vascular, and mixed rejections are more treatment resistant); (3) the recipient's ability to safely withstand powerful immunosuppressive regimens (elderly, frail patients with co-morbidities, persistent chronic infections such as bronchiectasis are relative contraindications); and (4) the underlying circumstances (acute rejection from transient situational problems versus more chronic disease and un-modifiable medication issues).

Overall nephron loss is best judged by the extent of tubulointerstitial damage, which guides prognosis (glomerulosclerosis is variable and misleading, some glomeruli are atubular). Transplant pathology should be viewed (not evaluated by only reading the report) to assess potential reversibility of the immune infiltrate. Balancing the recipient's risk of immunosuppression (primarily death from uncontrolled bacterial sepsis, invasive fungal infections, late CMV or PTLD, etc.) versus salvage of a damaged kidney (with potential stabilization or improvement of renal function) requires good clinical judgment. The default option is conservative treatment of pulse steroids, increased baseline therapy, and/or IVIG, accepting likely treatment failure with eventual return to dialysis.

Medication non-adherence

Non-compliance occurs in 23–50% of recipients at some time [139], varying from intermittent omission of some tablets (simple mistake or deliberate, or related to alcohol or drug use, chaotic life style, dementia, or forgetfulness), surreptitious dose reductions

or avoidance of particular medications (because of side-effects or other concerns), or complete cessation of therapy and presentation with acute rejection [77,140]. A history of poor medication adherence increases the risk of acute rejection and graft loss by sevenfold, contributing up to 36% of losses in some studies [139]. Medication non-compliance differentially effect recipients of higher immune risk, such as those with prior rejection, HLA mismatch, known DSA or regraft, where the margin for error is much less.

Risk factors include previous poor compliance with dialysis, psychiatric illness or personality disorders, substance abuse, high-risk behaviors, poverty, or when socioeconomic support is suboptimal. Children transitioning to adult transplant programs or adolescents are also vulnerable, and so bridging programs can help. Non-compliance increases with longer transplant duration and human complacency [141].

Clinical clues include missed appointments, fluctuating or undetectable CNI blood levels, differences between medication prescribed and dispensed, or an unexpected acute rejection episode—despite apparently adequate therapy. Often difficult to establish in practice, skipped medications can be clouded by overt denial (to avoid the clinician's displeasure), suspected by a pharmacist's medication audit, or acknowledged by guarded admission (sometimes to a nurse). Ask indirect, non-threatening questions and carefully listen to the patient's response (the "art of medicine"). Understanding the personal reasons for a patient's non-adherence and their situational stressors helps the formulation of a holistic strategy to minimize relapse.

Immunosuppressive therapies display differing non-renal toxicity profiles, influencing their use or misuse. Cosmetic side-effects, such as hirsutism in young women from cyclosporine (tacrolimus can be substituted), a moon face and acne (topical treatments or doxycycline can be used) with corticosteroids can be disconcerting and provoke non-adherence. Steroid withdrawal or minimization can reduce diabetes, cataracts, osteoporosis, fractures, growth retardation in children, and dyslipidemia. The benefits of better hypertensive and cholesterol control and less diabetes are balanced against increased rejection rates (10–15% in trials versus continued steroids) and worse renal function [142, 143]. Tacrolimus causes more post-transplant diabetes mellitus, tremor, headache, neurological symptoms, and diarrhea, compared with cyclosporine, which also causes gingival hyperplasia and dyslipidemia. mTOR inhibitors (sirolimus or everolimus) commonly have dose-dependent side-effects such as edema and mouth ulcers.

Preventive strategies include individualized and repeated education by a specialized transplant pharmacist, minimization of medication side-effects, complexity, and costs, and regular clinical support addressing patient's concerns [34,144].

Differential diagnosis of late acute dysfunction

Acute kidney injury from associated intercurrent illness

Transplant acute kidney injury may be triggered by medical or surgical events, such as myocardial infarction, sepsis, late CMV, abdominal emergencies, etc. Specific treatment is directed to the underlying acute illness, with optimization of cardiovascular medications, transient reduction of immunosuppression for severe life-threatening infections (resuming doses after recovery), and supportive intravenous hydration where needed. Transplant dysfunction usually resolves with physical recovery; however, incomplete recovery can follow a severe acute illness (Table 69.3).

Late transplant ureteric stenosis

As discussed above, this can present in settings following recurrent rejections, leading to ischemic injury to the distal ureter. BK virus also is an underlying cause of late ureteral stricture.

Late de novo or late recurrent glomerulonephritis

As described in Section Late-occurring glomerular diseases.

Late graft thrombosis

This is exceedingly uncommon, and related to underlying hypercoagulable states or conditions of severe systemic hypoperfusion.

Management of the recipient with a failing graft

Chronic transplant failure is often insidious and sometimes unpredictable. Many kidneys function for years at moderate or severe chronic kidney disease levels, before reaching stage 5 (<15 mL/min/1.73 m^2) when dialysis (re)institution is commenced. Many recipients with a failing graft are older (15% of wait-listed patients are over 65 years) and medically complex with excess cardiovascular disease, diabetes, prior malignancy, or chronic pulmonary disease contributing to mortality [145,146]. Recipients with a poorly functioning transplant are uremic, anemic, malnourished, and medically compromised, with excess mortality rates (HR 13.6 for the first week after failing, but remaining abnormal to 10 years) [146]. Late opportunistic infections from immunosuppression are often present and include CMV, and bacterial or fungal diseases requiring specific antimicrobial therapies. Surgical or cardiovascular emergencies can cause acute kidney injury and precipitate graft failure, especially in those with pre-existing poor function.

Early management of chronic kidney failure complications should commence. Treatment of anemia by erythropoietin, hyperphosphatemia and hyperparathyroidism by phosphate binders and vitamin D analogues, hypoalbuminemia with adequate nutrition, and hypertension with appropriate antihypertensive regimens should be undertaken [147,148]. Advance planning of dialysis access, appropriate psychological support, and careful tapering of immunosuppression, using expert multidisciplinary care, can minimize transitional morbidity and mortality.

Summary

Dysfunction of the transplanted kidney has an extensive differential diagnosis, which is weighted toward technical and ischemic-related problems early, transitions shortly after transplantation to immune rejection, and is dominated late by recurrent disease and consequences of inadequate or overly aggressive immunosuppressive drug use. Experienced clinical assessments, prompt use of diagnostic maneuvers including biopsies, and collaborative relationships with transplant pathologists are required to mitigate the many potential causes of allograft failure. While acute rejection is a transplant-specific diagnosis, numerous other pathologies should be considered prior to augmented immunosuppressive therapy.

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CHAPTER 70

Clinical Allograft Rejection Syndromes in Liver Transplantation

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Introduction

Liver transplantation is recognized as the ideal standard of care to treat acute non-recoverable liver injury, chronic end-stage liver disease, certain metabolic diseases, and hepatocellular carcinoma in the cirrhotic patient. In the past four decades, this procedure has evolved from an experimental high-risk procedure to one performed world-wide with excellent outcomes, including 1- and 5-year patient survival exceeding 90% and 75%, respectively [1]. While the improvement in outcomes after renal transplantation have been attributed in large part to a significant decrease in early acute rejection rates, this has not been the case in liver transplantation, where acute rejection rates continue to exceed 30% in most reported series and compiled data sets [2–5]. The vast majority of these acute rejection episodes occur within the first 3 months after liver transplantation, but both acute and chronic rejection can occur later after transplantation [6]. Compared to other solid organs transplanted, the incidence of chronic rejection is lower, perhaps due to the regenerative nature of the liver itself, such that injury does not accumulate over time but is efficiently repaired with preservation of structure and function [7]. This ability to repair injury through regeneration likely explains the dissociation between acute rejection and inferior graft survival that is unique in solid organ transplantation [3,8–10]. An additional difference between rejection of the liver compared to other allografts is the apparent relative unimportance of HLA antibody-mediated processes [11,12]. Cross-matching is routinely performed for all solid organ transplants except for the liver [4,12]. Moreover, the vast majority of acute rejection episodes are successfully reversed without consideration or treatment of antibody-mediated processes. The relative resistance to antibody-mediated damage by the liver may be due to the liver's ability to adsorb antibody, an intrinsic property of the liver that may protect syngeneic organs such as the kidney which is vulnerable to antibody-mediated damage [4]. This convention has recently been challenged by data implicating antibody-mediated rejection in graft loss and chronic rejection of the liver [13–16].

This chapter will build on prior chapters that have delineated the molecular and cellular basis of organ rejection to outline the clinical presentation of liver allograft rejection according to time of presentation and type of rejection. It will also present standard approaches to diagnose rejection among the other causes of liver allograft dysfunction. Treatment strategies for liver rejection will be presented. Lastly, controversies, specialized cases, and future directions in the diagnosis and management of liver rejection will be outlined.

Cellular basis for rejection

The molecular and cellular basis as well as the histopathological aspects of rejection are discussed in detail in Chapters 5–7, and Chapters 81–87 refer to the histopathological aspects. As a summary, the most common type of liver rejection encountered is acute rejection, manifested histologically by lymphocytic infiltration of the portal tract structures (bile duct, portal vein, and hepatic artery), with or without involvement of the central veins [17–20]. The preponderance of the infiltrating lymphocytes is $CD4^+$ and $CD8^+$ T cells accompanied by macrophages, leading to the term acute cellular rejection (ACR). $CD4^+$ T cells are the dominant infiltrating cells, although greater numbers of activated $CD8^+$ T cells are seen in clinical rejection [2,21]. Eosinophils are often present in the infiltrate along with natural killer (NK) cells [17,18]. The severity of ACR—mild, moderate, and severe—is graded according to Banff criteria (Table 70.1) [17,18].

The antigenic target of ACR is likely mismatched donor cell surface-bound major histocompatibility (MHC) antigens, although the degree of human leukocyte antigen (HLA) mismatch is not predictive of risk of rejection in human liver transplantation, as it is in other organs [2,4,22,23]. Hepatocytes and biliary epithelial cells typically express only MHC class I at low levels. However, biliary epithelial cells can up-regulate MHC class I and class II antigen expression under the influence of an inflammatory state [4]. Liver sinusoidal endothelial cells (LSECs) are the major non-parenchymal cells of the liver. They express not only MHC class II but also costimulation molecules, such that they can present antigen to both $CD4^+$ and $CD8^+$ T cells in experimental systems [4,24–27]. However, the donor LSECs may actually function as a barrier to lymphocyte infiltration by effectively forming a continuous lining of the sinusoids. Moreover, experimental evidence supports a tolerogenic role for the interaction between donor LSECs and recipient T cells via cross-presentation of endogenous antigen on MHC Class I, resulting in clonal deletion and T-cell anergy. Thereby, LSEC may play a role in the relative tolerogenicity ascribed

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Table 70.1. Grading of acute liver allograft rejection

Global Assessment*	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute rejection (see text)
Mild	Rejection infiltrate in a minority of the triads, that is generally mild, and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all of the triads
Severe	As above for moderate, with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

Note: Global assessment of rejection grade mode on a review of the biopsy and after the diagnosis of rejection has been established.
* Verbal description of mild, moderate, or severe acute rejection could also be labeled as Grade I, II, and III, respectively.
Source: Banff Schema Group 1997 [17]. Reproduced with permission from John Wiley and Sons.

to liver transplantation [25,28]. Kupffer cells, another nonparenchymal cell resident within the liver, comprise a major percentage of total body macrophages. Kupffer cells can also present antigen to recipient T cells. T-cell apoptosis induced by Kupffer cells is associated with liver graft tolerance in experimental systems [29,30].

Antibody basis for rejection

The liver appears to be relatively resistant to hyperacute antibody-mediated rejection [31–34]. While hyperacute rejection of a liver allograft can occur in sensitized animal models, it is extremely rare in human liver transplantation. As a result, the vast majority of liver transplant programs do not perform pretransplant cross-matching and many do not even characterize recipient or donor HLA type [35,36]. In ABO-incompatible liver transplantation, preformed antibodies to donor blood group antigens can induce early and severe rejection with higher rates of biliary and vascular complications, particularly in adult recipients. This indicates that antibodies can be pathogenic for liver grafts [37–40]. The ABO incompatibility barrier may be crossed in the setting of low antibody titers (more common in infants) with enhanced anticellular and antihumoral therapies, such as exchange transfusion, plasmapheresis, antibody adsorption with affinity columns, intravenous immune globulin, splenectomy, and/or anti-CD20 antibody therapy [38,41–44].

The biological mechanisms conferring relative resistance of liver allografts to alloantibody-mediated damage are poorly defined. Proposed mechanisms include the regenerative capacity of the liver itself, the shielding of liver parenchyma by LSEC and other endothelial cells, the secretion of soluble MHC class I molecules which may complex with and clear antibody, and potential direct cellular effects of the liver on immune cells [45,46]. None of these hypotheses have a proven role in human transplantation but remain areas of current investigation. The effect that the liver can have on antibody pathogenesis is demonstrated by the fact that auxiliary liver transplantation can facilitate transplantation of a positive cross-match kidney from the same donor [47]. This protective effect is not universal, however, and antibody-mediated rejection (AMR) of either the liver or the kidney has occurred with high antibody titers [4,48–50].

In ABO-compatible liver transplantation, cases of acute AMR have recently been described and successfully treated with antihumoral agents and therapies [11,13,36,48,51,52]. There has been substantial debate over decades about the utility of antibody detection and testing of liver recipients and whether, in fact, AMR occurs with any frequency [51,53–58]. One study identified antibodies against liver sinusoidal epithelial cells as highly associated with late acute rejection in prospectively studied patients, proposing that such antibodies may alter TGF-β expression and allow increased T-cell responses in the liver [59]. This debate continues today, focusing on C4d staining in the transplanted liver and the association of C4d staining with inferior liver graft survival [14,15,54,60–67]. Moreover, additional data has linked the presence of donor-specific antibody with inferior survival of primary and secondary liver allografts [34,68–70].

There has been recent interest in the hypothesis that alloantibody contributes to the pathogenesis of chronic rejection of the liver. The rarity of chronic rejection substantially increases the difficulty of delineating relevant mechanisms along with their relative contributions [4]. A number of these studies have shown that C4d staining and emergence of donor-specific antibody is associated with subsequent chronic rejection. This is certainly intriguing as the process of chronic rejection is still not well understood at the cellular and molecular level [11,14,60,66,67,71]. Finally, donor-specific antibody also poses concern in the context of immunosuppression minimization or withdrawal [4,72,73]. The presence and/or emergence of donor-specific antibody as drug dosing is reduced may signal an antidonor response that could limit the success of such approaches. However, until there is a clear understanding as to the functional significance of alloantibody in liver transplantation, particularly in the context of an allograft that is functioning normally, these concerns remain theoretical.

Thus, in summary, the clinical significance of alloantibody and humoral rejection pathways in liver transplantation remains controversial. There is a growing sense that alloantibodies may play a role in at least some cases of rejection, both acute and chronic. However, more clinical and pathological investigation is needed to properly document their presence and assess their importance in liver transplant recipients with normal and abnormal allograft function.

Histopathology and molecular pathology in diagnosis of rejection

While the differential diagnosis of liver allograft dysfunction changes with timing after transplant, the identification of liver graft rejection is made by direct histopathologic analysis of liver tissue [17,19,74]. There are a variety of ways to obtain an allograft biopsy after liver transplant. The most straightforward is a percutaneous biopsy of the liver that is typically performed with ultrasound guidance. Complications include bleeding, bile leak, creation of an intrahepatic vascular fistula, and injury to adjacent structures [75–78]. Transjugular liver biopsy is an interventional radiology procedure that has been increasingly utilized in the past decade, particularly in the setting of coagulopathy or ascites. The major advantage of this approach is to limit bleeding to the intravascular space unless the liver capsule or major biliary structure is punctured. Disadvantages include lower tissue yields and substantial procedural cost associated with this approach [79]. Open liver biopsy by wedge or needle is always possible when the patient is being explored for other reasons or no other safe approach exists. Fine needle aspiration (FNA) biopsy compromises cellular architecture and is currently inappropriate to assess rejection [80]. However, FNA may

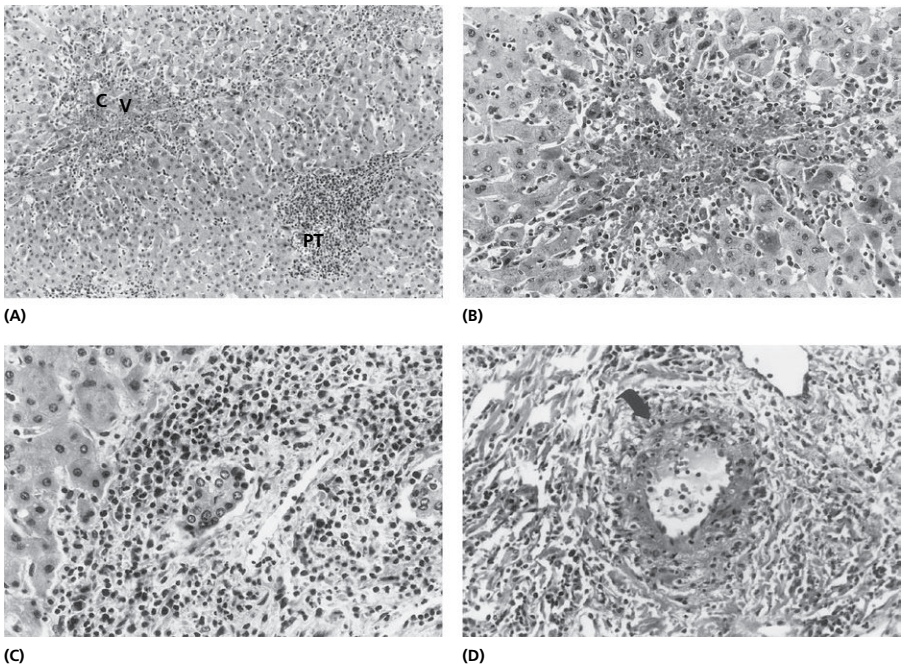


Figure 70.1. Low-power photomicrograph of a failed liver allograft with severe acute rejection. Note the prominent portal tract (PT) and central vein (CV) inflammation, associated with confluent perivenular necrosis, which is shown at a higher magnification in (B). These findings would elicit a diagnosis of severe acute rejection. (C) In the same liver allograft, the bile duct inflammation and damage was widespread and there was focal lumenal disruption, eliciting an RAI score of 3 for bile duct damage. Both the portal and venous endothelial inflammation was also scored as severe, or "3", resulting in a total RAI score of 9/9. (D) Sections from the hilum of this failed allograft also revealed clear-cut necrotizing arteritis (arrow), which is rarely detected with certainty in needle biopsies. Source: Banff Schema Group 1997 [17]. Reproduced with permission from John Wiley and Sons.

become a viable alternative if the diagnosis of acute rejection can be made with molecular rather than histological analysis [81]. Lastly, there have been attempts to diagnose rejection using bile cytology but this technique is unreliable [82,83].

The classification of histopathological findings associated with ACR of the liver were delineated by a Banff consensus conference in 1997 [17]. ACR is adjudicated on a scale of 0–3 (none, mild, moderate, severe) for three categories of inflammation: portal inflammation, bile duct inflammation and damage, and venous endothelial inflammation. The three scores are summed to yield a composite score from 0–9 that represents the rejection activity index (RAI). Mild acute rejection was diagnosed with RAI $\leq$4 and was not associated with firm treatment recommendations. Moderate or severe ACR with RAI $\geq$6 (Figure 70.1; also see Chapter 82 for extensive characterization of the cellular infiltrate) was generally associated with liver test abnormalities and treatment with additional immunosuppression is recommended (see Table 70.1 listing the grading of acute liver allograft rejection and Table 70.2 listing the rejection activity index, both from Banff 1997; also see Chapter 82 for additional characterization of the Banff grading system). The consensus conference acknowledged that early liver allograft injury from preservation and ischemia as well as effects of recurrent viral disease may complicate the diagnosis of rejection, particularly as multiple processes can be present concurrently.

Graft infection with hepatitis C virus (HCV) is universal in patients with viremia at the time of transplantation [84]. There is significant overlap in histological appearance between recurrent HCV and ACR and the differentiation of these phenotypes can be challenging. Both processes can also occur in the same recipient simultaneously. Treatment of these two conditions

Table 70.2. Rejection activity index

Category	Criteria	Score
Portal Inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile Duct Inflammation Damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear:cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolization of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal lumenal disruption	3
Venous Endothelial Inflammation	Subendothelial lymphocytic infiltration involving some, but not a majority of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

Note: Total Score = Sum of Components. Criteria that can be used to score liver allograft biopsies with acute rejection, as defined by the World Gastroenterology Consensus Document.
Source: Banff Schema Group 1997 [17]. Reproduced with permission from John Wiley and Sons.

requires differing approaches so this can be a crucial clinical distinction. Features favoring recurrent HCV infection include steatosis, lobular inflammation, spotty necrosis, and acidophil body formation [17,85,86].

An update to the Banff criteria was published in 2000 to account for the histopathology of chronic liver allograft rejection [18]. This classification emphasizes that the term "chronic rejection" is not meant to imply a late-developing process, as this injury pattern has been observed within months of transplantation and, on occasion, is associated with rapid progression to allograft failure. Chronic rejection is generally felt to result from unresolved acute rejection that leads to severe duct damage and, eventually, loss of bile ducts, hepatic arterial braches, and terminal hepatic venules. Chronic rejection is often associated with a diminution or resolution of the typical necroinflammatory portal infiltrates that are crucial for a diagnosis of acute rejection. The presence of active inflammation can denote reversibility with immunosuppression modulation, while structure loss in the absence of inflammation is a later phenotype that can denote irreversibility (Table 70.3 [84] ; also see Chapter 82) [18,87]. Finally, there has been a recent assessment, but no consensus, as to whether C4d staining and donor-specific antibody titers should be considered in the histopathologic analysis of acute and, in particular, chronic rejection [11,14,15,60,61,65,67,88].

Significant effort has focused on the non-invasive molecular diagnosis of liver rejection events using peripheral blood transcriptional biomarkers and serum microRNAs. The statistical validation of such approaches remains challenging but offers the potential to streamline the diagnosis of rejection. Moreover, validated biomarkers may facilitate ongoing monitoring for rejection and responses to antirejection treatment, which may allow for individualized tailoring of immunosuppression regimens including minimization and/or complete withdrawal [89–94].

Table 70.3. Features of early and late chronic liver allograft rejection (CR)

Structure	Early C.R	Late CR
Small bile ducts (<60 μm)	Degenerative changes involving a majority of ducts: eosinophilic transforma tion of the cytoplasm: increased N:C ratio; nuclear hyperchromasia; uneven nuclear spacing; ducts only partially lined by biliary epithelial cells Bile duct loss in <50% of portal tracts	Degenerative changes in remaining bile ducts Loss in ≥50% of portal tracts
Terminal hepatic venules and zone 3 hepatocytes	Intimal/lumenal inflammation Lytic zone 3 necrosis and inflammation Mild perivenular fibrosis	Focal obliteration Variable inflammation Severe (bridging) fibrosis
Portal tract hepatic arterioles Other	Occasional loss involving <25% of portal tracts So-called "transition" hepatitis with spotty necrosis of hepatocytes	Loss involving >25% of portal tracts Sinusoidal foam cell accumulation; marked cholestasis
Large perihilar hepatic arteiy branches Large perihilar bile ducts	Intimal inflammation, focal foam cell deposition without lumenal compromise Inflammation damage and focal foam cell deposition	Lumenal narrowing by subintimal foam cells Fibrointimal proliferation Mural fibrosis

Source: Demetris et al. 2000 [18]. Reproduced with permission from John Wiley and Sons.

Clinical presentation of liver rejection

We will separate our discussion regarding the clinical presentation of acute rejection into early and late using 6 months after transplant as the separation threshold. Suspicion of rejection is most commonly raised by elevations in aminotransferases, alkaline phosphatase, and/or bilirubin. No single pattern of abnormalities has proven to be diagnostic [95,96]. Histological severity of rejection has been correlated with higher serum levels of total and direct bilirubin and aminotransferases but not alkaline phosphatase or γ-glutamyl transferase at the onset of rejection [6]. Clinical signs, including fever, malaise, abdominal pain, and hepatomegaly, can also be observed, although these are less common and not individually diagnostic [6].

Early acute rejection

Differential diagnosis

Early acute rejection has been variably classified but we will define it as occurring within 6 months of liver transplantation [97]. The most recent published incidence of early acute rejection is approximately 25% and is equivalent for deceased-donor and living-donor liver transplants (Figure 70.2) [5]. When allograft dysfunction is identified by abnormal liver tests, a substantial differential diagnosis often exists. Figure 70.3 presents a schematic flowchart to guide diagnostic choices based on presentation and time from transplant. In the initial period after transplantation, it is incumbent to assess for vascular and biliary complications. Early after transplantation, hepatic artery thrombosis (HAT) can be associated with significant elevations of aminotransferases, severe allograft dysfunction approaching acute hepatic failure, biliary necrosis, and/or hepatic abscess formation, as collateral circulation has not yet developed. As such, retransplantation may be necessary. Current allocation policies specify criteria for listing status elevation or Model for End-stage Liver Disease (MELD) exception point allocation if HAT is diagnosed within 2 weeks of transplantation. Exception scores can be requested if a diagnosis is made outside of this time period and clinical circumstances merit. Less common vascular complications, such as portal vein thrombosis or hepatic vein obstruction, are also in the differential and each of these conditions can be

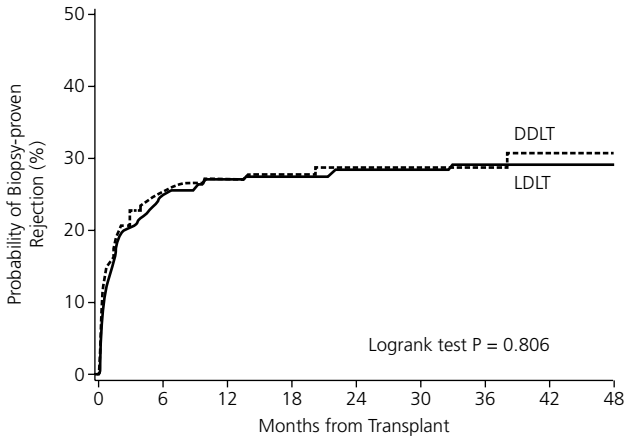


Figure 70.2. Incidence of early acute rejection. DDLT, deceased donor liver transplantation; LDLT, living donor liver transplantation. Source: Shaked et al. 2009 [5]. Reproduced with permission from John Wiley and Sons.

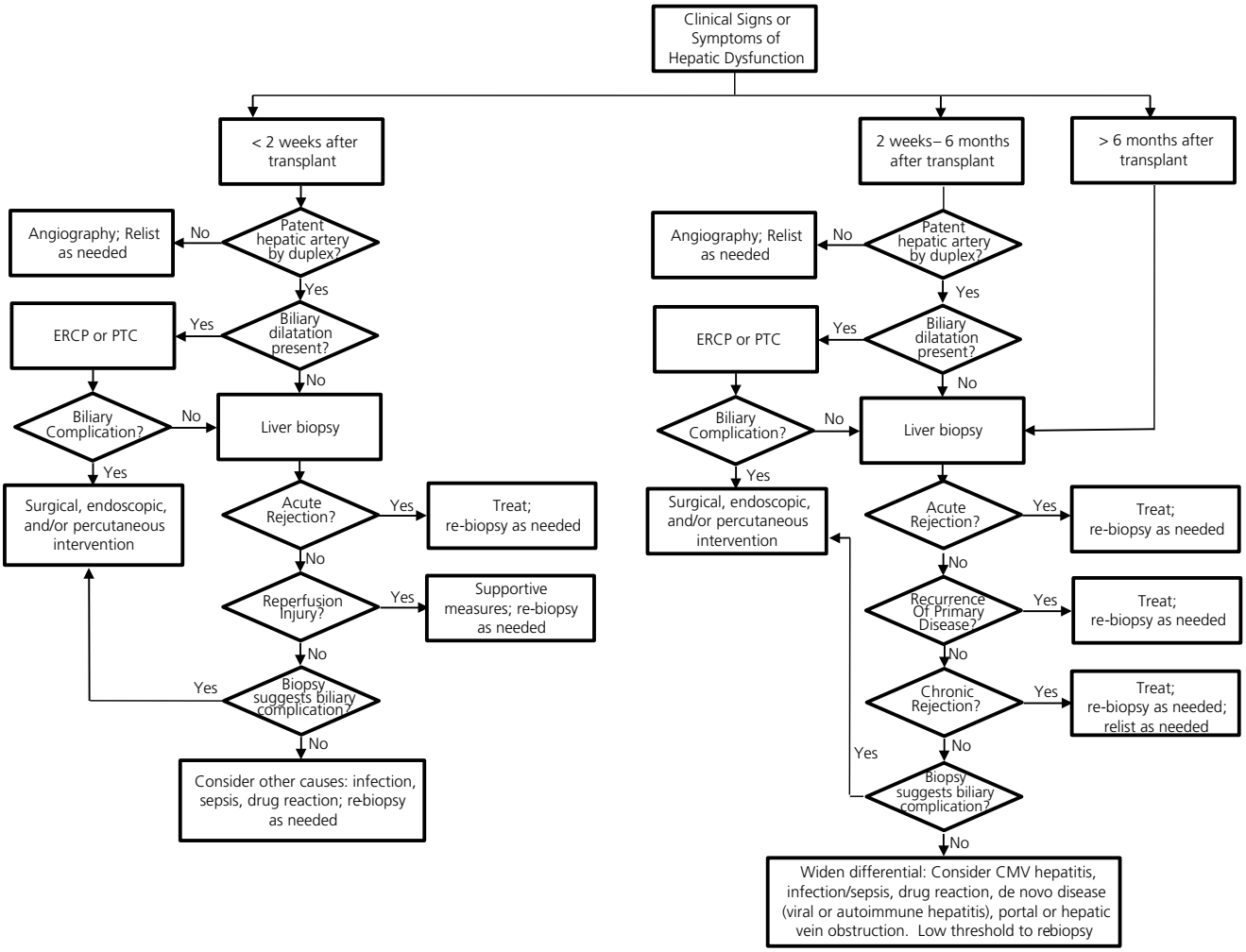


Figure 70.3. Schematic flowchart to guide diagnostic choices based on presentation and time from transplant. ERCP, endoscopic retrograde cholangiopancreatography; PTC, percutaneous transhepatic cholangiography.

suggested by duplex ultrasonography with definitive diagnosis by angiography.

The next major branch point in the differential is whether the liver test abnormalities may be secondary to a biliary complication. The relative likelihood of this will determine whether biliary interrogation or allograft biopsy is the next planned intervention. If ultrasound examination shows dilatation of the biliary tree, which is uncommon in the early post-transplant period, then biliary imaging should come next in the form of endoscopic retrograde cholangiopancreatography for duct-to-duct reconstruction and percutaneous transhepatic cholangiography (PTC) for Roux-en-Y reconstructions. These invasive approaches also offer the possibility of therapeutic intervention. Alternatively, magnetic resonance cholangiopancreatography (MRCP) or computed tomography cholangiography are non-invasive diagnostic approaches that depend upon bile excretion for image generation. Cholangitis or biloma may also accompany biliary leak and/or stricture and necessitate specific treatment. The timing and nature of intervention—surgical or interventional—with a fresh biliary anastomosis may vary based on individual discretion.

If the suspicion of biliary complications is low or if the biliary imaging does not identify a complication, the next step in the diagnostic algorithm is allograft biopsy. Active cholangitis is a relative contraindication to liver biopsy so identifying biliary complications prior to liver biopsy is preferred. The tissue may evidence acute rejection, preservation injury, recurrence of primary disease, or infection (viral hepatitis or cytomegalovirus [CMV]), and/or chronic rejection. Histological assessment can also suggest the presence of a vascular or biliary complication. It is also possible that two processes are occurring simultaneously, such as recurrent hepatitis C with acute rejection. Follow-up biopsy can be done if the initial biopsy was non-diagnostic or to assess efficacy of administered therapy. Adjuncts to diagnosis of early allograft dysfunction include obtaining hepatitis C RNA viral load in patients with known hepatitis C, CMV DNA titers, blood cultures, and cross-sectional imaging if there is evidence of infection or fever. Additional discussion of the differential diagnosis and recurrent disease is found in Chapter 78.

Immunosuppression regimens and rejection

Multiple studies have determined rates of ACR and steroid-resistant rejection associated with different immunosuppression regimens. According to a compendium of 16 randomized trials comparing cyclosporine-based to tacrolimus-based immunosuppression

regimens, the latter exhibited 20% lower risk of ACR and 50% lower risk of steroid-resistant rejection in the first post-transplant year [98,99]. Both mycophenolate mofetil and tacrolimus originally gained traction as rescue agents for the treatment of refractory rejection [100–102]. Mycophenolate mofetil has been shown to be more potent than azathioprine, particularly in combination with cyclosporine [103,104]. With the currently available armamentarium of medications, double or triple regimens comprised of corticosteroids and tacrolimus, with or without mycophenolate derivatives, are standard and associated with comparable rates of early ACR [105,106]. Induction immunosuppression with either an IL-2 receptor antibody or rabbit antithymocyte globulin is administered most frequently to facilitate complete corticosteroid avoidance or corticosteroid and/or tacrolimus minimization. This tradeoff approach has been associated with either similar or lower rates of early ACR, compared to standard, non-induction regimens [107–109]. The use of m-TOR inhibitors in maintenance immunosuppression regimens has evolved in response to the toxicity profile of calcineurin inhibitors (CNI), including renal dysfunction and increased malignancy risk. Conversion of transplant recipients with deteriorating renal function from a CNI-based regimen to an m-TOR-based regimen may stabilize or even improve renal function [110,111]. There is also interest in administering m-TOR inhibitors to recipients with a history of hepatocellular carcinoma or with hepatitis C infection secondary to the antiproliferative effects of m-TOR inhibitors that may deter recurrent malignancy or allograft fibrosis [112]. In the United States, sirolimus and everolimus are two m-TOR inhibitors approved for prophylaxis against kidney allograft rejection. While everolimus is now approved for liver transplantation, it should be noted that sirolimus carries a black box warning pertaining to increased risk of hepatic artery thrombosis [113].

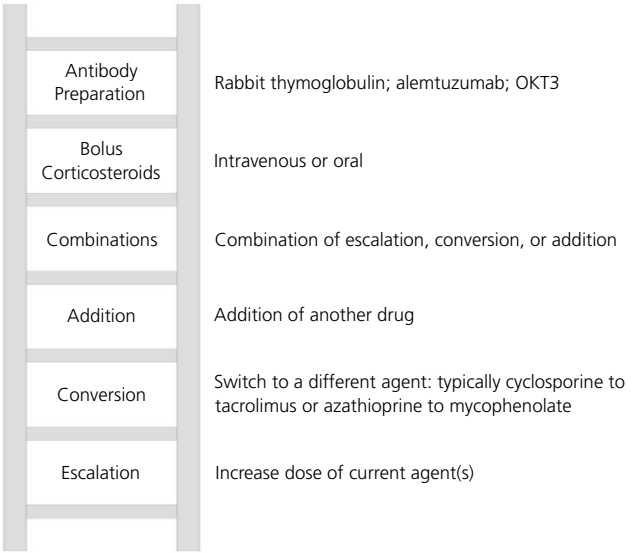


Figure 70.4. Treatment ladder for acute rejection after liver transplantation.

Treatment of early acute rejection

Once the diagnosis of ACR is made, the treatment decision must be made within the context of multiple factors. First, the clinical and histological severity of acute rejection should be considered. Second, background liver disease is important. Autoimmune etiologies of liver disease, including autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis, are associated with increased rejection risk and may motivate more potent treatment. In contrast, hepatitis C may motivate less potent treatment as over-immunosuppression can exacerbate recurrent disease. Other clinical morbidities and/or laboratory abnormalities can affect the choice of treatment, including renal dysfunction, proteinuria, diabetes, hypertension, hyperlipidemia, and cytopenias. Third, the intensity of immunosuppression at the time of the acute rejection episode merits consideration. Because episodes of acute rejection, particularly for recipients without hepatitis C, do not exert a negative impact on long-term outcomes, selection of a treatment strategy that is adequate but not excessive is highly desirable. However, incomplete resolution of acute rejection can predispose to chronic rejection, which is more difficult to reverse and can threaten graft survival. A balanced and judicious approach to the treatment of acute rejection can be associated with a more gradual normalization of laboratory abnormalities, requiring close monitoring and frequent assessment until complete resolution is achieved. For more general discussions of rescue immunosuppression, see Chapter 67.

The treatment options for acute rejection can be considered as a ladder with a stepwise increase from less to more aggressive options (Figure 70.4). The lowest rung is intensification of baseline immunosuppression, defined as an increase in the dose of one or more pre-existing drugs. The second rung is conversion, defined as a switch from a less potent to more potent drug, typically in the same class. This includes conversion from cyclosporine to tacrolimus, or from azathioprine to mycophenolate or to an mTOR inhibitor [98,101,102]. The third rung in the treatment of acute rejection is addition, defined as adding a new drug to the existing regimen and thereby going from a one or two-drug regimen to a two or three-drug regimen, respectively. These approaches are typically exercised in the setting of mild histological severity and modest elevations in liver tests. Historically, the fourth step, corticosteroids, was the starting point for the treatment of ACR. Dosing often begins with two or three doses of bolus intravenous corticosteroids and is typically followed by a tapering course of oral corticosteroids. Alternatively, solely the oral "recycle" of corticosteroids can be administered. Corticosteroids have been associated with increasing the aggressiveness of recurrent hepatitis C, so the risk/benefit ratio must be considered carefully in this patient population.

Rejection that fails to respond to corticosteroids is termed steroid-resistant rejection. T-cell-directed antibody therapies, such as monoclonal anti-IL-2 receptor antibodies (basiliximab), monoclonal anti-CD52 antibody (alemtuzumab), and polyclonal rabbit antithymocyte globulin (rATG), have all been used, although rATG is the most common choice [114–119]. Overall, 60–75% of steroid-refractory rejection is successfully reversed, with episodes that occur earlier after transplantation more amenable to treatment than those that occur later after transplantation.

The strategies delineated above are standard approaches to treat ACR. If humoral rejection is diagnosed, then different therapies targeting antibody presence and production should be considered. AMR-directed therapies include plasmapheresis and IVIG in tandem, as well as monoclonal anti-CD20 antibody (rituximab), which target predominantly B cells. Because this drug does not appreciably affect the repository of B-cell memory (plasma cells), agents directed at plasma cells are being actively evaluated in

kidney transplant recipients but have not been tested in liver transplantation.

Mitigating factors to consider in selecting treatment for early acute rejection

A primary considerations when determining the treatment strategy for early ACR after liver transplantation is the recipient's hepatitis C status. The aggressiveness and tempo of recurrent hepatitis C infection is determined by multiple donor, recipient, and viral factors [120–122]. The immune status of the recipient is believed to play a role in disease control. As such, treatment of ACR with pulse corticosteroids and, in particular, antibody preparations, has been associated with more rapid and severe recurrent disease [107,123–125]. It is also important to consider the histological ambiguity between ACR and recurrent hepatitis C, leading to the possibility of a mistaken diagnosis [86,126,127]. The presence of hepatitis C virus has therefore encouraged a balanced approach to treatment of ACR, motivating the selection of as low a rung on the treatment ladder as effective. Repeat biopsy to monitor the evolution of the rejection episode, the response to treatment, and the degree of virus-induced damage is encouraged [86,127–129]. The dilemma posed by the possibility of ACR within the hepatitis C context has intensified efforts to deliver and maintain adequate immunosuppression early after transplant to minimize the risk of ACR.

An additional, major consideration in the choice of antirejection treatment is renal dysfunction. The implementation of MELD allocation has increased the prevalence and severity of renal dysfunction, particularly early after transplantation, which often limits dosing of CNIs. Recipients with renal dysfunction are typically maintained on both corticosteroids and mycophenolate in addition to a CNI to complete the immunosuppression regimen. Therefore, if early ACR does occur, then the threshold to use bolus corticosteroids is lowered, even for hepatitis C recipients as the alternatives (intensification, conversion, and/or addition) are either impossible or unlikely to be sufficient to control ACR.

Another factor in the choice of antirejection treatment is bone marrow suppression in the early post-transplant timeframe. Relative neutropenia and thrombocytopenia are common. Contributing factors include residual hypersplenism and drug effect related to anti-CMV (valgancyclovir) and/or immunosuppression medications (mycophenolate mofetil, m-TOR inhibitors, or azathioprine). Reduction of immunosuppression dosing as a result of cytopenias may increase vulnerability to ACR. Currently, there is no readily available therapy for thrombocytopenia but filgrastim can be administered to treat leucopenia/neutropenia. Similar to renal dysfunction, cytopenias reduce antirejection treatment options, increasing the likelihood that bolus corticosteroids will be administered [130,131].

Special cases of early acute rejection

ABO-incompatible (ABOi) transplantation has historically been associated with poorer outcomes and higher rejection rates than ABO-identical or compatible liver transplantation for adults [42,44]. The hallmark histological feature of ABOi graft rejection is intragraft disseminated intravascular coagulation, leading to thrombosis and necrosis [41,132]. This pattern can appear histologically similar to humoral rejection in renal transplantation. This is despite the fact that, rather than anti-HLA antibody, the pathology is driven by anti-blood group antibodies. Much of the investigation into and practice of ABOi liver transplantation comes from living donor liver transplantation in Japan, a country where deceased-donor liver transplantation is rare. Adjunctive therapies that have been typically used in various combinations to mitigate antibody-mediated rejection of ABOi livers include plasmapheresis/exchange transfusion, intravenous immunoglobulin, anti-CD20 monoclonal antibody, splenectomy, and infusion of various agents into the portal vein and/or hepatic artery such as high-dose corticosteroids, prostaglandin E1 (vasodilator), and gabexate mesilate (a protease inhibitor to prevent platelet aggregation) [37–40]. In addition to these therapies unique to ABOi transplantation, standard immunosuppression is augmented such that four-drug maintenance regimens are often employed.

The risk of ACR after liver re-transplantation has not been well documented relative to the risk after initial liver transplant [133,134]. It is described that the risk of rejection is higher after renal re-transplantation [135–137]. One single-center series of 32 liver re-transplant patients described no ACR, although the overall 1-year survival in this group was low [138]. Models aiming to predict survival after liver re-transplantation did not include rejection during the initial transplant course as a prognostic factor for re-transplant graft outcome [134,139,140]. Overall, it there is not sufficient data to determine if rejection rates differ after primary versus non-primary liver transplantation.

Outcomes after early acute rejection

Unlike other transplanted organs, early ACR rejection in liver transplantation is not believed to exert an impact on long-term graft and patient survival [8]. In fact, univariate analysis has shown that early ACR rejection is associated with significantly lower post-transplant mortality [6]. The association was attributed to the better overall health status of recipients with rejection compared to those without rejection. Notably, this study was performed more than one decade ago and has not been replicated in the era of MELD allocation. However, reduction in early ACR rates has not translated into improved long-term outcomes, lending further credence to the concept that early ACR is less important to long-term outcomes compared to other factors such as hepatitis C status [141].

Late acute rejection

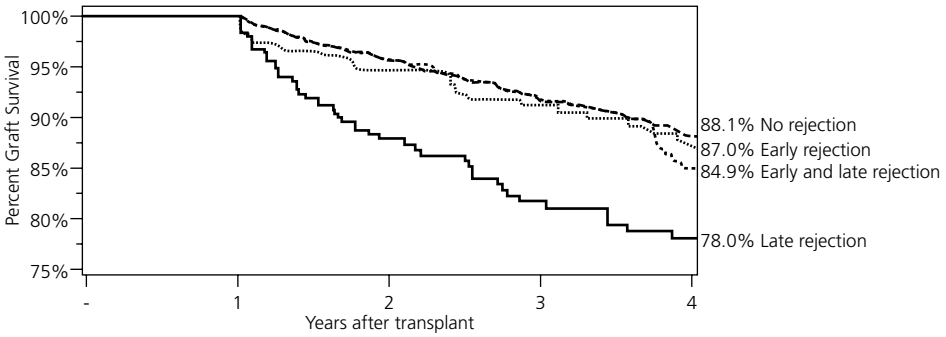
Definition

Late acute rejection (LAR) has been variably defined as acute rejection occurring more than 1, 3, 6, or 12 months after transplantation, with 6 months as the most common threshold. Reported frequency of LAR have varied widely from 7% to 23% depending on the timeframe considered, the diagnostic rigor (biopsy proven or not), and the follow-up duration [142–145]. Although broad assessments using national registry data have been reported [104], the literature on LAR is dominated by single-center studies that have tried to elucidate predisposing and/or precipitating factors, clinical and histological severity, therapeutic approaches and responses, and patient and graft outcomes [142–144].

Risk factors for late acute rejection

Predisposing recipient characteristics for LAR include demographics and etiology of liver disease. Younger age at transplant has emerged as a consistent association [97,142,144,145]. Autoimmune etiologies of liver disease such as autoimmune hepatitis, primary biliary cirrhosis, or primary sclerosing cholangitis are strong risk factors [97,143]. Interestingly and notably, the occurrence of early ACR has not exhibited a strong correlation.

As for immunosuppression practices, the occurrence of LAR clearly signifies insufficient immunosuppression for that patient at



Early rejection (0-6 mos)	451	451	426	248	246
Late rejection (6-12 mos)	255	255	217	143	107
Early and late AR	261	261	243	165	121
No rejection (0-12 mos)	3742	3742	3614	2613	1893

Figure 70.5. Detrimental impact of late acute rejection. Source: Wiesner et al. 2006 [145]. Reproduced with permission from John Wiley and Sons.

that time. Within the literature, there is strong speculation linking non-compliance with LAR [146,147]. Available data is, however, conflicting as to whether a specific agent or the overall intensity of maintenance immunosuppression poses increased risk for LAR. Some studies show that patients who suffer LAR, compared to those who do not, are more frequently maintained on triple therapy and have comparable trough levels of CNIs [97,142]. In contrast, other studies show that patients who suffer LAR, compared to those who do not, are more likely to be on cyclosporine as opposed to tacrolimus maintenance immunosuppression [142] or more likely to have undergone immunosuppression reduction (such as corticosteroid discontinuation or more substantial reduction secondary to post-transplant lymphoproliferative disorder) [97,144]. Finally, registry analyses of patient cohorts separated according to liver disease etiology have assessed the risk of LAR according to immunosuppression regimens at discharge from the transplant hospitalization. Patients discharged on two and three-drug maintenance regimens of corticosteroid and tacrolimus, with or without mycophenolate, were compared. For each diagnostic group—hepatitis C, hepatitis B, and non-viral disease—those discharged on the three-drug regimen compared to the two-drug regimen enjoyed greater freedom from LAR in multivariable analyses [145].

Diagnosis, severity, and treatment of late acute rejection

Since LAR typically occurs when follow-up transplant recipients are no longer followed at a high frequency or intensity, diagnosis may be delayed. When liver tests are elevated more than 6 months after transplantation, particularly if immunosuppression dosing has been recently reduced or there is evidence of insufficient immunosuppression exposure, liver biopsy is often performed as the first diagnostic test (Figure 70.3). Histologic severity of LAR episodes vary, with preponderance of mild ACR (79%) reported in one study [144] and moderate to severe ACR (51%) in another [143]. The treatment of LAR theoretically parallels that of early ACR with a hierarchy of options. However, lower-tier therapy such as dose escalation and conversion is infrequently administered. The more aggressive approach reflects concern that the diagnosis may have been delayed and that LAR is generally more refractory to treatment. The vast majority of patients are therefore treated with intravenous and/or oral corticosteroids [142–144]. Use of antibody preparations is not infrequent in the setting of severe and/or corticosteroid-resistant LAR.

Outcome of late acute rejection

In contrast to early ACR, it is widely believed that LAR compromises graft and patient survival. One primary mechanism is by predisposing to chronic rejection (ductopenic or vascular) which can necessitate re-transplantation and/or lead to patient death. The reported frequency of chronic rejection after LAR varies between 4.1 and 16.3% [97,143,144], although no chronic rejection was observed in patients with LAR after living donor liver transplantation [142]. Registry analyses have examined 4-year graft survival among patients alive 1 year after transplantation [145]. Defining ACR as early if it occurs within 6 months of transplant and as late if it occurs between 6 and 12 months of transplant, all patients were classified as having early ACR, LAR, neither, or both (Figure 70.5). Cox regression analyses showed that the hazard ratio for late graft loss, relative to patients with neither early ACR nor LAR, was 0.99 (95% confidence interval 0.75–1.31), 1.50 (95% CI 1.09–2.07) for patients with early ACR and LAR, and 1.99 (95% CI 1.50–2.66) for patients with LAR alone. While patients with no rejection or early ACR alone enjoyed comparable 4-year survival of 88.1% and 87.0%, respectively, those with both early ACR and LAR and those with LAR alone had 4-year survival of 84.9% and 78.0%, respectively.

Chronic rejection

Background of chronic rejection

It is well recognized that, among the solid organs that are transplanted, the liver is the most resistant to chronic rejection. Historically, in the cyclosporine era of the 1980s, the prevalence of chronic rejection was as high as 20–40% [7,148–152]. Since the 1990s, and coincident with the introduction of tacrolimus, chronic rejection occurs much less frequently with an estimated prevalence of 2–5% [87,150,152–155]. The natural history has also changed over time. Classically, chronic rejection of liver allografts occurred within the first post-transplant year and progressed rapidly, within 3 to 6 months, to graft loss. Currently, in the modern era of immunosuppression, chronic rejection occurs later after transplantation and

can run a more indolent course [61,156]. Moreover, unlike chronic rejection after kidney, heart, and lung transplantation that gradually and inexorably leads to graft loss, chronic rejection after liver transplantation can be reversible with intensification of immunosuppression, if diagnosed early in the process [18,61,154,157–160]. Nevertheless, chronic rejection remains an important cause of late graft dysfunction, which can lead to both significant mortality and morbidity for liver transplant recipients.

Risk factors for chronic rejection

The most potent risk factor for chronic rejection universally cited is the number and severity of ACR episodes [6,9,87,97,148–150,152,153,161]. Chronic rejection frequently evolves out of an episode of ACR that is resistant to corticosteroid treatment and does not fully respond to treatment [18,61,148,150,152]. A second strong association is cyclosporine-based, compared to tacrolimus-based immunosuppression regimens [87,150,152,153,162]. Three lines of direct and indirect evidence substantiate this association. Tacrolimus is superior to cyclosporine for the prevention of ACR [98,162]. Conversion to tacrolimus is an efficacious treatment of chronic rejection for patients on cyclosporine [157,163]. In addition, the University of Pittsburgh group reported that tacrolimus offered "virtual freedom from chronic rejection after primary liver transplantation" for both adult and pediatric liver transplant recipients [153,164]. Another factor associated with increased risk of chronic rejection is autoimmune disease (autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis), leading to liver transplantation [87,150,152,153,165]. Finally, reduction of immunosuppression, whether secondary to a routine management decision, to the development of a contraindication to immunosuppression, or to non-compliance, has also been associated with subsequent development of chronic rejection [150,152,153,166].

More recently, a potential association between chronic rejection and treatment of recurrent hepatitis C with interferon and ribavirin has been raised and debated. It is well known that interferon administration to renal transplant recipients can precipitate acute and chronic rejection of the renal allograft [167,168]. However, the association with liver allograft rejection, both acute and chronic, has been more controversial [169]. Multiple factors may contribute to blurring the relationship, including the different rates of ACR as a function of time after transplantation, the highly variable timing of initiating interferon therapy with the concomitant variability in immunosuppression intensity, and the inherently abnormal liver test profiles that likely reduced biopsy frequency. Moreover, there are multiple formulations, including pegylated versions which, in both theory and practice, can pose differential risks. Finally, the adjunctive use of ribavirin, an immunomodulatory agent, introduces yet another factor that might modify the association of treatment with rejection.

In spite of these caveats, several detailed single-center analyses have provided data that correlate interferon-based treatment with chronic rejection [170–173]. The reported rates of 8.7–17% are distinctly high, considering the fact that many patients initiated treatment years after transplantation. Chronic rejection typically developed soon after treatment initiation, within a few weeks to months, further lending credence to the association. And intriguingly, viral clearance has been suggested as a risk factor for the development of chronic rejection [170–172]. It has been postulated that successful treatment, by definition, signifies an efficacious antiviral response, which may have been mediated through enhancement of immunologic mechanisms that led to viral clearance.

Diagnosis and treatment of chronic rejection

Chronic rejection has been a difficult diagnosis to make, particularly early in its course when it is amenable to treatment [18,96,149,152,154,158]. As a result, clinicians have been warned to raise their index of suspicion such that any episode of ACR with an unfavorable profile (e.g. histological grading of severe or resistance to corticosteroids) merits consideration of impending or evolving chronic rejection. The classic diagnostic criteria of bile duct loss from 50% or more of the portal tracts or obliterative foam cell arteriopathy are late and potentially terminal manifestations, signaling the need for re-transplantation [18,61,149,152,154,158–160,174]. Moreover, these features are notoriously difficult to identify in the limited sample provided by a needle biopsy [18,62,154,160]. Bile duct loss cannot be accurately quantified unless the biopsy contains a sufficient number of portal tracts. Foam cell arteriopathy occurs predominantly in larger arteries than those typically sampled.

Nevertheless, there have been concerted efforts to delineate early histological features of chronic rejection [18,61,87,154,157,158,161,175]. Early biliary changes are dominated by degeneration with dysplasia or atrophy and with the variable presence of inflammation, rather than bile duct loss. These changes are thought to reflect direct immunologic attack on the biliary epithelium in combination with indirect ischemia induced by loss of small arterial branches. Additional arterial findings indicative of early chronic rejection include inflammatory lesions with the presence of lymphocytes and lipid-laden macrophages. Characteristics of early chronic rejection can also be found in the centrilobular regions with central perivenulitis along with ballooning and dropout of centrilobular hepatocytes, leading to mild perivenular fibrosis. As chronic rejection progresses, inflammation generally diminishes with bile duct disappearance, arterial obliteration, and severe fibrosis and cholestasis.

More recently, humoral mechanisms have been invoked as a potential association with the development of chronic rejection [14,15,58]. While the two hallmarks of antibody-mediated responses—donor specific antibodies [14,58] and C4d staining [14,15]—may be increased in the chronic rejection setting, the biological significance of these associations are insufficiently clear. There is, as yet, no prospectively collected cross-sectional or longitudinal data on large recipient populations to determine the prevalence of these two findings in normal allografts or in allografts involved in other processes, including acute rejection and recurrent disease.

Treatment of chronic rejection focuses on augmenting immunosuppression. One of the primary considerations is whether an acute inflammatory component is present that merits administration of bolus corticosteroids or antibody preparations—treatment options typically exercised for acute rejection. As discussed above, inflammatory changes are typically present early and wane as chronic rejection evolves. Beyond this important decision, treatment options are largely determined by the immunosuppression regimen in place when chronic rejection develops. For patients on cyclosporine, conversion to tacrolimus can be strongly recommended based on available data [157]. Mycophenolate has also been reported as a potential rescue agent either as an additional agent or as a more potent alternative to azathioprine [101,176–178]. More recently, sirolimus has also been cited as efficacious for the

treatment of chronic rejection, perhaps due to antifibrotic properties [113].

Outcome of chronic rejection

The outcome of chronic rejection substantially depends upon timely diagnosis and treatment at an early, reversible stage. Favorable clinical factors include lower aspartate transaminase and lower serum bilirubin [153,154,157]. Favorable histological factors include decreased bile duct and arterial loss, fewer foam cell clusters, and less severe perivenular fibrosis [153,154,157,158]. Overall, between 20 and 70% of chronic rejection spontaneously resolve or respond to treatment with long-term maintenance of allograft function [87,113,152,153,157,158,177]. Failure to reverse chronic rejection typically leads to graft failure, necessitating re-transplantation or death from sepsis or allograft dysfunction.

Summary

The manifestations of liver allograft rejection differ from those of alloimmunity directed toward other organs in their relative benign consequences and limited involvement of alloantibody-dependent mechanisms. However, this generally favorable profile is dependent on prompt and accurate diagnosis, and appropriate treatment. The prevalence of viral hepatitis confounds the diagnosis of rejection, and impacts treatment decisions to avoid over immunosuppression. The evolution of proper treatment algorithms is a clear factor in the generally excellent outcomes achieved in modern liver transplantation.

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CHAPTER 71

Clinical Allograft Rejection Syndromes in Heart Transplantation

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Introduction

In an early report after performing his second heart transplant, Dr. Christiaan Barnard described the manifestation of allograft rejection as a compilation of clinical findings:

> The immunologic attack of the recipient on the transplanted organ may be detected by (1) systemic changes such as fever, tachycardia and anorexia, among others; (2) local changes that result in enlargement of the transplanted organ; (3) clinical and laboratory evidence of deterioration in function of the transplanted organ; (4) evidence of parenchymal destruction; and (5) immunologic changes indicating response to allogeneic tissue [1].

It is essential to recognize that since the advent of clinical cardiac transplantation, the diagnosis of allograft rejection has not been made with a single lab test or clinical finding, but by analyzing a constellation of clinical symptoms, physical examination findings, electrocardiographic measurements, and laboratory data measuring immunologic activity and muscle injury [1–4]. As immunosuppressive agents have evolved, so have the clinical manifestations of cardiac allograft rejection. It is now recognized that there is heterogeneity in the clinical and histopathologic manifestations of the alloresponse: acute or insidious and chronic; cellular or antibody mediated or mixed variety; symptomatic or clinically quiescent.

Many of the newer immunosuppressive therapies target cellular rejection; as such, we now see less of this type of rejection in isolation. Instead, cellular rejection is most commonly observed in combination with the more prevalent form of rejection—antibody-mediated rejection (AMR). Because allograft rejection is still a significant concern, application of a multimodality surveillance regimen, including immunologic monitoring, clinical allograft performance evaluation, and direct histology of the cardiac allograft, continues to be an important part of contemporary practice.

In this chapter we review ways to diagnose cellular- and antibody-mediated rejection, new therapies to prevent rejection in high-risk individuals, and we briefly discuss the treatment of different types of rejection.

Cellular rejection

Acute cellular rejection (ACR) is a recipient T-cell-mediated attack of the donor allograft tissue which is most prevalent early after transplantation (see Chapter 5). The histopathology of cellular rejection (see Chapter 83) is notable for lymphocytic infiltrates, which in mild cases of rejection are localized to the perivenular regions. In more severe cases of cellular rejection, the infiltration of lymphocytes is more pronounced and progresses into the cardiac interstitium. Furthermore, other inflammatory cells, including neutrophils and eosinophils, are observed in addition to evidence of myocyte injury, intramyocardial hemorrhage, and/or vasculitis.

Myocyte injury is associated with the highest grades of rejection, manifesting as sarcoplasmic scalloping, hypereosinophilia, and nuclear pyknosis on endomyocardial biopsy samples.

In 1990, the International Society for Heart and Lung Transplantation (ISHLT) formulated criteria to standardize the pathologic grading and diagnosis of cellular rejection (Table 71.1) [5]. The initial system consisted of seven categories; however, it was criticized for having high rates of interobserver variability [6]. Furthermore, the clinical relevance of several of the subcategories was questioned. The diagnosis of focal, moderate rejection (grade 2) was frequently applied to Quilty lesions, which have never been found to have any clinical relevance [7]. Mild, diffuse rejection (grade 1B) was a rare diagnosis and difficult to distinguish from moderate, diffuse rejection (grade 3B). The pathologic criteria were eventually revised in 2004, when the grades 1A, 1B, and 2 were consolidated into the revised grade 1R; grade 3A became the revised 2R and the severe rejections grades of 3B and 4 were combined into the revised grade 3R (Figures 71.1 and 71.2A,B) [8].

Risk factors

An analysis of the Cardiac Transplant Research Database (CTRD) showed that the hazard ratio for ACR peaked at 1 month after transplantation, decreased until 1 year, and leveled thereafter [9]. Risk factors for rejection include female recipient, younger age, black race, cytomegalovirus infection, female donor, mechanical circulatory support, and greater donor–recipient human leukocyte antigen (HLA) mismatch [9–11].

Similar to kidney transplantation, HLA compatibility between the donor and the recipient may affect rejection rates and subsequent allograft survival after heart transplantation; however, the data are controversial. While smaller studies failed to show a

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 71.1. Histopathologic criteria for the diagnosis of cellular rejection

ISHLT working formulation 1990	Pathology findings	ISHLT working formulation 2004	Pathology findings
Grade 0	No evidence of cellular rejection	Grade 0R	No evidence of cellular rejection
Grade 1A (focal, mild acute rejection)	Focal infiltrate of inflammatory cells *without* necrosis	Grade 1R (mild, low-grade, acute cellular rejection)	Interstitial and/or perivascular infiltrate of mononuclear cells with up to 1 focus of myocyte damage, otherwise normal myocyte architecture
Grade 1B (diffuse, mild acute rejection)	Diffuse, but sparse, infiltrate of inflammatory cells *without* necrosis		
Grade 2 (focal, moderate acute rejection)	One focus of aggressive infiltration, typically with local myocyte damage		
Grade 3A (multifocal moderate rejection)	Multiple foci of aggressive infiltration, typically with local myocyte damage	Grade 2R (moderate, intermediate-grade, acute cellular rejection)	Two or more foci of infiltrate with associated myocyte damage, with normal myocardium in between
Grade 3B (diffuse, borderline severe acute rejection)	Diffuse infiltration of inflammatory cells with evidence of myocyte damage	Grade 3R (severe, high-grade, acute cellular rejection)	Diffuse infiltrate with multifocal myocyte damage; may be accompanied by edema, hemorrhage and/or vasculitis
Grade 4 (severe acute rejection)	Diffuse infiltration of inflammatory cells, with polymorphous collection of infiltrates, myocyte damage and edema, hemorrhage and/or vasculitis		

ISHLT, International Society for Heart and Lung Transplantation.

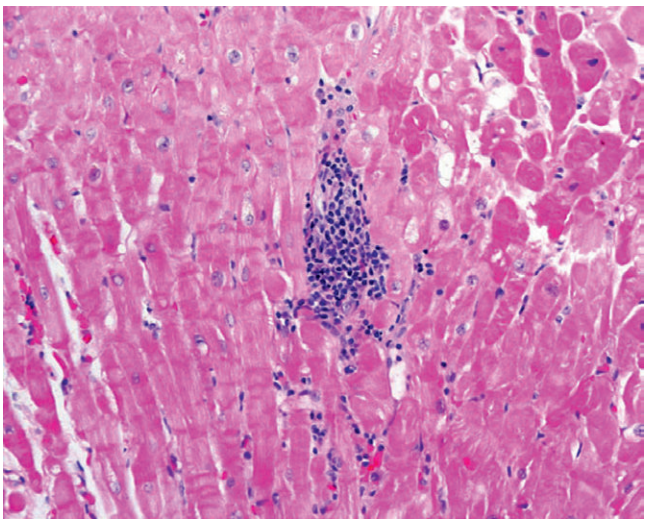


Figure 71.1. Hematoxylin and eosin stain showing a grade 1R (previously grade 2) rejection with single area of aggressive infiltrate. (Image provided by Dr. Margaret M. Grimes, Richmond, VA.)

protective effect of HLA matching of the donor and recipient, Opelz and Wujcik reported a study of over 8000 patients included in the Collaborative Transplant Study which showed improved allograft survival in patients who had two or fewer mismatches at the HLA-A, B, or DR loci [12–14]. The survival benefit in this study was primarily in the first 3 to 6 months after transplantation, suggesting a protective effect of HLA matching on the incidence of acute allograft rejection [12]. An analysis by the CTRD of nearly 1200 patients showed that individuals with two or fewer HLA mismatches had no rejection-related death or re-transplantation in the first year after transplantation [10]. The incidence of allograft rejection episodes (grade 2 or higher) in the first month after transplantation was especially increased (65% vs. 40%) in those patients who had two recipient donor mismatches at the MHC-II DR loci [15]. The incidence of rejection associated with HLA-DR mismatch is independent of induction therapy with OKT3 or IL-2 inhibitors; however, it may be reduced with utilization of antithymocyte globulin for induction, and tacrolimus instead of cyclosporine for maintenance immunosuppression [16–18]. Despite these findings, implementing HLA matching for heart allocation protocols has many practical limitations. Considering the time sensitivity of the operation, scarcity of organ donors, and critical nature and instability of the recipient, HLA matching algorithms are not currently used to determine organ allocation.

Cytomegalovirus (CMV) infection after heart transplantation increases the risk of both acute and chronic allograft rejection. Active CMV infection has a proinflammatory and immune-activating effect. Prophylaxis for CMV infection in selected patients has been shown to reduce episodes of acute and chronic rejection [19]. Typically, during an active CMV infection, immunosuppression is reduced to facilitate viral control, often leading to a heightened risk of cellular rejection in the post-CMV recovery phase.

There may be race-specific responses to immunosuppression as black heart transplant recipients have consistently been shown to have poorer survival than Caucasians due to increased rates of allograft failure and graft rejection [20]. However, further study is required to understand the demographic or biological mechanisms to account for the discrepancy. This risk may be mitigated by the use of more targeted immunosuppression in black cardiac transplant recipients [21].

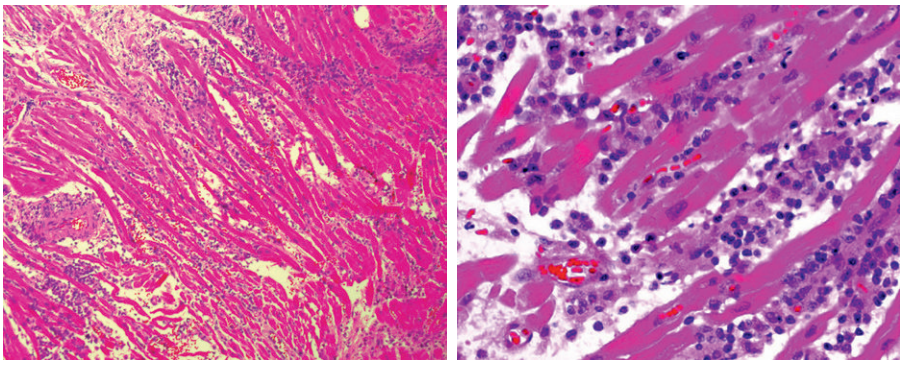
Recipients of female gender have also been shown to be at higher risk for allograft rejection. While previous pregnancy is an accepted risk factor for sensitization and increased risk of rejection, other gender-specific mechanisms may play a role in allograft rejection. Researchers have shown that gender matching may protect against rejection episodes independent of weight matching of the recipient and donor [22].

Diagnosis

Spectrum of symptoms

The signs and symptoms of cardiac allograft rejection are nonspecific. Therefore, the diagnosis of rejection is commonly made by the histologic assessment of protocol-driven, surveillance endomyocardial biopsies. Most episodes of acute rejection occur within the first 3 to 6 months after transplantation and most of those episodes are mild and usually asymptomatic.

Patients with severe rejection may present with signs and symptoms of heart failure, including decreased exercise tolerance, shortness of breath, fluid retention, orthopnea, paroxysmal nocturnal



(A) (B)

Figure 71.2. (A) Low magnification and (B) high magnification hematoxylin and eosin stain showing a grade 3R (previously grade 3B) rejection with diffuse infiltrates, myocyte necrosis, and disruption of cellular architecture. (Images provided by Dr. Margaret M. Grimes, Richmond, VA.)

dyspnea, and fatigue. Clinicians should always be wary of gastrointestinal complaints, including right upper quadrant tenderness, nausea, and vomiting, which may be related to right ventricular dysfunction and elevated central venous pressures. Any of the above findings, especially early after transplantation or in the setting of medication non-compliance, should be presumptively treated with intravenous steroids and worked up with an endomyocardial biopsy.

Since the transvenous bioptome was first devised in Japan and modified by Caves and colleagues at Stanford University, endomyocardial biopsy has been the gold standard for surveillance of the host's immune response toward the graft [23,24]. The contribution of surveillance endomyocardial biopsies to the overall success of heart transplantation, especially in the precalcineurin inhibitor era, cannot be overstated. However, despite the low risk of complications, the use of frequent endomyocardial biopsies has been criticized for high cost, patient discomfort, and questionable clinical utility in the modern immunosuppressive era. The incidence of biopsy-proven rejection during surveillance in clinically asymptomatic patients in the first year after transplantation was less than 2% with contemporary immunosuppression. Furthermore, the clinical significance of low and moderate grades of rejection discovered on surveillance biopsies is uncertain because nearly 85% of patients diagnosed with moderate, cellular rejection have spontaneous resolution of the histopathologic findings without any medical intervention [25]. Other issues include the fact that the distribution of inflammatory cells in the myocardium can be heterogeneous, which can lead to false-negative biopsies [26], and that—even when adequate samples are obtained—there can be high interobserver variability in the interpretation of moderate grade rejection [7].

In spite of these limitations, endomyocardial biopsy continues to be the primary tool for allograft rejection surveillance due to the lack of specificity and sensitivity in the available alternatives. However, these concerns and considerations have prompted further investigation into non-invasive and laboratory-based surveillance techniques, as described next.

Electrocardiography

While surveillance ECGs help identify conduction and rhythm disturbances associated with severe acute cardiac rejection, the findings are typically non-specific. Furthermore, the sensitivity of this method for early or moderate rejection, especially with contemporary immunosuppression, is poor [27,28]. During an episode of acute rejection, myocardial edema caused by inflammation and cellular infiltration may alter conduction properties of the myocardium. Rightward deviation of the QRS axis and decreased ECG voltage are classically described features of acute rejection [1–4].

Echocardiography

Two-dimensional and Doppler echocardiography of the allograft can identify changes in myocardial structure and function associated with acute rejection.

It is imperative to understand the normal echocardiographic findings after heart transplantation in order to appreciate changes associated with rejection. In comparison to age-matched normal controls, transplanted hearts have several distinguishing features. The ventricular mass is increased and the myocardial walls are at peak thickness immediately after surgery, and then gradually decrease over time. While the left ventricular cavity size and systolic function are usually normal early after transplant, the right ventricular cavity dimension can be dilated and the systolic function reduced. The reason for early right ventricular dysfunction is not clear but may relate to the fact that the right ventricle is more sensitive to ischemic injury, brain-death-mediated catecholamine injury, and the increased pulmonary resistance seen in some recipients. Usually, early right heart dysfunction is transient and improves over the first few weeks to months after transplantation.

If a bi-atrial anastomosis is performed, the atria will appear elongated and hourglass-shaped and an echodense cuff will identify the suture line. The intraventricular septum will have a paradoxical motion due to anterior swinging motion of the allograft which, unlike the native heart, is not adherent to the posterior thoracic wall [29–32].

Color flow interrogation of the tricuspid valve will frequently reveal significant tricuspid regurgitation immediately after transplantation [33]. As the allograft's right ventricular function improves and the recipient's pulmonary vascular resistance decreases, the severity of the regurgitation diminishes. Persistent tricuspid regurgitation resulting from continued pulmonary hypertension, endomyocardial biopsy-induced chordal injury, post-surgical annular remodeling, or right ventricular dysfunction is associated with a less favorable outcome [34].

Two-dimensional echocardiography can detect changes in cardiac structure and function; however, these parameters lack sensitivity (<50%) for the reliable early detection of rejection, making

it untrustworthy as routine screening tools [35]. Rejection causes an acute increase in ventricular mass and myocardial wall thickness [4,36]. Decreased left ventricular systolic function is usually apparent only in severe rejection. Right ventricular dysfunction may, however, be more pronounced even during mild episodes of rejection and a pericardial effusion often develops with inflammation [31,37,38].

During an episode of rejection, diastolic dysfunction precedes systolic dysfunction. Echocardiographic indices of diastolic filling properties may detect early acute rejection. Doppler measurements of blood flow properties reveal a profile similar to patients with a restrictive cardiomyopathy. Acute rejection has been associated with shortening isovolumetric relaxation time (IVRT), decreasing pressure half time (PHT) of the early mitral inflow velocity (Em), increasing ratio of early to late mitral inflow velocities, and sustained flow reversal of the atrial component of pulmonary venous flow [37–42]. However, these variables have limited reliability because they are influenced by loading conditions [42]. Tissue Doppler imaging is less influenced by hemodynamic factors, and measures decreased diastolic and systolic velocities of the mitral annulus and posterior left ventricular wall during acute rejection [43]. However, the specificity of theses indices is also limited because the diastolic properties of the allograft are modulated by other factors including immunosuppression-related hypertension, left ventricular hypertrophy, and fibrosis from previous rejection episodes. Accordingly, variable diagnostic accuracies have been reported and studies evaluating the role for screening serial echocardiographic evaluations have been inconclusive [40,44–48].

Magnetic resonance imaging

Cardiac magnetic resonance imaging (CMR) is emerging as a powerful diagnostic tool for cardiac disease. CMR has superior image quality and spatial resolution and has become the reference standard for evaluating ventricular mass and systolic function [49]. CMR is an emerging tool in patients with coronary disease for the assessment of regional wall motion, myocardial viability, and ischemia [50–52].

The utility of CMR in patients with heart transplantation, however, is yet to be defined. Cardiac magnetic resonance imaging, compared to echocardiography, has greater reproducibility for the measurement of allograft myocardial mass, systolic ejection fraction, and ventricular volumes [53]. However, changes in these parameters usually identify only late, severe rejection [54]. During acute allograft rejection, the increased water content of the myocardium and inflammation can also prolong the T2 relaxation time (measured by spin-echo techniques). Early animal studies showed that T2 relaxation times are prolonged and wall thickness is increased in dogs with severe rejection who are not receiving immunosuppression [55]. However, in humans treated with modern immunosuppressive therapies, studies with this technique have shown conflicting results precluding clinical application [56–59].

Magnetic resonance imaging techniques that detect the accumulation of the tagged immune cells within cardiac allograft are an area of active investigation [60,61].

Biomarkers

Elevation of markers of myocyte injury, including creatine kinase MB isoforms and troponin, have been observed to be increased in severe forms of allograft rejection [62]. Levels are seen to be increased uniformly in all patients early after transplant, presumably related to preoperative ischemic time [63,64]. Patients who continue to have elevated or detectable troponin concentrations—likely indicating microvascular disease and myocyte necrosis—are more likely to develop early angiographic coronary graft vasculopathy or allograft failure [63].

In general, markers of myocyte necrosis are detectable only in more severe cases of allograft rejection and are too insensitive for routine surveillance [65,66]. Contemporary ultrasensitive troponin assays may have clinical utility, but have yet to be comprehensively studied.

B-type natriuretic peptide (BNP) is an amino acid polypeptide secreted by myocytes in the heart in response to cardiomyocyte stretch. BNP and amino-terminal-BNP (NT-BNP) have been identified as useful markers for the diagnosis and prognostication of patients with heart failure. BNP concentrations have been observed to be elevated early after heart transplantation and seem to plateau by 4 to 6 months after surgery [67,68].

Elevated BNP concentrations correlate with echocardiographic findings of acute rejection, including increased wall thickness, ventricular mass, right ventricular dilation, and right ventricular dysfunction [69]. In patients with apparently normal ventricular systolic function, BNP concentrations correlate with hemodynamic derangements including elevated filling pressures, decreased cardiac output, and restrictive filling pattern [69,70]. However, BNP concentrations can be increased independent of echocardiographic hemodynamic measurements, suggesting alternative, stretch-independent pathways of peptide secretion [71]. In fact, BNP secretion in heart transplant patients is independent of other stretch-sensitive markers, including atrial natriuretic factor [72].

In an investigation of peripheral gene expression in clinically stable heart transplantation recipients with normal filling pressures and cardiac function, genes up-regulating immune activity, and specifically those related to the HLA system, mast cells, heat shock protein, and B-cell lineage, were correlated with BNP concentrations [73]. Cytokine-mediated BNP secretion may occur through a common p38 signaling pathway that links inflammation to cardiac peptide secretion [74]. Together, these data suggest that in addition to hemodynamic derangement associated with myocardial rejection, the inflammatory process modulates and up-regulates BNP secretion.

Elevated BNP levels portend a poor prognosis in allograft dysfunction in the first year and beyond after heart transplantation [75,76]. While increased BNP concentrations in heart transplant patients have been correlated with episodes of acute allograft rejection, the discriminatory power is poor (c-statistic: 0.71–0.76), much less than for diagnosing acute heart failure in a patient presenting with acute dyspnea [77,78]. Therefore, BNP has not replaced endomyocardial biopsy as a surveillance tool.

Invasive hemodynamics

Early after transplantation, patients often have a restrictive filling pattern with elevated filling pressure observed during invasive hemodynamic measurement. A non-compliant right ventricle may cause a Kussmaul's sign (increase in right atrial pressure during inspiration) and the right atrial pressure may often be elevated with prominent "a" and "v" deflections and a rapid "y" descent. These early hemodynamic abnormalities may be attributed to postoperative myocardial stunning, rejection, pre-existing recipient pulmonary hypertension, donor catecholamine surge, and/or intraoperative volume expansion [79–82]. Although intracardiac pressures usually approach the normal range within a few months of surgery, they

may not completely correct even in the absence of biopsy evidence of rejection or fibrosis [81]. The restrictive filling pattern typically disappears months after transplantation, but can be unmasked with volume loading [82].

During episodes of acute rejection, the thin-walled right ventricle, having less functional reserve, is more vulnerable to injury and becomes edematous and non-distensible. Findings consistent with restrictive filling may become apparent in hemodynamic tracings. Invasive hemodynamics often reveal elevations in filling pressures. The cardiac output is decreased in severe episodes of rejection. Improvement in filling pressures may accompany treatment of rejection and resolution of acute inflammation; however, hemodynamic abnormalities may persist, possibly related to irreversible myocardial injury and fibrosis [83,84].

Gene expression profiling

Gene expression profiling refers to the comparison of mRNA production from many genes to identify gene activation patterns associated with a disease process or a clinical event. Acute rejection-related up-regulation of gene expression in various signaling pathways has been observed in studies of allograft tissue and peripheral blood [85–89]. Through a gene-discovery process with microarray analysis, a clinically applicable scoring system and commercial test has been devised to predict cellular rejection in cardiac allografts. A set of 11 genes measuring macrophage activation, steroid responsiveness, platelet production, hematopoiesis, T-cell activation, and cell morphology were identified and a scoring system for gene expression was validated for predicting allograft rejection [90].

A non-invasive surveillance strategy for allograft rejection comparing gene expression profiling to endomyocardial biopsy was evaluated in the Invasive Monitoring Attenuation through Gene Expression trial (IMAGE) [91]. The IMAGE trial evaluated low-risk heart transplant patients who were predominantly greater than 1 year after orthotopic heart transplantation and without any history of rejection, vasculopathy, or allograft dysfunction. In the selected population, the two groups had similar event rates (14.5% vs. 15.3%) of the composite primary endpoint: greater than 25% decrease in left ventricular ejection fraction, any rejection with hemodynamic compromise, or all-cause mortality.

This was the first trial to compare an alternative surveillance mechanism to the long-accepted standard of endomyocardial biopsy; however, further study is necessary to implement this testing in higher-risk patients closer to the time of heart transplantation. The score is valid for surveillance of stable patients well after heart transplantation and should not be performed in close proximity to a blood transfusion or treated rejection. It is also invalid for patients receiving more than 20 mg of corticosteroids daily due to the steroid-responsive nature of the test. Further comparisons of gene expression profiling to serial biopsy could establish this surveillance modality as being advantageous to the patient (less invasive) and the hospital (less cost).

Antibody-mediated rejection

The concept and existence of non-cellular, antibody-driven cardiac allograft rejection has been proposed and debated for many years. In1983, Hess and Mohanakumar made the observation that antibodies directed toward B cells may play a role in acceleration of the atherosclerosis in the transplanted heart [92]. Herskowitz et al. went on to observe that there was a type of rejection associated with arteriolar vasculitis found on endomyocardial biopsy in patients with allograft failure [93]. Later, Hammond et al. described a pattern of rejection consisting of immunopathologic findings of immunoglobulin deposition and complement fixation on immunofluorescence, along with histopathologic findings of endothelial swelling and interstitial edema on routine light microscopy [94]. The pathologic findings of "humoral" rejection were associated with decreased survival compared to those with cellular rejection. These and other early works set the stage for years of debate, eventually leading to the growing acceptance of antibody-mediated rejection (AMR) as a clinically significant entity.

During AMR, antibodies specific for the donor allograft cause acute rejection by binding to the allograft and amplifying the immune response (see Chapter 6). In severe cases, this leads to acute allograft dysfunction and increased risk of coronary artery graft vasculopathy.

Due to the absence of practice guidelines for surveillance and diagnosis, AMR has been a diagnostic and therapeutic challenge after cardiac transplantation. The epidemiologic characteristics of AMR, including disease incidence, prevalence, and rate of progression, are unclear due to wide variation in surveillance patterns, histopathologic diagnostic criteria, and technical aspects of histopathologic and immunopathologic preparation of biopsy samples. The reported incidence of "non-cellular" rejection or AMR has been variable depending on clinical and histopathologic criteria employed; however, the estimated incidence varies from 8% to 20% with a higher prevalence in the first 6 months after heart transplantation [95,96]. Unlike the decrease observed in the incidence of cellular rejection, the incidence and morbidity associated with AMR has remained unchanged [97].

Patients with presensitizing events are at risk for AMR. For example, the incidence of AMR is higher in female patients, recipients with positive cross-matches, and/or elevated panel-reactive antibodies [95,96]. Other risk factors for AMR include blood product transfusions, mechanical circulatory support devices, pregnancy, and infection.

Diagnosis

The clinical and diagnostic criteria for cardiac allograft AMR are evolving. The inclusion criteria for early reports were variable. Most studies required that patients have evidence of antibody-mediated injury on endomyocardial biopsy, clinical diagnosis of allograft dysfunction, and/or serological evidence of circulating autoantibodies. Similar to cellular rejection, the current guidelines proposed by the ISHLT have made AMR a purely pathologic diagnosis, allowing for the diagnosis of clinically quiescent humoral rejection (Table 71.2).

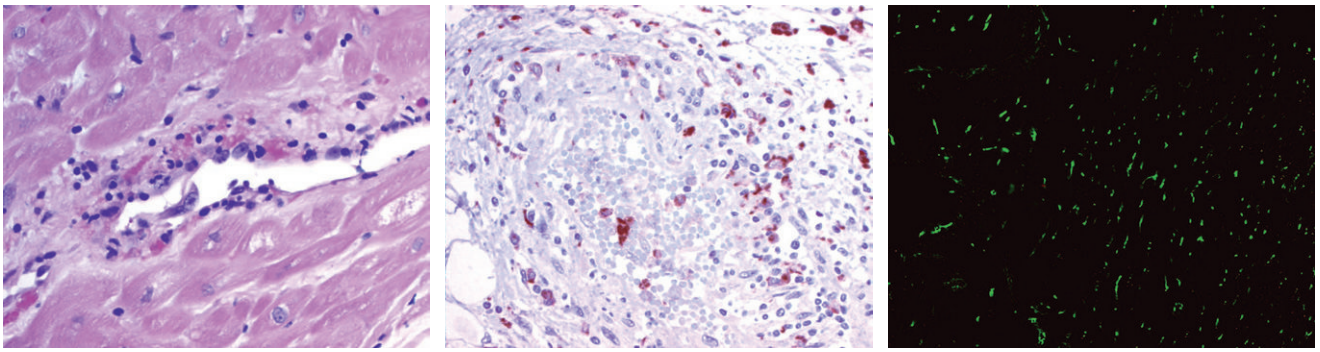
Clinical findings

The diagnosis of AMR has traditionally been considered after the development of hemodynamic derangements or unexplained allograft dysfunction. Early clinical signs and symptoms of AMR can be subtle, non-specific, and easily overlooked. Patients may present with fatigue, weakness, or nausea and vomiting. They may have right upper quadrant pain from liver capsule tenderness due to hepatic congestion. However, with better histopathologic markers, appreciation for early or asymptomatic antibody-mediated rejection has been growing. While the clinical relevance of this entity is yet to be established, there are growing data suggesting that the detection of early presymptomatic AMR on surveillance endomyocardial biopsy identifies patients at higher risk for development of

Table 71.2. Histopathologic criteria for the diagnosis of antibody-mediated rejection

ISHLT working formulation 2004	Pathology findings	Proposed criteria 2011	Pathology findings
Grade AMR 0	No evidence of antibody-mediated rejection	Grade pAMR 0	No evidence of antibody-mediated rejection
Grade AMR 1	Histologic criteria: evidence of endothelial swelling and intravascular macrophage accumulation; severe cases will have neutrophil invasion and interstitial hemorrhage Immunopathologic criteria: immunoglobulin and complement staining of the capillaries (IF) *OR* positive CD68 staining for macrophages + complement deposition the capillaries (IC), or fibrin staining in the vessels	Grade pAMR 1 (I+): histopathologic AMR alone	Only histologic criteria for AMR are present Although not specifically defined, may include: endothelial edema, intravascular macrophage accumulation, interstitial edema, hemorrhage, intravascular thrombi and myocyte necrosis
		Grade pAMR 1 (I+): immunopathologic AMR alone	Only immunopathologic criteria for AMR are present Yet to be defined, but may include IC and IF stains for immunoglobulins, complement and macrophages
		Grade pAMR 2 pathologic AMR	Both histologic and immunopathologic criteria are met
		Grade pAMR 3 severe AMR	Histopathologic findings of marked edema, interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis, or karyorrhexis

ISHLT, International Society for Heart and Lung Transplantation; AMR, antibody-mediated rejection; pAMR, pathological antibody-mediated rejection.



(A) (B) (C)

Figure 71.3. (A) Hematoxylin and eosin stain showing histologic evidence of AMR: endothelial edema and inflammation including neutrophils. (B) Immunoperoxidase staining in AMR that is positive for CD68, identifying the intravascular cells to be macrophages. (C) Immunofluorescence positivity for C4d in AMR, indicating complement deposition on the capillary walls. (Images provided by Dr. Margaret M. Grimes, Richmond, VA.)

cardiac allograft vasculopathy (CAV) and allograft-related mortality [98,99].

Circulating HLA and non-HLA antibodies

The development of circulating donor-specific alloantibodies (see Chapter 36) in the recipient blood is supportive, but not required, for the diagnosis of AMR. However, the presence of antidonor HLA antibodies after heart transplantation has been associated with risk of AMR and the development of allograft vasculopathy [100–102].

The widespread application of solid-phase assays has allowed for reliable identification and quantification of circulating donor-specific HLA antibodies. But the quantification techniques are not standardized and their clinical application has yet to be validated. Cell-based assays are used in a complementary manner to confirm whether detected antibodies are donor-antigen reactive and thus clinically relevant.

Antibodies can also develop against non-HLA antigens and pose a diagnostic challenge, possibly accounting for cases of unexplained allograft dysfunction. There are published reports of antibodies toward major histocompatibility class I-related chain A (MICA), antiphospholipid antibodies, endothelial cells, vimentin, nuclear antibodies, and myosin heavy and light chains [102–106]. Routine testing for non-HLA antibodies is not recommended at this time and may be reserved for scenarios of unexplained allograft dysfunction.

Endomyocardial biopsy

Early AMR is associated with capillary injury, endothelial swelling with nuclear enlargement, and accumulation of macrophages within the vasculature (see Chapter 83). The finding of vascular adherence of macrophages or endothelial edema (Figure 71.3A,B) has unacceptably low sensitivities to be meaningful as a screening test alone [107]. Findings of intravascular thrombi, fibrin deposition, myocyte necrosis, interstitial hemorrhage, and degeneration of endothelial cells, however, are typically seen only in the most severe form of AMR. Additional immunopathologic assessment is routine at the time of tissue evaluation for AMR. The most widely accepted standard for immunophenotypic evidence includes staining for complement split product (C3d, C4d) in myocardial capillaries, while some centers also stain for macrophages (CD68) and immunoglobulin (Figure 71.3C).

Despite years of debate, the ISHLT only in 2005 recognized AMR as a specific histologic entity [8]. In 2011, the ISHLT modified the pathologic diagnostic criteria and proposed a preliminary grading scale for AMR. The working group excluded requirements for allograft dysfunction or the presence of circulating donor-specific antibodies for the diagnosis of AMR, making it strictly a pathologic diagnosis [108]. The preliminary categories proposed have yet to be validated for clinical use, but are composed of a 0 to 3 grading scale. A grade 0 represents a biopsy sample without any histologic or immunopathologic features of antibody-mediated rejection. Grade 1 is given to biopsy samples with features of either

histopathologic or immunopathologic features of AMR. Grade 2 has features of both histologic and immunopathologic AMR, and grade 3 is the most severe pathologic grade, which would include findings of interstitial hemorrhage, inflammatory infiltrates, endothelial cell thickness, and marked edema.

The frequency, timing, and utility of biopsy-guided surveillance in asymptomatic patients are not yet defined. Early immunopathologic staining for complement and immunoglobulin can yield false-positive results in patients treated with immunoglobulin-meditated induction therapies, and thus should be interpreted with caution.

Treatment strategies (see Chapter 67)

Clinically "quiescent" ACR

Patients with asymptomatic grade 1R cellular rejection on routine surveillance biopsy are treated with observation and without intensification of immune modulating therapies. Grade 1R rejection typically resolves spontaneously without progression or impact on allograft survival. Winters et al. observed that 85% of focal mild rejection resolved without any intervention, while Loveras et al. showed that intervention with prednisone in mild or moderate rejection offered no clinical benefit [25,109]. However, if any foci of myocyte damage/necrosis are present (previously grade 2), patients may be at higher risk of progressing to clinically significant rejection and warrant heightened surveillance [110].

If the endomyocardial biopsy reveals multiple foci of infiltrating inflammatory cells along with evidence of myocyte damage (grade 2R), patients are treated with brief pulse dosage of either oral or intravenous steroids and possibly intensification of maintenance immunosuppression. Doses of prednisone vary from center to center but typically range between 3 and 5 mg/kg total dose over several days with return to the patient's baseline prednisone dose afterwards. In a retrospective review of 100 patients with moderate-grade rejection, Park et al. reported a 75% response rate with histologic improvement in rejection after a 3-day pulse dose of 100 mg of prednisone [111]. Treatment is followed by repeat endomyocardial biopsy in 1 to 2 weeks. If the rejection has not resolved, treatment with pulse dose intravenous methylprednisolone is recommended.

ACR with hemodynamic compromise

Patients who present with pathological or clinical evidence of rejection accompanied by decrease in myocardial function should be managed with the highest urgency because the mortality associated with this condition can approach 40% at 3 months. Those with primarily cellular rejection, however, have a better prognosis with a 2-year survival near 84% [112]. Early hemodynamic support with intravenous inotropes, chronotropic agents, intra-aortic balloon pump support, or ventricular assist device should be instituted as warranted. In addition to immediate administration of intravenous steroids, it is appropriate to treat the patient with lymphocyte-depleting antibodies. Utilization of the murine monoclonal antibody, OKT3, has largely been replaced by the better-tolerated antithymocyte globulin (ATG). OKT3, which binds to the CD3 antigen on the T-cell surface, is associated with a high frequency of serum sickness and sensitization which precludes repeat dosing of therapy. Antithymocyte globulin is a polyclonal preparation directed at various antigens on the T-cell surface. Although better tolerated than OKT3, ATG is also associated with allergic immune-mediated reactions during drug infusion. Patients are treated with steroids, antihistamines, and antipyretic agents prior to infusion. CD3 cell count monitoring can be used to guide ATG dosing with a target of achieving an absolute CD3 count of less than 25 cells/μL [113]. During therapy the patient should receive antifungal, antibiotic, and antiviral prophylaxis, along with close hemodynamic surveillance. Repeat endomyocardial biopsies should be obtained in subsequent weeks to ensure resolution of rejection. Recurrent or persistent episodes can be treated with repeat intravenous steroid doses and courses of antilymphocyte antibodies, albeit the clinician must be wary of the increased risk of malignancies associated with lymphocyte-depleting therapies.

AMR with hemodynamic compromise or mixed rejection (ACR plus AMR)

Patients presenting with clinical evidence of AMR in the presence of cellular rejection or along with allograft dysfunction should have treatment directed at eliminating or inhibiting circulating antibodies and suppressing further antibody production. Initial treatment with intravenous steroids may have some beneficial effect through multiple mechanisms. Plasmapheresis or plasma exchanges are rapid ways to remove alloantibodies from circulation. There are various frequencies and treatment protocols; however, close surveillance for antibody levels and re-formation and concomitant intensification of immune-modulating therapies need to be instituted because rebound antibody production is common.

Intravenous immunoglobulin (IVIG) or cytomegalovirus hyperimmunoglobulin are often used together with plasmapheresis to treat AMR. Intravenous immunoglobulin has multiple anti-inflammatory and immune-modulating effects and may be beneficial in the setting of antibody-mediated rejection. Antibody preparations are predominantly IgG from pooled collections of plasma. Intravenous immunoglobulin is thought to work by removing potential circulating alloantibodies from circulation and down-regulating the effects of inflammatory cytokines; it has been used widely to treat antibody-mediated rejection due to its low side-effect profile. However, its efficacy has never been systematically proven in the setting of acute cardiac allograft AMR.

In addition to depleting antibodies, intensification of T-lymphocyte-directed inhibition with increased maintenance immune medications or ATG is often used.

Therapies directed at depleting B-cell lymphocytes or inhibiting plasma cells may be considered for patients unresponsive to standard interventions. Rituximab is a monoclonal antibody against CD20, a protein localized to the surface of B-cell lymphocytes. Rituximab has been used to decrease panel-reactive antibodies in sensitized patients prior to solid organ transplantation, and there is growing literature on its use for treating antibody-mediated rejection in cardiac allografts [114–116]. The marginal benefit of rituximab is unclear from these published reports because it has been used with multiple background therapies. Randomized data for the drug are lacking and enthusiasm is somewhat muted as the medication's efficacy is limited to depleting memory B-cells, with no effect on plasma cells.

There is growing enthusiasm for bortezomib, a proteasome inhibitor that binds the catalytic site of the 26S proteasome, which regulates protein expression and may prevent degradation of proapoptotic factors, permitting activation of programmed cell death in plasma cells. The medication has been effective for treating plasma cell disorders including AL type amyloidosis and multiple myeloma, and there is growing experience in solid organ transplantation for vascular rejection and desensitization [117–119]. Common

side-effects with bortezomib include thrombocytopenia, GI complaints, and paraesthesias.

Evolving therapies for AMR include complement inhibition with drugs such as eculizumab and novel B-cell suppressing agents including belimumab and epratuzumab.

Clinically "quiescent" AMR

There is a growing acceptance that clinically quiescent or subclinical AMR is a clinically relevant entity. Kfoury et al. studied nearly 900 patients and found that individuals with asymptomatic AMR, with or without evidence of concomitant cellular rejection, were at markedly higher risk for cardiovascular mortality [98]. Wu et al. showed that patients with clinically quiescent AMR were at increased risk of developing CAV [99]. While the histopathologic and immunopathologic findings of AMR on biopsy in asymptomatic patients identify those at higher risk, there are currently no data that support any alternative to continued medical therapy and surveillance.

Recurrent or recalcitrant rejection (AMR or ACR)

Recurrent rejection (repeat episodes despite augmentation of immunosuppression) or recalcitrant rejection (persistent pathologic finding of rejection without intervening rejection-free biopsy) are rare but challenging scenarios. Total lymphoid irradiation (TLI) has been available for several decades as a potential treatment for refractory rejection and is effective in treating persistent cellular rejection after cardiac transplantation [120,121]. Total lymphoid irradiation involves exposing all lymph nodes, the thymus, and spleen to high doses of radiation with the intention of altering T-lymphocyte-mediated immune actions. Enthusiasm for TLI has been tempered due to late recurrent episodes of rejection and increased risk of myelodysplasia and leukemia [122].

Photopheresis is an immunomodulating therapy in which leukocytes are treated with a photoactivatable drug and subsequent ultraviolet light exposure resulting in preferential damage to rapidly proliferating T-cell lymphocytes. Photopheresis has been effectively used to treat the patient with recurrent refractory cellular allograft rejection, and side-effects seem to be minimal [123–125].

There may be a role for splenectomy as rescue therapy for recurrent AMR because most plasma cells reside in the spleen before migrating to the bone marrow. Splenectomy has been performed in ABO-incompatible organ transplantation and the treatment of severe acute AMR after kidney transplantation [126].

Mimickers of allograft rejection

Clinicians must be cognizant of pathologic processes that may be misdiagnosed as allograft rejection, particularly because intensification of immunosuppression may worsen many of these conditions. Myocarditis from enterovirus infections may result in allograft dysfunction. Cytomegalovirus myocarditis has been observed after heart transplantation and can be distinguished from rejection on microscopy by identifying nuclear inclusions composed of large basophilic nuclei surrounded by a pale artifactual halo [127,128]. The frequency of this diagnosis has decreased with introduction and implementation of prophylactic antiviral therapies in high-risk populations. Reactivation or transmission of *Toxoplasma gondii* can lead to fulminate, disseminated disease or only involve one or two organ systems [129]. When the parasite causes myocarditis after heart transplantation, endomyocardial biopsy typically reveals non-specific inflammatory infiltrates with or without intramyocytic toxoplasmic cysts. Immunohistochemical staining and electron microscopy identifying intracystic oval-shaped organisms with double-layered pellicle and an anterior-placed conoid can confirm the diagnosis [130]. Post-transplant lymphoproliferative disorder (PTLD) involving the heart is characterized by infiltration of atypical lymphocytes. Identification of the Epstein–Barr virus with in situ hybridization can help with the diagnosis [131].

Conclusions

Advances in immunosuppression have decreased the incidence of traditional cellular rejection and focused attention the emerging importance of AMR. Despite improvements in non-invasive gene and biomarker-based surveillance strategies, the new epidemiology of AMR is not adequately addressed in the diagnostic realm. Collective pathologic consensus is now emerging to classify and standardize the recognition of clinical AMR, and its prognostic attributes in the short and long term are only now emerging. The challenge of therapy targeted to antibody suppression and elimination continues to vex investigators and clinicians alike, but targeted strategies using novel molecules and pathways are now emerging [132].

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CHAPTER 72

Clinical Allograft Rejection Syndromes in Lung Transplantation

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Introduction

Similar to other solid organ transplants, lung transplant recipients face the hurdle of repetitive immunologic attack upon the allograft. Roughly 30 years of clinical experience with lung transplantation has led to an appreciation of the various types of rejection. It is widely known that lung allografts are more susceptible to immunologic injury relative to other transplant types. There are several reasons that potentially underlie the high risk of rejection syndromes in lung transplantation. First, the lungs directly interface with the environment. As a result, lungs are vulnerable to pathogens that are acquired via the respiratory tract, including viral, fungal, and bacterial species. Further, as a respiratory organ, the lung is susceptible to triggers such as air pollution, which may provide danger signals to the immune system. Second, lungs develop during embryology as an embryonic foregut derivative. The airway maintains continuity through the digestive tract in postnatal life, and as a result humans remain susceptible to injury from aspiration of oral or gastric contents. Such aspiration also may trigger activation of the immune system. Third, lungs are relatively vulnerable to early ischemia reperfusion injury, which leads to recruitment of inflammatory mediators and cells into the lung early after transplantation. Finally, the surgical anastomosis in the bronchi causes a mechanical disruption in the mucociliary elevator that impairs the ability of the lungs to clear pathologic substances. This chapter will review the clinical aspects of the distinct types of rejection faced in lung transplantation and the immunopathologic mechanisms underlying them. Two distinct types of rejection, acute and chronic, will be separately explored, but it is important to note that these processes are mechanistically related. Throughout this chapter, risk factors associated with both acute and chronic rejection will be noted. Finally, treatment strategies for the different types of rejection will be addressed.

Acute allograft rejection

Diagnosis of acute rejection

The histopathology of acute and chronic allograft rejection is described extensively in Chapter 84. The key aspects of the pathologic scoring of acute cellular rejection (ACR) are that lung biopsy pieces can be scored for both a perivascular component (A-grade) and a bronchiolar component (B-grade) as defined by the Lung Rejection Study Group (LRSG). The major unresolved issues with respect to lung transplantation pertain to how to apply clinically this widely utilized histopathologic scoring system. Specific issues include the understanding of what constitutes an appropriate schedule for performing lung biopsies and how to treat patients in the event that rejection is detected.

The use of flexible fiberoptic bronchoscopy has allowed for the ability to periodically obtain small pieces of alveolated lung tissue for histologic assessment. Forceps biopsy of a single lobe or multiple lobes can be obtained with a relatively low risk of complications. Reported adverse effects from transbronchial lung biopsy include pneumonia (8%), pneumothorax (<3%), mild reversible hypoxemia (10%), and clinically significant bleeding defined as more than 100 mL (10%) [1–4]. There are no deaths specifically reported with the use of bronchoscopy in lung transplantation, but there is a very low overall risk of mortality (<0.02%) in the general pulmonary population [5]. Lung transplant centers employ one of two strategies to detect rejection: either protocol-determined periodic lung biopsies (termed surveillance biopsies) or biopsies in the setting of clinical deterioration (termed for-cause biopsies). The potential advantage of a surveillance biopsy approach is that subclinical ACR can be detected early. The unproven assumption with this approach is that it leads to improved outcomes in the long term, such as improved survival and maintenance of lung function. The theoretical advantage of a for-cause approach is that patients would only be subjected to an invasive procedure when the pretest probability of a positive finding (ACR and/or infection) is high. This would limit costs to the patient and system as well as decrease the potential for adverse outcomes. Of note, a recent analysis of 224 bronchoscopy procedures done as surveillance showed that the rate of A2 or higher rejection was 18.7% and that the results of such procedures altered clinical management 32% of the time [1]. These findings suggest that the pretransplant probability for rejection is relatively high even in otherwise stable patients. Yet in spite of this relatively high pretest probability for rejection and infection, small pilot studies have suggested that patients being managed using a for-cause approach have equivalent medium-term outcomes compared to subjects in whom lung biopsy is performed at regular intervals. Hence, at the present time, the question of whether surveillance or a for-cause approach to detection of ACR results in improved patient outcomes remains unsettled.

For-cause lung biopsy of the transplant patient should be considered in cases of clinical decline. These may include declining

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spirometric values (discussed later), breathlessness, radiographic infiltrates such as ground glass opacities and pleural effusions, and systemic manifestations of an inflammatory process such as fever. In virtually all cases where for-cause bronchoscopy with lung biopsy is performed the differential diagnosis will include both ACR and lung infection.

The technique for performing transbronchial lung biopsies as surveillance or for-cause involves obtaining multiple pieces of alveolated lung tissue. This requires use of forceps placed via the bronchoscope and generally guided by uniplanar real-time fluoroscopy. While ACR could be involving any region of the lung, many clinicians feel the dependent portions of the lung have a higher incidence of ACR [6], and hence some biopsy pieces should be taken from one of the lower lobes. The total number of biopsy pieces to be taken is also uncertain. The LRSG recommends obtaining at least five pieces of alveolated lung tissue containing at least 100 alveolar air sacs, and processing this tissue immediately in formalin to preserve the alveolar architecture. In contrast, Snell and colleagues extrapolating from older literature recommend ten biopsies as a reasonable target [7,8]. An early study of lung transplant recipients reported that a mean of 17 biopsies were needed for a sensitivity of 94% and a specificity of 90% for the detection of ACR [7], a level of sampling which is not generally undertaken. An important aspect to considering transbronchial biopsy as the gold standard for rejection is understanding the consistency of interpretation between pathologists. The largest study to date aimed at assessing consistency of evaluation across different institutions showed that the kappa statistic for A grade rejection was modest at best (kappa = 0.426; a kappa of 0 indicates chance agreement and a kappa of 1 indicates perfect agreement) [9]. Further, this level of agreement exists only for biopsy samples felt to have adequate tissue for interpretation. Other smaller studies have mirrored the modest degree of interobserver agreement [10–12]. Hence, it is important to understand that when the pretest probability for ACR is high, lung biopsies that are reported as negative for ACR do not completely rule out ACR being present, particularly when the quality of the sample is poor.

Diagnosis of antibody-mediated rejection

The diagnosis of humoral rejection in lung transplantation is considerably less well defined and more controversial than that of other transplanted organs such as heart and kidney. Consensus exists within the transplant community that antibody-mediated rejection in solid organ allografts generally manifests as capillary endothelial cell injury. Hence much work has been devoted to understanding how endothelial antigens promote antibody binding, complement activation, and subsequent tissue injury [13,14]. Within the lung transplant community there is a growing consensus that the humoral component of the immune system does contribute to progressive loss of lung function over time. The primary controversy currently revolves around the concept of how to reliably detect antibody-mediated rejection within sampled lung biopsy histology specimens and whether an antibody-mediated acute rejection process exists as a separate entity distinct from ACR. The evidence that antibody responses are important for graft function is largely circumstantial, but convincing in aggregate. First, it has been shown in rare cases that hyperacute rejection characterized clinically by respiratory failure and pulmonary edema can be attributable in some cases to the presence of pre-existing antidonor antibodies [15–18] which were present but not detected prior to transplant. Second, antibody-mediated rejection is well described in both heart and lung transplant, as reviewed elsewhere in this textbook. Antibody-mediated rejection is known to contribute to loss of graft function for both heart and kidney allografts, so it is plausible that similar processes would exist in lung transplantation. Third, several studies have linked pretransplant panel reactive anti-HLA antibodies (PRA) to subsequent outcomes following lung transplantation. For example, in one large retrospective study of 656 subjects, patients who had a pretransplant PRA of >25% had a median post transplant survival of 1.5 years compared with 5.2 years in patients with lower or negative PRAs [19]. Similar findings were reached utilizing the UNOS registry of lung transplantation in which over 10 000 lung transplant cases were analyzed. In this analysis, high level PRAs conferred a 30-day mortality hazard ratio of 2.6 [20]. Fourth, the development of PRAs after transplant has been linked to ACR, lymphocytic bronchiolitis, and bronchiolitis obliterans syndrome (BOS) in numerous studies. Finally, the development of de novo donor-specific anti-HLA antibodies (DSA) has been strongly linked to the subsequent development of BOS [21]. Patients who were successfully treated with a protocol to decrease DSA had improved survival compared with patients in whom this treatment was unsuccessful.

Given the strong association between anti-HLA antibodies and various outcomes in lung transplantation, considerable interest has developed in trying to detect direct antibody injury in lung biopsy specimens and to develop a pathologic grading system for antibody-mediated rejection (AMR). At the present time the LRSG does not recognize a pathognomonic pattern of AMR, but there have been proposals to do so. The major pathologic hurdle to diagnosis of AMR in lung transplantation is that C4d deposition, which characterizes AMR in renal and cardiac transplant AMR lacks specificity in lung tissue. Early descriptions of AMR in lung transplantation relied on the use of immunofluorescence (IF) of frozen lung tissue. In these small studies utilizing relatively large biopsy samples, patients who had acute cellular rejection uniformly also demonstrated septal capillary C4d deposition [22]. These findings have been difficult to validate by other groups. For example, a relatively large study of 68 lung transplant patients, which utilized immunohistochemistry (IH) of previously paraffin embedded lung transplant biopsies, demonstrated that AMR with isolated C4d deposition in the capillaries and with relative sparing of venous or arterial endothelium was quite rare and did not correlate with acute cellular rejection. Further, similar lung samples from non-transplant subjects showed a comparable degree of diffuse C4d deposition, indicating that this phenomenon is not specific for an antibody-mediated rejection process [23].

Despite the lack of clear diagnostic criteria for AMR in lung transplantation, several case reports have highlighted the features of what the syndrome might look like clinically. Girnita published a case report on two patients who developed donor-specific anti-HLA antibodies and, on transbronchial lung biopsy, showed subendothelial capillary C4d deposition [24]. Both of these patients demonstrated worsening graft survival, which was refractory to augmented immunosuppression and eventually experienced total allograft loss. A similar case reported by Morrell described early graft worsening in a patient who developed donor-specific antibodies. Lung pathology showed C4d endothelial deposition, and the patient responded clinically to therapies implicated in antibody removal: IVIG, plasmapheresis, and anti-CD20 monoclonal antibody therapy [25]. Finally, Astor reported on a pediatric lung recipient whose lung biopsy showed C4d deposition associated with significant B cells populating the graft and the development of

antidonor HLA antibodies in the circulation. This patient also responded to antibody-lowering therapy [26]. Collectively, what these cases demonstrate is that while detection of AMR by histology may be difficult, AMR should be considered in the context of donor-specific anti-HLA antibodies, clinical worsening, a lack of responsiveness to traditional therapy for rejection, and a lung biopsy that does not show features of typical ACR.

Risk factors for acute rejection

An extensive literature links many patient factors to a risk of developing ACR. In broad terms these risk factors include immunosuppression, genetic variability, antigenic disparity between donor and recipient, and post transplant clinical factors. With respect to immune suppression, the vast majority of lung transplant recipients are treated with maintenance immunosuppression including a calcineurin inhibitor. Two studies have demonstrated superiority of tacrolimus (TAC) over cyclosporine (CSA) with respect to ACR risk, although this effect is modest—a 7–22% reduction in ACR incidence in the first year post transplant [27,28]. A significant ongoing question in lung transplantation centers is what constitutes the optimal lymphocyte depletion therapy at the time of transplant. Various types of induction agents are used across transplant centers, including polyclonal antithymocyte globulin, selective anti-IL2 receptor monoclonal antibodies such as basiliximab and daclizumab, and the potent panlymphocyte depleting agent alemtuzumab. Some centers do not employ any induction therapy. With respect to risk of ACR, several studies support the concept that induction is associated with a decreased incidence of ACR [29–32], and the ISHLT registry data show that patients who received induction therapy with anti-IL2 receptor agents had a modest decreased rate of ACR compared with no induction [33]. Similar to other solid organ transplants, a significant degree of HLA antigenic disparity between donor and recipient exists in the vast majority of cases. The degree of HLA mismatch of HLA A, B, and DR alleles has been linked to the incidence of ACR [34–36]. Several genetic polymorphisms with high frequency within the general population have been linked to ACR incidence. Genetic polymorphisms involving the toll-like receptor 4 protein, the CD14 lipopolysaccharide receptor, the CCL4L chemokine, and the cytokine IL-10 have been linked to alterations in ACR risk [37–40]. Finally, there is now evidence that gastroesophageal reflux disease (GERD), a risk factor for chronic rejection, may also increase the incidence of ACR [41].

Treatment of acute rejection

The vast majority of patients with ACR can be successfully managed by augmenting immune suppression. Nearly every center will elect to treat A2 and higher grade ACR episodes, with the main point of controversy being how to manage A1 rejection. Given the association of even minimal rejection and chronic lung failure (discussed later), some programs have taken an aggressive stance to minimal rejection. First-line therapy consists of intravenous methylprednisolone dosed at 10–15 mg/kg per day for 3 days. While this regimen is used across multiple centers worldwide, there exist no data to prove that this is the optimal dosing strategy. One prospective study has shown that time after transplant negatively correlates with steroid responsiveness: 85% of patients within the first year of transplantation demonstrated a significant response to high-dose corticosteroids, while only 55% responded to steroids for the treatment of ACR in patients more than 2 years out from their transplants [42]. The major adverse effects of such pulse corticosteroids are transient hyperglycemia, which may require insulin therapy, as well as a temporary increase in latent CMV reactivation. Most programs will institute temporary CMV prophylaxis for serologically at-risk patients in the weeks following such steroid pulses.

Persistent ACR, defined as any grade of rejection after conventional high-dose corticosteroids, can be managed with a variety of treatment modalities, including a second round of high-dose corticosteroids. Other therapies that have shown promise include the use of polyclonal antithymocyte globulins (ATG), monoclonal antibody to CD3 (OKT3), as well as the humanized monoclonal antibody to CD52 (alemtuzumab). All of the reports on rescue agents for refractory ACR are limited to small groups of patients, and as a result there remains no standard second-line agent for ACR beyond steroids. In addition to targeted antilymphocyte therapy, some clinicians recommend changing the baseline immune suppression in cases of refractory ACR: for example, exchanging CSA to tacrolimus or exchanging azathioprine to mycophenolate [43,44]. For very severe cases of refractory rejection, additional adjunctive rescue methods have been described including the use of total lymphoid irradiation and extracorporeal photopheresis [45,46].

Chronic allograft rejection

The majority of patients who undergo lung transplantation enjoy a significant increase in their quality of life. This is related to a decrease in the degree of breathlessness experienced as well as liberation from the need for chronic exogenous oxygen therapy. Spirometric testing of lung transplant recipients is the main monitoring tool used to measure lung function over time, with two specific measures, the forced expiratory volume in 1 second (FEV_1) and the forced vital capacity (FVC) being the most widely utilized. A third spirometric measure, the forced expiratory flow between 25% and 75% FVC ($FEV_{25–75}$) is a third value sometimes utilized. The majority of lung transplant patients achieve peak lung function between 6 and 12 months following transplantation. Chronic rejection occurs when lung function begins to decline after such a peak has been attained. The classic histopathologic finding in chronic rejection is luminal obliteration of the terminal (non-cartilaginous) airway. This is termed bronchiolitis obliterans (BO). Importantly, the pathology of BO is distinct from the pathology of A or B grade ACR. BO lesions are discreet anatomically, involving the terminal airway, without involvement of larger cartilaginous airways, the alveolar airspace or the pulmonary interstitium. Further, while ACR is characterized by an influx of perivascular or peribronchiolar lymphocytes, BO is characterized by a relative paucity of lymphocytes. The obstructing lesions of BO may be concentric, or eccentric. Additionally, foamy macrophages are sometimes associated with BO lesions. Because of the extensive fibrotic nature of the BO lesions, much work has focused on determining the origin of these fibroblasts, which are sometimes referred to as myofibroblasts due to their elongated structure and significant actin content. Theoretically, the myofibroblasts could arise from several sources. First, they may derive from donor resident fibroblasts present from the time of transplant. Second, they could be recipient-type cells derived from a circulating bone marrow-derived precursor termed the fibrocyte. Third, because they are present at areas where airway injury may have previously occurred, they may derive from previous bronchiolar epithelial cells in a process termed epithelial–mesenchymal transformation. None of these possibilities is mutually exclusive and there is circumstantial evidence that all the processes may be involved.

BO lesions are sometimes seen on transbronchial lung biopsy specimens, and have therefore been assigned a grading schema by the LRSG. However, it is important to note that the pathologic lesions of BO can be quite patchy. As a result, transbronchial lung biopsy has a relatively poor negative predictive value for the diagnosis of BO. This limitation has necessitated the development of a surrogate clinical designation for the diagnosis of chronic rejection termed bronchiolitis obliterans syndrome (BOS). The classification system for BOS as determined by the ISHLT has undergone two iterations to date, most recently in 2003 [47].

As shown in Table 72.1, BOS is defined as being present when the post-transplant FEV_1 drops below 80% of the best post-transplant value. Because early BO causes partial luminal obstruction, the loss in FEV_1 is generally out of proportion to the *FVC*, which is a much more resistant to changes in airflow resistance. Hence, one clue that BOS is developing will be a gradual loss in 1-second airflow by spirometry, which is manifest as an inward bowing of the expiratory limb of the flow volume loop, as shown in Figure 72.1. Many processes distinct from fibroblastic obliteration of the terminal airway can result in a decreased FEV_1. Therefore, in order to make the diagnosis of BOS, other etiologies must be ruled out. Common causes of airflow loss include entities such as ACR as well as infectious etiologies. Thus a standard approach to a patient experiencing a loss of airflow is to perform radiologic studies as well as bronchoscopy. The later affords the opportunity to assess for both an infectious etiology as well as rejection. It essential to remember that many other etiologies can result in a loss of airflow and should be considered prior to labeling a patient as having BOS. Airway stenotic lesions, typically at the bronchial anastomotic site, can result in a loss in FEV_1. Lung transplant recipients are at risk of developing focal narrowing at the sites of the bronchial anastomoses, as the bronchial arteries feeding these areas are not re-anastomosed during transplantation. Such anastomotic strictures can be appreciated on CT imaging as well as bronchoscopy (Figure 72.2). Body habitus can contribute to a patient's spirometry. Significant obesity will often result in decreased FEV_1. One clue that worsening obesity is the cause of declining spirometry is that both the *FVC* and FEV_1 will be reduced to a similar degree, but the $FEF_{25–75}$ is unchanged. Of all the spirometric values used to monitor lung transplant patients, the $FEF_{25–75}$ is the most

Table 72.1. Staging criteria for bronchiolitis obliterans syndrome

Stage	Criteria
BOS 0	$FEV_1 > 90\%$ of baseline and $FEF_{25–75} > 75\%$ baseline
BOS 0p	FEV_1 81–90% baseline and/or $FEF_{25–75} < 75\%$ baseline
BOS 1	FEV_1 66–80% baseline
BOS 2	FEV_1 51–65% baseline
BOS 3	$FEV_1 < 50\%$ baseline

BOS, bronchiolitis obliterans syndrome; FEV_1, forced expiratory volume in 1 second; $FEF_{25–75}$, forced expiratory flow between 25% and 75% *FVC*.

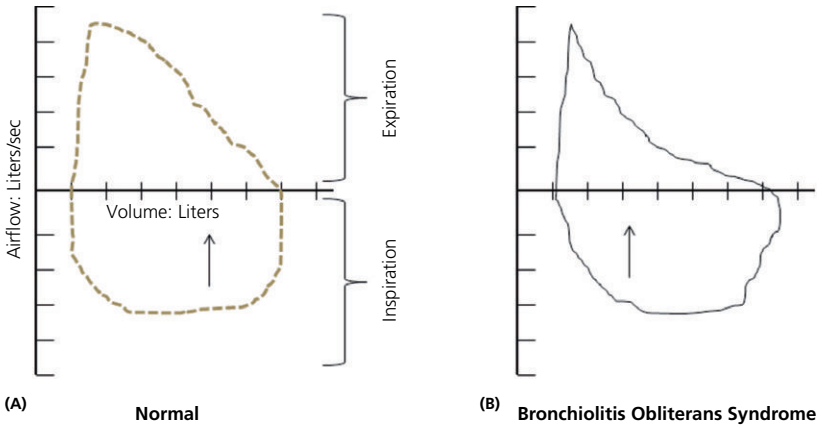


Figure 72.1. Spirometry trends after transplant. The flow/volume loops compare lung volumes (x axis) as a function of airflow (y axis). The tracing above the x axis depicts expiration while the tracing below depicts inspiration. In the normal patient (A), the FEV_1 (arrow) occurs at a relatively high lung volume. In the setting of small airway obliteration, as seen in bronchiolitis obliterans syndrome (B), there is an abrupt loss of airflow as a function of lung volume such that the FEV_1 (arrow) occurs at a smaller volume. With progressive loss of airflow, the expiratory limb converts from a convex to a concave configuration.

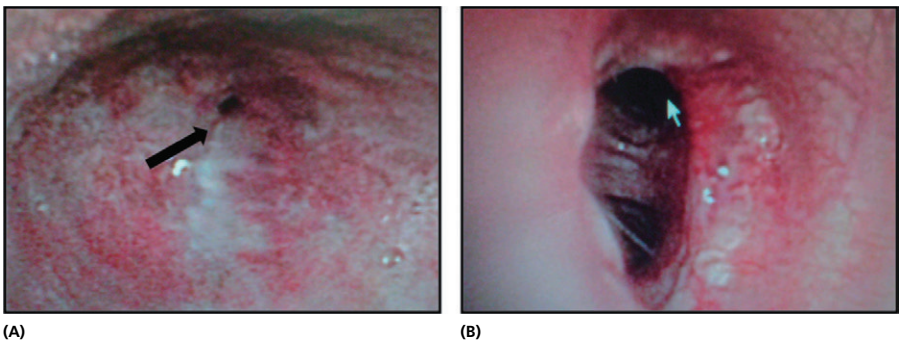


Figure 72.2. Focal airway narrowing. A lung transplant recipient presented with an abrupt loss in FEV_1. Bronchoscopy revealed pinpoint narrowing of the right mainstem bronchial anastomosis, shown by the arrow in (A). After bronchoscopic balloon dilatation, the airway was widely patent (B) and the FEV_1 returned to baseline.

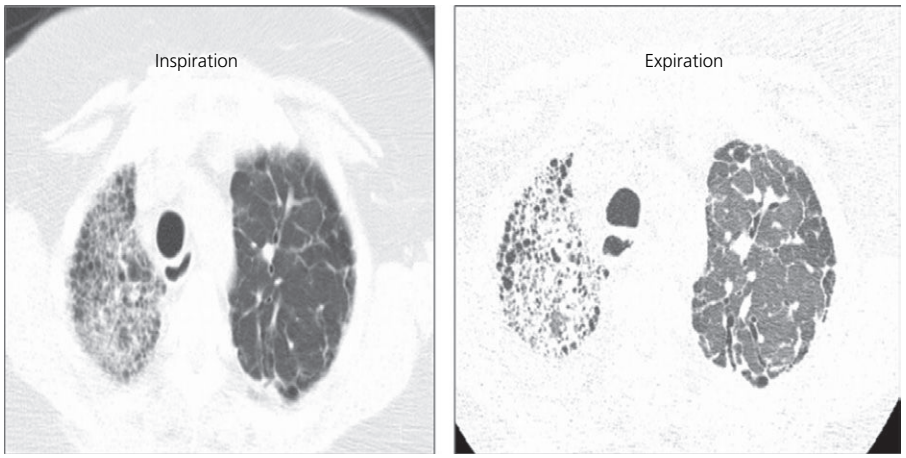


Figure 72.3. Radiographic appearance of bronchiolitis obliterans. A patient with a demonstrated loss in FEV_1 7 years after left single lung transplantation; (A) shows the inspiratory view with a limited degree of mosaic attenuation within the left lung; (B) shows the same region during exhalation with a significant variability in attenuation of the secondary pulmonary lobules consistent with areas of air trapping.

sensitive to small changes in airflow resistance. As noted in Table 72.1, an additional classification termed BOS0p (for potential BOS) exists. The criterion for BOS0p, which was added to the 2002 classification, is a 10% decline in the FEV_1 or a 25% decline in the $FEF_{25–75}$. The rationale for including BOS0p in the classification scheme was to alert clinicians when a particular patient's lung function was declining. A retrospective assessment of the two variables comprising BOS0p, FEV_1 and $FEF_{25–75}$, showed that only the drop in FEV_1 had a suitable prognostic value in determining BOS for recipients of bilateral lung transplants [48]. Similar conclusions were reached in a retrospective study of single lung transplant recipients, with the combined use of FEV_1 and $FEF_{25–75}$ showing a slightly higher prognostic value over the FEV_1 alone [49]. Given the fact that the majority of lung transplant patients do ultimately meet the criteria for BOS, and that treatment options for BOS remain limited, the clinical utility of BOS0p remains unclear aside from serving as an endpoint for investigational studies.

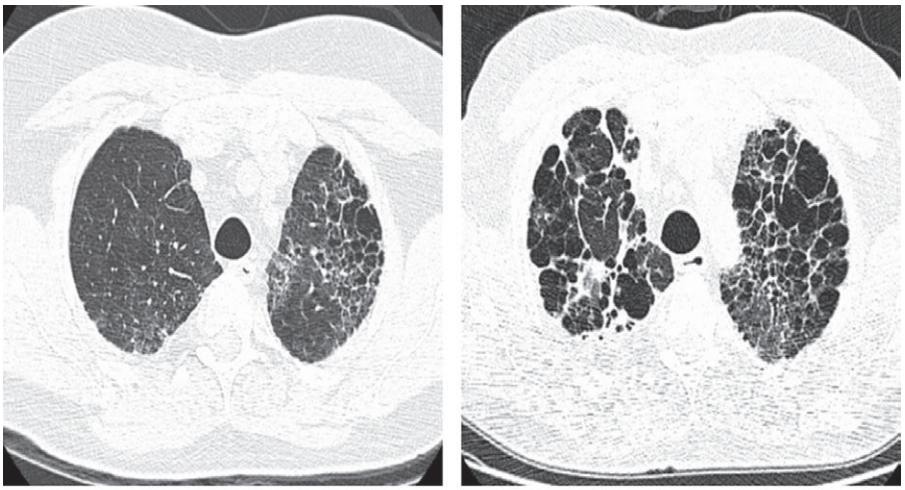
Clinical presentation of bronchiolitis obliterans syndrome

Every lung transplant recipient is at risk for the development of BOS. The most recent iteration of the ISHLT registry, which tracks the largest cohort of lung transplant patients worldwide, indicates that the rate of freedom from BOS is 90% at 1 year, 51% at 5 years, and 25% at 10 years post transplant [33] . Collectively, these data indicate a very high overall incidence of BOS, which tends to occur several years after transplantation. The symptoms of BOS can be quite variable. Most patients may begin with an asymptomatic presentation only detected by changes in spirometry, while some patients do present as acute dyspnea similar to a typical respiratory infection. Some investigators have classified patients according to the tempo of their BOS. In a study of 204 lung transplant patients, patients described as having an acute drop in the FEV_1 were compared to patients with a more linear, chronic drop in the FEV_1. The acute-drop patients experienced a median survival of 29 months compared with 58 months in the chronic group [50]. The most extensive study to date modeling the tempo of BOS over time showed that in most cases when BOS occurs, the drop in FEV_1 is most abrupt within the first 6 months of onset. After the abrupt loss of FEV_1, the rate of further decline in the next 18 months was much lower [51]. This non-linear relationship between FEV_1 loss and time is important to consider when assessing any treatment effects for BOS. Not surprisingly, patients who had a more precipitous decline into meeting the clinical criteria for BOS also demonstrated steeper further declines in FEV_1 and lower FEV_1 at 2 years post onset [51].

The radiologic studies in patients with BOS are generally subtle. Plain chest radiographs typically demonstrate normal to hyperinflated lungs without evidence of airspace opacity. High-resolution CT studies can provide additional details. For example, comparison of inspiratory to expiratory imaging on CT may reveal air trapping in the form of mosaic attenuation between adjacent secondary pulmonary lobules (Figure 72.3). Further, since OB involves the terminal bronchioles, patients with BOS may demonstrate tree-in-bud opacities in the periphery of the lung. It is important to note that many infectious processes that cause bronchiectasis can also result in tree-in-bud opacities.

Other clinical manifestations of chronic allograft injury

While the classic pattern of chronic rejection involves fibromyxoid luminal obliteration of the terminal airway resulting in airflow obstruction, other forms of chronic lung dysfunction have been described. These include entities termed restrictive allograft syndrome (RAS), neutrophilic reversible allograft dysfunction (NRAD), and follicular bronchiolitis. RAS is characterized by a decline in both the FEV_1 (>20%) and the total lung capacity (TLC, >10%). Some patients with RAS demonstrate upper lobe fibrosis, including radiographic honeycombing and pleural thickening (Figure 72.4), although these findings are not seen in all patients with RAS. NRAD refers to a phenotype characterized by: (1) FEV_1 loss, (2) bronchoalveolar lavage (BAL) neutrophilia, and (3) improvement in FEV_1 after the initiation of azithromycin (AZM) [52]. Follicular bronchiolitis is a rare small airway disease seen in non-transplant patients. It is characterized by lymphoid hyperplasia of surrounding the airway, and clinically characterized by progression to bronchiectasis. Two publications have reported the finding of follicular bronchiolitis in lung transplant patients, with the major findings including a strong association with prior



(A) 2 years post transplant (B) 5 years post transplant

Figure 72.4. Radiographic appearance of restrictive allograft syndrome (RAS). The patient developed progressive loss of lung volume with a 10% decrease in the total lung capacity (TLC) at 2 years post transplant and a 40% decrease in the TLC by 5 years post transplant. The decline in TLC was associated with development of apical honeycomb changes, which progressed over time.

Aspergillus infection, airway neutrophilia, and improvement following treatment with AZM [53,54]. Collectively, chronic lung dysfunction encompassing one of these patterns including OB has been termed chronic lung allograft dysfunction (CLAD) by some authors. When distinct types of CLAD have been compared, several studies suggest that CLAD with a BOS phenotype is associated with an improved survival compared with CLAD with a RAS phenotype.

Risk factors for bronchiolitis obliterans syndrome

The pathophysiology of BOS is thought to be multifactorial. Many risk factors have been linked to the subsequent development of BOS and mechanistic studies on these risk factors have moved the field of lung transplantation closer to a more complete understanding of how these factors are related. ACR is considered a strong risk factor for the development of BOS, as indicated by multiple studies [55–59]. These studies demonstrate that even minimal grade rejection is associated with some future risk of BOS. Further work has focused extensively on the relationship between lymphocytic bronchiolitis (LB), the B score of ACR grading scheme. The rationale for these investigations is that LB lesions and OB lesions occupy the same anatomic location, even if they are distinct histologically. This rationale is buttressed by animal tracheal transplant models showing a close temporal relationship between lymphocytic airway involvement and subsequent airway obliteration [60]. Several small studies in the early days of lung transplantation observed a similar close association between LB and subsequent OB [61–63]. More recently, Glanville examined the relationship between LB and BOS in an extensive longitudinal study of 341 lung transplant recipients [64]. These investigators demonstrated, in a multivariable model, the highest B-grade rejection score was associated with subsequent BOS and death. A-grade (vascular) rejection did correlate with subsequent risk of BOS in the univariable model, but fell out as a risk factor in the multivariable model [64].

Not every patient who experiences ACR goes on to develop BOS and some patients with BOS have no antecedent episode of ACR. Hence, either the diagnosis of ACR is imprecise, as noted earlier, or other factors may participate in increasing BOS risk. These factors, which are considered "non-alloimmue," include ischemia–reperfusion injury (also termed primary graft dysfunction [PGD]), gastroesophageal reflux disease (GERD), and respiratory infections. These three major factors are discussed below.

Table 72.2. Consensus grading scheme for primary graft dysfunction (PGD)

Grade	PaO_2/FiO_2	Radiographic infiltrates consistent with pulmonary edema
0	>300	Absent
1	>300	Present
2	200–300	Present
3	<200	Present

In addition to the grade of 0–3, PGD is subject to grading at several time intervals post transplant. Lung transplant patients have a PGD score determined at 0–6 hours, 24 hours, 48 hours, and 72 hours post transplantation.

Primary graft dysfunction

Injury to the transplanted allograft begins prior to implantation. Brain death itself results in activation of the neuroimmunologic axis, potentially resulting in subclinical epithelial and endothelial injury to an otherwise normal lung. Additionally, lung donors are at risk of aspiration and trauma from mechanical ventilation. Procurement followed by the transplant procedure results in a period of cold and warm ischemia. As a result, many normal-appearing transplanted lungs demonstrate significant non-cardiogenic pulmonary edema in the first days following lung transplant. Consensus guidelines have been developed to grade PGD [65]. As shown in Table 72.2, there are four potential grades of PGD derived at four time points (0–6, 24, 48 and 72 hours). Cardiogenic edema needs to be excluded for a patient to meet the criteria of having PGD.

This grading system has allowed investigators to assess the contribution of PGD to risk of BOS in several studies. A large single-center retrospective study of 337 lung transplant patients concluded that the severity of PGD was associated with a significant increased risk of subsequent development of BOS, both at early and later time

points [66,67]. A large international registry study demonstrated that survivors of severe PGD experienced increased mortality even when censored for those patients who lived at least a year, although there was only a trend toward increased BOS in the PGD group. While it is largely unclear how PGD predisposes to BOS given that these two events occur at distinctly different time points post transplantation, a recent finding by Bharat shows that patients who previously experienced PGD are at a significant risk for the development of antidonor HLA antibodies directed against MHC class II epitopes. Hence, it is possible that the pulmonary epithelial injury response during PGD contributes to increasing the antigenicity of the lung. Additionally, it is possible that PGD results in revealing previously cryptic self-antigens to the immune system. One such example of a cryptic antigen is type V collagen (Col V). Col V is a relatively rare constituent of collagen, which is usually intercalated within mature collagen bundles and therefore not accessible to T-cell surveillance. Rodent transplantation even in the absence of antigenic mismatch is associated with release of Col V into the airway, and leads to accumulation of T cells within the graft specific to Col V [68]. Some human subjects with idiopathic pulmonary fibrosis prior to transplant demonstrated a high frequency of T cells reactive to Col V in a single-center study. Severe PGD was significantly more common (55% vs. 22%) in patients with pre-existing Col V reactivity [69]. Hence, one potential mechanism linking PGD to subsequent development of BOS is that the early lung injury leads to exposure of previously cryptic autoantigens, which drives subsequent immune reactivity to the lung even after the allograft has recovered from the PGD.

Gastroesophogeal reflux disease

Many factors put lung transplant recipients at risk for GERD and subsequent aspiration. The surgery and postoperative course appear to impair normal swallowing. A study by Atkins et al. found laryngeal penetration or aspiration of thin liquids on swallowing evaluation in a third of patients [70]. Many of these patients had clinically silent aspiration. Patients have a high rate of gastroparesis. Factors that may impair GI motility and predispose to retrograde gastric reflux include vagal nerve injury during surgery and the use of calcineurin inhibitors, which may delay gastric emptying [71,72]. Decreased mucociliary clearance related to a blunted cough reflex and scar tissue formation at the anastomotic site also contribute to a heightened risk of exposure of the pulmonary epithelium to gastric contents. GERD is estimated to be present in at least 50% of patients [73]. Additionally, constituents of gastric fluid, such as pepsin and bile salts, are often found in bronchoalveolar lavage fluid of transplant patients [74,75]. Patients with GERD experience a higher incidence of ACR [41]. Because of these associations, some groups have begun routinely performing surgical gastrofundoplication on lung transplant recipients in the early postoperative period. While these studies are uncontrolled, they are associated with improved outcomes compared to historical experience. Interestingly, in the aftermath of surgical correction, a significant decrease in the number of activated CD8 T cells was observed in a small cohort of lung transplant patients [76]. Hence, GERD with aspiration of gastric contents into the lung may induce an injury response leading to increased immunity against the allograft. A study of both pre- and postlung transplant survival found that patients with GERD had increased T-cell reactivity to Col V; post transplantation, patients with GERD and Col V T cells developed BOS at an increased rate [77]. These findings thus may potentially link aspiration events to the development of autoimmunity.

Infection

Lung transplant patients are at high risk of developing a multitude of respiratory infections given that they are highly immunosuppressed and the lung is susceptible to environmental exposure. General discussions of the infectious risks of transplantation are covered in depth in Chapters 92–94. A diverse range of microbial pathogens has been linked to subsequent BOS development, including bacterial infections with pseudomonas [78], atypical bacterial infection with *Chlamydia* [79], fungal infection [80,81], community-acquired respiratory viruses (CARV) [82,83], activation of chronic CMV infection [84,85], and human herpes virus 6 [86]. A significant body of work has assessed the clinical sequela of CARV. CARV typically studied include influenza, parainfluenza, respiratory syncytial virus, metapneumovirus, adenovirus, and rhinovirus. Nearly all of the studies to date reporting on the association between CARV and outcome have been single-center retrospective reports. From these studies, CARVs appear to be strongly associated with respiratory symptoms such as cough and dyspnea. On the other hand, these single-center studies reach divergent conclusions regarding the association between CARV and ACR or BOS. One large, prospective, single-center study, utilizing very sensitive molecular tools for respiratory virus, showed a strong association between CARV infection and A2 rejection, while at least three others have not. Regarding the risk of BOS, most of the studies to date reporting BOS incidence contain a very small number of total cases, although a risk of BOS was found in three of the four studies. A meta-analysis of the literature to date on CARV and lung transplantation found that the variability between studies was so great that BOS risk related to virus could not be calculated with any appropriate statistical analysis. Collectively, these studies indicate that CARV infection is potentially harmful, but more controlled studies are required to fully quantify the risk. An important point to consider with respect to viral infection is that few agents exist with which to treat the majority of these infections. Influenza virus can be managed with agents such as oseltamivir, which decrease the length of viral shedding, and respiratory syncytial virus can be treated with ribavirin (inhaled or oral), but in general management of viral illnesses generally involves reduction of baseline immune suppression and supportive care. With respect to the 2009 pandemic of novel H1N1 influenza, the experience from Australia suggests that this outbreak was associated with an overall increase in BOS in influenza survivors [87].

In addition to CARVs, which are ultimately cleared by the infected individual, another potential threat to lung transplant recipients are the intrinsic viruses that set up latency in the host. The most extensively studied latent virus is cytomegalovirus (CMV), a member of the beta herpes family of viruses. Roughly 80% of the general population is serologically positive for CMV and hence at risk for reactivation and viral replication in the setting of immunosuppression. Of all the latent viruses, CMV appears to have the highest predilection for replication within the lung. In the early decades of lung transplantation, CMV was noted to confer a significant risk for BOS. This notion was based in part on international registry data showing that patients who were CMV naïve but received a lung transplant from a CMV positive donor—termed a CMV mismatch—were at increased risk for developing BOS compared to patients who had pre-existing immunity to CMV prior to transplant. CMV infection in the lung can result in CMV pneumonitis, resulting in recruitment into the lung of inflammatory cells as well as increasing the immunogenicity of the respiratory epithelium. Hence the mechanism by which

CMV infection leads to BOS would be related to chronic injury to the respiratory tract. One mechanistic study in human lung transplant recipients showed that CMV pneumonia was associated with elevated BAL levels of CCL2 and MCP-1, two chemokines with known association to risk of BOS [88].

In the last decade, improved therapies for CMV have become standardized. The most utilized is oral valganciclovir, which strongly inhibits viral replication. The use of valganciclovir for all patients at risk of CMV for some period of time, generally 3–12 months, after transplantation has resulted in a significant reduction in the risk of BOS attributable to CMV infection. This is best appreciated in the recent ISHLT registry, which compares mortality after transplantation among different combinations of CMV status between donor and recipient. In the new era of CMV prophylaxis, there is no longer a statistically different survival between CMV mismatched patients (D+/R−) and patients who are not CMV mismatched. This does not mean that in certain individuals, CMV replication is benign and there are compelling recent data that CMV pneumonia is indeed linked to a risk of subsequent BOS. Snyder demonstrated, in a large retrospective cohort of 231 lung transplant patients, that CMV pneumonia was associated with a twofold increased risk of subsequent BOS development [89]. Similarly, Paraskeva, utilizing a very sensitive PCR assay, showed in a study of 192 patients that CMV replication detected in BAL fluid was also associated with a twofold risk of BOS development [90]. Given these findings, significant effort has been devoted to defining the optimum prevention strategy for CMV disease in lung transplant recipients. A randomized placebo-controlled study from 11 US centers compared 3 months of oral valganciclovir prophylaxis to 12 months of prophylaxis. The key finding from this study was that patients receiving longer-term prophylaxis had a significantly decreased rate of CMV disease at 13 months (4% vs. 32%) [91]. It remains unclear whether longer-term prophylaxis post transplant will translate into a lower risk of BOS, though the long-term results of prospective trials will ultimately answer this question.

Treatment strategies in chronic rejection

Given that BOS remains highly prevalent following lung transplantation and contributes significantly to the mortality experienced in lung transplant recipients, it is fair to say that treatment methods for chronic rejection remain disappointing. In view of the significant association between ACR and subsequent BOS, many reports have cited some improvement in BOS outcomes through manipulation of standard immune suppression regimens, such as conversion from azathioprine to mycophenolate, from cyclosporine to tacrolimus [92–96], or through the use of cytolytic therapy [97–99]. None of these reports have convincingly shown a major improvement in lung function once BOS is established, but rather suggest that in some patients stabilization of lung function may be achieved. Because of the uncontrolled and retrospective nature of these studies it is unclear if their findings simply reflect the natural history of BOS.

The histology of BO in lung transplant is similar to a rare pulmonary disease termed diffuse panbronchiolitis (DPB). DPB can ultimately lead to end-stage lung disease. Further, one of the most effective class of agents used to treat DPB is the macrolide antibiotics [100]. Subsequent studies in patients with cystic fibrosis confirmed a clinical benefit from the use of the macrolide azithromycin (AZM) [101]. These findings formed the rationale for early pilot studies examining the potential of AZM to stabilize lung function in transplant patients with BOS. The first case report showed a trend toward stabilization of FEV_1 in five of six patients with established BOS [102]. A subsequent larger single-center study of 81 patients with BOS showed that treatment with AZM improved FEV_1 by 10% in 24 of the patients [103]. Responders were noted to have a higher pretreatment level of BAL neutrophilia than nonresponders. Some have suggested that response to AZM in the setting of BAL neutrophilia defines a subclass of CLAD distinct from BOS, termed NRAD as described earlier. The potential benefit of AZM has been assessed with one randomized, prospective trial with 40 patients in the AZM group and 43 patients in the placebo group. Treatment with AZM beginning at the time of transplantation was associated with a threefold decrease in BOS prevalence at 2 years post transplant, but survival was equivalent between the groups [104]. Given that AZM is generally well tolerated by patients, the use of AZM in patients with developing airflow obstruction following transplantation appears to be a reasonable approach as there are no other proven effective therapies for BOS treatments.

Multiple additional therapies for BOS have been proposed, which may have some therapeutic efficacy but yet do not have the weight of strong experimental evidence at this time. These include the use of antibody-lowering therapy for DSA, aerosolized CSA, photopheresis, and infusion of donor hematopoietic cells. As noted previously, a single-center study found that patients who developed de novo DSA, and had successful depletion of this DSA with a combination of rituximab and IVIG, had lower rates of BOS then patients in whom this therapy failed to lower the DSA [21]. Hence, it is tempting to speculate that antibody-lowering therapy in patients with DSA might reduce the risk of BOS. Inhaled CSA has been proposed as a potential therapy to decrease allospecific responses in the airway. A single-center prospective study of inhaled CSA, where a reduction in ACR was the primary endpoint, failed to show a treatment effect for ACR. Interestingly, retrospective analysis of that study showed that patients who received inhaled CSA had improved survival and decreased BOS [105]. Hence, results from future studies on inhaled CSA are anticipated. Extracorporeal photopheresis (ECP) involves treatment of patients with a photosensitizing agent followed by perfusion of the patient's blood over a pheresis circuit where leukocytes are subjected to UV light. ECP has shown promise in the treatment of allogeneic bone marrow transplant associated OB. In lung transplantation, one study has shown that ECP is associated with a stabilization of lung function in patients with BOS, although there was no control nontreatment group in this study [106]. Mixed hematopoietic chimerism may be one strategy to induce donor-specific tolerance in lung transplant recipients. One clinical study to date has explored this: 26 patients who received lung transplants and infusion with donor bone marrow were compared to 13 patients who received only a lung transplant. Five per cent of the donor marrow infused patients developed OB compared with 33% in the control group [107]. Importantly, the degree of donor chimerism waned to the threshold of detection by 1 year post transplant, so it is uncertain if true stable chimerism was achieved. These results will need to be validated by future studies before infusion of donor bone marrow becomes an established tool to prevent BOS.

Summary

Rejection, both acute and chronic, is common in lung transplant recipients, and proper management requires proactive surveillance, including protocol biopsies or biopsies triggered by a high index of suspicion. Close collaboration with a trained histopathologist is

required to differentiate alloimmune causes of graft inflammation from other maladies in the differential diagnosis. Prompt treatment and aggressive follow-up are similarly required.

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CHAPTER 73

Clinical Allograft Rejection Syndromes in Pancreas and Islet Transplantation

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Immunogenicity of the pancreas allograft—a historical perspective

Modern-day transplantation of the pancreas began on December 17, 1966 at the University of Minnesota when Drs. William Kelly and Richard Lillehei transplanted a simultaneous duct ligated segmental pancreas allograft with a kidney allograft into a 28-year-old woman [1]. Over the last four decades, transplantation of the pancreas has evolved to serve as an effective treatment option for patients with diabetes mellitus with or without end-stage kidney disease. As of December 2010, more than 35 000 pancreas transplantations had been reported to the International Pancreas Transplant Registry, with the largest proportion (24 000) occurring within the United States [2]. Of the pancreas transplants performed, 75% were with a simultaneous kidney transplant (SPK), 18% were pancreas transplants performed following a previous kidney transplant (PAK), and 7% represented isolated pancreas transplants alone (PTA) (Figure 73.1).

The refinements in operative technique, patient care management, and an expanding arsenal of immunosuppressive medications have allowed for the progressive improvement in both patient and graft survival. The rate of early loss of a pancreas graft due to surgical technique has decreased to 8%. However, the most significant advances contributing to improved results have been the ability to protect against both alloimmune and autoimmune destruction. The rate of immunological graft loss at 1 year is 1.8% for SPK recipients, 3.7% for PAK, and 6.0% in PTA recipients. These results reflect dramatic improvements in the ability to control the immune response against the highly immunogenic pancreas transplant [2,3]. The overall effects of the effort in optimizing pancreas transplantation have resulted in patient survival rates of 95% at 1 year post-transplantation, and 83% at 5 years post-transplantation. The best graft survival has been found in SPK recipients, with 86% pancreas and 93% kidney graft function at 1 year. PAK graft function reached 80%, and PTA pancreas graft function reached 78%, at 1 year post-transplantation. Long-term outcomes for pancreas transplantation are covered in more depth in Chapter 107.

The vast majority of pancreas transplant programs use induction with a T-cell depletion agent (alemtuzumab or thymoglobulin) for all recipients regardless of the degree of sensitization (Figure 73.2). This reflects the early experience with extremely high rejection rates following pancreas transplantation. Concomitant with induction via T-cell depletion is the administration of steroids, mycophenolate mofetil (MMF), and tacrolimus for maintenance therapy (Figure 73.3). Many centers are now maintaining unsensitized recipients of SPK on tacrolimus and MMF dual therapy and rapidly tapering steroids during the first week following transplantation. For the recipients at higher immunologic risk, including sensitized SPK, PAK, or PTA, maintenance therapy often consists of triple therapy including steroids. In patients who have had early problems with rejection, or recipients at an extremely high risk for rejection, limited duration quadruple therapy with the inclusion of sirolimus has been used.

Clinical presentation: pancreas graft dysfunction

The clinical presentation of pancreas graft dysfunction is often asymptomatic, discovered by incidental elevations in serum amylase and lipase levels and/or the presence of hyperglycemia. Although broad consideration for the potential etiologies for graft dysfunction should be entertained (Table 73.1), the clinical assessment should be standard for all recipients of pancreas allografts and should include an ultrasound with Doppler of the pancreas graft, or magnetic resonance imaging or computed tomography, depending on the expertise available at the center, and the degree of suspicion for technical complications. A complete laboratory panel including amylase and lipase also should be performed. In SPK recipients, a kidney ultrasound should also be obtained. Attention to the serum levels of amylase, lipase, HbA1c, C-peptide, creatinine (in the setting of SPK transplantation), and immunosuppression drug levels is warranted. A thorough physical exam should be performed, and blood cultures and CMV levels obtained if a high degree of suspicion for an infectious etiology exists. It should be recognized that clinical signs could be muted substantially in a heavily immunosuppressed patient. Thus, positive physical findings could be useful, but negative physical findings are less informative. For the patient presenting with severe hyperglycemia, hydration with concomitant insulin therapy is warranted.

Elevations in serum amylase and/or lipase following pancreas transplantation can be attributed to various non-specific or specific inflammatory reactions, as well as a host of infectious states, anastomotic leak, or drug-induced toxicity. During periods of pancreas rejection, mean serum amylase and lipase values have been demonstrated to be increased by 3.6 and 8.3-fold, respectively, and

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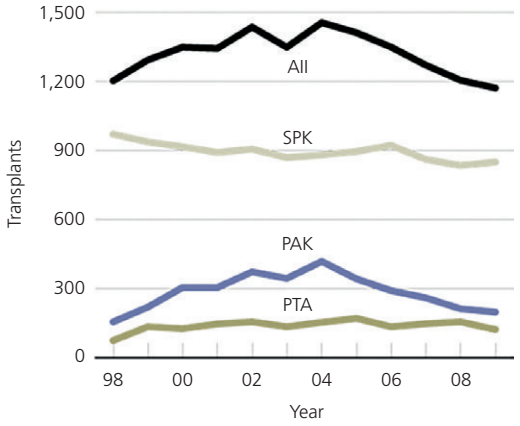

Figure 73.1. Total number of adult pancreas transplants. From: Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR). OPTN/SRTR 2010 Annual Data Report. Rockville, MD: Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, 2011.

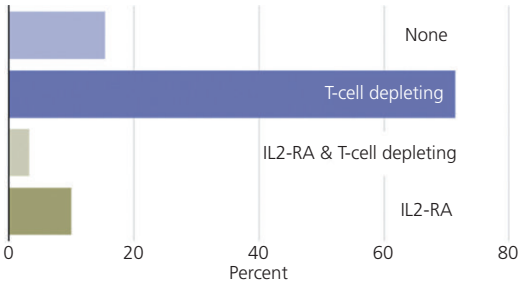

Figure 73.2. Induction agents used at time of pancreas transplant, adult recipients 2009. From: Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR). OPTN/SRTR 2010 Annual Data Report. Rockville, MD: Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, 2011.

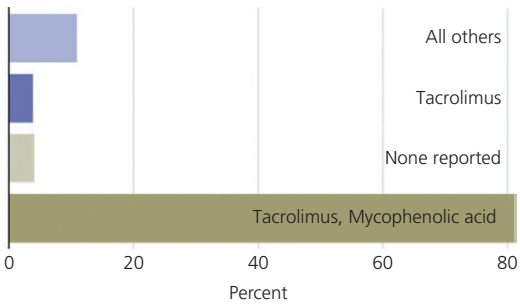

Figure 73.3. Initial immunosuppression regimen in adult pancreas transplant recipients, 2009 (steroids not considered). From: Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR). OPTN/SRTR 2010 Annual Data Report. Rockville, MD: Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, 2011.

correlate with biopsy-proven rejection in 80% of cases [4]. Other laboratory findings that should increase suspicion for graft dysfunction include a trend in increasing levels of HbA1c. The kidney allograft in SPK recipients may serve as a surrogate marker of pancreas rejection, particularly when an elevation in serum creatinine levels occurs concomitant with elevations in serum markers of pancreatic acinar cell injury. This form of synchronous rejection has been noted to occur in up to 73% of dual organ transplant recipients. Isolated pancreas or kidney allograft rejection has been noted to occur in 3% and 23%, respectively, of rejection episodes in SPK recipients [5].

Ultrasound assessment of the transplanted pancreas allows for the assessment of the pancreatic parenchyma, the detection of peripancreatic fluid collections, the presence or absence of venous or arterial blood flow, and allows for image-guided percutaneous needle biopsies to be performed [6] (Figure 73.4A). Although ultrasound assessment of the pancreas allograft vasculature avoids the need for intravenous contrast administration associated with computed tomography (Figure 73.4B) and magnetic resonance imaging, limitations associated with this imaging modality include operator variability, as well as difficulty with assessment of the pancreatic allograft given its intraperitoneal location with overlying bowel gas capable of obscuring adequate visualization.

Perhaps, the greatest application of ultrasonography for pancreas transplant recipients is the ability to facilitate percutaneous needle biopsy [7]. Given the lack of specificity of serum biochemical markers in allowing for the diagnosis of pancreas allograft dysfunction, allograft biopsy has emerged as an essential adjunct for the management of the pancreas transplant recipient. Pancreatic allograft biopsy is performed by insertion of an 18 or 20-gauge core biopsy needle through the peritoneum and directly into the pancreas body or tail under sonographic guidance. Doppler imaging is used to reveal pertinent vascular structures. Following tissue sampling, a sonogram is obtained immediately following biopsy to assess for any immediate biopsy-related complications. Pancreatic allograft biopsy has a high success rate, with up to 88% of tissue samples being adequate for histological evaluation. There is a low complication rate (2.8%) that includes bleeding following biopsy, biochemical evidence of postbiopsy pancreatitis, and the inadvertent biopsy of adjacent structures [8,9]. Poor visualization of the pancreas allograft secondary to adjacent bowel, patient body habitus, or a small allograft can result in increased risk of technical failure with ultrasound-guided biopsy. If an adequate window cannot be obtained secondary to overlying bowel gas, the ultrasound can be repeated after short intervals until an adequate biopsy window becomes available. Alternatively, CT-guided biopsy remains an option. As a last resort, operative pancreas allograft biopsy can be performed either through an open or laparoscopic approach [10].

A special note should be made of the differences in surveillance and diagnosis of rejection in bladder-drained versus enteric-drained pancreas allografts. Although currently the majority of grafts are drained via the enteric system (80%), the 20% drained via the bladder allow for the assessment of allograft function by evaluation of urinary amylase levels. Indeed, it has been suggested by some that stable urinary amylase levels reliably rule out rejection; this point is controversial, however. Although a decrease in urinary amylase can serve as a marker for acute rejection, this is a nonspecific finding and should be supplemented by pancreas allograft biopsy. As opposed to ultrasound-guided percutaneous biopsy of the pancreas allograft, bladder drained allografts are amenable to cystoscopic transduodenal biopsy [11].

Table 73.1. Pathological changes "other" than rejection in pancreas needle biopsies

Diagnosis	Main histological findings	Clinical presentation
Post-transplant ischemic pancreatitis	Inflammation: neutrophils, foamy macrophages Location: septal if mild or diffuse if severe Other features: fat necrosis, edema and interstitial hemorrhage Patchy coagulation necrosis of clusters of acinar cells may be present No fibrosis, the septa may be expanded due to edema/fat necrosis	Increase in amylase and lipase in serum Decrease in urinary amylase* Hyperglycemia if there is extensive necrosis
Peripancreatitis/ peripancreatic fluid collection	Inflammation: mixed (lymphocytes, plasma cells, eosinophils, neutrophils) Location: septa and periphery of lobules Other features: dissecting bundles of active fibroblastic proliferation with obliteration of septal structures, relative preservation of the center of lobules ("cirrhotic appearance")	Local or systemic infectious symptoms, abdominal pain, peritonitis Peripancreatic fluid accumulation Increase in amylase and lipase in serum
Cytomegalovirus pancreatitis	Inflammation: mostly mononuclear Location: septal and acinar, patchy Other features: cytomegalovirus cytopathic changes in acinar, endothelial or stromal cells	Increase in serum amylase and lipase Decrease in urinary amylase* Systemic symptoms if generalized disease Other: duodenal cuff perforation
Post-transplant lymphoproliferative disorder	Inflammation: ranging from polymorphic with lymphoblasts, plasma cells, eosinophils in low-grade disease, to monomorphic, predominantly lymphoid in high-grade disease (lymphoma) Other features: lymphoid proliferation is nodular, expansive Necrosis may be present	Asymptomatic, or increase in serum amylase and lipase Lymphadenopathy Tumor mass May coexist with acute rejection
Bacterial or fungal infection	Inflammation: variable; acute, chronic, purulent, necrotizing (abscess), granulomatous Location: random Other features: same as bacterial and fungal infections in other organs	Systemic and/or localized infectious symptoms Peritonitis, duodenal cuff perforation Increase in serum amylase and lipase
Recurrent autoimmune disease/ diabetes mellitus	Inflammation: islet-centered lymphocytic inflammation (isletitis) No inflammation in late stages after disappearance of beta cells Other features: immunohistochemical stains for insulin and glucagon demonstrate absence of insulin producing beta cells in some or all islets depending if early or late disease	Acute or chronic deterioration in glucose metabolism with increasing need for insulin Although not pathognomonic, islet cell autoantibodies typically present (i.e. GAD 65, IA-2, etc.)
Acute calcineurin inhibitor toxicity	Absence of inflammation Variable degrees of islet cell injury (cytoplasmic swelling, vacuolization, islet cell drop-out formation of empty spaces (lacunae), apoptotic fragments) Immunoperoxidase stains: markedly diminished staining for insulin in comparison to controls and to glucagon stain Electron microscopy: loss of insulin dense core granules with preservation of glucagon dense core granules	Acute hyperglycemia High levels of cyclosporine or tacrolimus with return to normoglycemia with adjustment of drug dose or discontinuation

* In bladder-drained grafts.
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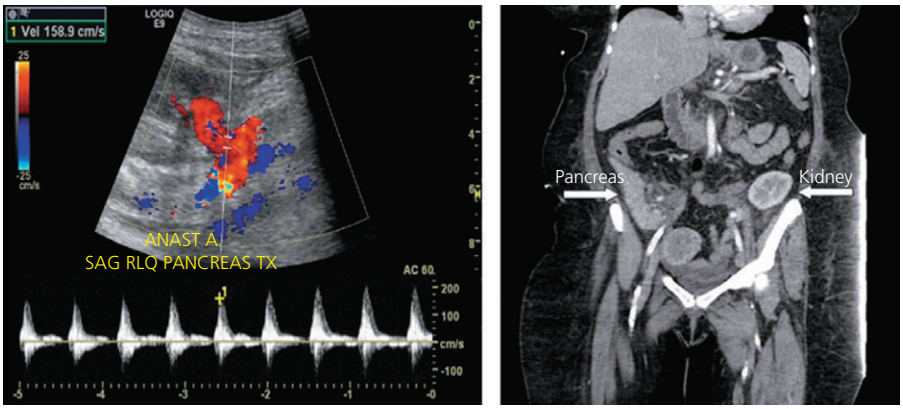

(A) (B)

Figure 73.4. (A) Ultrasound assessment of a pancreas transplant vasculature. (B) Computed tomography scan of a simultaneous kidney transplant recipient.

Acute pancreas rejection

Acute pancreas rejection can be seen in up to 35% of pancreas transplant recipients [12,13]. The histopathological diagnosis of pancreas allograft rejection is detailed in Chapter 85. Although several features of the clinical presentation of rejection are unique to the pancreas, the pathophysiology is similar to that seen in other transplanted organs, and treatment approaches are similar to that of the rejecting kidney, with the potential caveat that treatment escalation tends to proceed at a faster pace in pancreas transplant rejection. General comments about rescue therapy for allograft rejection can be found in Chapter 67. At 1 year post-transplant, SPK recipients demonstrate lower rates of acute rejection (25%), when compared to PAK recipients (36%), and PTA recipients (40%) (Figure 73.5). Although analysis of these rejection episodes has demonstrated that the majority occur within the first 6 months post-transplant, the late (>6 months) rejection episodes occurred primarily in PAK and PTA populations. Indeed, it is the PAK and PTA groups that demonstrate the highest rate of graft failure

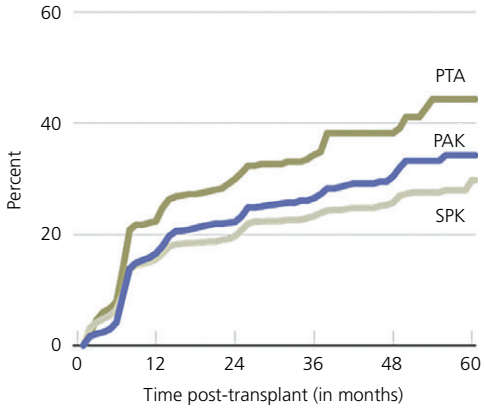

Figure 73.5. Incidence of first acute rejection among adult patients receiving a pancreas transplant in 2005–2009. From: Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR). OPTN/SRTR 2010 Annual Data Report. Rockville, MD: Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, 2011.

associated with acute rejection (1.8% and 2.9%, respectively) when compared to SPK recipients (0.3%). The need for appropriate and timely institution of antirejection therapy for pancreas transplant recipients is highlighted by the significantly increased risk of chronic rejection in those who have had multiple acute rejection episodes. Thus, with the associated increased rates of acute rejection seen in PTA and PAK recipients, there has been a demonstrated higher risk for chronic rejection in these recipients when compared to SPK recipients. The latter may be attributed to the absence of a surrogate marker for rejection in PTA and PAK recipients, as compared to the kidney allograft in SPK recipients, which may allow for a more expeditious appreciation of acute rejection and thus prevent delays in diagnosis or treatment of acute rejection. The cumulative effect of acute rejection episodes on the development of chronic rejection has been demonstrated. Despite improvements in immunosuppression protocols allowing for a reduction of rejection rates in recipients of solitary pancreas transplants [14], this population of patients is thought to benefit from prospective monitoring with protocol biopsies to allow for the earlier detection of allograft rejection, with the hopes of improving long-term graft and patient survival [15]. Although the timing of protocol biopsies varies with institutional protocols and expertise, biopsies at 3, 6, and 12 months should be considered.

Cellular-mediated rejection

Acute cellular-mediated rejection in a pancreas allograft, which has been well defined in 2008 by a consensus panel, is graded as mild (grade I), moderate (grade II), or severe (grade III) based upon histological assessment [16]. Prior to obtaining histological results, initial therapy should be directed at maximizing drug levels of calcineurin inhibitors and MMF, so as to not delay institution of therapy. Confirmation of the presence of acute cellular rejection should be treated by an additional round of T-cell depletion therapy for grade II and III rejection, whereas grade I rejection can be initially managed by pulse steroids. Failure of response in the latter scenario should prompt use of T-cell depletion for management of grade I rejection. Hyperglycemia associated with the rejection episode, or iatrogenic from high-dose steroid administration, should be managed with institution of insulin therapy with associated diet modification. Achievement of normoglycemia, normalization of amylase/lipase serum levels, as well as resolution of additional associated symptoms of rejection (graft pain, swelling, fever), should serve as indication of effective therapeutic intervention. Response to therapy for patients with grade I acute cellular rejection is nearly 90%, dropping to 71–85% for grade II rejection, and less than 50% for patients presenting with grade III rejection. Failure of symptomatic resolution, or concern for partial response, should prompt: (1) consideration for antibody-mediated rejection (see below), (2) further escalation of immunosuppression, and/or (3) an additional pancreas graft biopsy.

Antibody-mediated rejection

The effects of antibody-mediated rejection have gained wider appreciation with an improved understanding in not only pancreas transplantation, but other solid organ transplants as well. The Banff Grading Schema for pancreas rejection was updated in 2011 in order to address antibody-mediated rejection [17] (see Chapter 85). Routine pretransplant cross-matching and ABO matching has essentially eliminated antibody-mediated hyperacute and delayed hyperacute rejection. Of greater concern is acute antibody-mediated rejection, which typically manifests within the first few weeks following transplantation [18]. The presence of (1) allograft dysfunction, (2) donor-specific antibodies in the recipient's serum, and (3) capillary C4d deposition (consistent with complement deposition) allows for the diagnosis of acute antibody-mediated rejection to be established. Consensus on the significance of either isolated donor-specific antibodies or isolated C4d deposition, without the presence of the other or graft dysfunction, has yet to be determined. Currently, treatment should be reserved for those patients who present with at least two of the three above-mentioned criteria.

Once the diagnosis has been established, therapy aimed at attenuation of the antibody- mediated graft injury should be instituted. Current therapy often consists of various combinations of enhanced T-cell depletion with thymoglobulin, in conjunction with antibody-mediated therapy with plasmapheresis, IVIG, and rituximab administration. Utilization of additional antibody-mediated therapy (bortezomib, eculizumab, belatacept) have yet to gain wider acceptance [19]. Following graft rescue from acute antibody-mediated rejection, serial monitoring for the recurrence of donor-specific antibodies should be considered at 6-month or yearly intervals, with additional consideration for allograft biopsy for assessment of C4d deposition.

Chronic pancreas rejection

Cellular-mediated rejection

Pancreas graft survival following transplantation has gradually improved due to both advances in surgical techniques to limit graft loss from technical complications, and refinement in immunosuppressive protocols to prevent and manage episodes of acute rejection. With this success there has been a growing population of patients with long-term pancreas graft survival, and a subsequent increased appreciation of chronic allograft rejection as a source of pancreas graft loss [12]. The clinical presentation of chronic rejection is often manifested by the gradual loss of glycemic control during a late time course following transplant. In contrast to acute pancreas rejection, elevation of serum amylase/lipase is less prevalent [13]. A decline in C-peptide levels and elevation in HbA1c levels will further delineate the chronicity of graft loss. Pancreas

allograft biopsy will demonstrate graft sclerosis and loss of functional parenchyma in chronic rejection. The return of a hyperglycemic state late after transplant, in the absence of graft fibrosis on biopsy, but with evidence of insulitis and beta cell loss, should raise concerns for recurrent autoimmune disease as the source of allograft loss. There are single-center reports of temporary salvage of the pancreas allografts affected by recurrent autoimmune disease through enhanced immunosuppression [20]; however, there remains to be identified any therapeutic intervention capable of reversing chronic rejection. The latter further emphasizes the need for aggressive management of acute rejection, as the number and severity of acute rejection episodes are associated with the increased risk of chronic rejection and graft loss [13].

Antibody-mediated rejection

Chronic rejection of a pancreas allograft through antibody-mediated mechanisms is determined by graft biopsies demonstrating chronic graft sclerosis, concomitant with C4d-positive staining in parenchymal capillaries and the presence of donor-specific antibodies [17]. The clinical presentation is similar to that of chronic rejection mediated through cellular mechanisms, with the patient's gradual return to a hyperglycemic state. Unfortunately, like cellular-mediated chronic rejection, there currently are no therapies available and current efforts should be directed at aggressive detection and management of acute rejection episodes.

Immunogenicity of the islet allograft—a historical perspective

The origin of islet replacement in diabetic patients began in the late 19th century, with the reports of the transplantation of sheep pancreatic extracts without clinical success [21]. Although the clinical development of modern-day islet transplantation began in close parallel with pancreas transplantation, its establishment into the clinical armamentarium has been slow and limited in application. The demonstration, in 1972 by Ballinger et al., of the reversal of streptozocin-induced diabetes in rats by the transplantation of islet isografts helped establish the foundation on which current islet replacement is based [22]. Translation to a clinical reality ensued, with demonstration at the University of Minnesota of autologous islet transplantation success in patients undergoing near-total pancreatectomies [23,24]. Subsequent success across allogeneic barriers was demonstrated [25], with the largest advancement being improved success through utilization of a steroid-free immunosuppressive protocol, termed the Edmonton Protocol. However, insulin independence relied on the use of multiple pancreas donors for success [26]. Unfortunately, long-term follow-up of these islet transplant recipients who were initially rendered insulin-independent found that over 80% had returned to insulin dependence within 2 years. Furthermore, many of these recipients became sensitized, likely as the result of the use of multiple donors compounded by allograft rejection. Further understanding of appropriate donor selection, and advancements in islet isolation, have allowed for success rates utilizing single pancreatic donors for the achievement of insulin independence. In addition, calcineurin inhibitor/steroid-sparing immunosuppressive regimens that rely on costimulation blockade (belatacept or efalizumab) have demonstrated long-term islet allograft survival without the associated renal and beta cell toxicity associated with calcineurin-inhibitors [27]. The latter protocols have consisted of T-cell depletion at the time of islet transplant, and subsequent maintenance immunosuppression with MMF or sirolimus, as well as a costimulatory blocking antibody. A more comprehensive discussion of islet transplantation can be found in Chapter 61.

Islet allograft dysfunction—rejection versus islet "burn-out"

Although the clinical success of islet transplantation can be assessed by the reversal of the diabetic state, histological assessment of the islet graft following portal vein injection has remained elusive. Although percutaneous liver-needle biopsy is feasible following islet transplantation, it has been demonstrated to have limited practical application, with islet identification in only 31% of biopsy samples [28]. Non-invasive imaging of islet grafts following transplantation, with correlation of islet function or rejection, has yet to be formally established [29]. The inability to ascertain a histological assessment of islet engraftment has thus led to measures of islet transplant success based on degree of insulin requirement post-transplant, as well as serum C-peptide, HbA1c, and glucose levels.

The inability to accurately image islets infused into the portal system has made it difficult to resolve the question of whether islet dysfunction is related to rejection versus non-immune mediated islet loss (i.e. "burn-out"). It has been speculated that the islet loss may be related to the inability of islets to replace beta cells in the absence of a precursor population, or stem cell. However, recent success with long-term islet allografts following induction with lymphocyte depletion has suggested that the early graft dysfunction noted in the Edmonton trial may have been related to inadequate immunosuppression, rather than early islet burn-out. Indeed, single-center results with utilization of more rigorous immunosuppression protocols have demonstrated insulin independence for a mean of >3 years in up to 66% of islet recipients [30]. Most current clinical islet trials are using induction with lymphocyte depletion and more aggressive maintenance regimens, reflecting the concern with controlling the immune response following beta cell replacement in the type I diabetic recipient. This is analogous to the evolution of more aggressive immunosuppression regimens following solid organ pancreas transplantation, and may be related to the necessity to control both the alloimmune and autoimmune response. Long-term insulin independence rates following islet transplantation with lymphocyte depletion are approaching that achieved following PTA.

Islet rejection

Although a large body of literature on rodent and large animal models of islet transplantation and rejection exist, there remains limited information of the histological assessment of acute or chronic islet allograft rejection in humans. Based on studies of rodent islet transplant, it appears that islet rejection is driven principally by the indirect pathway of antigen presentation (see Chapter 5 for complete discussion of indirect pathway recognition). Although there is an obvious lack of data concerning the path of rejection in human islet transplantation, the potency of the alloimmune response can be implied based upon the problematic sensitization seen in clinical trials of islet transplants [31].

The diagnosis of clinical islet rejection, whether acute or chronic, is solely based upon the return to a hyperglycemic state, with the rate of return post-transplant determining the identification of an acute or chronic process. Intensification of immunosuppression is warranted, with consideration of therapy directed at antibody-mediated rejection should donor-specific antibody be detected in the postoperative period [32].

Summary

Whole-organ pancreas transplantation has emerged as a safe and durable treatment for the diabetic patient. Continued aggressive surveillance and treatment of acute rejection episodes, especially in pancreas-alone transplant recipients who remain at higher risk for acute rejection, is currently the only effective strategy to address late allograft loss from chronic rejection. Although islet transplantation remains experimental and hindered by the inability for postoperative histological assessment of the cellular grafts, there continues to be progress in long-term success rates utilizing single-donor pancreata for islet isolation concomitant with aggressive immunosuppression protocols. Further refinements in islet isolation, investigations into various islet sources (stem cell-derived beta cells), as well as novel safe and effective immunosuppressive agents, will increase the option for beta cell replacement in the type I diabetic recipient.

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CHAPTER 74

Clinical Allograft Rejection Syndromes in Intestinal Transplantation

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Introduction

Rejection is one of the foremost complications of intestinal transplantation; it is second only to sepsis (which frequently follows treatment of rejection) as the leading cause of mortality, graft loss, and morbidity [1–5]. Severe, unrelenting acute rejection can progress to the most feared form—known as the exfoliative form—in which large segments of the intestinal mucosa slough off as a result of a massive epithelial damage [6,7]. Predictably, such forms of severe rejection have been associated with the worst outcomes for patients.

The intestinal graft is at particular risk for the development of rejection for multiple reasons, including the diversity of tissues and cell types within the intestinal wall, the large amount of immune-competent hematopoietic and lymphoid cellular mass in the mesentery and intestinal submucosa, and the presence of bacteria within the lumen of the intestine, which can trigger immunological responses [8,9]. Additionally, as the rejection process ensues, the loss of integrity of the epithelial intestinal mucosa allows for translocation of the intraluminal bacteria and subsequent development of sepsis. The latter is at times the only heralding sign of an acute rejection episode [10]. Rejection also impairs the absorption of immunosuppressive medications, thus thwarting attempts to bring it under control.

Development of acute rejection in the intestinal graft can occur at any time after transplant and usually presents as either cellular rejection or humoral rejection. Chronic rejection can develop as an unexpected long-term complication or, more commonly, follow recurrent episodes of acute rejection. The clinical symptomatology and treatment of the various forms of intestinal rejection are discussed herein. The histopathological details of intestinal rejection are discussed in detail in Chapter 86.

Acute cellular rejection

The most common form of rejection observed in recipients of an intestinal transplant is acute cellular rejection (ACR). In the earliest published series, up to 60–70% of all patients experienced at least one episode of ACR [1,2,5]. More recent series show that rejection rates can be reduced to as low as 30% when induction therapy is utilized [3,4]. ACR can occur at any time after transplantation, including many years after transplant, but it is usually a complication seen within the early postoperative period. The initial episode of acute rejection is typically seen around 2–4 weeks. More than 70% of all rejection episodes are observed within the first year of transplant [11]. It is widely documented that the presence of a liver graft within the intestinal transplant confers the intestinal graft some degree of protection from developing ACR as compared to intestinal graft alone [2,4,12,13]. There is evidence that the multivisceral graft may provide some protection even in the absence of a liver [3,11].

The severity of ACR is usually graded as mild, moderate, or severe based on pathologic findings [14,15]. Pathological readings of "indeterminate" rejection (suspicious but not clear-cut findings to meet criteria for "mild") need to be correlated with endoscopic and clinical findings and, if necessary, endoscopy and biopsy are repeated for a definitive diagnosis. "Subclinical" rejection is a pathologic diagnosis in the absence of endoscopic or clinical signs. It has to be attended carefully because, if untreated, it has been shown to be associated with inferior outcomes [16].

The length of each rejection episode is directly related to its severity. Mild rejection episodes usually last for about 1 week and for each worsening grade the duration of the episode doubles, with moderate ACR episodes lasting about 2 weeks and severe rejection episodes lasting about 1 month [11]. Prolonged periods of rejection lasting over 45 days are significantly correlated with graft loss from rejection [11].

Symptoms

The initial stages of intestinal graft rejection are usually asymptomatic. Symptoms are typically associated with advanced stages and include increased stoma output, abdominal distension and pain, fever, and sepsis. Stoma output not only increases in volume but also can change its characteristics, becoming watery. Reduced and bloody stoma outputs are late, grave signs. In the most serious cases of exfoliative rejection, gelatinous casts of fibrin and intestinal mucosa are expelled through the stoma. Abdominal pain is acute, persistent, and poorly responsive to pain medications. Abdominal distension is often present in such patients due to ileus, mesenteric, and intestinal wall edema. Fever almost always accompanies the insurgence of ACR, either from the systemic inflammatory response or due to superimposed infection from bacterial translocation. Such symptoms cannot be underestimated and patients

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experiencing such symptoms should undergo emergent endoscopy, since even a 24-hour delay could significantly affect the outcome. The same symptoms can also be seen during infection episodes, especially viral enteritis; therefore, it is important that diagnosis be made as soon as possible by endoscopy and biopsy.

Diagnosis

The gold standard for diagnosis of ACR is endoscopic evaluation of the bowel mucosa followed by pathological analysis of multiple biopsy specimens [1–5,8,11]. According to the interval of time from transplant to current episode, the patient will receive either endoscopy through the ileostomy or natural orifice endoscopy. The goal is to reach and evaluate the mucosa of the distal ileum, whenever possible, since the ileum is the most affected area during ACR. Indeed, different segments of the gastrointestinal tract develop rejection with different frequency. In a paper describing the relative frequency of rejection in multivisceral transplant organ explant specimens, overall detection of ACR in the small intestine was in 25.5% of cases, 12.2% in the stomach, and 16% in the large intestine. In patients undergoing ACR, the relative frequencies were 80.0% in the small intestine, 38.5% in the stomach, and 33.3% in the large intestine [17]. If the distal ileum cannot be reached, the proximal jejunum can be sampled as an alternate site.

Even when rejection affects the distal ileum, it may leave some areas intact ("skip zones"); therefore ileoscopy should be carried for a good length of intestine (at least 15–20 cm) and multiple biopsies obtained at different levels. Any area of abnormality should be sampled, as should normal-looking mucosa for comparison. On areas where ulcerations are present, a biopsy at the edges of the lesion will yield better results than the center of the ulcer. Diagnostic endoscopy alone can help to rule out gross changes in the bowel mucosa. In patients who can accommodate large scopes, zoom endoscopy of the intestinal mucosa allows for an in-depth evaluation of the intestinal villi and defines alterations in their height, shape of the villous tip, erythema of the mucosa, and friability [18].

Serum citrulline levels, which can also be measured on a dry blood spot, may be useful for the diagnosis of ACR. These levels stabilize at approximately 3 months after transplantation [19,20]. A sudden drop from a stable baseline level or absolute levels below 13–15 μmole/L is strongly associated with episodes of moderate and severe rejection [19,21]. Similar to what we observe with blood urea nitrogen and creatinine in kidney transplantation and liver function tests in liver transplantation, these changes are not pathognomonic, as they can be associated with other causes such as infections or ischemia. They should prompt endoscopy and biopsy to confirm the diagnosis. Calprotectin levels in stools have also been proposed as an alternative non-invasive marker [22].

Recently, analysis of the trend in donor-specific antibodies (DSA) and panel of reactive antibodies (PRA) has been correlated with the presence and response to treatment of acute cellular, as well as mixed cellular, and humoral rejections [23,24].

In search of alternative markers for rejection, more recent studies have been performed both on peripheral blood and on the mucosal biopsies from intestinal transplant recipients [25,26]. In the study of peripheral blood, samples were taken and compared to simultaneous mucosal biopsies during different stages of rejection. The authors showed that there is a significant down-regulation in the expression of several genes implicated in translation-related proteins, with the ribosomal protein L13a (RPL13a) being the most affected during episodes of rejection [25]. The hypothesis is that such events may occur early during the course of rejection and that, therefore, this assay could be used as a potential non-invasive marker for rejection.

In another study, formalin-fixed paraffin-embedded samples were examined in patients with and without biopsy-proven ACR. Significance analysis of microarray (SAM) of the mRNA from such biopsies revealed distinct gene patterns between patients undergoing rejection and patients without rejection. During rejection episodes, there was an over-expression of leukocyte-specific surface markers as well as several chemokines and costimulatory molecules [26].

None of these markers can yet replace pathological analysis of the intestinal mucosa biopsy, which remains the gold standard for the diagnosis.

The differential diagnosis of ACR includes infectious enteritis, whether viral or bacterial, and in such cases the pathology of the intestinal biopsy is of assistance. Testing for viral infections, both systemic including Epstein–Barr virus (EBV) or cytomegalovirus (CMV), respiratory (viral panel for respiratory viruses), and enteroviruses should be performed. Stool specimens should be collected to test for common pathogens such as *Clostridium difficile*, *Cryptosporidium*, *Giardia*, and other parasites.

The final diagnosis of ACR is based on the combination of clinical findings, symptoms, and pathological evaluation of the intestinal biopsy. Once diagnosis has been established, treatment should be started immediately.

Treatment

Empiric treatment of rejection with a bolus of corticosteroids is accepted practice in cases when the patient cannot be readily transferred to the transplant center or when endoscopy is not immediately available.

Treatment of ACR is based on three simultaneous principles: increase in baseline immunosuppression, treatment course with steroids and/or antilymphocyte agents, and antibiotic prophylaxis for opportunistic infections.

First, primary immunosuppressive agents (usually tacrolimus) are increased from baseline levels to levels similar to the immediate post-transplant period (15–20 ng/mL). It has to be noted that tacrolimus levels may rapidly increase during an episode of rejection, due to a more rapid absorption of the drug through a permeable intestinal mucosa as well as a decreased tacrolimus metabolism at the level of the enterocytes.

Second, boluses followed by a weaning cycle of steroids are used to control mild episodes of rejection. For moderate and severe rejection, antilymphocyte agents such as thymoglobulin or alemtuzumab are used, for periods ranging from a few days up to 2–3 weeks, until clinical and histological resolution of the ACR episode. Additional therapies for resistant rejection include the use of antitumor necrosis factor monoclonal antibodies, such as infliximab [27]. In those episodes of rejection where a vascular component is present, and in which there is a suspicion of antibody-mediated rejection (often accompanied by an increase of DSA/PRA), several strategies to decrease humoral response are utilized. These include: the use of rituximab, a monoclonal antibody against the CD20 marker present on B lymphocytes; plasmapheresis, which removes circulating antibodies, coupled with the use of intravenous immunoglobulin (IVIG); and bortezomib [28,29]. Addition of a second baseline immunosuppressive agent such as sirolimus is an option for those patients who may need prolonged higher levels of immunosuppression, as well as for

those in which chronic rejection may be ensuing, because sirolimus inhibits fibroblast proliferation and can possibly slow down fibrosis in a previously injured graft [30].

The third principle of management of ACR is the use of adequate antibiotic coverage. Patients are at a very high risk of developing infections during an episode of ACR not only because the integrity of the intestinal mucosa is altered and bacterial translocation is common, but also because the use of strong immunosuppression will expose the patient to opportunistic infections. Patients being treated with antilymphocyte agents should be covered with empiric antifungal and antiviral therapy to prevent CMV or EBV infection or reactivation. Additional antibacterial therapy is often added and should target the enteric flora, including anaerobe and possible antibiotic-resistant bacteria, such as vancomycin-resistant *Enterococcus* (VRE). It has to be noted that many patients develop sepsis as a first sign of ACR and therefore empiric antibacterial coverage is often started even before final diagnosis of ACR is made.

In those cases where rejection cannot be controlled and where the patient's clinical status is deteriorating, graft enterectomy is a therapeutic option, presenting the following advantages: removal of a non-functional graft that is causing bacterial translocation and infectious complications; a rapid wean off immunosuppression; and the opportunity to prepare for re-transplant when the patient's clinical condition allows [4,7]. In cases of liver–intestine or multivisceral transplantation, removal of the whole graft is not possible; however, removal of the small intestine alone can be performed while the rest of the allograft (liver for liver–intestine grafts and liver–stomach–pancreas for multivisceral grafts), which is generally less susceptible to rejection, can be left in place. Successful re-transplantation of the intestinal component alone can be performed at a later time after graft enterectomy. Lastly, when intestinal graft enterectomy is considered, a useful presurgical procedure embolization of the graft arterial supply by interventional radiology will allow for a decrease in intraoperative blood loss, as well as a clear demarcation of the transplanted graft from the native intestine [31].

Outcomes

Mild rejection episodes usually resolve with short courses of treatment and no residual dysfunction of the intestinal graft. On the other hand, severe ACR episodes, prolonged moderate ACR episodes, and repeated occurrences of ACR have been clearly associated with worse outcomes, both in graft and patient survival [11]. The worst form of severe rejection, exfoliative rejection, results in graft loss in over 50% of cases with a significant increase in patient morbidity and mortality [4,6,7].

Infectious complications are the most common immediate cause of death in patients being treated for ACR. The most common sites of infection are pulmonary (especially in pediatric patients), either viral or bacterial, and central blood stream infections associated with central venous catheters and/or driven by bacterial translocations.

Although an episode of ACR may be successfully treated, medium- and long-term consequences are always of concern: for instance, the incidence of viral infections weeks or months after treatment is quite high and post-transplant lymphoproliferative disorder (PTLD) can develop if there is a reactivation of EBV viremia.

Lastly, ACR can lead to the development of chronic graft rejection, which will be detailed elsewhere in this chapter.

Other forms of acute rejection

Antibody-mediated acute rejection

Antibody-mediated rejection is driven by allospecific antibody and predominantly targets the intestinal graft endothelium. This results in micro- and macrothrombosis and consequent ischemic injury to the graft [32]. High alloantibody levels are found in 18% to 30% of patients waiting for intestinal transplantation [33]. These patients, as well as others who mount de novo donor specific antibodies (DSA) following intestinal transplantation, are prone to develop humoral and mixed cellular/humoral rejections. If a patient was previously sensitized, for instance a patient undergoing re-transplant who lost their graft from previous episodes of rejection, this patient will often have high titers of preformed antibodies or DSA [34,35].

Hyperacute humoral rejection in the operating room presents with sudden congestion of the intestinal graft, discoloration, edema, and loss of arterial pulsatility. The classic hyperacute rejection syndrome that occurs minutes after perfusion of the graft is rare. The few reported cases were associated with discoloration, congestion, and rapid loss of the graft. Interestingly, we have witnessed one such case with documented humoral rejection that responded to treatment with alemtuzumab, rituximab, and plasmapheresis. This patient had an uneventful subsequent course and is well with a perfectly functioning graft 5 years after transplant [36].

The more common syndrome, which is increasingly being recognized, appears several days or a few weeks after transplant and is associated with a rapidly evolving cellular rejection, concomitant rise of PRA and DSAs and characteristic pathologic findings. Accelerated acute rejection can happen a few days after transplant and presents with abdominal distension and tenderness, bloody stoma output or no stoma output, endoscopic appearance of mucosal congestion, and erythema with friability.

Humoral (acute antibody-mediated) rejection is not as well defined in pathological specimens as is ACR, but there is a growing body of histological evidence that antibody-mediated damage at the level of the small- and medium-caliber vessels can contribute to organ damage and either worsen a concurring episode of ACR or alone cause subclinical damage to the graft and lead to chronic rejection. There are no definite clinical signs or symptoms of humoral rejection; however, pathological findings of vascular congestion, deposition of complement components C4d and C3d within the lamina propria of the vessels, or deposition of immunoglobulins along the vessels, can suggest an ongoing process, especially when the patient develops an increased titer of DSA or PRA [34].

A third syndrome, which is being increasingly recognized, is the gradual rise of the antibody levels without evidence of rejection. In the absence of established criteria for treatment, we follow these levels very carefully and treat patients who have been hypersensitized in the past or with a history of repeated rejections.

Treatment of the humoral rejection usually follows concurrent treatment of the episode of ACR, but targeted attack to humoral immunity mechanisms has to be included, with a combination of plasmapheresis, IVIG, and rituximab or bortezomib. Persistence of either cellular rejection or high antibody titers is ominous.

In those rare cases of hyperacute or accelerated acute rejection, graft removal is almost always required; if pharmacological therapy is attempted, it should include aggressive use of antithymocyte agents, in addition to plasmapheresis, rituximab and other agents that target antibody-mediated responses.

Chronic rejection

Approximately 15% of patients receiving an intestinal transplant will develop chronic rejection [4]. Risk factors include the number and length of previous episodes of ACR, especially moderate and severe episodes, viral infections, and previous episodes of PTLD [4,37].

Signs and symptoms are not always easy to detect. Patients usually develop: chronic malabsorption, with increased stool output and occasionally steatorrhea; frequent dehydration episodes requiring intravenous replacement; and repeated episodes of abdominal distension and pain. Upon endoscopy the mucosa may appear normal or near normal, though the intestinal villi are often flat. Intestinal absorption tests will be abnormal in such cases and patients often require parenteral nutrition supplementation. Chronic rejection also leads to the development of strictures in the bowel graft and recurrent episodes of small bowel obstruction.

Radiological studies will show thick, matted loops of bowel with loss of mucosal folds and poor motility with a lead tube appearance of the graft. Encapsulating peritoneal sclerosis has also been described in a few cases, although this finding is usually evident only in the operating room [38]. Most studies of chronic rejection have focused on the histology of explants after death or graft enterectomy [17,37]. This is due to the fact that intestinal mucosal biopsies are too superficial to evaluate the small and medium-sized vessels located in the muscularis mucosae layer of the intestinal wall. Therefore the diagnosis of chronic rejection can be made only indirectly if significant mucosal fibrosis with crypt loss is observed or at the time of the organ removal when the whole thickness of the bowel wall can be evaluated [39].

Some patients will develop only focal areas of intestinal strictures, especially in the distal ileum; in such few selected cases, it is possible to perform partial small bowel resection of only the affected areas and save the rest of the intestinal or multivisceral graft. In many cases, however, removal of the graft will be required. In cases of complete enterectomy, preoperative embolization of the intestinal graft is advisable because it demarcates the graft, facilitates the resection, and helps to reduce blood loss by clearly demarcating native and transplanted bowel [31].

Summary

It is clear that rejection is one of the most significant issues in the management of any intestinal transplant patient. The significant morbidity and mortality associated with the development of rejection episodes results in poor long-term survival rates. Important advancements have been made over the years and rejection rates have improved significantly. It is universally accepted that induction therapy has to be given at the time of transplant, whether with antilymphocyte agents or interleukin-2 receptor inhibitors. It is also clear that a very high index of suspicion must be maintained at all times, not only during the first year after transplant. Definitive diagnosis of any rejection episode requires endoscopy and biopsy; non-invasive markers of rejection, although helpful, have not yet been able to replace histological analysis. Early and aggressive treatment of rejection episodes prevents progression of severity and potential graft loss. Infection complications must be tackled simultaneously with treatment of any episode of rejection. It is yet unclear how chronic rejection can be prevented and how to best treat antibody-mediated rejection episodes. Monitoring and modulation of the recipient humoral response is rapidly becoming a critical issue as compared to the past. DSA/PRA levels, cross-match at the time of transplant, desensitization protocols for presensitized patients, and use of agents like rituximab and bortezomib are all tools that should now become standard of care when treating patients after intestinal transplantation.

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CHAPTER 75

Clinical Allograft Rejection Syndromes in Vascularized Composite Allotransplantation

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Introduction

With the number of hand and face transplants approximating 100, accumulating data indicate that even though the skin, a highly immunogenic tissue, is part of the graft, hands and faces are not irreversibly rejected after transplantation [1–8]. Graft survival at 1 year after such transplantations appears to be consistent with that in other organ transplants. Induction therapy with monoclonal or polyclonal antibodies (as used in most cases) together with a triple maintenance immunosuppression regimen is sufficient to prevent early graft loss [1–8]. However, episodes of acute skin rejection are observed in most patients at rates that appear higher than those associated with other organs and, indeed, these rejection episodes can lead to graft loss: one hand was lost due to late acute rejection (AR) after stopping immunosuppression, and a second was lost in response to "skin inflammation" that was not further specified [1,8,9]. Furthermore, steroid-resistant rejections are frequently observed and require treatment with antithymocyte globulin (ATG), basiliximab, or alemtuzumab [1,8–10]. Scientific reports indicate that the majority of patients demonstrate at least one episode of AR in the first year and that skin was the primary target of the immune response [1–10]. Repeated episodes are observed in some patients beyond the first year after transplantation [1,5–10].

The high frequency and severity of AR in hand transplantation has been attributed to the high immunogenicity of the skin, which forms a major component of the graft. The high antigenicity of the skin can in part be related to the high proportion of potent antigen-presenting cells (Langerhans cells) and keratinocytes, which express MHC I constitutively and MHC II, ICAM-I, and proinflammatory cytokines upon stimulation [11–20]. Also, viral infections, in particular cytomegalovirus (CMV), have been postulated to trigger the episodes [21]. It also has been postulated that the higher incidence of AR reported in hand transplants is due to the ability to visually detect rejection (resulting in an increased positive prevalence). Accessibility of the skin component of a hand allograft enables biopsies to be taken routinely and whenever clinical suspicion warrants histopathologic confirmation. Thus, visual monitoring allows for diagnosis of conditions in hand transplants that may often be missed in organ transplants.

Clinical allograft rejection syndromes are a consequence of an immune response toward one or more of the tissues comprising a vascularized composite allograft (VCA). While skin rejection is most frequently observed after human hand and face allotransplantation, an immune response toward tissues other than the skin has been documented [22–24].

Within the first year post-transplant, approximately 85% of recipients experienced at least one episode of acute skin rejection [1]. The clinical relevance and long-term implications of skin rejection on graft survival and functional outcome remains unknown. An evaluation of over 170 skin biopsies taken from five human hand or forearm transplant recipients revealed that most skin rejections were histologically mild (grade I) [25].

This chapter presents the clinical appearance, diagnosis, and treatment of skin rejection following transplantation of a VCA. Given the low number of transplants performed to date, most data are anecdotal compared to other organ systems. The histologic appearance is covered in greater depth in Chapter 87.

Diagnosis, definition, and mechanisms

Skin rejection

Observations made in the first decade of human hand and face transplantation point toward the skin as a prominent target for rejection [1–10]. More recent findings, however, indicate that also the vasculature of the graft may be affected and proliferation of the myointima can occur [23]. Indeed, these vascular effects may be more consequential with regards to long-term graft survival. Skin rejection usually manifests with maculopapular skin lesions, which can be restricted to small areas or spread over the whole allograft. They vary in color and location and present with or without edema and onychomadesis [5,10,26]. In contrast to solid organ transplantation, rejection of skin-containing VCAs can be diagnosed by visual inspection. In combination with the histopathology of skin biopsies, this allows for rapid diagnosis of rejection, but may also confound the clinical situation as other non-alloimmune processes such as fungal infection and autoimmune diseases can present as a rash. The main histopathologic finding of skin rejection is an immune cell infiltrate, varying in location, distribution, and

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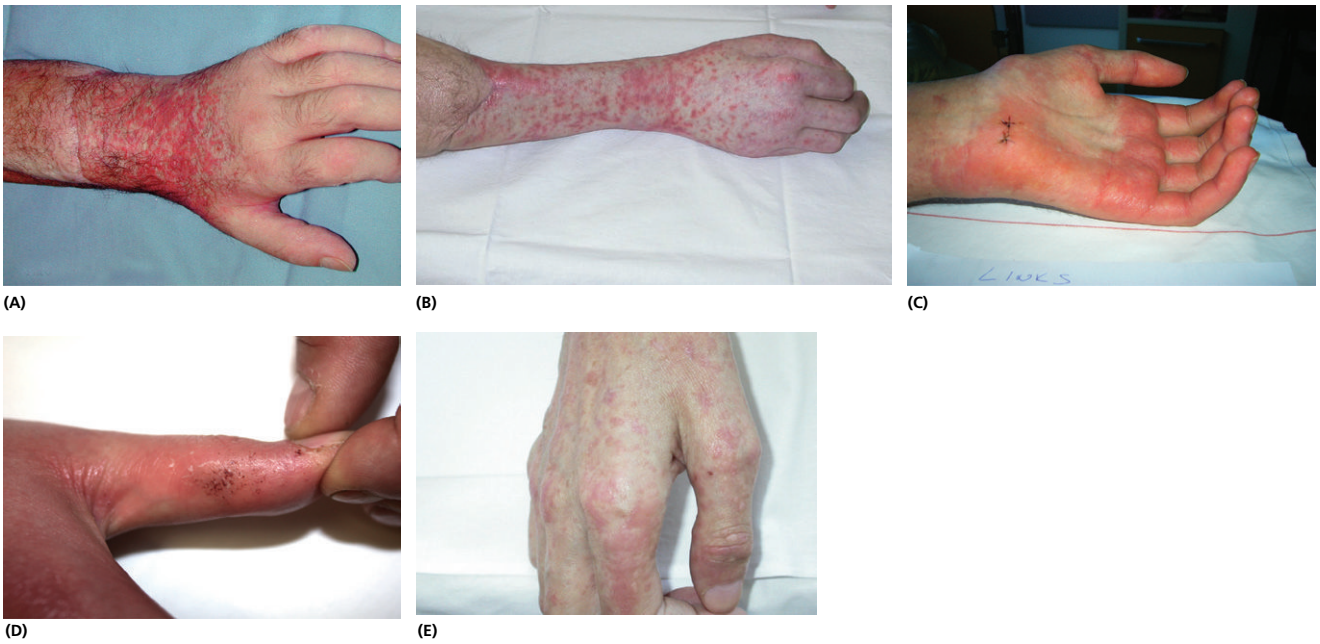

Figure 75.1. The heterogeneity of the appearance of skin rejection in hand transplantation. Very commonly, rejection appears on the forearms with confluent maculopapulous lesions on the dorsum of the forearm (A). Lesion can be heterogeneously scattered over the entire graft (B) or have a specific localization such as the palm of the hand (C) in different types of rejection. Upon mechanical stress and stimulation, rejection may appear on exposed areas (D). Late after transplantation the appearance may differ from rejections early after transplantation and be brighter in color and less prominent (E).

expansion, depending on rejection severity, as well as apoptotic and necrotic cells/tissue in more advanced stages [9,22,26].

The "classic" lesions of skin rejection in hand transplantation affect the dorsal and volar aspects of the forearm and wrist and, in some cases, the dorsum of the hand. The appearance of skin rejection varies interindividually, and even within one patient the presence of rejection can differ from one episode to another one (Figure 75.1). AR has been characterized as a maculopapular erythematous skin rash that may be diffuse, patchy, or focal, with or without burning pain. The location, color, size, and progression of the lesions can differ.

While the palm of the hand and the nails, usually are spared, an "atypical" pattern of rejection involving the palmar skin and the nails has been observed in a series of four patients [10]. In these cases, all patients were young and exposed to repetitive and persistent mechanical stress of the palm. Characteristic features of these rejections included a desquamative rash associated with dry skin, red papules, scaling, and lichenification localized to the palm. Skin lesions were associated with nail dystrophy, degeneration, deformation, or loss. This type of rejection also seems to differ with regard to the heterogenic pattern of rejection on the palmar skin and appendages like nails. The involvement of the nail bed/matrix by rejection leads to weakening/thinning of nail plate followed by loosening of the nail from its bed. Although also observed in conventional types of rejection, resistance to steroids seems to be more frequent in this type of rejection. Histology of the skin and nail bed revealed a lymphocytic infiltrate with predominance of T cells ($CD3^+$, $CD4^+$, $CD8^+$) with small numbers of B cells ($CD20^+$, $CD79a^+$) and a low number of $FOXP3^+$ cells. The lesions persisted over weeks to months, responded poorly to steroid treatment, and were managed with antithymocyte globulin, alemtuzumab and/or intensified maintenance immunosuppression.

Repetitive and significant mechanical stress could not only initiate but also sustain an inflammatory and immune response in the skin of the palm. A neutrophil-mediated induction and acceleration of AR has been described previously and might also be a relevant mechanism in this context [27].

At this point, any erythematous skin rash on a VCA independent of specific appearance size, location, progression has to be considered a skin rejection until proven otherwise. Differential diagnoses include skin infection, pemphigoid, and other skin inflammatory disorders and hyperemia (usually not associated with a rash); a skin biopsy should be performed in any event.

Mechanisms of skin rejection

Based on experimental, clinical, and histologic assessment, AR in VCA seems to be a predominantly cell-mediated immune response directed against the skin—most prominently the epidermis. While the dominance of $CD4^+$ or $CD8^+$ $CD3^+$ T cells has shown some diversity between centers and reports, the phenotype of the cellular infiltrate is dominated by CD3 T cells in any case of skin rejection in VCA [28–30]. Macrophages comprise 10–50% of the infiltrate in mild to moderate skin rejection. In grade IV rejection, $CD68^+$ macrophages are the dominating cell type.

In-depth histopathologic examination of skin biopsies of human hand and face allografts indicates that skin rejection is a dynamic and multistep process. After transmigration through the vessel wall the T cells form a dermal immune cell infiltrate (mainly composed of $CD3^+$ T cells), located in the perivascular areas [29]. Inflammatory cells then spread within the dermis. Guided by a number of cytokines, the cell infiltrate starts to migrate toward the epidermis as rejection progresses. Keratinocytes are known to secrete numerous proinflammatory cytokines when activated [29] and this may further enhance the recruitment and attraction of inflammatory

cells. The induction of apoptosis and necrosis of single keratinocytes, followed by dermal–epidermal separation and finally necrosis of the epidermis, is the final step in the cascade of skin rejection [9,26,29]. In animal models where the size of the graft and the skin defect is limited, regeneration starting at the margins of the graft results in reconstitution of the skin and coverage of the defect [30].

Investigation of adhesion molecules in human hand allografts revealed an up-regulation of E- and P-selectin, which are responsible for leukocyte rolling along the vessel wall, and also ICAM-1 on the vascular endothelium and its counterpart LFA-1 on lymphocytes, which enable cell adhesion to the vessel wall [29]. Expression of all adhesion molecules is particularly up-regulated during severe rejection, indicating that adhesion molecules mediate leukocyte and lymphocyte rolling and adhesion to the vessel wall in the dermis as one of the initial steps in skin rejection [29].

The clinical relevance of antibody-mediated rejection (AMR) in VCA is not well defined [31]. In two different studies and a large set of skin biopsies taken from human hand and face allografts, $CD20^+$ B cells were sparse or absent [29,31,32]. Aggregates of B cells located in the dermis and subcutis were found only in one case of severe allograft rejection [28].

C4d deposition was found in the capillaries of two human hand allograft recipients in the presence of cellular rejection [33], but was also found in the absence of clinical rejection. When analyzed in a large set of skin biopsies, C4d staining was positive in 50% of specimens from six human double hand/forearm transplant recipients showing rejection [29]. Donor-specific alloantibodies (DSA, HLA class I/II) were negative in all cases reported [28,33,34].

Grade I skin rejection

Mild skin rejection represents a particular problem in VCA. While it is commonly observed, its clinical relevance is not well defined. A comprehensive histologic and immunohistochemical analysis of mild rejection revealed a $CD8^+$-prominent cellular infiltrate with sparse $CD20^+$ B lymphocytes and $CD68^+$ macrophages. Adhesion molecules LFA-1, ICAM-1, E-selectin, P-selectin, and VE-cadherin are up-regulated and C4d staining was mainly positive in samples at time points later than 1 year. There was a tendency toward increased indoleamine 2,3-dioxygenase (IDO) expression and more $Foxp3^+$ cells at later time points [25]. A tolerogenic phenotype of a proportion of the infiltrating cells (IDO or Foxp3 expression) was observed during grade I rejection and the percentage of these cells increased at later time points. This observation suggests that regulatory mechanisms are present within the skin of the allograft and may contribute to self-limitation of the alloimmune response [25].

Assessment of skin rejection

When the first hands were transplanted, it was assumed—based on observations in experimental limb transplantation—that the skin might be the primary target for AR. Biopsies of the skin were therefore obtained routinely or whenever clinically indicated. After the first hand transplants had been performed, it became apparent that an erythema with maculopapulous erythematous lesions on the skin represented the macroscopic picture of rejection and correlated well with histomorphologic findings [2–10].

Rejection occurs affecting a heterogeneous pattern of the skin; biopsies taken close to the lesions but not exactly from the affected areas may show normal histology. Therefore, the exact site of skin sampling is of utmost importance. Biopsy specimens are collected as per protocol or whenever clinically indicated. In hand and arm allografts, the forearm or upper arm as sites for taking biopsies are considered anatomically less problematic when compared to the hands. In any case, biopsies need to be taken from the lesions. In face transplant patients, biopsies may be taken from a "sentinel skin flap" often transplanted together with the face. The correlation between skin rejection on a sentinel skin graft and a facial allograft, however, needs to be more convincingly established before biopsies taken from such a distant site can be considered reliable. Histologic and immunohistochemical investigation of skin biopsy samples should be performed according to the 2007 Banff Classification for skin-containing VCA (see Chapter 87).

In summary, the skin seems to be the most evident target for an immunologic response after hand transplantation with early rejection additionally characterized by some minor mononuclear cell infiltration in other compartments, primarily the perineurium and connective tissue. The role of alloantibody remains speculative, but likely to emerge with additional clinical experience. Vascular injury, be it from cellular- or antibody-mediated mechanisms, is concerning both for its prognostic implications and the general inability for it to be assessed directly on biopsy.

Grading of acute skin rejection

Currently, the skin is the component of a VCA routinely monitored for rejection. An erythema comprised of maculopapulous lesions scattered irregularly over the transplanted hand is the clinical feature of AR. So far, such an erythema has always been associated with a mononuclear cell infiltrate as the histologic sign for rejection; in our experience, however, absence of macroscopic lesion does not necessarily indicate normal skin histology. In relation to what has been described previously in kidney transplantation, this is referred to as "subclinical rejection."

The main histologic feature of cellular rejection is a mononuclear cell infiltrate. It first appears in the perivascular space of the dermis. If rejection progresses, the infiltrate spreads to the interphase between dermis and epidermis and/or adnexal structures. A cellular infiltrate within the epidermis is typical for a moderate rejection with the immunologic response reaching the outermost layer. When rejection is not successfully treated at that stage, the inflammatory character of this process shifts toward destructive features; necrosis of single keratinocytes can be observed resulting in focal dermal–epidermal separation. With further progression, necrosis and loss of the epidermis as the ultimate stage of skin rejection is assumed to be irreversible.

The composition of infiltrating cells does not allow for distinction of rejection of a hand transplant from dermatitis; however, when lesions are clinically limited to the graft, rejection can be assumed. In addition, it is rather unlikely that an unspecific dermatitis would occur under immunosuppression.

Rejection of tissues other than skin

Limited information is available about the involvement of components other than the skin in AR. Biopsy samples from bone, vessels, nerves, or muscles have not been routinely obtained clinically. Based on clinical reports, tissues other than the skin are affected to a lesser extent, but this reflects some selection bias with regard to sampling [1,3–6,8,9,22].

Probably the best individual example is the systematic histopathologic assessment of the hand amputated 2.5 years after transplantation in Lyon. Although the hand revealed erosive and necrotic areas over the skin, only mild inflammation in muscles and tendons and no changes in bones and joints were observed [22].

Additional evidence from deep tissue biopsies confirmed a dominance of the immune response toward the skin but also revealed cellular infiltrates in muscle and connective tissue. Infiltrates in larger vessels and a perivascular infiltrate in muscle, tendons and nerves together with a lymphocytic infiltrate in the bone marrow, however, indicate that other compartments can be affected to some extent [22–24]. Potential long-term effects of such limited lymphocytic activity remain to be elucidated and tools other than biopsies are needed for monitoring these compartments. However, in some patients the vasculature, too, has demonstrated hyperplasia of the intima indicating vasculopathy and resulting in loss of the hand in one case [23].

In summary, there is good evidence that a VCA in aggregate induces less immune response than the individual components; this is convincing data supporting the concept that the skin elicits the strongest immune response, and weak evidence that tissue mass has an impact on allograft rejection.

Chronic rejection

A 1-year graft survival of 100% achieved in hand transplantation is better than any outcome achieved in solid organ transplantation. However, this is to be interpreted in light of the fact that in other organs, survival is defined functionally, not by the presence of the graft. For VCA, functional parameters remain to be defined in large part due to the lag involved in re-innervation, and the general lack of intrinsic muscle function in distal graft elements. Nevertheless, well-designed immunosuppression protocols together with the chance of early detection of AR by visual inspection may be responsible for these satisfactory early results. It remains unclear at this point if long-term graft survival may be impacted by the development of VCA vasculopathy and degeneration. Experimental evidence suggests that this is a valid concern.

Unadkat et al. have published a systematic analysis of an orthotopic hind limb allotransplantation model in rats in which multiple AR episodes resulted in development of myointimal proliferation and morphologic signs for chronic rejection (CR) [35]. In this model, repeated AR episodes were treated with cyclosporine A and dexamethasone. At postoperative day (POD) 90 animals had gone through 19 episodes of AR and myointimal proliferation associated with concentric luminal occlusion and perivascular fibroses similar to vasculopathy in cardiac transplantation was observed.

In vascularized bone transplantation, six human vascularized allogeneic knee transplantations were all lost within the first 56 months. A histomorphologic assessment of the latest case resulted in detection of diffuse concentric fibrous intimal thickening and occlusion of graft vessels [36]. Findings are comparable to cardiac allograft vasculopathy (CAV). In cases of bone transplantation, the lack of adequate tools for monitoring rejection might have allowed repeated or ongoing AR of the bone, eventually triggering myointimal proliferation and occlusion of graft vessels.

In hand transplantation, one case was described recently in which unmanageable ischemia in the hand resulted in amputation. The histopathologic assessment of the limb revealed significant myointimal hyperplasia in the graft arteries. Hyperplasia was confluent in all studied arteries restricted to donor vessels.

In solid organs, myointimal proliferation and luminal occlusion of graft vessels—the hallmark for CR—have been observed as early as 1 year after transplantation (e.g. cardiac or kidney). Similar findings in one center, where there was of carefully monitoring patients after hand transplantation with high-resolution ultrasound techniques, gave rise to the hypothesis that graft vessel might be the major target of CR also in VCA. In other centers, however, ultrasound, angiography, and CT-angiography scan together with skin biopsies failed to identify early signs of luminal narrowing or occlusion. In conclusion, myointimal proliferation and impairment of perfusion were observed in a small number of patients after hand transplantation, and careful monitoring of the vasculature after hand and face transplantation remains mandatory. With regard to pharmacologic prevention of CR, a beneficial effect has been described for rapamycin [37]. It therefore may be reasonable to include rapamycin in a long-term immunosuppression protocol.

Management

Prophylaxis of rejection

Clinically applied therapy

Since VCAs are derived from genetically disparate deceased donors, recipients need to be immunosuppressed for life to prevent rejection of the transplant. A unique feature of a VCA is that such grafts, unlike solid organ transplants, are composed of a wider variety of tissue components such as muscle, tendon, nerve, blood vessels, bone, and skin. In particular, due to the fact that the skin is thought to be highly antigeneic, historically, VCA has been considered an immunologic challenge [38].This high immunogenicity of the skin has been mainly attributed to skin-specific antigens in the epidermis as well as the presence and high frequency of antigen-presenting cells (APCs), which could act in an immunostimulatory manner by direct presentation of alloantigens [39]. Thus, broader clinical application of hand allotransplantation has been hampered by particularly strong rejection of the skin component, necessitating long-term high-dose multidrug maintenance immunosuppression. This led to considerable concern for adverse effects of those immunosuppressive drugs that are required to sustain the graft. Such risks include, but are not limited to, infection, diabetes, hyperlipidemia, nephrotoxicity, or malignancy and remain the single most important obstacle to routine application of reconstructive transplantation in the clinic.

The current immunosuppressive protocols applied to hand transplantation are extrapolated from regimens used in solid organ transplantation. An in-depth coverage of maintenance immunosuppressive therapy is also found in Chapter 66. However, no standard immunosuppressive protocol has been established for reconstructive transplantation. The overall amount of immunosuppression required to ensure graft survival is comparable or even slightly higher than for kidney transplantation. However, as aforementioned, the use of maintenance immunosuppression in hand transplantation has resulted in a high graft survival at 1 year after transplantation (in patients compliant with immunosuppressive medication) [1]. Such conventional immunosuppressive strategies rely, for the most part, on agents that halt the robust immunologic attack on the graft in a non-specific manner. According to the International Registry on Hand and Composite Tissue Transplantation the majority of hand transplant patients received either polyclonal (antithymocyte globulins, ATG) or monoclonal (alemtuzumab, basiliximab) antibody preparations as an induction agent, followed by a high-dose triple drug combination for maintenance therapy, including tacrolimus, mycophenolate mofetil (MMF), and steroids [1,40]. The level of tacrolimus was adjusted to 10–15 ng/mL during the first 1–3 months and 5–10 ng/mL thereafter by most centers. With regard to steroid management, prednisone doses were rapidly tapered in the early post-transplant period and

then maintained on lowered doses (5–15 mg/day) for 6 to 12 months in most of the patients. Of the 33 patients included in the most recent report of the international registry, all recipients received tacrolimus as the baseline immunosuppressive agent, 26 received mycophenolate mofetil, and 27 received steroids. During the follow-up period, eight patients were converted from tacrolimus to the mTOR inhibitor sirolimus with the rationale to minimize renal side-effects, improve glycemic control, and to potentially avoid chronic vascular changes (myointimal hyperproliferation) and neurotoxicity [41]. In five cases, steroids were withdrawn; two recipients received steroids and low-dose of tacrolimus and everolimus; and two patients received sirolimus and MMF [1,40]. Of those patients who did not receive induction therapy ($n = 2$) or were not started on triple immunosuppressive regimens ($n = 2$), topical steroid and tacrolimus ointments were applied in addition to systemic immunosuppressive medication. As of now, induction therapy followed by maintenance immunosuppression with at least a dual-drug combination at optimum dose can be considered the most widely used treatment protocol for reconstructive transplantation.

Overall, such conventional regimens have proven sufficient to prevent early immunologic graft loss but were not able to prevent AR so that 85–90% of all hand transplant recipients experienced at least one AR episode within the first year after transplantation. This incidence is significantly higher than what is currently seen in solid organ transplantation, where AR rates are less than 10% in selected series during the first year after renal transplantation [42]. However, this is likely influenced by the fact that the grafts in reconstructive transplantation are visible, and this enables immediate diagnosis of rejection based on even minor changes in appearance. Episodes of AR were always macroscopically characterized by erythematous, maculopapular, heterogeneously scattered cutaneous lesions, that correlated well with the histomorphologic findings of mononuclear cellular infiltrates [43].

The other components of a hand allograft like muscle, nerves, tendon and bone do not seem to be subject to significant damage when episodes of skin rejection occur. However, available information on the involvement of these components is still very limited and more data are needed before a final conclusion can be made that the skin is the sole and prime target of rejection in reconstructive transplantation (see also Section Rejection of tissues other than skin).

Chronic multidrug immunosuppression is expensive and causes substantial long-term costs. Furthermore, due to the amount of daily oral medication required and its resulting high patient burden, such regimens frequently lead to non-compliance. However, considering these obvious downsides of multidrug immunosuppression and its various, sometimes severe side-effects, there is an evident need for novel concepts of systemic immunosuppression in VCA to prevent rejection. In this regard, hand transplantation might offer some unique advantages because continuous monitoring of the graft in contrast to solid organ transplants can be performed by simple visual inspection of the skin, this being the main target of rejection. This allows for directed biopsies and unbiased pathologic confirmation of the earliest stages of AR and subsequent timely intervention, treatment, and precise adjustments of immunosuppression on an individualized basis. When treated adequately and effectively, AR does not seem to impair graft function or long-term survival. Therefore, novel strategies to minimize immunosuppression or even to achieve the ultimate attainable clinical goal of transplantation to induce immune tolerance are particularly appealing in hand transplantation and VCA in general. Studies from our own group demonstrated that a whole limb allograft elicited a less intense alloimmune response than did allografts of each of its individual components, thereby challenging the relative scale of tissue antigenicity [44]. In addition, certain types of VCA contain immunocompetent elements such as bone marrow and lymph nodes that may hasten the rejection processes or result in graft-versus host-disease (GVHD). These factors not only govern the immune reactivity of these allogeneic tissues, but also define potential immunomodulating strategies that are different from those currently used in solid organ transplantation.

Experimental therapy

Historically, routine therapy (as outlined above) has been a non-specific suppressive multidrug regimen, administered to dampen the recipient's immune system. Side-effects of such therapy, however, include an increased risk for opportunistic infections, metabolic disorders, or malignancies. For this reason, research has focused on developing new drugs and biologic agents that aim to specifically target key pathways and receptors during allogeneic T-cell activation, in order to avoid the unwanted negative effect on other immune lineages. This significantly reduces undesirable complications while still enabling graft survival [45].

Furthermore, various modifications have been applied over the years to the immunosuppressive protocols used in hand and face transplantation such as steroid sparing/avoidance attempts, alemtuzumab induction, cell-based immunomodulatory protocols, conversion from tacrolimus to the mTOR inhibitor sirolimus for long-term therapy, or the use of topical steroid and tacrolimus ointments to reduce the overall amount of systemic immunosuppression [46].

Interestingly, a small number of organ recipients have been documented to tolerate their grafts, allowing withdrawal of immunosuppression without promoting rejection [47]. This phenomenon of spontaneous donor antigen-specific immunologic tolerance has given rise to the realization that there is a feasible way to overcome histocompatibility barriers not by means of immunosuppression, but rather immunomodulation or immune regulation. In addition, spontaneous tolerance, achieved in experimental organ transplant models, is often the result of an exhausted host-versus-graft immune response. Such a state of operational tolerance is achieved when the immune response toward the transplant is exhausted because the specific cell clones mediating rejection are deleted. Furthermore, maintenance of engraftment has been postulated to be facilitated when a small number of donor leukocytes persist in the recipient (microchimerism) long term [48]. Such strategies would be particularly appealing for the field of VCA since those transplants, even though significantly improving the quality of life, are not considered life saving and have inherent unique biologic features. Nevertheless, the "risk–benefit balance" in VCA needs to be most carefully addressed, probably even more so than in any other type of solid organ transplantation. To date, several small and large animal studies show highly encouraging results when transplanting vascularized composite allografts without the need for long-term maintenance immunosuppression [49,50]. Strategies applied in these experimental models include the use of total lymphoid irradiation, costimulatory blockade (CD28/B7 and CD154/CD40 pathways), selective depletion of alloreactive recipient T and B cells (e.g. $\alpha\beta$-T cell and CD20-specific monoclonal antibodies), inhibition of lymphocyte trafficking, infusion of

$CD4^+CD25^+Foxp3^+$ regulatory T cells and tolerogeneic APCs, as well as donor bone marrow infusion and chimerism induction [29,51–57]. Indefinite graft survival without the need for long-term systemic immunosuppressive treatment was achieved with the use of an immunomodulatory concept employing donor bone marrow cells in complete MHC-mismatched swine heterotopic hind-limb transplant models [58,59]. This concept has been translated into a novel clinical cell-based treatment protocol for reconstructive transplantation by a joint team at Johns Hopkins University School of Medicine and the University of Pittsburgh [60]. In the initial experience, five patients received alemtuzumab induction and maintenance immunosuppression in the form of tacrolimus monotherapy as well as an infusion of unmodified donor bone marrow cells. This study is currently ongoing but has demonstrated thus far that the protocol is feasible and has allowed reconstructive transplantation in upper extremity amputees with low-dose tacrolimus monotherapy.

Along these lines, Mathes et al. have developed a translational protocol that combines a non-myeloablative induction approach and VCA in a canine model that has led to tolerance to all components of the VCA, including skin, without the need for long-term maintenance immunosuppression [61]. Thus, when considering development of novel therapeutic strategies for minimization or avoidance of maintenance immunosuppression following reconstructive transplantation, cell-based protocols including donor bone marrow or stem cells are promising candidates due to the unique nature of VCA. This trend is further fueled by recent advancements in solid organ transplantation, where both cell-based therapies and non-cell based protocols have resulted in reduction or elimination of long-term immunosuppression [62–64]. VCAs can include vascularized bone and bone marrow and might thereby function as a vascularized bone marrow transplant by itself [65]. Such a graft could be a continuous source of donor-derived stem cells, which have been demonstrated in animal models to favorably modulate the host immune response [66]. In this experimental setting, induction of donor-specific tolerance was attributed to the bone marrow component and to specific immunomodulatory protocols permissive for bone marrow engraftment. This recently led to an intense search of optimal bone marrow-based protocols to prolong VCA survival and to induce donor antigen-specific immune tolerance. Along these lines, Barth et al. developed a non-human primate model utilizing selectively mismatched cynomolgus macaques of facial segment allotransplantation to elucidate the unique pathophysiology, immunogenicity, and immunosuppressive requirements of VCA with addition of concomitant vascularized bone marrow [67]. Heterotopically transplanted facial segment VCA with vascularized bone marrow treated only with tacrolimus and MMF but no radiation or T-cell depletion for induction demonstrated prolonged rejection-free survival (205–430 days), compared to VCA without vascularized bone marrow that experienced early rejection episodes and graft loss by postoperative day 7–15 [67]. While VCA with vascularized bone marrow demonstrated sporadic low-level macrochimerism, AR and CR and graft loss occurred after discontinuation of maintenance immunosuppressive therapy. However, radiologic, phenotypic, histologic, and genotypic evidence supported the persistence of viable donor bone marrow within the transplanted vascularized bone marrow component. Interestingly, vascularized bone marrow in this model prevented allosensitization and the development of IgM and IgG alloantibodies even after withdrawal of immunosuppression [67]. Data from this translational study further indicate an important immunoregulatory role of the vascularized bone marrow component in VCA with the potential to reduce the need for immunosuppressive medication.

Despite these advances, overcoming CR still seems to be a formidable task in most transplant cases for solid organs and continues to limit the best possible long-term outcomes. However, if similar concerns are warranted for VCA, they still need to be determined. Thus far, only a few patients have a follow up beyond the 10-year mark and the incidence of CR in VCA seems to be low in this pioneering cohort. There is only one report in the world series of VCA of graft loss in a patient compliant with immunosuppression that showed vascular lesions such as intima hyperproliferation that are reminiscent of CR in solid organ transplantation. This underscores the importance of close long-term surveillance and standardized follow-up protocols in VCA in particular, with more and more experimental minimization and tolerance-inducing protocols emerging.

Treatment of rejection

Clinically applied therapy

Almost all hand and face transplant recipients to date have experienced AR episodes regardless of their induction or maintenance immunosuppressive treatment protocol. Again these rates of skin rejection are higher than those observed after kidney, liver, or heart transplantation. All episodes of skin rejection encountered and diagnosed clinically were confirmed with skin punch biopsies. Similar to maintenance immunosuppressive regimens, treatment of AR mirrors therapies in solid organ transplantation. Additional coverage of rescue therapy in general can be found in Chapter 67. According to the International Registry on Hand and Composite Tissue Transplantation, treatment of acute skin rejection episodes included high-dose bolused intravenous steroids in 60% of all reported cases. In 30% of the recipients, however, increasing oral steroids was sufficient to reverse rejection. Only in 7.5% of patients, in which rejection episodes were steroid-resistant, treatment consisted of antithymocyte globulins, basiliximab, or alemtuzumab [1]. In addition to systemic treatment, topical application of immunosuppressive ointments (tacrolimus, corticosteroid, or flumix) was performed, taking advantage of the unique possibility of treating skin rejection locally. As under-immunosuppression was thought to be the most important cause for sustained rejection, immunosuppression was increased at the same time in most cases.

When a second rejection episode was encountered, steroids were given intravenously with or without topical ointment in several patients. Alternatively, ATG, basiliximab, or topical drugs were given only in a few patients. In one case of a steroid and ATG resistant rejection, alemtuzumab (Campath-1H, Genzyme, Cambridge, MA, USA) was administered and resulted in full restoration of normal skin histology [6]. Thus far, no graft has been lost due to acute irreversible rejection in the world experience with reconstructive transplantation. The definition of graft function as it relates to a VCA differs from that related to, for example, a heart, where mild degrees of damage would be intolerable clinically.

In hand transplantation most rejections were treated successfully with the modalities described above. In one case, namely the first hand transplanted in France in 1998, progressive AR following non-compliance with immunosuppression led to lichen-like lesions of the skin and progressive loss of function and prompted the patient to request amputation of the hand at 2 years and 4 months after transplantation [22]. Although it has been speculated that

rejection might have been reversible, treatment with a steroid bolus and an anti-CD25 antibody did not prevent progression of rejection. In the second case, a severe "skin inflammation" resistant to "any" treatment resulted in amputation of the hand at 3 years after transplantation [1].

Experimental therapy

Topical application of immunosuppressants has been routinely applied for maintenance immunosuppression as well as treatment of AR in most cases of hand transplantation [1–10]. Interestingly, the first report illustrating the concept of topical/local immunosuppressive therapy appeared as early as 1951, in which Billingham et al. found that topical application of cortisone acetate at a dosage that was ineffective when administered systemically prolonged skin allograft survival in a rabbit model [68]. Further examination of local immunosuppression, however, was then abandoned for almost two decades, with the exception of experimental and clinical studies demonstrating the efficacy of local graft irradiation. With advances in drug delivery systems and a more thorough understanding of both the cellular events during allograft rejection and the pharmacokinetics of target-aimed drug delivery such strategies have recently regained particular interest in the field of reconstructive transplantation.

Several delivery techniques including controlled drug release micro- and nanoparticles or other bioengineered matrices, liposomes, gene transfer and cellular co-transplantation have been used in experimental transplant models to direct immunosuppressive agents and molecules to the site of interest within the graft [69]. Hautz et al. characterized the cellular infiltrate and the mechanisms of cell trafficking in acute skin rejection in skin biopsies taken from human hand and forearm transplants [29]. In this sample set, expression of ICAM-1 and E-selectin correlated closely with the severity of rejection. E-selectin was found predominantly in the endothelium of the vessel wall in both mild and advanced rejection. Endothelial E- and P-selectin are known to mediate tethering and rolling of immune cells along the vessel wall as part of the complex multistep cascade that leads to recruitment of alloreactive T cells and other immune cells to the skin. The concept of therapeutically targeting adhesion molecules has also been pursued in inflammatory skin diseases with, for example efalizumab, which has been applied successfully for the treatment of psoriasis [70]. In a proof-of-concept experimental small animal study this group demonstrated that limb allograft survival is significantly prolonged by local administration of a specific selectin blocker (efomycine M) in combination with amyotrophic lateral sclerosis (ALS) induction and low-dose tacrolimus maintenance therapy. Such a combination of lymphocyte depletion and targeting lymphocyte trafficking in the skin of a VCA seems to be an appealing approach for the treatment of both AR episodes as well as to minimize/spare maintenance immunosuppression. However, one downside of such topical approaches might be that it leaves rejection of other tissue components of VCA undetected and allows progression of an immune response within deeper tissues such as muscle and bone. Although an immunosuppressive agent limited to the graft would be desirable, rejection represents a systemic response and it is unlikely that topical treatment alone would be sufficient to prevent or treat rejection. Therefore, although promising, therapeutic strategies employing topical drug administration to treat acute skin rejection in reconstructive transplantation warrant further evaluation in translational animal models before they can be safely introduced in humans.

The ideal immunosuppression

The ideal situation and ultimate attainable clinical goal in transplant medicine would be the ability to induce donor antigen-specific immune tolerance. This by definition would facilitate avoidance of any type of immunosuppression and could significantly improve long-term graft outcomes [46]. Complete discussions of tolerance from a mechanistic and clinical implementation standpoint can be found in Chapters 11 and 76, respectively. However, tolerance induction has not been routinely achieved thus far and it is not very likely that it will become a clinical reality, broadly available in the near future. Nevertheless, recently introduced innovative protocols appear to provide the opportunity to wean patients from maintenance medication altogether or allow reduction of long-term immunosuppression to minimal levels [62–64]. However, whether such tolerogenic protocols will be ultimately effective in VCA remains to be shown.

For now, clear evidence for superiority of one immunosuppression regimen over another can be derived only from prospective, randomized clinical trials. For hand or face transplantation, a field that is still in its infancy, such data will unfortunately not be available for several decades. Therefore, an immunosuppression regimen needs to be based on the limited experience made in those patients transplanted to date, building on the progress and advances made in solid organ transplantation and data obtained from experimental VCA studies. For those reasons, it is mandatory to implement standardized follow-up protocols and outcome measures to enable comparison of data across institutions and different protocols as well as to continue to pursue multicenter prospective trials despite the limited number of patients enrolled in such trials.

When designing an optimized immunosuppressive protocol for VCA, several differences from organ transplantation need to be taken into account: (1) VCA patients, apart from their amputation or devastating craniofacial tissue loss, are otherwise healthy recipients and usually lacking co-morbidities that might increase the risk of infection and possibly development of malignancy; (2) transplantation of a hand or face is not considered life saving, nor does it positively influence long-term patient survival; (3) the skin component of a VCA is constantly visible and rejection can be detected in most cases by simple visual inspection and biopsies can be taken from lesions under direct vision; (4) repeat acute skin rejection episodes when adequately treated seem not to negatively impact long-term allograft function; (5) the relevance of DSA and AMR at least at this time seems to be less when compared to solid organ transplantation; and (6) virtually all AR episodes thus far in VCA occurred during the first postoperative year.

These factors might be particularly relevant and should be considered with regard to the development and design of novel therapeutic strategies or the ideal protocol for VCA. Hence, based on the world experience with reconstructive transplantation and the available outcome data reported, we make the following recommendations for an immunosuppressive protocol for VCA:

1. An induction therapy with a depletional biologic such as ATG or alemtuzumab may help to prevent early rejection and keep the requirement for maintenance immunosuppression low.
2. Tacrolimus should be part of the immunosuppression regimen during at least the first 2 years because it has demonstrated support of nerve regeneration via its stimulatory effect on the synthesis of axotomy-induced growth-associated protein (GAP-43).
3. During the first 2 years a triple therapy including steroids and mycophenolic acid seems to be effective in preventing AR.

Steroids should be tapered and can finally be withdrawn within that same time period.

4 At 2–3 years after transplantation, calcineurin inhibitors should be replaced with a TOR-inhibitor for their favorable toxicity profile, their antiproliferative and potential protective effect on CR, as well as their antitumor properties.

Summary

In conclusion, when planning the design of new immunosuppression protocols, specific requirements for a VCA mentioned above need to be taken into consideration. Despite the fact that great advances have been made in choosing the most favorable combination of medications from the multitude of available agents to prevent solid organ allograft rejection, the same regimen may not always be optimal when used or attempted in reconstructive transplantation due to the unique biologic/immunologic features outlined in this chapter. In addition, immunosuppression minimization strategies designed for solid organ transplantation should be carefully reviewed and applied with due diligence to VCA.

Because there is no "magic bullet" or breakthrough in conventional immunosuppression on the horizon, we need to continue to optimize combination strategies of the currently available agents and compare data with other centers to create protocols with low toxicity profiles and inhibitory effects on chronic myointimal hyperproliferation and CR. Adherence to these guidelines will ultimately favor the risk–benefit balance of these life-changing transplants and advance this novel era in transplantation.

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CHAPTER 76

Clinical Transplantation Tolerance

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Introduction

Clinical tolerance, defined broadly as the functional acceptance of an allograft without the requirement for ongoing immunosuppression, has been a fundamental goal of transplantation since the initial studies by Medawar that conceptually galvanized the possibility for allotransplantation (see Chapter 11). It is generally and reasonably assumed that the induction of allograft tolerance will improve long-term results of organ transplantation. However, it is only recently that this goal has been prospectively achieved. It is clear that tolerance requires that patients undergo treatments beyond those required to prevent allograft rejection. As such, the short- and long-term risks of those treatments must not outweigh the benefits conferred by tolerance provided by current standard of care. This chapter summarizes the ethical considerations, indications, and strategies for tolerance induction in clinical organ transplantation.

Risk of long-term immunosuppression

Since the first successful human kidney transplantation by Murray et al. in 1956 [1], numerous immunosuppressive regimens have been developed for clinical application. As these more efficacious immunosuppressive drug combinations have successfully prevented or treated acute allograft rejection, short-term survival of organ transplants has significantly improved, resulting in solid organ transplantation becoming the therapy of choice for many end-stage organ diseases [2,3].

However, chronic use of immunosuppressive drugs results in significantly increased risks of cardiovascular disease [4–6], infection [7–9], and malignancies [10–13]. Immunosuppressive medications also cause various side-effects, such as nephrotoxicity, de novo diabetes [14–16], dyslipidemia [17–20], and neurotoxicity [21,22]. Cardiovascular disease has become the most common cause of mortality, accounting for 30–48% of the deaths after kidney transplantation [10]. Infection is also a common complication and remains the second most common cause of mortality after transplantation [7–9]. The risk of many common cancers has been observed to be higher in chronically immunosuppressed transplant patients. Such risk is particularly high in non-melanoma skin cancer and melanoma/leukemia, which are 30-fold and 3-fold higher, respectively, than that of the general population [11,23]. Some studies report that the overall incidence of cancer after kidney transplantation is as high as 40% after 20 years of immunosuppressive therapy, in contrast to a 6% cumulative risk for cancer in an age-matched, non-transplanted control population [13,24]. Because of these morbidities, death with a functioning graft accounts for 16% of graft losses over the first 10 years after kidney transplantation [25]. Chronic nephrotoxicity from calcineurin inhibitor (CNI) treatment is also a serious side-effect, not only for kidney allografts but also for native kidneys in extrarenal transplant recipients. The incidence of chronic CNI nephrotoxicity in non-renal transplant recipients has been reported to be as high as 18%, with half of these patients progressing to overt renal failure requiring replacement therapy within 10 years [26]. Unfortunately, despite these toxicities that accompany the potent immunomodulatory effects of current therapeutic protocols, development of chronic rejection is not consistently prevented. Approximately 15–30% (dependent upon the assay utilized) of renal transplant recipients receiving maintenance immunosuppression eventually develop anti-HLA antibodies [27]. Chronic rejection, often in association with de novo alloantibody detection, accounts for about 20% of graft losses by 10 years after kidney transplantation [25]. In summary, the net effect of these limitations of currently available chronic immunosuppression protocols is an inexorable loss of previously functioning transplanted organs at an annual rate of about 5–7% [28], with no significant improvements of renal graft half-life in the last 10 years [2,3,29] (Figure 76.1).

Rationale of tolerance induction

Induction of tolerance has been considered one strategy to improve the long-term results of organ transplantation. Operational tolerance (defined as the absence of a destructive immune response to a transplanted tissue without ongoing immunosuppression) can be observed in up to 20% of liver transplant recipients following complete immunosuppression withdrawal, 4 to 10 years after the transplant procedure [30,31]. In contrast, such spontaneous tolerance is very rare in kidney transplant recipients and likely less common in heart or lung transplant recipients. These observations have emphasized that additional conditioning or treatment is required for consistent induction of tolerance across major histocompatibility complex (MHC) barriers. In order to justify clinical application, development of such additional conditioning/treatment regimens must utilize approaches with short and long-term risks that do not outweigh the benefits over current standard of care. Presumably, if tolerance could be induced consistently, chronic

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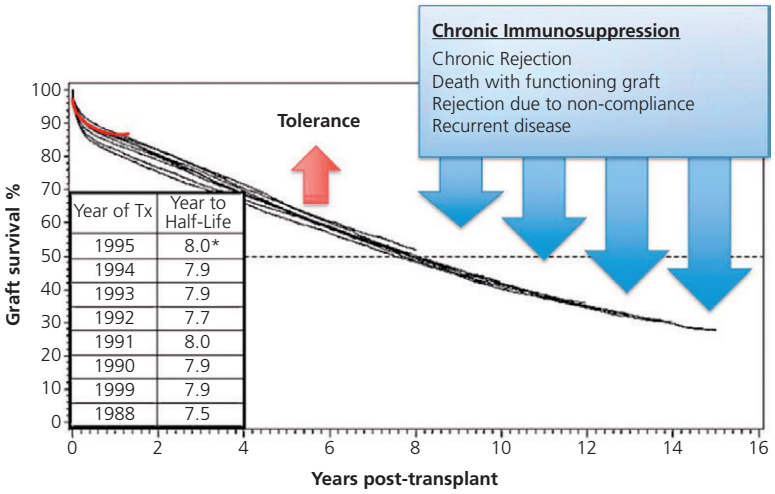

Figure 76.1. Currently available chronic immunosuppression protocols lead to an inexorable loss of functioning transplanted organs at an annual rate of about 5–7% [28], with no significant improvement of renal graft half-life in the last 10 years (lower survival curves, in black). Among the major causes for this graft loss, acute cellular rejection, chronic rejection, and rejection due to non-compliance may all be eliminated if tolerance can be consistently induced (projected survival curve in red). In addition, chronic rejection would be eliminated and the risks of cardiovascular disease, infection, and malignancy should be considerably minimized. * Years to 51.8% survival. (Modified from [36] Meier-Kriesche HU et al. Am J Transplant 2004;4:1289, with permission from Wiley.)

rejection would be eliminated and the risks of cardiovascular disease, infection, malignancy, and other drug-related complications should be considerably minimized. These advantages must be balanced against the potentially increased risks of infection and treatment-related morbidities in the early post-transplant period, which may be higher after administration of the conditioning regimens required for tolerance induction. It must also be weighed cognizant of the potential for recurrent disease in patients whose original disease was autoimmune in nature and for whom post-transplant immunosuppression may carry benefits beyond its requirement for alloimmunity.

In our experience with combined kidney and bone marrow transplantation, discussed below, these requirements appear to be obtainable, indicating that a tolerant state is feasible in some settings. Following our induction regimen, antithird-party responses have been regularly observed to completely recover by 6 months, while donor-specific non-responsiveness persists [32]. In addition, it has been reassuring that no serious infectious complications have been encountered in either non-human primate recipients followed for up to 13 years or in human recipients followed for up to 9 years (unpublished observations). The potential risk of malignancy following tolerance conditioning must also be considered, and this concern is particularly relevant in considering the use of ionizing irradiation during the conditioning regimen. For example, if total body irradiation (TBI) or total lymphoid irradiation (TLI) is included in the induction regimen, as it has been in some of the clinical trials discussed below, the incidence of irradiation-induced malignancy should be compared with the risk of malignancy resulting from long-term immunosuppression. Epidemiologic studies have established that low-dose TBI (2–2.5 Gy) increases the life-time risk of malignancy by a factor of 1.2 over that observed in individuals without prior exposure to irradiation [33]. In contrast, as described above, long-term immunosuppression is associated with a 3- to 30-fold increased risk of cancer [13,24]. Such data would appear to justify the use of low-dose irradiation for tolerance induction. An additional consideration involves strategies using donor bone marrow transplantation (DBMT). Graft-versus-host disease (GVHD) at any level would not be acceptable in organ transplant recipients unless they were simultaneously being treated for malignancy [34,35].

Indications for tolerance induction

Who are the most appropriate candidates for tolerance induction and are there specific contraindications? Because the current graft half-life following deceased-donor kidney transplantation has been unchanged for over a decade, at about 9–10 years [3,36], young kidney transplant recipients may be anticipated to require re-transplantation several times during their lifetime. However, the number of donors is limited and HLA allosensitization by the previous transplants typically makes serial re-transplantation difficult to accomplish. The high prevalence of non-compliance with post-transplant medications that is regularly encountered in pediatric recipients also defines these individuals as highly appropriate candidates for tolerance induction, providing this does not interfere with the follow-up required by the induction protocol. Non-compliant behavior has been reported in 5% to 80% of pediatric allograft recipients, with adolescents having the highest rates [37–40]. As a result, long-term outcomes in the adolescent age group are typically observed to be worse than in other age groups [41,42]. Thus, successful tolerance induction would appear to be highly advantageous for adolescent recipients. Likewise, in view of the significantly increased risk of malignancy in chronically immunosuppressed recipients, patients with a past history or strong family history of malignancy may also represent a group who would achieve additional benefit from tolerance induction. Tolerance induction in patients presensitized to HLA antigens has proved to be exceptionally difficult [43]. In our initial clinical trial, a patient, who had high panel-reactive antibodies (PRA) but no detectable donor-specific antibodies (DSA) by ELISA, developed aggressive humoral rejection within 2 weeks of the transplant procedure [32]. Therefore, we currently exclude any patient with detectible PRA from enrolling into our tolerance induction trials.

Whether patients with high probability of recurrence of their original disease are appropriate candidates for tolerance induction remains to be determined [25]. In the Stanford trial, which enrolled HLA-matched kidney transplant recipients, one patient developed recurrent disease, which led the investigators to discontinue the planned withdrawal of immunosuppression [44]. In our clinical trial, one patient developed recurrence of his original membranoproliferative glomerulonephritis 7 years after immunosuppression had been withdrawn, requiring reinstitution of low-dose immunosuppression. Although there is some possibility that tolerance

induction directed to alloantigens may also suppress autoimmunity, tolerance induction apparently neither enhanced nor prevented the recurrence of the original disease in these anecdotal cases. Currently, no non-human primate (NHP) models have been developed to assess autoimmunity but ongoing studies of mechanistic pathways, such as Tregs (regulatory T cells), may help to evaluate this potential obstacle to tolerance induction.

Strategies for tolerance induction

Since the landmark study by Billingham, Brent, and Medawar that defined a means to achieve neonatal tolerance [45], numerous strategies for induction of tolerance have been developed in rodent models [46–53]. However, only a limited number of these have been successfully translated to NHPs [54–62] and even fewer to humans [32,44,63–66]. The discrepancy in the ease of tolerance induction between rodents and primate models may be attributed to differential expression of MHC antigens, especially class II [67,68], as well as to the presence of heterologous memory T cells in recipients not housed in environmentally controlled conditions [69–71]. Recent studies have emphasized that alloreactive memory T cells can inhibit tolerance induction approaches that utilize costimulatory blockade [72] or mixed chimerism [69]. Nevertheless, at least some recent studies in non-murine models have provided encouraging observations of relatively consistent induction of allograft tolerance in NHP and even in human allograft recipients. Strategies for tolerance induction that, to date, have been extended to clinical transplantation include: (1) profound T-cell depletion, (2) costimulatory blockade, and (3) DBM infusion/transplantation. These strategies appear to depend upon combinations of the three major avenues of tolerance induction: (1) T-cell depletion, (2) anergy, and (3) regulation (Figure 76.2). An example of such a combination of mechanistic pathways is the induction of tolerance through mixed chimerism, in which profound T-cell depletion by anti-T-cell antibodies, which has been reported to expand regulatory T cells [73], has been a necessary part of the non-myeloablative conditioning regimen [74]. Mechanistic studies in clinical trials have also intimated a critical involvement of regulatory T cells in the allograft tolerance induced by transient mixed chimerism [32,75].

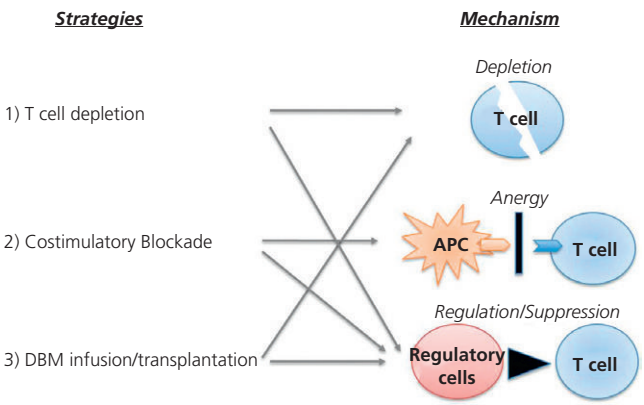

Figure 76.2. Strategies for tolerance induction that have been extended to clinical transplantation include: (1) profound T-cell depletion, (2) costimulatory blockade, and (3) DBM (donor bone marrow) infusion/transplantation. Effective strategies generally depend on combinations of the three major mechanisms of tolerance induction, that is depletion, anergy, and regulation. APC, antigen-presenting cells.

T-cell depletion

Depletion of donor-reactive T cells has been achieved by: (1) irradiation or (2) potent anti-T-cell antibodies or recombinant proteins.

1 Total lymphoid irradiation (TLI). Tolerance induction by TLI was initially studied in the1980s in large-animal models and humans. The Stanford group applied TLI with a cumulative dose of 2800–4500 cGy in canine experiments [76]. Myburgh et al. subsequently demonstrated prolonged renal and liver allograft survival in baboons with a reduced cumulative TLI dose of 1600 cGy [77]. In that study, DBM was also infused in some recipients, but prolonged survival was achieved even in recipients without DBM infusion. The Stanford group subsequently evaluated TLI in a pilot clinical kidney transplant trial, using a cumulative dose of 2000 cGy. Although successful withdrawal of immunosuppression was initially reported in three patients [65], two of these patients eventually lost graft function due to ureteral stenosis and chronic rejection [66]. One-year renal allograft survival of all patients receiving this regimen was 76% [78], which was inferior to graft survival with conventional immunosuppression. The complexity of the regimen as well as failure to show a significantly improved graft survival discouraged further studies with this approach until recently (see below).

2 Anti-T-cell reagents. Anti-CD3 (cluster of differentiation 3) immunotoxin (IT), which is an anti-rhesus CD3 monoclonal antibody (mAb) conjugated with the mutant diphtheria toxin protein (CRM9) [79], has been shown to effectively deplete rhesus monkey peripheral blood and lymph node T cells and prolong renal allograft survival [59,60,80]. However, DSA production was not prevented, ultimately resulting in alloantibody-mediated rejection of the kidneys [60]. A clinical trial based upon this strategy for tolerance induction has been attempted by Calne et al., who suggested that in human renal allograft recipients, a condition called "prope (almost) tolerance" could be achieved consistently after profound T-cell depletion with alemtuzumab (Campath-1H[Genzyme, Cambridge, MA], anti-CD52 mAb) [81,82]. In these patients, who were weaned to low-dose cyclosporine monotherapy, minimal evidence of rejection was observed, but no attempt was made to completely withdraw maintenance immunosuppression. Starzl et al. attempted to induce tolerance in 90 kidney transplant recipients who were treated with alemtuzumab or rabbit thymoglobulin [83]. In these studies, spaced weaning of immunosuppressants was achieved in selected patients, though complete withdrawal was not successful. There was no improvement in patient or graft survival compared with historic controls, and chronic allograft nephropathy progressed at the same rate in both groups [84]. Results with attempted tolerance induction using alemtuzumab have been inconsistent in other trials as well. Kirk et al. encountered acute rejection in their recipients treated with alemtuzumab, despite marked depletion of T cells. These lymphopenic rejections were notable for prominent graft infiltration with monocytes and macrophages [85]. Based on encouraging results in NHP [86], Kirk et al. subsequently added deoxyspergualin (DSG), a potent inhibitor of monocyte activation [87], to their alemtuzumab protocol [88]. However, despite profound T-cell depletion and therapeutic DSG dosing, all patients treated with alemtuzumab/DSG and attempted immunosuppression weaning developed

reversible rejection that was similar in frequency to that seen in patients treated with alemtuzumab alone [88]. Several chemokine gene transcripts were observed to be markedly increased in the biopsies of those allografts, which may have resulted in attracting residual lymphocytes to the kidney graft and led to rejection. Most investigators have now concluded that T-cell depletion alone cannot consistently induce tolerance in humans.

Costimulatory blockade

Among the costimulatory signals that have been identified, blockade of the CD28/CD80-CD86 [89,90], CD40/CD154 [61,62,91], and lymphocyte function associated antigen (LFA)-1/ICAM-1 [92] pathways have been tested in NHP. Although blockade of CD80 and CD86 has not significantly prolonged allograft survival in monkeys [90], blockade of the CD40/CD154 pathway with humanized anti-CD154 mAb (humanized 5c8) induced prolonged allograft survival in over half of recipients after discontinuation of all immunosuppressive treatment [58,61,62]. These encouraging observations suggested that this approach might be promising for human renal transplant trials. However, in the only clinical trial of this regimen, similar immunosuppressive effects were not observed and five of seven patients developed rejection episodes [93]. Further clinical trials of this antibody were suspended when an unexpectedly high incidence of thromboembolic complications was found to be associated with this treatment [94]. The ultimate clinical applicability of anti-CD154 mAb therefore remains questionable at this point.

CTLA4Ig (a protein consisting of the extracellular domain of CTLA4 [cytotoxic T-lymphocyte-associated protein-4] fused to the Fc domain of human IgG1) was designed to target the blockade of CD28/CD80-CD86. This agent only moderately prolonged allograft survival in NHP [95,96]; however, a modified version of CTLA4Ig, LEA29Y (belatacept), in which two amino acids were substituted to provide slower dissociation rates from both CD80 and CD86 ligands, has demonstrated more significant immunosuppressive effects in NHP and humans [97–101] and was recently approved for clinical use. Although the results of LEA29Y have been encouraging, attempts to completely discontinue immunosuppressive treatment following its administration have been unsuccessful [102]. However, belatacept is the first of these new classes of therapeutic agents to be approved for clinical kidney transplantation and its application in clinical tolerance induction trials may be forthcoming. We have recently tested belatacept in place of anti-CD40L mAb in our mixed chimerism conditioning regimen in an effort to develop a clinically applicable costimulatory blockade strategy and the preliminary results have been encouraging (Kawai et al., manuscript in preparation).

In a more complex trial that used costimulatory blockade ex vivo, Bashuda et al. reported successful induction of renal allograft tolerance in three of six monkey recipients who received adoptive transfer of anergized T cells, induced by co-culture with donor alloantigen in the presence of anti-CD80/CD86 antibody [103]. Although induction of allograft tolerance was not consistent, this study represents the first report of renal allograft tolerance induction by adoptive transfer of anergized cells in NHP. However, in their subsequent clinical trial, successful withdrawal of immunosuppression was not accomplished in any of the patients treated with this strategy [104].

Donor bone marrow infusion/transplantation

Strategies using DBM can be divided into those attempting to achieve microchimerism, detectable only by molecular assays, and those striving for mixed chimerism, readily detectable at a macroscopic level. These two approaches appear to operate through different immunologic mechanisms. In the microchimerism approach, DBM has been infused together with conventional immunosuppression, with or without T-cell depletion. Levels of chimerism induced were minimal (<0.1%), and were detectable only by polymerase chain reaction (PCR) analysis (hence the term "microchimerism") [105–108]. In contrast, in the mixed-chimerism approach, either more potent cytotoxic drugs and/or irradiation have been used to provide more complete T-cell depletion, which leads to DBM engraftment and induces higher levels of chimerism, detectable by flow cytometry.

Microchimerism strategy

Attempted tolerance induction by infusion of DBM with antilymphocyte serum (ALS) was first reported by Monaco et al. [46,109] in the 1970s. This strategy was subsequently tested in NHPs [110] and humans [111]. Despite favorable results in NHP studies, immunosuppression was not successfully discontinued in any of the 57 participants in the initial clinical trial. Chimerism in these patients was not evaluated. The concept of microchimerism as an explanation for allograft acceptance in patients treated with conventional immunosuppression, without administration of bone marrow, was first proposed by Starzl et al. in 1992. In that report, the investigators had used sensitive cytostaining and PCR techniques to detect small numbers of donor leukocytes (microchimerism) in 30 recipients of livers or kidneys with long-term stable graft function after all immunosuppression had been discontinued [105]. They hypothesized that responses of coexisting donor and recipient cells lead to reciprocal clonal exhaustion, followed by peripheral donor-reactive clonal deletion [107]. Based on the previous animal studies, as well as Starzl's observations, DBM infusion with conventional immunosuppression has been tested in multiple clinical organ transplant protocols [111–114]. To date, no convincing evidence has been provided to suggest that this approach will have a beneficial effect on patient or allograft survival, although a lower incidence of acute rejection and lower required maintenance immunosuppressive drug dosages have been reported [111–113,115].

Mixed chimerism

In contrast to the myeloablative regimens utilized when DBMT is performed to treat malignancies, the conditioning regimens used for induction of mixed chimerism have generally been non-myeloablative, implying that recipients would recover from the therapy-induced pancytopenia even without engraftment of DBM. The chimerism induced after such non-myeloablative regimens is generally characterized as "mixed chimerism," a state in which both donor and recipient hematopoiesis stably co-exist [47,50,74,116–121]. The presence of lymphohematopoietic elements of both host and donor origin distinguishes such mixed hematopoietic chimerism from full (i.e. 100%) chimerism observed after myeloablative conditioning. The advantages of the mixed-chimerism approach are that the recipient remains more immunocompetent and is less likely to develop GVHD [117].

Mixed chimerism approach in HLA-identical kidney transplantation

Using TLI and DBMT, the Stanford group reported successful induction of stable mixed chimerism and renal allograft tolerance in HLA-identical kidney transplant recipients [44,64]. The protocol consisted of TLI (80–120 cGy × 10), antirabbit thymocyte globulin

(1.5 mg/kg × 5, days 0–4), followed by HLA-matched peripheral blood $CD34^+$ stem cell infusion on day 11. These patients received mycophenolate mofetil (MMF) for 1 month, and cyclosporine starting at day 0 and continued for at least 6 months. All 12 patients receiving this regimen developed persistent mixed chimerism and eight patients were reported to have been completely withdrawn from immunosuppressive medications, with the longest allograft survival exceeding 3 years. Four patients continued to receive immunosuppressive drugs because of recurrence of focal segmental glomerulosclerosis in one, and rejection episodes during the tapering of cyclosporine in the others. To date, this approach has not been successfully applied to HLA-mismatched kidney transplants (see below). A potential advantage of this strategy might be applicability to deceased donor transplantation, because all treatments in the conditioning regimen are initiated after organ transplantation.

At Massachusetts General Hospital, ten patients with renal failure secondary to multiple myeloma who received conditioning with a non-myeloablative regimen and HLA-identical combined kidney and bone marrow transplantation (CKBMT), as well as subsequent donor lymphocyte infusions to provide additional antimyeloma effects, have been observed for periods of 3 to 14 years. The preparative regimen consisted of cyclophosphamide (60 mg/kg × 2), local thymic irradiation 700 cGy, horse antithymocyte globulin (ATG), and a 60-day course of post-transplant cyclosporine administration [34,35,122,123]. All ten patients developed mixed chimerism, which became undetectable by day 100 after CKBMT in four of them. Stable remission of myeloma has been observed in six of the 10 patients. Three patients died after 4 to 7 years, due to recurrence of myeloma, and two surviving patients have required ongoing medical treatment for recurrent myeloma. Of the six patients with stable remission of myeloma, four have successfully discontinued all immunosuppression, while two with stable chimerism have required low-dose immunosuppression to control minor symptoms of chronic GVHD. All kidney allografts were accepted, with no evidence of rejection, although early recurrence of myeloma prevented one patient from completing therapy.

Mixed chimerism in HLA-mismatched kidney transplantation

DBMT with a TLI-based regimen was attempted by the Stanford group in HLA-mismatched kidney transplant recipients [63]. In this clinical trial, three of four recipients developed transient multilineage chimerism, but rejection developed when immunosuppression withdrawal was attempted [63]. These investigators concluded that, despite its successful application to recipients of HLA-identical kidneys, this protocol did not induce allograft tolerance in HLA-mismatched recipients [124].

At Massachusetts General Hospital, based on the studies in NHPs [54–56,125,126] and the experience in HLA-matched CKBMT for myeloma [34,122,123], we have applied a non-myeloablative conditioning regimen to HLA-mismatched kidney transplant recipients [32]. Unlike the situation for myeloma patients, we considered GVHD at any level to be an unacceptable complication for renal transplant recipients without underlying malignant disease. Therefore, ATG, which we had used for the HLA-identical transplants (described above) and which had been used for DBMT in patients with myeloma and lymphoma [127], of whom several experienced GVHD, was replaced with an anti-CD2 mAb (Medi-507). The latter antibody was known to have potent T-cell-depleting properties as well as costimulatory blockade properties [128,129] and had not led to GVHD in patients receiving mismatched DBM for treatment of malignancy [130]. The protocol for recipients of HLA-mismatched allografts was also modified by adding an anti-CD20 mAb (rituximab) to prevent humoral rejection, after the occurrence of an antibody-mediated rejection in one patient (see above). A total of 10 subjects have been enrolled into these studies and maintenance immunosuppression was successfully tapered off in eight subjects by 8–14 months after transplantation. One of these eight subjects developed an episode of acute cellular rejection, which occurred 7 weeks after immunosuppressive therapy had been withdrawn. Following re-institution of immunosuppression, his renal allograft function improved, but has remained compromised and he underwent re-transplantation 3 years later. The clinical courses of the two recipients who were not able to be weaned from immunosuppression as planned were complex. The first developed irreversible humoral rejection during the second post-transplant week, as previously reported [32]. Although this patient was highly sensitized (PRA >40%), pretransplant DSA was not detectable by ELISA. Subsequent Luminex assays suggested that this patient was presensitized to donor HLA specificities. The second unsuccessful clinical course occurred in a patient who developed progressive thrombotic microangiopathy, thought to be the consequence of tacrolimus toxicity, although some element of cellular rejection could not be ruled out. Calcineurin inhibitor immunosuppression was discontinued, but the allograft eventually failed and she was returned to dialysis, 6 months after transplantation. Stable renal allograft function without ongoing immunosuppression has been maintained in the remaining seven patients for follow-up periods to date of 2.5–9 years. Of these seven patients, one patient developed recurrence of his original disease (membranoproliferative glomerulonephritis type 1) after 7 years. Although kidney function remained stable, low-dose MMF was added in an attempt to delay the progression of his recurrent disease. Another of these patients developed antidonor HLA class II antibody shortly after withdrawal of his immunosuppression. Serial biopsies did not show any evidence of chronic rejection until 5 years after discontinuing immunosuppression when the protocol biopsy showed duplication of basement membranes, suggestive of early chronic rejection. Although his kidney function remained stable with no proteinuria, immunosuppressive therapy was instituted after 6 years. These patients, who successfully discontinued their immunosuppression, consistently showed donor-specific unresponsiveness with in vitro immunological assays. However, the mechanism of tolerance induced by this approach has not been fully elucidated. Because the mixed chimerism induced in these patients has been transient, lasting only for 2–3 weeks, the involvement of peripheral tolerance mechanisms has been postulated to maintain the tolerance after the disappearance of chimerism [32,75]. In order to extend clinical applicability of this approach, further clarification of tolerance mechanisms as well as additional modifications of the protocol to decrease morbidity of the procedure are in progress.

Fully allogeneic chimerism in HLA-mismatched kidney transplantation

Direct evidence that persistent donor chimerism permits successful organ transplantation in patients was initially provided in the unique setting of patients who underwent myeloablative HLA-matched bone marrow transplantation for hematologic disorders and years later developed renal failure requiring transplantation. With the kidney donor being the same individual who previously donated bone marrow, kidney transplants succeeded without the

need for immunosuppression, affirming the principle that donor chimerism carried with it tolerance of other tissues and organs transplanted from the same donor [131]. However, as complete replacement of recipient myeloid and lymphoid lineages by donor cells may compromise immunocompetence and incurs the risk of GVHD, full chimerism for induction of allograft tolerance has, until very recently, been considered unlikely to have clinical applicability. However, Leventhal et al. at Northwestern have recently reported use of a conditioning strategy consisting of total body irradiation (200 Gy), fludarabine, and cyclophosphamide, together with administration of donor hematopoietic stem cells, for treatment of eight HLA-mismatched kidney transplant recipients [132]. Although the conditioning regimen was close to myeloablative and all recipients developed fully allogeneic chimerism, no GVHD was reported, purportedly as the result of administration of novel "tolerogenic $CD8^+/TCR^-$ facilitating cells." Although the follow-up period has been brief, the results are striking; multilineage full donor chimerism was achieved in all subjects at 1 month; and, in five of eight patients, it persisted long term, allowing weaning from all maintenance immunosuppression by 1 year. Kidney function has been maintained for 4–18 months without apparent rejection or evidence of GVHD. In the three cases in which tolerance was not successful, weaning was halted with development of membranous nephropathy in one case, presumed rejection in one case, and loss of the renal allograft to thrombosis in the third case, following what was believed to be an episode of sepsis. Considering the fact that this last patient exhibited a high level of chimerism and that the presumed sepsis followed a transient skin rash, it would appear possible that GVHD may have also played a role in the unfortunate outcome for this patient.

These remarkable results reported by Leventhal et al. may be attributable to the addition of a population of bone marrow-derived cells termed "facilitator cells", which include precursor plasmacytoid dendritic cells [133]. In animal models, these cells have been found to improve engraftment and to avoid GVHD, possibly through induction of regulatory T cells [134,135].

Operational tolerance in liver transplantation

Since Calne et al. first reported spontaneous tolerance of liver allografts in swine in 1969 [136], a considerable number of experimental and clinical studies on liver allograft tolerance have been reported. The swine used in these studies were outbred and not typed for MHC antigens. Subsequent data on liver transplantation in MHC-characterized miniature swine demonstrated that such spontaneous tolerance only occurred when the animals were MHC-matched [137], making it likely that Calne's animals, which were farm bred, shared MHC antigens. Nevertheless, the liver is generally considered the most tolerogenic of transplanted organs, with its as yet undefined tolerogenic effects being speculated to be multifactorial. Postulated mechanisms for the tolerance induced by the liver allograft include: (1) soluble MHC class I antigen produced by the liver, which potentially neutralizes graft-specific antibodies or inhibits graft-reactive cytotoxic T cells [138,139]; (2) presence of microchimerism from donor lymphoid cells in the liver transplant, causing a limited graft-versus-host reaction that counteracts the rejection response [105]; (3) clonal deletion/apoptosis of alloreactive cells in the liver after rapid T-cell activation [140–142]; (4) abundant sinusoidal antigen-presenting cells, such as liver sinusoidal endothelial cells, Kupffer cells, and liver dendritic cells (DC), which may present antigen in a tolerogenic manner to naïve T cells [143–145]; and (5) involvement of T regulatory cells, caused to proliferate by the liver [146].

The first large clinical study of liver transplant tolerance was reported from Pittsburgh in 1997 [30]. In this study, 95 recipients (64 adult and 31 pediatric), who were clinically stable for more than 5 years after transplantation, were chosen for withdrawal of immunosuppression. While 18/95 (19%) successfully discontinued immunosuppression for 10 months to 4.8 years, 18/95 (19%) developed rejection during immunosuppression withdrawal. At King's College in London, immunosuppression withdrawal was attempted in 18 adult recipients. Five of 18 recipients (28%) were initially reported to have successfully discontinued immunosuppression for up to 3 years [147], but later studies revealed that only 2/18 remained immunosuppression-free after 10 years [148]. In a study of hepatitis C-positive patients, however, stable long-term (up to 6.5 years) liver function has been reported in 7/34 recipients (20%) [149,150].

In pediatric patients, Kyoto University initially reported that a significantly higher proportion of patients (38%) achieved immunosuppression-free status [151]. However, in their more recent study, with a larger number of patients, approximately 15% (87/581) were reported immunosuppression free [31]. Furthermore, their most recent study revealed a higher incidence of fibrosis in protocol biopsies of immunosuppression-free patients, despite absence of clinical markers of rejection [152]. While studies in Kyoto included relatively older pediatric patients (up to 18 years old with the median age 12), Feng et al. at UCSF attempted to withdraw immunosuppression in much younger liver transplant recipients, (5.5–9.1 months at transplant and 6–11 years at study enrollment) who received parental living-donor liver transplantation [153]. Additional differences from the Kyoto study included longer time from transplant (4 vs. 2 years) and requirement of normal biopsy prior to study entry. Immunosuppression was gradually withdrawn over a minimum of 36 months, and 60% (12/20) of these recipients successfully discontinued their immunosuppression and maintained normal allograft function for a median of close to 3 years. Despite detection of de novo donor-specific antibodies in some tolerant recipients, follow-up biopsies obtained more than 2 years after complete withdrawal of medication showed no significant change compared with baseline biopsies. In this study, although HLA mismatch, sensitization status, and presence of donor-specific antibodies were not relevant to operational tolerance, tolerant patients initiated immunosuppression withdrawal significantly later after transplantation compared with non-tolerant patients (median of 100.6 vs.73 months).

As described above, Starzl et al. reported successful immunosuppression weaning in kidney, liver, and pancreas transplant recipients after profound T-cell depletion by large doses (5 mg/kg) of rabbit thymocyte globulin [83]. In that study, about 70% (14/17) of liver recipients achieved minimized doses of tacrolimus monotherapy. However, this thymoglobulin induction therapy failed to achieve complete immunosuppression withdrawal. Eason et al. attempted to withdraw immunosuppression from 18 patients, 6 months after transplantation, but only one patient successfully achieved immunosuppression-free status, with the majority of patients (14/18) developing rejection. Based on the hypothesis that microchimerism induces allograft tolerance through clonal deletion/exhaustion [105], the Miami group attempted to induce liver allograft tolerance by perioperative infusion of donor bone marrow cells (DBMCs) [113]. Although rejection rates were reported to be significantly lower in the recipients with DBMC, there was no

difference in overall graft survival. In this study, approximately 20% of patients successfully achieved immunosuppression-free status for 1–3 years, regardless of whether or not they had received DBMC.

In conclusion, in contrast to renal allograft recipients, spontaneous induction of tolerance following withdrawal of all immunosuppression can probably be achieved in up to 20% of liver allograft recipients. Long-term consequences of attempted immunosuppression withdrawal in those patients who do not become tolerant remain to be determined. To date, there have been no reported clinical trials attempting to demonstrate or induce operational tolerance in heart or lung transplant recipients.

Biomarkers for tolerance

It would be of great value to identify biomarkers that could accurately identify patients who are already in immunologically tolerant state while still on immunosuppression. Such arrays might help identify which patients can decrease or discontinue immunosuppressive medications and could also provide important insights into the mechanisms of tolerance induction.

Liver transplantation

Most studies of tolerance biomarkers have been carried out on liver transplant recipients because, as described above, the number of recipients who achieve tolerance after discontinuation of immunosuppression is higher for transplantation of the liver than for any other organ. Early studies showed that operationally tolerant recipients had increased numbers of certain lymphocyte subsets, compared to healthy volunteers and recipients maintaining immunosuppression or experiencing rejection. Mazariegos et al. demonstrated increased numbers of plasmacytoid DC precursors (DC2 or $CD11c^{-}CD123^{hi}$) relative to the frequency of monocytoid DC (DC1 or $CD11c^{+}CD123^{-/lo}$) in recipients who achieved immunosuppression-free graft survival [154]. Other studies have shown that operationally tolerant pediatric and adult liver transplant recipients have significantly higher peripheral blood $CD4^{+}CD25^{high}$ T cells and γδT cells (Vδ1/Vδ2 ratio), compared to non-tolerant recipients or healthy individuals [155,156]. A subsequent study also demonstrated a significantly higher level of $FOXP3^{+}CD4^{+}CD25^{high}$ T cells in liver transplant recipients who achieved immunosuppression-free graft survival than in those who developed rejection [157]. More recently, gene-expression profiling of operationally tolerant liver recipients has demonstrated a unique genomic signature set that was preferentially expressed in natural killer, natural killer T, and γδT cells [156,158].

Kidney transplantation

In contrast to the situation in liver transplantation,, it has been difficult to identify tolerance biomarkers for kidney transplantation, because of the scarcity of recipients who achieve spontaneous operational tolerance. Brouard et al. analyzed the blood T-cell repertoire usage in five renal transplant recipients with operational tolerance. They found higher CDR3 length distribution (LD) of Vβ families compared to normal control and stable recipients with conventional immunosuppression. The recipients with low-dose steroid monotherapy also showed similarly high CDR3-LD of TCR Vβ [159]. However, these results have not been reproduced by other groups. The same group subsequently analyzed peripheral lymphocytes of tolerant renal transplant recipients and reported significantly lower absolute counts of B cells, $CD25^{high}CD4^{+}$ cells, and lower levels of Foxp3 transcripts in recipients with chronic rejection than those in tolerant recipients [160]. This group further performed microarray analysis of the peripheral blood in the same five tolerant recipients to identify specific gene expression patterns in tolerant recipients. They identified a 49-gene set specific to tolerant recipients by microarray with 33 of the included genes correctly segregating tolerance and chronic rejection [161]. However, one patient who fulfilled the full clinical description of operational tolerance later developed rejection with antidonor antibody. The authors suggested that the operational tolerance gene-expression signature is likely a metastable rather than permanent status.

More recently, two studies have reported genomic biomarkers of renal allograft tolerance in such individuals. In the first study, sponsored by the Immune Tolerance Network (ITN) and performed in the United States, 25 kidney transplant recipients who had not rejected their graft despite stopping of immunosuppressive medications, were identified and their genetic and cellular signatures were studied [162]. The study found that a set of three B-cell differentiation genes distinguished tolerant from non-tolerant recipients, although not from normal individuals, raising the question of whether non-tolerant recipients showed lower expression of these genes because of ongoing immunosuppression. This B-cell signature was associated with elevated numbers of peripheral blood naïve and transitional B cells in the tolerant recipients. These results were independently verified by another ITN-sponsored study, which was performed in Europe. The European study also demonstrated a bias toward differential expression of B-cell-related genes, which were associated with significantly higher percentages of peripheral blood B and natural killer cells. The study also showed a significantly lower percentage of circulating activated $CD4^{+}CD25^{int}$ cells. Although there was no significant difference in circulating $CD4^{+}CD25^{hi}$ cells, expression of Foxp3 to α-1,2-mannosidase genes was found to be significantly higher in the peripheral blood of tolerant recipients [163]. These studies on biomarkers for renal allograft tolerance have been based on a limited number of patients who achieved operational tolerance of the renal allograft, and further confirmative studies with larger numbers of patients will be required to validate these initial results and to show that there is also a meaningful difference between biomarker expressions in normal versus tolerant individuals.

Summary

Spontaneous cases of allograft tolerance in patients who stopped taking their immunosuppressive medications have been described anecdotally for liver transplants and even for kidneys [159,164,165]. However, it is only during the past 10 years that the goal of intentional induction of allograft tolerance has been brought from animal models to the clinic for both HLA-matched and HLA-mismatched kidney transplantation. Although induction of tolerance by combined kidney and DBMT has thus far been the only effective means of inducing tolerance in the clinic, modifications of current protocols as well as additional approaches should be pursued in both basic laboratory and preclinical studies, to reduce morbidity of the preparative regimen and to make tolerance induction the standard of care in routine organ transplantation.

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CHAPTER 77

Recurrent Disease after Kidney and Pancreas Transplantation

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Introduction

The aim of every transplant program is to achieve long-term patient and graft survival whilst minimizing the adverse effects associated with immunosuppression. Advances in transplant immunosuppression and anti-infective prophylaxis has impacted favourably on short-term outcomes, with early acute rejection rates below 15%, and 1-year graft survival greater than 90% [1]. Despite this, impact on long-term graft function has been disappointing with late allograft failure now major causes of end-stage kidney disease (ESKD). Whilst causes of graft dysfunction are numerous, recurrent disease has been largely ignored as an important contributor.

Better understanding of disease pathogenesis, together with improved diagnostics, has revealed recurrent disease in the kidney to be a much greater challenge to the transplant community than previously appreciated. Reported as the third most common cause of graft failure (after death with a functioning graft and interstitial fibrosis and tubular atrophy), recurrent disease is an important cause of graft dysfunction [2]. Furthermore, data from surveillance biopsy studies suggest that the true incidence of recurrent disease is likely to be much greater, while many patients with slowly deteriorating graft function may never be biopsied and are often incorrectly diagnosed with "chronic rejection." The challenge of recurrent disease lies not only in an accurate diagnosis. For many of the primary glomerulonephritides, recurrence within a graft is unpredictable, with limited therapeutic options available once the diagnosis is confirmed. Moreover, the true impact of recurrent disease on graft survival is unclear in many circumstances. Some may cause a rapid deterioration in graft function with early graft failure, while others appear to have little or no impact on long-term graft survival.

Simultaneous pancreas kidney transplantation (SPK) is the treatment of choice for appropriately selected patients with ESKD due to type 1 diabetes mellitus. Over the past three decades, graft survival for both the pancreas and the kidney have been steadily improving and registry and outcome studies show that patients with type 1 diabetes who receive a pancreas graft with their renal graft have improved survival outcomes compared to those that receive a cadaveric renal graft alone [3–5]. Whilst protecting the renal graft from the recurrence of diabetic nephropathy, the pancreas remains susceptible to recurrent autoimmune disease and it is a recognized cause of graft failure. Although reported to be an uncommon cause of pancreatic failure, true prevalence is unknown, as definitive diagnosis requires a pancreatic biopsy, which is not routinely carried out in most transplant centres.

This chapter will review the aetiology, clinical presentation, and investigation of recurrent disease in the kidney and pancreas grafts after transplantation. Recurrence of specific forms of glomerulonephritis and autoimmune disease in the pancreas is addressed individually, together with a review of current understanding on disease pathogenesis and an evaluation of current therapeutic options.

Recurrent disease after kidney transplantation

Background

Transplantation is the treatment of choice for the majority of patients with end-stage kidney disease, including those with primary or idiopathic glomerulonephritis (GN). However, this therapy does not usually eliminate the underlying cause of renal failure and as such should be regarded as a treatment rather than a cure.

Short-term graft survival has improved substantially in recent years, with a marked improvement in early acute rejection rates [1]. Although the long-term impact has been less impressive [6], overall graft survival has increased and, with this, the likelihood of disease recurrence. Specific discussions of long-term outcomes following kidney and pancreas transplants are found in Chapters 103 and 107, respectively.

Recurrence of primary GN has been considered since the beginnings of successful transplantation [7,8]. Previously thought to be of little significance as a cause of graft failure, prolonged allograft survival, in combination with improved diagnostics, has revealed recurrent GN to be an important factor influencing allograft outcome and survival.

Risk of recurrence and consequent impact on graft survival differs for each category of GN. Reported recurrence rates range from 10 to 20%, with up to 50% losing their graft on long-term follow-up [2,9]. These are likely to be an underestimate, as much

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disease is subclinical and immunofluorescence and electron microscopy are not routinely performed on allograft biopsy. Furthermore, the introduction of rapid steroid withdrawal or complete avoidance in recent years has resulted in an increased incidence of recurrent GN with a reported tripling of risk of recurrent GN [10].

Epidemiology

To better understand recurrent GN after transplant, it is important to recognize the epidemiology of primary GN leading to native kidney disease. GN is the cause of ESKD in 10% to 25% of patients, with a greater incidence in Caucasian patients and children [11]. High incidence in children is likely due to the low prevalence of diabetic nephropathy as a cause of ESKD, while variance in racial distribution between registries may in part explain differences in type of native kidney disease. For example, the United States Renal Data System (USRDS) includes a larger cohort of Black patients compared with the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA). Lower prevalence of reported GN in Black patients may in part relate to paucity of biopsy information, as well as a higher incidence of hypertensive nephrosclerosis and diabetic nephropathy.

Registry analyses in the US and Australia show the prevalence of recurrence rates following transplant for primary GN varies from 2.9 to 12.1% and is inversely proportional to the recipient's age and directly proportional to duration of follow-up [11]. The Renal Allograft Disease Registry (RADR) was introduced in the USA to better understand the prevalence of recurrent immunological and metabolic diseases and their impact on graft function. Reports confirm an increased risk of recurrent disease with time, rising from 2.8% at 2 years to 9.8% and 18.5% at 5 and 8 years, respectively [12]. Once established, graft survival has been shown to decline more rapidly in patients with recurrent or de novo GN. In a US study, median graft survival with recurrent disease was 1360 days versus 3382 days without recurrence ($P < 0.0001$) [13].

The true incidence of recurrent GN is difficult to establish. Numbers reported within analyses are influenced by the duration of follow-up and whether or not patients with recurrent disease and a functioning graft were included together with those who have lost their graft. Recurrence rates may also be underestimated, as much disease is subclinical. Surveillance biopsies confirm a greater prevalence of certain GN than reported previously [14,15]. Moreover, an accurate diagnosis may be lacking in many patients, in whom recurrent disease is not confirmed histologically or misdiagnosed.

Definition and classification of recurrent disease

Recurrent and de novo glomerular disease may be classified according to clinical and histological criteria (Table 77.1). In order to establish an accurate post-transplant diagnosis of recurrent GN, it is crucial to ascertain a precise primary disease diagnosis, and to exclude donor disease. This requires a biopsy of both the native kidney (or implantation biopsy if GN was not the primary diagnosis) and the transplant kidney. Many cases cited in the literature, do not meet these criteria. As a result, some patients with presumed GN (not biopsy proven), go on to develop biopsy-proven GN after transplant, which is labelled recurrent GN when it may be recurrent or de novo. Furthermore, some patients with deteriorating renal function and proteinuria following transplant may never be biopsied, and are misdiagnosed as "chronic rejection". Implantation biopsies are also informative as they may reveal an unsuspected primary GN in the donor that should be considered when describing subsequent allograft biopsies.

Table 77.1. Classification of recurrent glomerular diseases

Clinical classification	
True recurrent glomerulonephritis	Histology of both the native and transplant kidney confirms the same glomerulonephritis
De novo glomerulonephritis	Biopsy-proven glomerular disease that occurs within the kidney allograft after transplantation that was not present in the native biopsy
Transplant glomerulopathy with unknown primary	Biopsy-proven glomerular disease within the transplant kidney may be recurrent or de novo but histology from native kidney is not available to confirm the diagnosis
Donor glomerulopathy	Biopsy-proven glomerular disease within the kidney allograft after transplant was present in the donor kidney at time of transplant (confirmed by implantation biopsy)
Histological classification	
Recurrence of primary glomerulonephritides	Focal segmental glomerulosclerosis IgA nephropathy Membranous nephropathy Mesangiocapillary glomerulonephritis Anti-GBM disease
Recurrence of secondary glomerulonephritides	Lupus nephritis Henoch–Schönlein purpura Haemolytic uremic syndrome ANCA vasculitis Scleroderma
De novo disease	Haemolytic uremic syndrome Transplant glomerulopathy Anti-GBM disease in patients with Alport's syndrome Membranous glomerulonephritis
Recurrence of metabolic or storage disorders/ glomerular deposition disease	Diabetic nephropathy Oxalosis Cystinosis Amyloidosis Fabry's disease Fibrillary or immunotactoid glomerulonephritis

ANCA, antineutrophil cytoplasmic antibody; GBM, glomerular basement membrane.

For this reason, recurrent diagnosis may be classified in four ways:

1. True recurrent GN: histology of both the native and transplant kidney confirms the same GN.
2. De novo GN: biopsy-proven glomerular disease that occurs within the kidney allograft after transplantation which was not present in the recipient's native kidney biopsy.
3. Transplant glomerulopathy (unknown primary disease): biopsy-proven glomerular disease within the transplant kidney may be recurrent or de novo but histology from the native kidney is not available to confirm the diagnosis.
4. Donor glomerulopathy: biopsy-proven glomerular disease within the kidney allograft after transplant was present in the donor kidney at time of transplant (confirmed by implantation biopsy).

Clinical features

Clinical diagnosis is usually prompted by new-onset haematuria and/or proteinuria. Elevation in serum creatinine often accompanies abnormal urinalysis; however, renal function may be normal or stable at the time of recurrence. Rate of progression of renal disease varies considerably depending on the underlying disease and potential concomitant pathologies such as interstitial fibrosis and tubular atrophy or calcineurin inhibitor (CNI) toxicity, which may also impact on graft function.

The clinical course and severity of recurrent disease often replicates that of the original disease, with the exception of lupus nephritis and vasculitis, which is invariably controlled by transplant

immunosuppression. Timing of recurrence and impact on graft outcome also varies according to the primary disease. Focal segmental glomerulosclerosis (FSGS) may recur within days to weeks from transplantation, while other glomerulonephritides, such as IgA, may not recur until many years from transplant, with little or no deleterious effect on graft outcome.

Extra renal features associated with the primary disease may accompany recurrent disease in the transplant, such as pulmonary involvement in recurrent Wegener's granulomatosis or haemolysis and thrombocytopenia in recurrent haemolytic uremic syndrome (HUS).

Investigation

Urinalysis

New-onset and persistent proteinuria, or haematuria and proteinuria (in the absence of infection or mechanical cause), are suggestive of de novo or recurrent GN and should prompt further investigation. Proteinuria (quantified by either spot or 24-hour protein excretion) is present in up to 45% of transplant recipients and, regardless of underlying cause, is a powerful independent risk factor for graft loss and patient survival [16]. It represents a composite endpoint of many possible pathologies including transplant glomerulopathy, non-specific IF/TA, and tubular proteinuria, as well as glomerular proteinuria from a de novo or recurrent GN. Urinary protein excretion also increases with hyperfiltration, obesity, and hypertension, and from iatrogenic causes such as mTOR inhibitors. Conversely, proteinuria may be rescinded by renin-angiotensin blockade, CNI therapy, and through reduced glomerular filtration rate (GFR) resulting from ischaemia or poor transplant function.

The threshold for pathological proteinuria is less clear for the transplanted kidney compared with native kidneys. Many centres consider 0.5 g/24 hours or higher (which may delay diagnosis and intervention) while others have shown 0.15 g/24 hours is associated with worse outcome [17]. Mounting evidence from protocol and diagnostic biopsy studies suggests a specific disease is responsible for proteinuria in most patients [18]. For this reason, a pre-emptive approach to proteinuria is recommended, allowing early diagnosis and timely management.

Serology

Serology may support diagnosis of recurrent disease, such as with antiglomerular basement membrane (GBM) antibody in Goodpasture's disease and antineutrophil cytoplasmic antibody (ANCA) associated with Wegner's granulomatosis and microscopic polyangiitis. Others such as anti-dsDNA are less informative. Hepatitis B or C antigenaemia is helpful when considering recurrent mesangiocapillary GN (MCGN) or membranous nephropathy. Raised lactate dehydrogenase (LDH) and low haptoglobin together with evidence of microangiopathic haemolysis on a blood film, may support a diagnosis of recurrent HUS, but the absence of these features do not reliably exclude it.

Renal biopsy

Differential diagnosis of post-transplantation GN often poses a diagnostic challenge to pathologists, given the plethora of potential histological findings and often multiple pathologies co-existing within the graft, many with overlapping histological features.

Renal biopsy is crucial for the diagnosis of recurrent GN. Full evaluation of histology includes light microscopy, immunofluorescence, and electron microscopy. Samples should contain at least ten glomeruli and two arteries to reliably represent graft pathology. Some pathological features are often patchy, and two cores of cortex are recommended. Histology should be processed similarly to native biopsies, where light microscopy helps differentiate recurrent from de novo GN, acute rejection, BK virus-associated nephropathy (BKVAN), glomerulitis, CNI nephrotoxicity, and hypertensive nephropathy, while also defining the extent of chronic allograft damage. Periodic acid–Schiff stain defines the basement membrane, silver stain detects the double contours of transplant glomerulopathy and membranous nephropathy, while trichrome stains detect collagen deposition and determines the extent of fibrosis. Light microscopy features, such as reduplication of the glomerular basement membrane, may occur in transplant glomerulopathy, making it difficult to distinguish from recurrent MCGN. The use of immunohistochemistry (to detect immune deposits) and/or electron microscopy (to assess basement membrane and deposits) is often essential to help clarify the diagnosis.

Clinical features and differential diagnosis of recurrent glomerulonephritis

Focal segmental glomerulosclerosis

Background and pathogenesis

Primary FSGS leads to ESKD in a high proportion of adult cases and is the leading cause of acquired renal disease causing ESKD in children [19–22]. The risk of recurrence after transplantation is high, with reports ranging from 20 to 50% [23–25], rising to 80 to 100% in the second transplant if the first was lost due to recurrent FSGS [26]. Without intervention prognosis is poor, with 13–20% grafts failing by 10 years [2] (Table 77.2). Two patterns of clinical presentation are described; the first (and most frequent) occurs within hours to days after transplantation and is characterized by early nephrotic-range proteinuria. The second follows a more insidious course, presenting months or even years from transplant. Risk factors for recurrence include rapid progression to end-stage renal

Table 77.2. Risk of recurrent disease and graft loss

Primary glomerulonephritis	Recurrence (%)	Graft loss due to recurrence (%)
FSGS	20–50	13–20‡
IgA	9–61	9.7‡
Membranous nephropathy	10–30	10–15‡
MCGN type I	20–50	15‡–40
MCGN type II	50–100	15–30§
ANCA-associated GN	7*	rare
Anti-GBM		
Histological recurrence	15–50	rare
Clinical recurrence	rare*	rare
Lupus nephritis	2–9	2–4
HUS		
Overall	4–60	31
Factor H mutation	80–100	74–100
Factor I mutation	80–100	100
MCP mutation	rare	rare
Scleroderma	1.9–20	20–21
Fibrillary GN	50†	rare
Fabry's disease	rare†	rare

FSGS, focal segmental glomerulosclerosis; IgA, immunoglobulin A; MCGN, mesangiocapillary glomerulonephritis; ANCA, antineutrophil cytoplasmic antibody; GN, glomerulonephritis; GBM, glomerular basement membrane; HUS, haemolytic uremic syndrome; MCP, membrane co-factor protein.

* Providing disease is in remission at time of transplant.

† Histological diagnosis.

‡ At 10-year follow-up.

§ After 5-year follow-up.

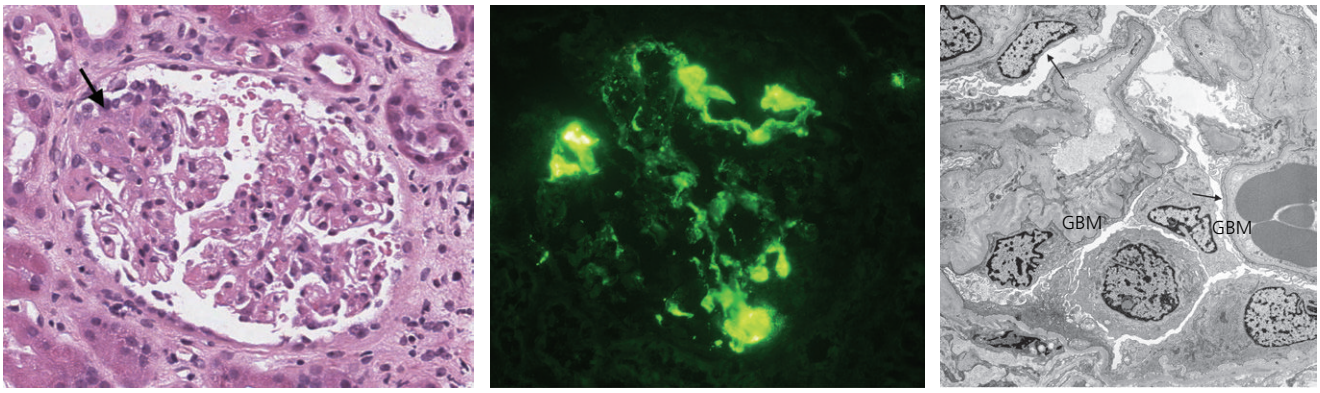

Figure 77.1. Renal transplant biopsy in a patient with recurrent FSGS. (A) Light microscopy demonstrating the characteristic features of segmental sclerosis (black arrow) and mesangial expansion (H&E). (B) Immunofluorescence showing deposits of IgM within sclerotic segments (*immunofluorescence with anti-IgM antibodies marked with fluorescein*). C3 and occasionally IgG may also be deposited within glomerular sclerotic segments and may represent non-specific deposition of serum protein. (C) Electron microscopy demonstrating effacement of the foot processes (black arrows) with rarefaction and thinning of the glomerular basement membrane (GBM). All pathology photographs were prepared by the Institute of Clinical Pathology and Medical Research, Westmead Hospital, Sydney, Australia.

failure with primary disease, young age, Caucasian race, and recurrence in a previous allograft.

Proteinuria is the hallmark of clinical presentation and may precede biochemical evidence of graft dysfunction. Histology is characterized by sclerosis in a segment of the glomerular tuft, along with podocyte effacement and fusion of foot processes on electron microscopy. Light microscopy may be normal in the early stages of disease recurrence and in this circumstance electron microscopy is crucial to confirm the diagnosis (Figure 77.1).

The pathophysiology of primary FSGS remains unclear but emerging evidence suggests a circulating factor plays a role in breaching glomerular permeability. An in vitro assay developed to detect this permeability factor showed that patients with a high level of activity were more likely to develop recurrence [27]. Evidence supporting a role for a circulating factor includes: induction of albuminuria in rats following the transfer of serum from patients with recurrent FSGS [28]; the observation of transient nephrotic syndrome in newborn infants in women with FSGS [29]; and the resolution of disease using therapies known to remove large proteins from the circulation such as plasma exchange or immunoadsorption [30,31]. Identification and characterization of this permeability factor has proved elusive for many years. Its molecular weight is thought to be between 30 and 50 kDa, depending on glycosylation and proteolytic cleavage [32,33]. A protein postulated to alter glomerular permeability, FS permeability factor (FSPS) protein, has been shown to bind with high affinity to galactose and may be eliminated by galactose affinity columns [34]. Serum soluble urokinase receptor (suPAR) has been identified as a circulating factor that may cause FSGS [35]. Elevated concentrations of suPAR were identified in two-thirds of patients with primary FSGS. High serum concentrations of suPAR were associated with recurrence after transplantation and in experimental models suPAR was found to activate podocyte β3 integrin leading to foot process effacement, proteinuria, and FSGS-like glomerulopathy, thereby providing a mechanistic basis for the disease [35]. Whilst this study has provided substantial evidence that suPAR correlates with disease, there have been several other soluble factors proposed as mediators of FSGS in the past. Proof of these findings will come from prospective multicentre studies to diagnose and monitor primary FSGS [36].

Separate studies in humans and experimental animal models of idiopathic nephrotic syndrome (INS) suggest a role for T lymphocyte dysfunction in disease pathogenesis, although treatments targeting T cells (anti-CD3, CNI, anti-CD53) have been largely unsuccessful at preventing or treating FSGS recurrence [31]. Resolution of recurrent FSGS following treatment with the anti-CD20 humanized monoclonal antibody, rituximab, has raised the possibility of B-lymphocyte involvement, although direct evidence is lacking [37]. While a clear trigger underlying disease pathogenesis remains elusive, the role of podocyte injury in disease pathogenesis now seems clear. Podocyte injury certainly appears to be the end point in this complex pathophysiological process, where it is proposed that circulating factor (with or without the involvement of immune cells) interacts with podocytes, leading to loss of nephrin and/or podocin with effacement of the foot processes [31].

Treatment strategies and outcomes

Idiopathic FSGS typically occurs early, with 80% of these cases occurring within the first 4 weeks after transplant [38]. Patients with a primary diagnosis of FSGS should be screened more frequently in the early post-transplant period for proteinuria (Table 77.3). Knowledge of pretransplant proteinuria informs interpretation of proteinuria in the early post-transplant period. Although accepted that the contribution of native kidneys to urine output (and associated proteinuria), declines after transplant, time to disappearance is variable [38].

Evidence supporting the successful treatment of recurrent FSGS has emerged in the last few years. Uncontrolled series and case reports have shown a substantial reduction in proteinuria following plasma exchange [39–41]. Early disease recurrence and initiation of plasma exchange may be associated with response to treatment although proteinuria may recur on cessation of therapy. Relapse may be prevented or reversed with long-term plasmapheresis with or without the addition of cyclosporine or cyclophosphamide. Cyclosporine acts directly on podocytes, blocking calcineurin-mediated dephosphorylization of synaptopodin, stabilizing the cytoskeleton [42]. Though limited by a small population size and historical control group, a non-randomized study achieved complete remission in 90% of patients using a combination of high-dose

Table 77.3. Screening for recurrent disease and potential therapies (adapted from Kidney Disease: Improving Global Outcomes Guidelines)

Disease	Additional Screening*	Minimum screening frequency	Diagnostic tests†	Potential therapies
FSGS	Proteinuria	Daily (1 week) weekly (4 weeks) 3 monthly (1 year) then annually		Plasmapheresis, steroids, cyclosporine, or cyclophosphamide Rituximab‡ ACEI/ARB if proteinuria
IgA	Proteinuria, microscopic haematuria	Once in the first month, every 3 months in the first year, then annually		No effective therapeutic measures ACEI/ARB if proteinuria
Membranous nephropathy				No effective therapeutic measures ACEI/ARB if proteinuria
MCGN			C3,C4 levels	No effective therapeutic measures Exclude/treat secondary causes (hepatitis B or C)
ANCA-associated GN			ANCA	Defer transplant until clinical remission Cyclophosphamide, steroids Consider additional therapies (plasmapheresis, rituximab)‡
Anti-GBM		unknown	Anti-GBM antibody	Defer transplant until clinical remission Cyclophosphamide, steroids, and plasmapheresis§
Lupus nephritis		unknown	Anti-dsDNA, C3,C4	Defer transplant until clinical remission No effective therapeutic measures MMF‡
HUS	Proteinuria, platelet count	During episodes of graft dysfunction	Platelet count, LDH, haptoglobin, blood film	Plasmapheresis with FFP IVIG or rituximab‡ eculizumab‡

FSGS, focal segmental glomerulosclerosis; IgA, immunoglobulin A; MCGN, mesangiocapillary glomerulonephritis; ANCA, antineutrophil cytoplasmic antibody; GBM, glomerular basement membrane; HUS, haemolytic uremic syndrome; LDH, lactate dehydrogenase; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MMF, mycophenolate mofetil; FFP, fresh frozen plasma.
* In addition to serum creatinine.
† Excluding renal biopsy.
‡ Evidence limited to single case reports.
§ Recurrence with high anti-GBM titres and cellular crescents on biopsy.

steroids, intravenous followed by oral cyclosporine, and an intensive and prolonged course of plasma exchange, compared with 27% in the control group who received a combination of therapies [43].

Pre-emptive plasmapheresis carried out perioperatively has been reported to reduce recurrence in children [44] and adults [41] although study numbers were small and uncontrolled. Furthermore, this approach is practically challenging with cadaveric transplants and carries the increased risk of hypocoagulation and bleeding peri- and postoperatively.

Rituximab has been used to treat recurrent FSGS refractory to plasmapheresis in a number of case reports. Different regimens have been described in each report with mixed results ranging from complete and persistent remission to no response and early graft failure [45–48]. Randomized controlled studies with sufficient numbers are required before the role of these therapies in modulating disease may be clearly elucidated.

Other novel therapies that may have a role in treating this condition include anti-TNF-α therapy and anti-CTLA4 therapy. Clinical trials are underway evaluating retinoic acid (NCT00098020) and galactose (NCT00814255) to treat primary FSGS in native kidneys.

IgA nephropathy

Background and pathogenesis

IgA nephropathy (IgAN) is the most common GN worldwide [49], leading to ESKD in 30 to 50% of patients after 25 years follow-up and is the most common recurrent GN after transplant [50]. Disease pathogenesis is complex and remains incompletely understood. Current understanding links altered production of abnormally glycosylated IgA1 in patients with primary disease, which cause glomerular inflammation and injury if deposited within the mesangium (Figure 77.2).

A recent review of the literature documented recurrence rates of approximately 30%, although incidence varied between studies, with reports ranging from 9 to 61% [51] (Table 77.2). Differences in duration of follow-up, threshold for biopsy, inclusion of surveillance biopsy, as well as racial and geographical variation, may explain the wide range of recurrence rates between reports. Surveillance biopsy in the absence of clinical signs report recurrence rates in 50 to 60% of patients [52,53].

Clinical presentation is similar to primary IgAN, with microscopic haematuria, low-grade proteinuria, and slow decline in renal function the most common features. Onset may occur early after transplant (implantation biopsy is necessary to exclude IgA within the donor kidney in such circumstances). More commonly, disease recurs from 3 years after transplantation [9]. Once established, disease progression is often benign, at least initially. Indeed, allograft survival for the first 5 years from transplant appears to be better in patients with primary IgAN compared with other disease [54–56]. Reasons are unclear but suggested mechanisms include protective alloreactive IgA anti-HLA antibodies blocking the effects of pathogenic IgG and IgM antibodies on the graft, or protective aberrations in immune function in patients with IgAN [57,58]. Graft survival becomes equivalent or inferior beyond 10 years from transplant when compared with other primary disease. Graft loss from recurrence may in part account for this, with an estimated 9.7% of grafts lost due to recurrent disease at 10 years [2].

Risk of recurrence in a second graft is high (20 to 100%) if the first graft was lost within a few years as a result of recurrent IgAN [51,59,60]. In this situation, alternatives to living donor transplant should be considered. Patients who lost grafts to recurrent IgA beyond 10 years should be regarded as low risk and live donation should not be precluded in this situation. There is no other single

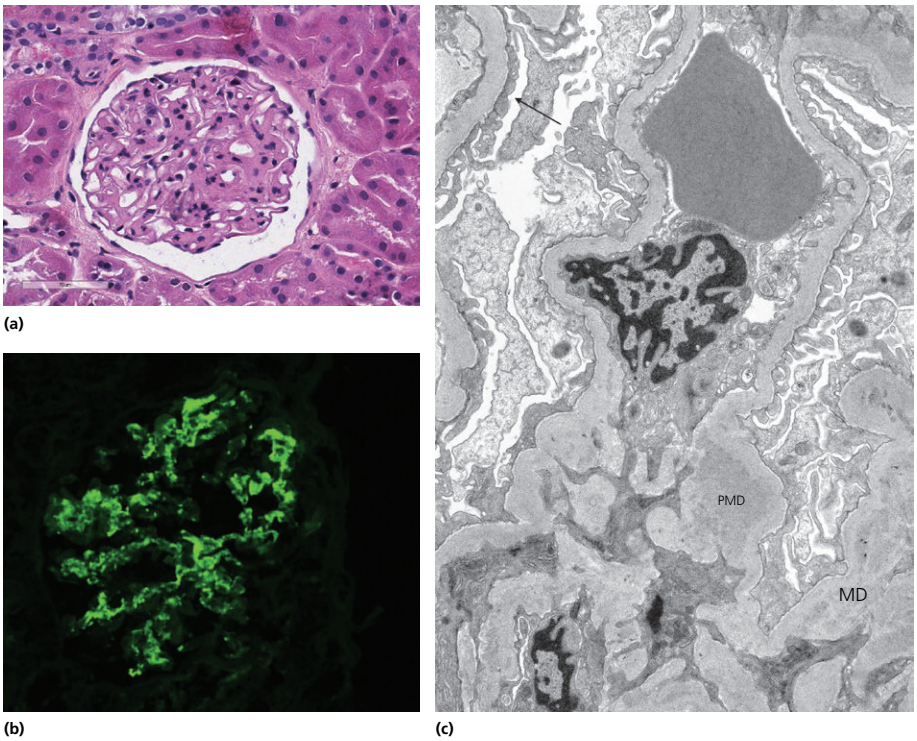

Figure 77.2. Recurrent IgA nephropathy. (A) Light microscopy showing mesangial expansion and mild hypercellularity (H&E). (B) Immunofluorescence demonstrating diffuse mesangial immunostaining for IgA *(immunofluorescence with anti-IgA antibodies marked with fluorescein). (C) Electron microscopy showing mesangial (MD) and paramesangial (PMD) electron dense deposits, with focal effacement of the foot processes (black arrow).* All pathology photographs were prepared by the Institute of Clinical Pathology and Medical Research, Westmead Hospital, Sydney, Australia.

predictor for recurrent disease, although recurrence tends to occur with greater frequency in young donors or in those who originally presented with more aggressive disease in the native kidneys. It has been postulated that risk of recurrence is greater in patients receiving a kidney from a live donor; however, this is unsubstantiated and data are conflicting.

Treatment strategies and outcomes

There is no effective therapy to prevent recurrence of IgAN. Treatment should focus on control of blood pressure with renin-angiotensin blockade. Newer immunosuppressive agents appear to have little impact on disease recurrence and reports of lower recurrence rates with mycophenolate mofetil (MMF) are anecdotal and uncorroborated. Although steroid free or rapid steroid withdrawal has been reported not to affect risk of recurrence [61], a recent survival analysis from ANZDATA reported steroid use was strongly associated with a reduced risk of recurrent IgAN [62]. ACE inhibitors and angiotensin receptor blockers reduce proteinuria and may preserve kidney function in recurrent IgAN [63].

Membranous glomerulonephritis

Background and pathogenesis

Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults, leading to ESKD in up to 50% of patients. Reported to recur in 10 to 30% of patients after transplant [64], accurate assessment of true recurrence rates are hampered by the frequency of de novo MN and the wide variability in threshold for biopsy between centres. Surveillance biopsies suggest that recurrent MN is probably more frequent than previously estimated [14].

Graft survival in patients with idiopathic MN appears to be similar to patients transplanted with different renal diseases (including other GN). Prognosis following recurrent MN after transplantation is less clear, with reports of 60 to 65% of cases progressing to ESKD within 4 years after recurrence [65,66], while others report no significant difference in graft failure, death, or renal function between patients with recurrent MN and those without recurrence [67]. The Australia and New Zealand Dialysis and Transplant Registry reports graft failure from recurrence between 10 to 15% at 10 years [2] (Table 77.2).

To date, there are no clinical or histological factors to reliably predict risk of recurrence. While some groups have reported more frequent recurrence with recipient of live-donor allografts [68,69], others have reported the opposite [70].

Clinical presentation is usually between the first and second year from transplant and is typically heralded by proteinuria (often within nephrotic range) with or without renal dysfunction. Presentation may occur earlier and surveillance biopsies have shown that clinical manifestations of proteinuria or elevated creatinine may be mild or absent in the early stages of disease recurrence [14]. Renal biopsy is characterized by uniform thickening of the glomerular basement membrane (GBM) resulting from immune complex deposits in the outer or subepithelial surface of the GBM. Differentiating recurrent from de novo MN requires an accurate diagnosis of the original renal disease, and review of the recipient's native kidney biopsy may be necessary to assist diagnosis. Clinical clues supporting de novo MN include histological features of transplant glomerulopathy, capillaritis, C4d deposition in the peritubular capillaries, and the presence of donor-specific antibody, all of which

result from either acute or chronic antibody-mediated rejection rather than recurrent idiopathic MN.

Although the pathophysiology of idiopathic MN remains uncertain, there is evidence that circulating autoantibody directed against podocyte enzymes M-type phospholipase-2 receptors, manganese superoxide dismutase, and aldose reductase may play a role in disease pathogenesis. Subepithelial immune complexes, which contain terminal complement, are deposited into podocyte membranes leading to sublytic cellular activation with oxidant and protease production and destruction of the underlying glomerular basement membrane. There is a paucity of data in patients with recurrent idiopathic MN but recent clinical observations have demonstrated the presence of circulating autoantibodies to antiphospholipase A2 receptor, which react with exposed epitopes in the donor kidney [71].

Treatment strategies and outcomes

There is no evidence to support the therapeutic advantage of one immunosuppressive agent over another. Specifically, cyclosporine and MMF (which have been shown to be beneficial in the treatment of primary MN) do not prevent or alter the course of recurrent disease. Reports of rituximab therapy in recurrent MN are few, and limited to case reports or series. Outcomes have been variable and numbers too small to reach any firm conclusion as to its efficacy in achieving partial or complete remission. Non-immunosuppressive therapies include ACE inhibitors and angiotensin receptor blockers to reduce proteinuria, lipid-lowering agents, diuretics to treat symptomatic oedema and anticoagulation for severe nephrotic-range proteinuria.

Mesangiocapillary or membranoproliferative glomerulonephritis

Background and pathogenesis

Mesangiocapillary glomerulonephritis (MCGN) describes a histological pattern of injury rather than a specific disease. Characterized by mesangial hypercellularity, endocapillary proliferation, and double contour formation along the glomerular capillary walls, recurrent MCGN should be distinguished from de novo transplant glomerulopathy, which demonstrates similar histological features. In such circumstances, recurrent MCGN is suggested by the presence of crescents on light microscopy, with strong staining for C3 and the presence of dense subendothelial deposits on electron microscopy [72], while transplant glomerulopathy is supported by the presence of C4d staining and the presence of circulating donor-specific antibodies. Primary MCGN may be idiopathic but more commonly arises from an array of underlying secondary causes including infection (hepatitis B and C the most common infective cause), autoimmune disease (systemic lupus erythematosus, Sjögren's syndrome and rheumatoid arthritis), and dysproteinaemias.

MCGN type I

Disease pathogenesis arises from mesangial and subendothelial glomerular deposition of IgG and C3, presumably representing immune complexes following exposure to endogenous self-antigen (DNA in SLE) or exogenous non-self antigen (hepatitis B or C antigen). As these antigens are not eliminated following transplantation, it is predictable that recurrence rates are high (20 to 50%) [58,73,74], with reports of graft loss in up to 40% of those with recurrence [73,74] (Table 77.2). Clinical manifestations include graft dysfunction, microscopic haematuria, and proteinuria. Risk factors associated with recurrence include severity of histological lesions in the native kidneys, live-related transplants (HLA-identical recipients appear to be at particular risk), HLA-B8DR3, and previous graft loss from recurrent disease (where risk rises to approximately 80%) [73,75]. No formal therapy has been proven useful, although the underlying cause of MCGN type I may influence therapy. Recurrent disease related to hepatitis B antigenaemia may respond to antiviral therapy while liver transplantation has reported short-term success with hepatitis C associated MCGN. Other forms of type I MCGN have been treated with immunosuppression with or without plasma exchange but all therapies have met with limited success.

MCGN type II

Type II MCGN (or dense deposit disease) is confirmed by electron-dense transformation of the glomerular basement membrane and deposition of C3 (and other complement protein) but without IgG deposition. It claims an even greater rate of recurrence at 50 to 100% of grafts, usually presenting with non-nephrotic range proteinuria, haematuria, and gradual graft dysfunction within the first year from transplant. Graft loss to recurrence occurs in 15–30% of grafts beyond 5 years [76] (Table 77.2). Male gender, heavy proteinuria, and cellular crescents on biopsy appear to correlate with worse outcome and higher risk of graft loss. It has been proposed that the superimposition of cellular crescents, interstitial fibrosis, and mesangial proliferation (i.e. severity of disease) better explains the higher risk of recurrence rather than disease subtype itself [75]. Regardless, type II MCGN appears to present with more aggressive histological features than type I, likely explaining the greater risk of recurrence and worse outcome in this group of patients. Immunosuppression and plasmapheresis have been used in case reports but no successful therapy is known.

MCGN type III

Often considered a variant of type I MCGN, histological diagnosis is supported by the presence of both subendothelial and subepithelial deposits. Data on this disease subtype are too sparse to reliably inform recurrence risk or prognosis, suffice to say that transplantation has been successfully performed with recurrence described in one case report at 13 months from transplant with graft loss at 7 years [77].

Thrombotic microangiopathy

Background and pathogenesis

Thrombotic microangiopathy (TMA) is characterized by endothelial injury and is manifested histologically by intimal cell proliferation, thickening and necrosis of the vessel wall with narrowed lumens and thrombi in the glomerular, arteriolar, or interlobular arteries. It is seen typically in the renal biopsies of patients with haemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura. Recurrent disease should be differentiated from de novo TMA following renal transplant that is secondary to infection (e.g. polyoma virus), iatrogenic (related to calcineurin inhibitors, mTORi, or OKT3), or immunological causes (acute humoral rejection).

Recurrent TMA typically occurs in patients who developed renal failure from the non-diarrhoeal form of HUS. It is particularly frequent in patients with autosomal recessive or dominant familial or atypical HUS (aHUS) where the disease arises from mutations or polymorphisms in genes encoding complement regulatory proteins factor H (CFH), membrane cofactor protein (MCP), and factor I (FI). Data are now emerging that patients with mutations

in the complement activating components factor B and C3 are also at risk of recurrence. Circulating complement factor proteins, factor H (the most common mutation), factor I, factor B, and C3, are all produced by the liver, explaining why recurrence rates are so high in these patients following renal transplant. In contrast, MCP is a membrane-associated regulator that limits complement activity at the cell surface. It is ubiquitously expressed and therefore present on endothelial cells within the kidney allograft. For this reason, TMA rarely recurs in patients with MCP mutations.

It is thought that kidney endothelial cells in patients with complement deficiency or anti-CFH autoantibodies with aHUS are more vulnerable to early ischaemic injury, triggering complement activation and thrombotic microangiopathy. Post-transplant viral or bacterial infections that activate complement, and injury that may potentiate inflammation and injury such as acute rejection, may also further increase the risk of triggering recurrent TMA.

Historical reports of HUS patients undergoing renal transplantation quote recurrence rates of 4 to 60%. The low incidence of recurrence in some reports is probably explained by the inclusion of patients who received transplants for HUS following a diarrhoea prodrome and most likely had Shiga-toxin producing *Escherichia coli* (STEC)-associated HUS (Stx-HUS). STEC-released toxins are the cause of Stx-HUS and re-exposure to toxin is required to induce recurrence. As most patients develop neutralizing antibodies to Stx following infection, recurrence is rare.

A review of 118 paediatric patients who received 137 renal transplants after HUS following a diarrhoea prodrome (most of whom were presumably Stx-HUS), found only one had recurrence after transplant. In a separate study of 62 children who were known to have had Stx-HUS, there was no recurrence of HUS and graft survival was superior at 10 years when compared to children transplanted for other causes [78,79].

Conversely, outcome is poor following transplantation for atypical HUS (i.e. STEC infection excluded). A review by Bresin et al. found approximately 60% of patients experienced recurrent disease, 91.6% of which progressed to graft failure. One-year graft survival was 32% for patients receiving a deceased donor kidney transplant and 50% for living donor transplants. The presence of CFH or FI mutations were associated with a high incidence of graft failure (74% and 100%, respectively) [80]. Data from USRDS confirm poor prognosis, reporting survival rates of only 50% at 3 years [81]. Time to recurrence may be within days from transplant up to 2 years, but the majority (60%) present within the first month [79].

Clinical presentation may vary from mild allograft dysfunction, with or without evidence of microangiopathic anaemia and thrombocytopenia, to rapidly progressive graft dysfunction in the absence of extrarenal manifestations. Platelet count should be closely monitored during periods of graft dysfunction in patients with atypical HUS, with a low threshold to assess peripheral blood film (for fragmented cells), haptoglobulin, and lactate dehydrogenase to look for evidence of haemolysis (Table 77.3). Renal biopsy is invariably required to confirm the diagnosis, although caution should be extended in the setting of severe thrombocytopenia.

Treatment strategies and outcomes

There is no evidence to suggest that avoiding agents associated with TMA, such as CNI and mTOR inhibitors, will reduce the risk of recurrence. Some have shown that early use of cyclosporine increased the risk of recurrent TMA [82] while others found no effect [83]. Although some advocate the avoidance of CNI early after transplant if possible [84], recurrent HUS has been reported in patients with CFH or CFI mutations receiving CNI-free immunosuppression. Active treatment strategies include plasmapheresis with fresh frozen plasma (FFP) (to increase levels of factor H and I), IVIG, and rituximab [85,86]. Reports of success are limited to case reports, with variable outcomes. Simultaneous liver and kidney transplant, with perioperative plasma exchange using heparin and FFP, has permitted successful transplantation of a handful of cases with factor H mutations [87–89]. Data have been reported on the successful use of the humanized anti-C5 monoclonal antibody, eculizumab, to treat atypical HUS in native kidneys and rescue therapy for recurrent HUS after transplant. Of five published reports using eculizumab to treat recurrent disease after transplant, four showed complete recovery and one partial recovery [90] (Table 77.3).

ANCA-associated glomerulonephritis

Background and pathogenesis

Advances in early recognition and treatment of ANCA-associated GN have resulted in improved patient survival and progressive renal disease. Despite this, a proportion of patients still progress to ESKD. Providing clinical remission has been achieved and maintained, transplantation is considered safe and the risk of disease recurrence is low. True recurrence rates are unclear, as data remain sparse. A pooled analysis, published in 1999, reported a recurrence rate of 17% with follow-up ranging from 4 to 89 months. Of these, 57% demonstrated renal involvement but only two lost their grafts due to recurrence [91]. In an era of tacrolimus and MMF-based immunosuppression, more recent analysis reported lower recurrence rates of 7%, with 94% graft survival at 5 years [92]. Briganti et al. reported a 10-year incidence of allograft loss of 7.7% in patients with ANCA-associated vasculitis, although not all these grafts were lost to recurrent disease [2] (Table 77.2).

Clinical renal presentation typically involves graft dysfunction accompanied haematuria and proteinuria. Histological features include changes of a pauci-immune necrotizing vasculitis, although acute arteritis may also be present and obstruction from ureteral stenosis resulting from granulomatous vasculitis has been reported.

Other than evidence of clinically active disease prior to transplant, there are no clear predictors of disease recurrence after transplantation. Pretransplant disease course, disease subtype (Wegner's, microscopic polyangiitis, Churg–Strauss), cANCA or pANCA subtype, and pretransplant antibody titres (without clinical disease), type of donor, or duration of follow-up all fail to reliably predict recurrence.

Treatment strategies and outcomes

Current therapies used to treat vasculitis in native kidneys also appear effective in allografts. A pooled analysis and several case series show a good response to cyclophosphamide therapy for the treatment of relapses [93]. Case reports and small case series have also reported successful remission using other treatments including plasma exchange, rituximab, and intravenous immunoglobulin [94–98].

Antiglomerular basement membrane disease

Early case series reported a high rate of recurrence (up to 50%) [99] after transplant. Recurrence of Goodpasture's disease is rare anyway, but current therapies, together with a general policy of delaying transplant until anti-GBM antibodies have been undetectable for

several months, probably explains only one case report of recurrent anti-GBM disease in the last 15 years [100]. Good treatment response was reported with pulse steroid, cyclophosphamide, and plasmapheresis.

Lupus nephritis

Background and pathogenesis

Lupus nephritis leads to ESKD by 10 years in 5 to 10% of patients diagnosed with the disease [101] and accounts for 1 to 2 % of all patients with ESKD who require transplantation [102]. There is no clear consensus on rate of recurrence after transplant. Traditionally thought to be low, more recent data report rates between 2 and 9% [2,103] (Table 77.2). Histological data (including reports from surveillance biopsies) report recurrence rates of 30 to 54%, although graft loss is rare [15,102]. Mean time to recurrence is approximately 3 years but may occur within days from transplant. There are no markers to reliably predict recurrence. In particular, serology and duration of dialysis before transplant are not predictive. Reports on graft survival in patients with lupus nephritis are conflicting. Pooled data have suggested equivalent outcomes in three studies, while six reported worse outcomes in patients with lupus nephritis despite their younger age [103]. Nevertheless, graft loss due to recurrent lupus nephritis is uncommon (2–4%) and more recent data report comparable patient and graft survival when compared with recipients with different underlying disease [102,104] (Table 77.2). The caveat to this is the greater risk of thrombotic events (including graft thrombosis) in lupus patients with known antiphospholipid antibodies.

Treatment strategies and outcomes

Management of recurrent lupus nephritis has not been formally studied. Post-transplant immunosuppression does not routinely differ from standard therapy. There are anecdotal reports on the efficacy of MMF for the treatment of recurrent lupus nephritis; however, these are currently limited to case reports [105,106].

Systemic sclerosis/scleroderma

Background and pathogenesis

Scleroderma renal crisis occurs in approximately 10% of patients with scleroderma, leading to progressive renal disease in many patients despite current therapy. Nevertheless, survival rates have improved in recent years and renal transplantation is an option for those patients who progress to ESKD.

Risk of recurrence was previously thought high, at 20 to 50%, with resultant graft loss occurring in 20% of cases [107]. In contrast, more recent data from the United Network for Organ Sharing (UNOS) report a recurrence rate of 1.9% in 260 patients transplanted between 1987 and 2004 for scleroderma or progressive systemic sclerosis. Graft survival was 79% at 1 year, 57% at 5 years, and 27% at 10 years [108]. Reasons for such disparity in recurrence rates may include publication bias of more complex cases in early case series, while incidence of recurrent disease by UNOS may have been under-reported (not all graft losses were reported and cause of graft loss was unknown in 32% of cases in the UNOS study).

Risk factors for scleroderma renal crisis in the native kidney include use of steroids, disease duration less than 4 years, progressive skin thickening, new-onset anaemia, pericardial effusion, or congestive cardiac failure. No such risk factors have been studied in the renal allograft after transplant and it is not known whether the use of steroids may precipitate recurrent disease in the allograft.

Treatment strategies and outcomes

For those who develop recurrent scleroderma, time to onset is unknown; however, limited data suggest onset is more commonly reported within the first few months to the second year from transplant and late presentation is rare. Management of recurrent scleroderma is not well described. The use of ACE inhibitors to treat hypertension would appear reasonable but there is no evidence clearly demonstrating a beneficial effect in this patient cohort.

Fibrillary glomerulonephritis and immunotactoid glomerulopathy

Fibrillary GN is a recognized histological entity that occurs in approximately 1% of native kidney biopsies in studies from Western countries. It is characterized by glomerular accumulation of randomly arranged fibrils, which differ from amyloid by their size and lack of reactivity with Congo red. In contrast to immunotactoid GN (which is histologically similar), fibrillary GN is rarely associated with underlying systemic disease. Progression to ESKD occurs within several years of diagnosis in approximately 50% of patients and no effective therapeutic intervention has been trialled. A number of these patients have been successfully transplanted. Fibril deposition has been reported in greater than 50% of renal allografts, although recurrent disease appears to follow a relatively benign course [109] (Table 77.2).

Fabry's disease

Background and pathogenesis

Fabry's disease is a rare X-linked glycosphingolipid storage disorder resulting from a deficiency of the lysosomal enzyme α-galactosidase A. It results in gradual accumulation of glycosphingolipids within the lysosomes of various cells and tissues, including renal epithelial cells, leading to proteinuria, renal dysfunction, and eventual ESKD in the majority of affected patients. Renal transplantation is the treatment of choice for these individuals, although data are lacking on the incidence of recurrent disease, graft survival, and the effects of enzyme replacement therapy following transplantation. The US Organ Procurement and Transplantation Network registry study examined 197 renal transplant recipients with Fabry's disease and found 74% had a 5-year graft survival, compared to 64% in patients transplanted for other reasons [110]. Similarly, data from the European Dialysis and Transplant Association/European Renal Association registry found graft survival was similar at 3 years in 33 patients with Fabry's disease compared to other patients with different nephropathies (72% vs. 69%) [111].

Evidence of disease recurrence after transplantation is sparse and limited to case reports, which have shown disease may recur in the kidney within 6 to 8 months from transplant [112], while deposition of glycolipid in tubular epithelial and endothelial cells has been demonstrated up to 14 years after transplant [113]. The presence of these lesions appear insufficient to compromise graft function.

Treatment strategies and outcomes

The introduction of enzyme replacement therapy (ERT), agalsidase-α or agalsidase-β, has been shown to reduce the rate of decline in kidney function in native kidneys; however, it is unclear whether therapy improves graft survival or reduces complications related to Fabry's disease in transplant recipients. A recent European study demonstrated that ERT was safe, well tolerated, and graft function remained stable over the 2-year study period. Whether it impacts on long-term allograft survival is not known [114].

Recurrent disease after pancreas transplantation

Background and pathogenesis

Recurrence of diabetes after pancreas transplantation was reported first by David Sutherland's group in the 1980s. They reported a cohort of ten patients who had been transplanted with the tail of the pancreas from living donors. Five were from HLA-identical twins and five from HLA-identical siblings. There was a prompt return to diabetes when immunosuppression was reduced or stopped. Graft biopsies confirmed disease recurrence with the presence of isletitis and selective β-cell destruction [115–117].

However, for SPK or pancreas after kidney (PAK) recipients the donors are not HLA-identical and they remain on lifelong immunosuppression. This combination is thought to protect the majority of recipients from recurrent diabetes after pancreas transplantation. The lack of MHC compatibility means that memory autoreactive CD4 and CD8 T cells have limited affinity for the newly transplanted β cells. In addition these diminished autoreactive responses seem adequately suppressed with the combination immunosuppression used to prevent rejection. For the majority of pancreas transplant recipients, the data would seem to support the hypothesis. Once the pancreas survives the early technical complications, the long-term graft survival is excellent. At our institution, the 20-year pancreas graft survival exceeds that of the kidney graft from the same donor, suggesting that autoimmune disease recurrence does not have a major impact on graft survival. Furthermore, long-term graft survival is excellent with normal glucose tolerance tests and HbA1c persisting beyond 10 years with no reduction in function over time [118]. Studies that have looked for autoimmune recurrence after pancreas transplantation have provided mixed results. A retrospective evaluation of pancreas histology, which was a combination of biopsy samples, pancreatectomy, and autopsy results, from a 100 pancreas transplant recipients, showed no definitive evidence of autoimmune recurrence [119]. In HLA-mismatched pancreas grafts isletitis was identified in 19% of grafts but was only seen in the context of associated graft rejection. Although disease recurrence could not be ruled out, none of these patients developed recurrent diabetes [119,120]. In a follow-up publication of 200 pancreas transplants by the same group, they failed to identify a single case of recurrent autoimmune diabetes provided the graft was mismatched for MHC and the recipient remained on immunosuppression [117]. Hence in their experience, disease recurrence was seen primarily in patients who received HLA-identical segmental pancreas graft from a sibling and minimal or no immunosuppression.

More recently, evidence of autoimmune disease recurrence has been demonstrated as a cause of graft loss in a small number of pancreas transplant recipients. Initially, there was a report of two cases of histologically proven autoimmune induced recurrent diabetes [121]. The two patients received HLA-mismatched pancreas grafts and were maintained on cyclosporine, azathioprine, and prednisolone. After initial good graft function, the pancreas graft failed whilst maintaining good renal graft function 2 years and 29 months after transplantation [121]. Histology showed isletitis without rejection and selective loss of β cells consistent with autoimmune destruction. A second study looked at the association of major islet autoantigens and pancreas graft outcome [122]. They looked at islet autoantibodies in 110 recipients and followed 75 of these sequentially for up to 11.2 years (median follow-up 6.3 years). They found that pancreas graft survival was not associated with the presence of GAD or IA-2A antibodies prior to transplantation. The majority (59%) of patients remained persistently autoantibody negative after transplantation; 34% of patients had stable or declining levels of autoantibodies after transplantation and it was not a predictor of loss of function. However, in five cases (7%) there was an increase in IgG1 antibody titre and graft function was lost in four of these patients, suggesting that in a minority of patients increasing titres of islet autoantibodies was a strong predictor of graft failure and return to insulin [122].

The most definitive proof of recurrence of diabetes was provided by researchers at the University of Miami. Using very sophisticated studies in a small number of individuals they were able to confirm recurrent autoimmunity as a cause of graft loss. In three individuals they showed: hyperglycaemia without rejection, the presence of isletitis with selective β-cell loss, the persistence or reappearance of islet autoantibodies, and the presence of circulating islet autoreactive CD4 and CD8 T cells at the time of graft loss [123–125]. The assays used to identify islet-specific autoreactive T cells are difficult to perform and the technology is available only in one or two research laboratories worldwide. Hence, it is not suitable as a routine screening test. Nevertheless, these studies show definitively that autoimmune disease recurrence does occur in HLA-mismatched pancreas transplants despite immunosuppression. Whilst the condition is thought to be relatively uncommon, there are suggestions it may occur in up to 5% of recipients [126]. Further work is required to more accurately determine the size of the problem and to validate diagnostic markers that would point to the diagnosis prior to loss of function.

Clinical features

The clinical feature suspicious of autoimmune recurrence is loss of c-peptide in the absence of rejection (Table 77.4). In SPK patients this is relatively easy to confirm as rejection is diagnosed by monitoring renal function and renal biopsy is standard of care for diagnosing rejection. In patients who have received a pancreas transplant alone or a PAK transplant this is more difficult as pancreas rejection can occur in the absence of kidney rejection and a pancreas biopsy is necessary to determine whether autoimmune recurrence or pancreas rejection is the cause.

Investigation

The early features of autoimmune diabetes is isletitis with mononuclear cells and selective loss of β cells [127]. With time there may be variable loss of other cell types such as α cells but this is variable and the loss of β cells is constant. In addition, the acinar tissue and blood vessels are normal without a cellular infiltrate. By contrast, rejection consists of a mononuclear cellular infiltrate within all compartments of the pancreas (acinar, islets, and vascular) with or

Table 77.4. Features of recurrent type 1 diabetes after pancreas transplantation

	Features in recurrent type 1 diabetes
Incidence	Uncommon Up to 5% of transplants
Clinical features	Gradual loss of c-peptide and recurrence of diabetes in absence of rejection
Pathology	Isletitis with selective loss of β cells with normal acinar and vascular histology
Diagnosis	Reappearance or increase in titre of anti-GAD and anti-IA2 antibodies Pancreas biopsy
Treatment	None shown to be of benefit Maximize immunosuppression if under immunosuppressed

without an endoluminal vasculitis. Whilst a mononuclear infiltrate of islets can occur in pancreas rejection, there is not a selective loss of β cells and other compartments of the pancreas, such as acinar tissue, are similarly involved [119].

Whilst definitive proof of autoimmune recurrence relies on a histological diagnosis, there are other laboratory features that are suggestive of this possibility. Loss of function from recurrence of disease is gradual and there is a progressive loss of c-peptide over months to years [125]. In addition, the reappearance or increase in titre of anti-GAD and anti-IA2 antibodies has been shown to be associated with recurrence. However, the data regarding this is limited and it is not clear whether autoimmune diabetes can recur in the absence of autoantibodies and many patients remain disease free despite the persistence of autoantibodies. Hence, diagnosis depends on longitudinal measurement of the titre of autoantibodies over time—something that is not done routinely.

Treatment strategies and outcomes

There is no effective treatment of autoimmunity associated with type 1 diabetes. Various immunosuppressive and short-term depleting T-cell therapies have been tried but none have been shown to be more than transiently effective [128,129]. There has been a small trial of autologous haematopoietic stem cell transplantation at the time of presentation of type 1 diabetes [130]. Whilst there was evidence of benefit, the long-term outcomes and a proper evaluation of the risks have yet to be undertaken. Translating this to a transplant setting has not been done and would be more difficult as there would be substantial additional risks for patients already on long-term immunosuppression.

Because the diagnosis of recurrent diabetes after pancreas transplantation has been limited to case reports, it is very difficult to say anything definitive about treatment. The patients are already on immunosuppression and this should be reviewed and optimized to ensure that adequate immunosuppression is achieved. This is a reasonable approach because the initial reports were in transplants from HLA identical siblings, where immunosuppression was minimized or abolished. Some centres have treated the patients with T- and B-cell-depleting antibodies upon diagnosis. Whilst it was reported that there was temporary abolition of autoreactive T cells and stabilization of c-peptide, there was eventual recurrence of autoreactive T cells and ultimate loss of function. Given the potential risks involved with such an approach it could not be recommended as routine therapy.

Summary of recurrent diabetes after pancreas transplantation

Recurrent type 1 diabetes after pancreas transplantation can occur and is responsible for loss of function and return to diabetes after successful transplantation. It should be suspected in patients who have a progressive loss of c-peptide months to years after transplantation in the presence of stable renal graft function. A reappearance or increase in titre of anti-GAD and anti-IA2 antibodies should raise the suspicion of the diagnosis, which would then be made on pancreas biopsy. The actual prevalence is not really known although it is uncommon. Clearly this is an area requiring ongoing investigation and research.

Concluding remarks

Despite overall progress in renal and pancreas transplant research, our understanding of the pathogenesis of recurrent disease, and its true prevalence in both organs, remains unclear while therapeutic interventions are limited.

Part of this problem lies in our poor understanding of primary disease in the native organ. However, rather than being seen as a deterrent, perhaps this should be regarded as a unique opportunity to provide insight into native disease in the setting of different persistent immunosuppressive regimens. Furthermore, the advent of new molecular methods, originally targeted to allow non-invasive monitoring of allograft pathology, provide the chance to apply techniques such as gene microarray, proteomics, and metabolomics to study the aetiology and pathogenesis of primary disease that may recur in the graft.

Collaboration is required to better inform the true prevalence of recurrent disease, risk of recurrence, and impact on graft survival. Given the relative rarity of many of the diseases in question, large multicentre trials are necessary if any hope of progress in prevention and therapeutic intervention is to be achieved.

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CHAPTER 78

Recurrent Disease after Liver Transplantation

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Introduction

For many patients with chronic liver disease, orthotopic liver transplantation (OLT) offers the best, if not only, hope for survival and long-term quality of life. For some patients, liver transplantation cures the offending disease, offering the recipient a chance for life without concerns about disease recurrence. For others, recurrence of the original disease, or manifestation of previously unidentified disease processes, present ongoing postoperative challenges. In this chapter, we limit our discussion to disease recurrence acknowledging that, in some cases, such as the differentiation of ischemic ductal disease from recurrent primary sclerosing cholangitis, or the development of de novo autoimmune disease, diagnosis may not be so precise. Much space has been devoted to recurrent hepatitis C commensurate with the effort required to manage these patients in the outpatient clinic and perhaps motivated by a consistently lower survival rate in these patients. On the other hand, the once feared recurrence of hepatitis B might soon become a footnote in transplant history. This chapter covers the common diseases with substantial recurrence risk to liver transplant recipients, and describes their differential diagnosis and treatment. It complements Chapter 70 covering the clinical identification and management of liver allograft rejection, and provides clinical context to the more in-depth discussion of liver transplant histopathology found in Chapter 82.

Viral disease

Hepatitis C

Recurrence of hepatitis C virus (HCV) infection is the leading cause of allograft failure and death in HCV patients undergoing OLT [1]. Forman reviewed over 11 000 OLT cases from the United Network for Organ Sharing (UNOS) database and compared outcomes of HCV+ patients with HCV– patients. Liver transplantation in HCV+ patients was associated with higher death and allograft loss rates than HCV– patients in this analysis, in contrast to other, smaller studies at various centers [2–5]. Outcomes for retransplantation for recurrent HCV infection are lower than for primary transplantation [6,7], leading some centers to refuse retransplantation, especially if renal dysfunction exists. Outcomes following retransplantation of HCV+ patients compared with HCV– patients may not differ when the degree of liver dysfunction and patient performance status are similar and when HCV+ patients are transplanted promptly upon development of allograft dysfunction [8].

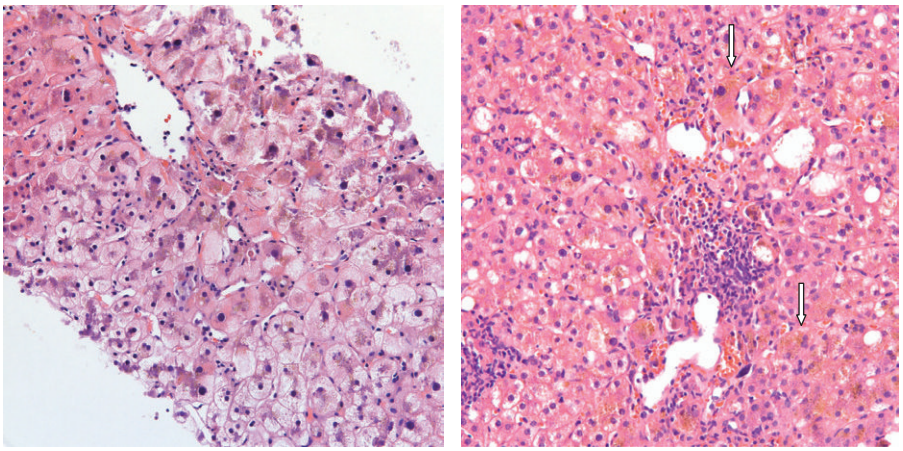
Viral kinetics

Recurrence of HCV infection after OLT usually occurs quickly and in essentially every case when the patient is viremic at the time of OLT. HCV RNA levels drop significantly in the anhepatic phase and for a short time after reperfusion [9]. Up to 90% of patients become viremic within 1 week after OLT, while peak viral loads are often not realized for up to 7–8 weeks. Mean viral loads in transplant patients are higher than those seen prior to transplantation due to immunosuppression. Gane *et al.* [10] noted many years ago that pulsed corticosteroids for acute cellular rejection were associated with higher viral loads and more severe histologic disease.

Disease course

Once allograft infection occurs, the clinical course is variable. Approximately 5–7% of patients will develop an ominous fibrosing cholestatic hepatitis, a term applied first to allograft infection with recurrent hepatitis B. These patients usually display high viral loads and relentless hepatonecrosis and jaundice. Histologic findings include centrilobular ballooning hepatocyte degeneration, cholestasis, and a characteristic paucity of lobular inflammation (Figure 78.1). The aggressive course and poorer outcomes associated with this type of disease represent perhaps the best evidence of a direct cytopathic effect of the virus on the hepatic allograft [11]. Most patients develop lobular hepatitis within 3–6 months after OLT. Approximately 25% of patients experience an aggressive recurrence of disease that leads to allograft cirrhosis or advanced fibrosis within 3–5 years. Ciccorossi et al. [12] correlated high viral load at 1 week after OLT with poorer outcomes. Sreekumar et al. [13] noted that higher HCV RNA levels at 4 months after OLT ($>10^9$ copies/mL or >log 1 meq/mL) were associated with higher grades of inflammation and stages of fibrosis at 1 and 3 years post-OLT. Approximately two-thirds of patients will experience a somewhat less aggressive but variable course, with little or slower fibrosis progression. A typical course includes mild lobular hepatitis within the first few months after allograft infection (Figure 78.2) followed by the appearance of more significant portal infiltrates and chronic active hepatitis (Figure 78.3). Patients with recurrent HCV infection after OLT often display significant impairment in quality of life and physical functioning [14]. Histologic evidence of recurrent HCV infection is often confused with, or obscured by findings of acute cellular rejection [15]. More obvious evidence of HCV recurrence is often evident after treatment of acute cellular rejection and resolution of its associated histologic findings. Table 78.1, and the

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.



(A) (B)

Figure 78.1. (A) Ballooning hepatocyte degeneration associated with fibrosing cholestatic hepatitis C virus (HCV) recurrence. (B) Cholestasis (arrows) associated with fibrosing cholestatic HCV recurrence.

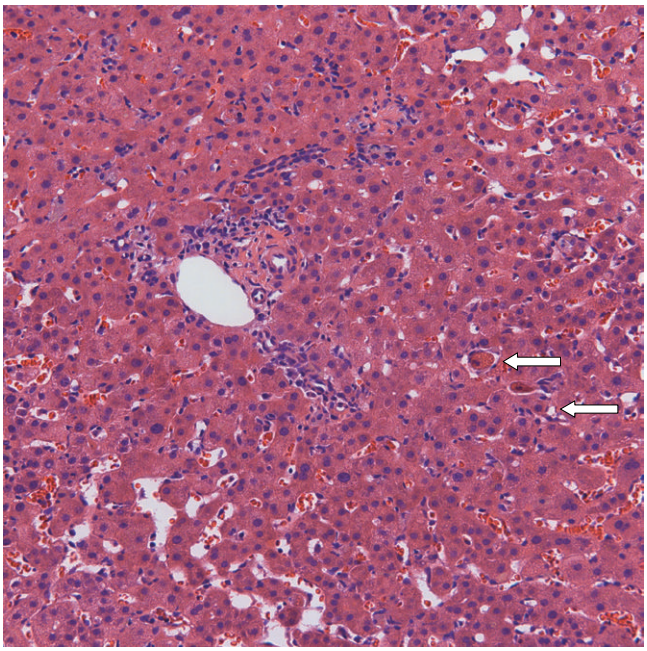


Figure 78.2. Early HCV recurrence with lobular inflammation and Councilman bodies (arrow).

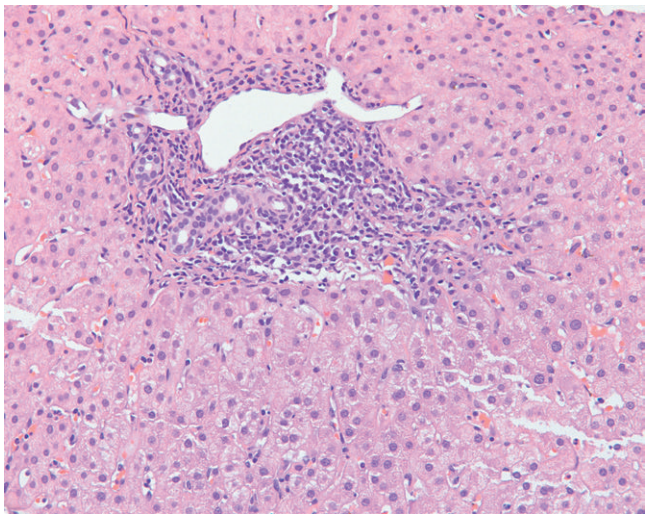


Figure 78.3. Lymphocytic portal infiltrate and interface hepatitis of established recurrent HCV.

sections that follow, summarize these and other factors that may predict more severe HCV recurrence.

Recipient factors and virus characteristics

Recipient factors associated with worse outcomes for HCV after OLT include pretransplant viral load and, in some studies, recipient age, gender, ethnicity, and HCV genotype [1,10,12,16]. Of these variables, high pretransplant viral load may represent the highest risk for graft loss and mortality [1]. Virus characteristics such as genotype and viral heterogeneity may have a role in disease severity after transplantation, but these findings have little practical application. Quasispecies noted pre-OLT do not necessarily persist after OLT, leading to further uncertainty about the significance of their existence [17]. Genotype 1b may be associated with more severe HCV infection after OLT [10] but other studies have shown no such discrepancy [3,18,19]. In a small study, genotype 4 was not associated with different outcomes when compared with outcomes of patients with other genotypes, but fibrosis development was more rapid and severe in the patients with genotype 4 [20]. A separate study from Australia, however, suggested that patients with genotype 4 have inferior outcomes [21]. Heterogeneity of patient cohorts and immunosuppression regimens make data comparison difficult with regard to these variables.

Donor factors

No donor factor has a stronger proclivity for more severe recurrence of HCV than donor age. Donor age greater than 50 years, and especially greater than 65 years, has been shown unequivocally to lead to worse outcomes for these patients, both in Europe and in North America and whether or not the donor is living or deceased

Table 78.1. Risk factors for more severe recurrence of hepatitis C virus (HCV)

Factor	Strength of evidence
Pretransplant	
Donor age (linear for age >35)	+++
Living donor	+
Donor/recipient HLA matching	+
Genotype 1B	+
HIV coinfection	+++
Female gender	+
Epoch (more recent)	+
Donor steatosis	+
Operative	
longer cold ischemic time (>12 h)	++
Donor genetic factors	+
Post-transplant recipient	
Advanced age (>50 years)	+++ (patient/graft survival)
Non-white race/ethnicity	+++ (patient survival)
Biliary tract complications	++
Viroiogic variables	
Higher pretransplant viral load	+++
Higher post-transplant viral load	+++
Immunosuppression	
OKT3, pulsed corticosteroids	+++
Short time to recurrence	+++
Treated cytomegalovirus infection	+++

Adapted from [49] Watt et al. (2009), with permission from John Wiley and Sons Ltd.

[22–24]. Donor organ steatosis has been blamed for more frequent and earlier HCV recurrence after OLT [25] but the effects of donor steatosis must be analyzed independently of prolonged ischemia time and associated organ reperfusion injury [26].

Other factors

It is fair to say that for the patient with HCV infection to have a favorable outcome after liver transplantation, a number of complications must be absent or averted. Excessive immunosuppression must be avoided. Yet acute cellular rejection, which does not generally adversely affect outcomes in non-viral patients, is associated with poorer outcomes in patients with HCV infection [27]. Just how to achieve optimal immunosuppression in the patient with HCV infection is a challenge for all transplant providers and a topic of much debate. Immunosuppression in these patients is covered in more detail later. Cytomegalovirus (CMV) infection, another factor directly related to immunosuppression levels, is associated with more rapid progression of recurrent disease, although its association with graft survival is not clear [28,29]. Although difficult to separate from reperfusion injury and prolonged ischemia times, biliary complications may contribute to poorer outcomes in patients with HCV. One carefully performed retrospective review of over 700 patients from two institutions showed that although biliary complications adversely affected 1-year graft survival rates, the combination of biliary complications and HCV did not, thereafter, affect fibrosis rates [30].

Treatment challenges

Sustained clearance of HCV infection through treatment was at first expected to be tenuous in the patient undergoing subsequent immunosuppression therapy but sufficient data exist to prove that sustained viral responses (SVR) are durable when achieved before and after OLT [31–33]. A persistent portal hepatitis in some patients rendered virus-free after transplantation has led to concerns about persistent virus in liver tissue [34]. However, modern assays for viral quantitation and viral detection as well as appropriate follow-up must be employed to determine the significance of such findings [35]. Some investigators have made a strong case for early treatment of recurrent HCV regardless of fibrosis score, noting the risk for progression of fibrosis and portal hypertension [36]. Since some patients with HCV infection do not progress rapidly after OLT and given the concerns about treatment-mediated chronic rejection and autoimmune phenomenon (see later), other programs initiate therapy when documented disease progression compels treatment [37,38]. Such reports of dire outcomes associated with antiviral therapy usually include only small numbers of patients and, while calling for caution, have not dissuaded the transplant community from taking an aggressive approach to treatment. At this time, the accepted treatment regimen of HCV after transplantation is standard of care (SOC) therapy with pegylated interferon and ribavirin as direct acting antiviral agents are only just being introduced to these patients. Treatment is less well tolerated by these patients compared with the general population and dose reductions, especially because of anemia, are much more common. Rates of SVR vary widely but are approximately 30% across many studies and are only rarely seen when treatment is not continued for at least 48 weeks [39]. As recently as 2005, no prospective randomized trials of post-OLT HCV treatment had been conducted. In a multicenter study, Chalasani et al. performed two randomized controlled trials, a prophylaxis trial and a treatment trial, to evaluate the safety and efficacy of peginterferon alfa-2a in the treatment of HCV recurrence after OLT. Peginterferon alfa-2a treated patients had significantly lower HCV RNA levels and more favorable changes in hepatic histologic features than untreated controls. However, only two treated patients in the prophylaxis trial (8%) and three patients in the treatment trial (12%) achieved a sustained virologic response. In the prophylaxis trial, eight patients (31%) in the peginterferon alfa-2a group and nine patients (32%) in the untreated group were withdrawn prematurely; whereas in the treatment trial, 10 patients (30%) in the peginterferon alfa-2a group and six (19%) in the untreated group were withdrawn prematurely. The incidence of acute rejection was similar in the treated and untreated groups in both the prophylaxis (12% versus 21%; $P = 0.5$) and treatment (12% versus 0%; $P = 0.1$) trials [40]. The importance of IL28B status and response to treatment after OLT is uncertain. A recent study from Barcelona suggests that successful treatment of patients for HCV infection after OLT may be influenced by the IL28B status of both recipient and donor [41].

Concerns about treatment-induced allograft rejection exist but the data are conflicting [42]. In one study, interferon-induced rejection, when it occurred, was easily treated [43]. In a French study of 70 treated patients, 21% developed rejection, five mild, nine moderate and one severe, during IFN-based therapy. Almost all responded to treatment of rejection and there were no allograft losses [44]. Previous history of rejection prior to interferon therapy and use of pegylated interferon were significantly associated with rejection. Finally, Berardi et al. have described de novo autoimmune hepatitis attributed to antiviral therapy with interferon and ribavirin in 9 of 44 patients despite viral clearance [45]. The occurrence of autoimmune and/or plasma cell hepatitis will be expanded in the following section.

The emergence of new viral-specific agents to include boceprevir and telaprevir offer the potential for less morbid and more effective therapeutic options. While data regarding these and other agents post-transplant are sparse at present, the use of more targeted antivirals will certainly occupy a substantial part of the future of HCV disease recurrence.

Plasma cell hepatitis

Ward and others have raised concern about the occurrence of de novo plasma cell hepatitis in patients with HCV after OLT [46]. It is unclear if this is a variant of autoimmune disease or a peculiar type of allograft rejection. These questions have focused attention on a broader challenge: the accurate interpretation of liver allograft biopsy findings in patients with HCV. Differentiation of acute rejection from HCV recurrence is difficult when the two processes share characteristics including periportal hepatitis and lymphocytic cholangitis. Effective treatment of acute cellular rejection may simply make residual findings of HCV more obvious. These challenges were brought nicely into focus by Demetris and Sebagh [47]. Their editorial in 2008 concentrated on the importance of the finding of centrilobular necroinflammatory activity and its association with acute and chronic rejection, the presence of plasma cell infiltrates, and the possible importance of autoimmune phenomena. The presence of autoimmune phenomena in patients with HCV in the general population is well described, including cryoglobulinemia and vasculitis, production of autoantibodies and serum rheumatoid factor, Sjögren's syndrome, glomerulonephritis, and B-cell lymphoma [48]. In the liver transplant patient, the use of steroids in some programs has approached anathema although experience clearly shows that corticosteroids often provide benefit in HCV+ liver transplant patients with autoimmune features. Nevertheless, some HCV+ transplant patients with autoimmune features will likely experience more aggressive disease and realize worse outcomes. The inconsistent nature of the post-transplant course, biopsy findings, and response to corticosteroids and antiviral therapy in HCV+ allograft recipients calls attention to the heterogeneous nature of these patients and the pitfalls of treating them all in the same way. Nevertheless, a general treatment algorithm is useful [42,49].

Immunosuppression considerations

Tacrolimus and HCV antibody testing were introduced at roughly the same time in the early 1990s. Since that time, there has been intense interest in the effects of immunosuppression on the severity of recurrence of HCV after OLT. Berenguer et al. [50] reported in 2000 that the fibrosis progression rate of HCV infection after liver transplantation has increased in more recent years, suggesting that perhaps changing immunosuppression may affect outcomes in these patients. Two groups have suggested, based on *in vitro* data, that cyclosporine may have inhibitory effects on HCV [51,52]. This notion has not been strongly supported by clinical data. By 1998, Wiesner [53] showed that patients with HCV infection receiving tacrolimus had significantly lower 5-year mortality rates than patients receiving cyclosporine. More recently, Irish examined the UNOS/OPTN (Organ Procurement and Transplantation Network) database and noted that in 8809 patients receiving either cyclosporine or tacrolimus between 2000 and 2007, with a median follow-up time of 5 years, patients receiving cyclosporine were more likely to develop allograft failure and death within 2 years post-transplant [54]. The authors point out the need to examine survival beyond 1 year when examining the effects of different types of immunosuppression. Some transplant programs convert patients with HCV infection to cyclosporine from tacrolimus when initiating antiviral therapy. In the time of direct acting agents (DAAs), such conversion may be justified because the interactions of cyclosporine and the DAAs will be much easier to manage, but only highly successful antiviral therapy may justify the switch away from tacrolimus. Manousou et al. [55] prospectively compared single-drug therapy with tacrolimus and triple-drug therapy with tacrolimus, azathioprine, and short-term prednisone, and noted slower fibrosis progression in the patients treated with triple-drug therapy. A single-center study by Jain et al. [56] prospectively compared tacrolimus and prednisone with tacrolimus, prednisone, and mycophenolate (MMF) and noted no differences in graft or patient survival, rejection, or rate of HCV recurrence based on biochemical changes and histologic findings. More recently, McKenna et al. [57], in a retrospective analysis, showed slower fibrosis progression in a cohort of sirolimus patients than a control group of patients not exposed to sirolimus. Conversely, Watt et al. [58] analyzed over 26 000 patients in the Scientific Registry of Transplant Recipients (SRTR) database including over 12 000 patients with HCV infection, and noted an association between sirolimus use and an increased risk of death and graft loss after liver transplantation in patients with HCV infection not seen in patients without HCV. The authors concluded that tacrolimus was associated with superior outcomes and that sirolimus should be used sparingly in recipients with HCV infections. In the multicenter HCV3 trial, patients were prospectively randomized to three arms: tacrolimus/corticosteroids; tacrolimus/corticosteroids/MMF; and a steroid-free arm using tacrolimus/MMF and daclizumab induction [59]. After 2 years, the steroid-free arm proved safe and was also associated with fewer patients with diabetes mellitus but there was no difference between the groups with regard to severe recurrence of HCV. Steroid avoidance in these patients is therefore feasible. On the other hand, given that acute cellular rejection (ACR) is to be avoided, and in consideration of de novo autoimmune disease, steroids should not be assiduously avoided.

In conclusion, interest in the importance of immunosuppression with regard to the severity of HCV infection progression after transplantation remains high, but few conclusions can be drawn at this time.

Hepatitis B

The early experience with liver transplantation for hepatitis B virus (HBV) infection was not encouraging as recurrence of the virus was usual, often with predictably unacceptable results. The term fibrosing cholestatic hepatitis, now more often associated with HCV after OLT, was first used to describe some cases of post-transplant HBV infection. The difference in outcomes of patients with or without measurable HBV DNA prior to OLT also became obvious once reliable and sensitive assays became available. Liver transplant programs were especially wary of transplanting patients with ongoing replicative HBV infection until Samuel et al. [60] demonstrated the effectiveness of passive immunoprophylaxis of such patients with hepatitis B immunoglobulin (HBIg). Their European multicenter trial of 334 patients showed a viral recurrence rate of 36% in treated patients versus 75% in untreated patients. Soon thereafter, teams from the Universities of Virginia and California San Francisco demonstrated the improved reliability of HBIg when used in higher and fixed doses sufficient to maintain threshold hepatitis B surface antibody (HBsAb) levels [61,62]. Using these dosing regimens and with compulsive attention to antibody monitoring, programs began to demonstrate only rare viral breakthrough including patients with hepatitis B E antigen (HBeAg) positivity prior to OLT. Over time, although effective, the use of HBIg proved expensive and inconvenient. The eventual emergence of evidence that intramuscular administration of HBIg is as effective as intravenous infusion did little to distract the transplant community from these concerns [63,64]. As oral drugs such as lamivudine and adefovir became

Table 78.2. Diagnostic criteria for recurrent autoimmune liver disease after liver transplantation

	Diagnostic Criteria	Exclusion Criteria
Autoimmune hepatitis	• Elevated transaminases • Hypergammaglobulinemia • Compatible histopathology – Portal inflammation – Lymphoplasmacytic inflammatory infiltrate – Interface hepatitis • Response to steroids	• Viral hepatitis • Drug-induced liver injury • Acute cellular rejection
Primary biliary cirrhosis	• Elevated alkaline phosphatase (variable) • Persistence of serum AMA (not required) • Histopathology – Typical: Florid duct lesion (granulomatous cholangitis) – Compatible: Lymphoplasmacytic portal infiltrates or lymphocytic cholangitis	• Acute or chronic rejection • Drug-induced liver injury • Biliary obstruction
Primary sclerosing cholangitis	• Cholangiogram with intra- and/or extrahepatic biliary stricturing, beading and irregularities of bile ducts • > 90 days post-LT • Histopathology – Fibrous cholangitis – Fibro-obliterative lesions with or without ductopenia, biliary fibrosis or cirrhosis	• Acute or chronic rejection • Hepatic artery thrombosis/stenosis • Anastomotic strictures • Nonanastomotic strictures <90 days post-LT • ABO incompatibillity

AMA, antimitochondiral antibody; LT, liver transplantation.

available, they were combined with HBIg to reduce the risk of HBIg breakthrough [65]. Lamivudine alone was not as effective in preventing HBV recurrence after OLT [66]. Nevertheless, in patients treated with lamivudine and HBIg, scattered cases of successful withdrawal, or ill-advised discontinuation of HBIg, did not result in recurrent viremia. The use of emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) in patients with coinfection with HIV and HBV has been highly effective and provided sufficient encouragement for investigators to consider prospective HBIg withdrawal protocols. Teperman et al. [67] treated 40 patients transplanted for HBV for 24 weeks with HBIg and FTC/TDF. Once viral suppression was documented, patients were randomized 1 : 1 to continue both drugs or to have HBIg withdrawn. Forty-eight weeks after randomization, no patient experienced viral breakthrough. Stravitz et al. [68] simply stopped HBIg after 6 months of therapy in 21 patients, and initiated FTC/TDF, again with excellent results and an estimated savings of $12 469 per year per patient compared with monthly intramuscular HBIg and lamivudine.

More recently, liver transplant programs have begun to replace HBIg early after OLT with either tenofovir or entecavir with increasing confidence.

Autoimmune disorders

Autoimmune hepatitits

Autoimmune hepatitis (AIH) is a chronic necroinflammatory liver disease of unknown etiology characterized by the presence of circulating autoantibodies, hypergammaglobulinemia, and histologic evidence of lymphoplasmacytic interface hepatitis. Treatment with immunosuppression, most commonly prednisone and azathioprine, is effective in preventing disease progression; however, up to 10% of patients fail medical therapy and require liver transplantation. AIH is a relatively uncommon indication for liver transplantation, accounting for 2–3% of pediatric and 4–5% of adult liver transplants performed in Europe and the United States [69]. Overall outcomes with liver transplantation are very favorable, with 5-year and 10-year patient survival rates of approximately 75% [69].

Despite the use of immunosuppressive agents post-transplantation, AIH can recur at rates ranging from 12% to 46% [1]. The wide variation of reported recurrence rates is likely the result of different diagnostic criteria, immunosuppressive regimens, length of follow-up and performance of per protocol liver biopsies. In a study by Gautman et al. [70] reviewing 25 publications, AIH recurrence was found in 23% of patients after a median interval of 26 months. Although AIH can recur in the early post-transplant period, most cases occur after 12 months, and the frequency tends to increase with time after transplantation.

As there is no one specific diagnostic marker of the disease, the diagnosis of AIH is based on a constellation of features, which also holds true for AIH recurrence (Table 78.2). The American Association of the Study of Liver Diseases (AASLD) has put forth the following diagnostic criteria for AIH recurrence:

1 Elevation of serum AST or ALT levels;
2 Persistence of autoantibodies;
3 Hypergammaglobulinemia;
4 Compatible histopathologic findings;
5 Exclusion of alternative etiologies; and
6 Responsiveness to steroids [69].

Defining AIH recurrence may often be a diagnostic dilemma as hypergammaglobulinemia and autoantibodies may persist after transplantation, without necessarily implying recurrence. As such, studies have pointed to histopathology as the most appropriate diagnostic marker of AIH recurrence. Compatible histologic evidence of recurrent AIH includes portal and/or lobular hepatitis in the presence of lymphoplasmacytic infiltrates, and the absence of histologic changes suggestive of alternative diagnoses. Histologic features of AIH recurrence appear to precede clinical or laboratory evidence of disease and may be found despite normal liver biochemistries, suggesting post-transplant protocol biopsies may be beneficial in identifying AIH recurrence early [71,72].

While the pathogenesis remains undefined, several studies have identified risk factors for developing AIH recurrence; however, most risk factors have not been validated and remain controversial. Transplant recipients positive for HLA DRB1*03 or HLA DRB1*04 have been identified as a risk factor in some but not all studies [71,73–77]. HLA-DR locus mismatching between donor and recipient has also been found to be a risk for recurrence [70,76]. In contrast to type 2 AIH, type 1 AIH incurred a sevenfold increased rate of recurrence based on a review of 89 cases [74,77–79]. Not only are patients undergoing transplantation for AIH at

higher risk of both acute and chronic rejection, but acute cellular rejection may also be associated with an increased risk of disease recurrence [72,80]. Increased severity of explant inflammation has been found to be a strong predictor of recurrence, in addition to high serum levels of IgG prior to transplantation [72,81]. While differences in transplant immunosuppressive regimens have been implicated, a systematic review of 13 studies reported no significant difference in disease recurrence between primary immunosuppression with either cyclosporine or tacrolimus [70]. Discontinuation of corticosteroids in patients with AIH following transplantation remains controversial. Several studies have associated disease recurrence with discontinuation of steroids, therefore supporting low dose steroid maintenance after transplantation [74,78]. Conversely, others have demonstrated successful withdrawal of steroids without impact on recurrence, but these studies lacked protocol liver biopsies and silent disease may have been missed [82,83].

The mainstay of treatment for AIH recurrence remains intensification of immunosuppression; however, randomized controlled trials are lacking. Most patients respond to reintroduction or increasing dosages of corticosteroids [73,75,78]. Optimization of calcineurin inhibitor levels and the addition of azathioprine or mycophenolate may provide added benefit. In the setting of a immunosuppressive-unresponsive course, substituting tacrolimus for cyclosporine or replacing sirolimus for calcineurin inhibitors may be efficacious [84,85]. Given the risk of allograft loss, discontinuation of corticosteroids after successful treatment of AIH recurrence is not recommended. For the most part, recurrent AIH can be effectively treated with immunosuppression and has limited impact on graft or patient survival. Some patients will progress to cirrhosis, allograft failure, and need retransplantation, although the true frequency of graft loss resulting from recurrence remains to be determined [86].

Primary biliary cirrhosis

Primary biliary cirrhosis (PBC) is a cholestatic autoimmune liver disease characterized by highly specific antimitochondiral antibodies (AMAs) and lymphocytic cholangitis of small- and medium-sized bile ducts that may eventually lead to biliary cirrhosis. Ursodeoxcholic acid (UDCA) has been shown to prolong survival, free of liver transplantation; however, approximately one-third of patients do not respond to therapy, and a proportion of these patients will require liver transplantation. In the 1980s, PBC was the leading indication for liver transplant, but the number of patients requiring transplant for PBC has declined by 20% in the last decade, possibly in part because of increased use of UDCA [87]. Outcomes following transplant for PBC are excellent, with 5-year patient survival rates of up to 85% [81].

Recurrent PBC (rPBC) was first described in 1982 and subsequent studies have reported prevalence rates ranging widely from 9% to 35% [88–96]. The average time to recurrence ranges between 3 and 5.5 years [88–90,95,96]. The rate of recurrence appears to increases over time with a cumulative incidence rate of 21–37% at 10 years and as high as 43% at 15 years [88,89,95].

Unlike PBC of the native liver, laboratory features of rPBC are not reliable diagnostic markers (Table 78.2). The majority of patients will have normal or clinically insignificant elevations of liver enzymes at the time of diagnosis [89,90,95,96]. In addition, AMA levels follow an unpredictable course post-transplant, as levels may decline, remain elevated, or re-emerge after becoming undetectable [97,98]. There does not appear to be a correlation between the presence of serum AMA and the development of rPBC. The diagnosis of rPBC relies heavily on liver biopsy and histologic analysis. The diagnostic hallmark of rPBC is the florid duct lesions of granulomatous cholangitis which are present in approximately 60% of initial diagnostic biopsies [89]. Other less specific features include lymphoplasmacytic portal infiltrate or lymphocytic cholangitis, and it has been suggested that these features may be predictive of eventual disease recurrence [91,93,94,97,99]. Although often challenging, differentiation between histologic changes of rPBC from other causes of bile duct damage, including acute and chronic rejection, ischemic cholangiopathy, or drug hepatotoxicity, is important.

Risk factors for rPBC remain poorly understood, and while several studies have sought to identify risk factors, findings have been conflicting. Some of the common risk factors identified are increased donor and recipient age, male recipient gender, increased warm and cold ischemia times, and HLA mismatching [76,88,89,95,96]. Multiple studies have demonstrated an increased risk of disease recurrence with the use of tacrolimus compared with cyclosporine-based regimens. Tacrolimus also has been associated with a significant reduction in time to recurrence, up to 50% in one study [88–90,99]. On the other hand, a systematic review including 16 original studies with a total of 1241 patients who received transplantations for PBC found an overall rPCB rate of 16% after a median time of 46.5 months. While there was a trend toward increased recurrence with tacrolimus compared with cyclosporine, statistical significance was not reached. Furthermore, thus far cyclosporine-based immunosuppression has not been shown to influence long-term survival following liver transplantation for PBC compared with tacrolimus [89,96]. No studies have identified a clear correlation between use of azathioprine, MMF, or corticosteroids and rPBC.

Although no established treatment guidelines exist, UDCA is widely used to treat rPBC given its proven pretransplant efficacy, minimal adverse effects, and the opportunity to initiate therapy in the early stages of disease. The assessment of UDCA efficacy is challenging, as many patients with rPBC have normal or near normal liver enzymes; however, Guy et al. [100] found an improvement in alkaline phosphatase levels among 75% of patients with rPBC treated with UDCA. Similarly, Charatcharoenwitthaya et al. [89] demonstrated 52% of patients with rPBC normalized liver enzymes with UDCA treatment compared with only 22% of those untreated. These sample sizes were small and there are no conclusive data that UDCA influences patient or graft survival.

Fortunately, rPBC appears to have little impact on patient or graft survival; however, rPBC can lead to graft failure in rare circumstances and require retransplantation. In a study of PBC patients transplanted in Birmingham, UK, 5.4% of all graft losses were attributable to recurrent disease. The median time from diagnosis of recurrence to graft loss was 7.8 years (range 22–181 months) [86].

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by inflammation and fibrosis of both intrahepatic and extrahepatic bile ducts leading to the formation of duct obliteration and biliary cirrhosis. PSC is a disease without any proven medical, endoscopic, or surgical therapies to impact disease progression. OLT remains the only curative therapy for patients with end-stage liver disease due to PSC, and the disease currently accounts for approximately 4–5% of adult liver transplantations

performed in Europe and the United States [101]. Similar to PBC, post-transplant outcomes for PSC are excellent, with 5-year patient survival rates of approximately 80% [102–104].

Recurrence of PSC after OLT was first described by Lerut in 1988 [105] and is now a well-established entity, with recurrence rates of 20–25% within 5–10 years following transplantation [102,106,107]. The diagnosis of rPSC can be challenging, and while there are no gold standard criteria, the most widely accepted definition of rPSC was proposed by the Mayo Clinic in 1999:

1 Confirmed diagnosis of PSC before OLT;
2 Cholangiography showing evidence of intrahepatic and/or extrahepatic biliary stricturing, beading and irregularities as least >90 days following transplant; and/or
3 Histologic finding of fibrous cholangitis and/or fibro-obliterative lesions with or without ductopenia, biliary fibrosis, or cirrhosis;
4 Exclusion of alternative diagnoses (hepatic artery thrombosis or stenosis, ductopenic rejection, ABO blood type incompatibility between donor and recipient, solitary anastomotic stricture, ischemic strictures, bacterial or fungal cholangitis with strictures, and CMV or cryptosporidium infection) (Table 78.2) [102].

Several risk factors for rPSC have been reported including, recipient age, male gender, donor–recipient gender mismatch, HLA DRB1*08, active ulcerative colitis prior to liver transplantation, especially requiring steroids, episodes of acute cellular rejection, steroid-resistant acute cellular rejection, steroid-resistant acute cellular rejection requiring OKT3, presence of cholangiocarcinoma, maintenance corticosteroids, and use of extended donor criteria grafts [107–115].

The impact of rPSC on graft survival remains controversial. Campsen et al. [106] found the median survival time before retransplantation for rPSC was 39.1 months. In an analysis of 3309 patients from the UNOS database transplanted for PSC, Maheshwari et al. [116] found a higher retransplantation rate and lower survival when compared with patients transplanted for PBC, which became apparent 7 years after OLT. The being said, most published studies have failed to demonstrate that rPSC has an impact on patient survival [106,107,111–115]. As for the native liver, there is no established medical therapy for rPSC. Although many centers use UDCA, there are no established data that UDCA is beneficial in the treatment of rPSC. The focus of treatment in rPSC remains symptomatic relief of biliary strictures, and patients with graft failure caused by recurrent disease should be considered for retransplantation.

Alcoholic liver disease

Alcoholic liver disease (ALD) is the second most common indication for liver transplantation in the Western world, accounting for approximately 20% of primary transplants performed [117]. Liver transplantation for ALD remains a controversial topic which stems from public perceptions that ALD is a self-inflicted disease with a risk of post-transplant recidivism or non-compliance, all in the setting of an increasing demand for donor organs. In fact, approximately 95% of patients with alcoholic end-stage liver disease are never formally evaluated for liver transplantation [118]. Although initial reports indicated poor post-transplant patient survival (20% at 3 years), more recent data suggest similar if not better survival than for those who undergo transplantation for other indications, with 1 and 5 year survival rates of 86% and 74%, respectively [119,120].

Unfortunately, alcoholism is a disease of relapses and remissions, and recurrence of disease post-transplant due to recidivism is frequent. There is significant variation in the reported rates of alcoholic relapse following OLT, ranging from 10% to 90% [121]. This high variability is the result of differing definitions of alcohol relapse, and the difficulty of obtaining accurate data regarding alcohol use from patients. Much of the data deriving recidivism rates are based on a definition of relapse as any alcohol use, instead of more appropriately distinguishing between occasional lapses or slips from harmful or addictive drinking [122]. It is estimated that 20% of patients transplanted for ALD resume harmful drinking, whereas most patients remain abstinent or consume only small amounts of alcohol occasionally [123,124]. This is an important distinguishing factor, as heavy drinking has been associated with a decrease in long-term patient survival [125]. Although early studies did not suggest an impact of recidivism on patient or graft survival, these studies were small in size with short periods of follow-up [126,127]. With longer follow up, there is increasing evidence of poorer outcomes in patients who return to drinking due to recurrence of ALD [125,128,129]. Pfitzmann et al. [130] demonstrated a significantly reduced patient survival in subjects who resumed abusive drinking compared with those who remained abstinent or had occasional "slips." Recurrent ALD was responsible for the majority of deaths (87.5%) among patients who resumed abusive drinking. Whereas patients transplanted for ALD who remain abstinent most commonly die from cardiovascular disease or malignancy, recurrent ALD accounts for the majority of deaths among recipients who resume abusive drinking after transplantation [128,129].

Several factors have been associated with alcohol relapse after transplantation (Table 78.3). One such factor that remains heavily debated is the time period of abstinence prior to transplant. Longer periods of abstinence are associated with lower rates of post-transplant recidivism, but no one optimal time period has been established [131,132]. In the United States, 85% of transplant centers require at least 6 months of abstinence from alcohol use before being considered for transplant listing, the "6-month rule" [133]. Nevertheless, studies have indicated that 6 months of abstinence alone is an inadequate predictor of relapse post-transplant, and it may take up to 5 years to achieve true predictive sobriety [131,132,134]. Drinking patterns pretransplant have been shown to have a role in risk of relapse, with a history of alcohol abuse carrying a lower risk than alcohol dependence [131]. Inadequate social support has been consistently linked to alcohol recidivism, the most common being lack of a spouse or poor marital relationship

Table 78.3. Risk factors for alcohol relapse after liver transplantation

Lower Risk	Higher Risk
• Diagnosis of alcohol abuse • Good social support • History of good adherence to treatments, medications and appointments • Lower risk for psychosocial risk factors – Mood disorders under treatment – Substance abuse amenable to rehabilitation	• Diagnosis of alcohol dependence • Poor social support • Pattern of non-adherence • Higher risk psychosocial risk factors – Psychotic disorders – Personality disorders

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[130,131,135]. Other risk factors for alcohol relapse after transplantation include younger age, non-adherence with alcoholism treatment, medication non-compliance, family history of alcoholism, polysubstance abuse, and the presence of psychiatric co-morbidities (personality disorder, psychotic disorder, depressive disorder) [136–138]. Unfortunately, studies have led to mixed conclusions regarding risk of relapse, and no one predictor thus far can reliably identify recidivism.

Given the poor long-term outcomes associated with a relapse to heavy drinking post-OLT, it is imperative for transplant centers to take necessary steps to prevent recidivism. This begins at the transplant evaluation stage, with careful psychosocial assessment for risk of relapse by social workers and trained mental health specialists. Those patients at risk for recidivism should be referred for appropriate substance abuse or psychiatric counseling and treatment before proceeding with transplantation. Following liver transplantation, detecting recidivism can be quite challenging. Several strategies have been suggested to monitor and identify alcohol use post-OLT, including biochemical markers, psychiatric interviews, questionnaires, and screening tools [122,139,140]. Many transplant centers have mental health specialists who follow patients post-transplant to provide ongoing relapse prevention and support. No single method for identifying recidivism is truly specific; therefore a combined use of methods is suggested to increase the detection of relapse. When relapse of alcohol use is identified, prompt implementation of a treatment plan should be sought; however, there is a paucity of data in the literature regarding the treatment of recidivism post-OLT. Weinrieb et al. [141] demonstrated the difficulty of engaging patients in treatment for recidivism with a failure to recruit participants into a trial of naltrexone for patients with alcoholism post-OLT. Not only did patients feel they did not require treatment, but they were also unwilling to take potentially hepatotoxic medications. Bjornsson et al. [142] were able to implement a successful transplant alcohol treatment program, including assessment by a skilled psychiatrist, encouragement to participate in motivational enhancement, and the use of an abstinent contract. This resulted in a decreased alcohol relapse rate of any drinking over a 4-year period (22% versus historic control of 48%). Although alcohol relapse post-OLT poses significant hurdles, transplant centers must try to implement programs to prevent, identify, and treat recidivism in order to limit recurrent ALD.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the developing world, affecting approximately one-third of the general population [143]. NAFLD encompasses a spectrum ranging from benign bland hepatic steatosis to steatosis accompanied by varying degrees of necroinflammation and fibrosis, or non-alcoholic steatohepatitis (NASH). An estimated 3% of individuals from developed countries have NASH, and up to 20% of these individuals may progress to cirrhosis [144,145]. Furthermore, up to one-third of patients with NASH will die from complications of liver disease or require OLT. NAFLD is associated with obesity, dyslipidemia, and diabetes mellitus and is considered to be the hepatic manifestation of the metabolic syndrome [146]. NAFLD accounts for 5–10% of OLT per year, and the disease is ultimately expected to surpass HCV infection as the leading indication for OLT in the future [147,148]. In general, post-transplant outcomes are good and similar to that of other indications, with 1- and 5-year patient survival rates of 86% and 71%, respectively [149].

The first reported case of recurrent fatty liver disease following OLT was in 1992, and multiple subsequent studies have confirmed that recurrence of NAFLD is common [150]. Incidence of post-transplant NAFLD recurrence has ranged from 25% to 60%, although most studies have been small with limited follow-up [151–155]. In a prospective histologic study, Maor-Kendler et al. [155] reported 60% of recipients with NASH had steatosis grade 2 or higher post-OLT, with half of these patients meeting histologic criteria for NASH. Another study similarly demonstrated 70% of patients developed recurrent steatosis at a mean follow-up of 18 months, with 25% having NASH and almost 20% having stage II or greater fibrosis [156]. Comparatively, a recent study found lower rates of recurrence with the probability of developing recurrent steatosis at 1-, 5-, and 10-years post-OLT to be 8%, 25%, and 33%, respectively [149]. It has previously been suggested that approximately 10% of patients undergoing OLT for NASH have recurrence that leads to cirrhosis and approximately half of these patients will develop graft failure, although more recent studies have not demonstrated graft loss due to NASH recurrence [157,158].

Several risk factors have been associated with NAFLD recurrence following OLT. Not surprisingly, most of these risk factors are closely linked to the metabolic syndrome and include obesity, diabetes, dyslipidemia, and hypertension pre- or post-OLT [156, 159,160]. It is possible that post-OLT immunosuppression may also impact disease recurrence, because corticosteroids, calcinuerin inhibitors, and sirolimus can be associated with an increase in obesity, hypertension, and insulin resistance; however, no one particular immunosuppression regimen has been implicated in NAFLD recurrence. There is increasing evidence that steroid avoidance or minimization of calcineurin inhibitors are safe and reduce the frequency of metabolic complications post-OLT [161,162]. It is yet to be determined whether these strategies may lead to a decreased incidence of NAFLD recurrence.

The diagnosis of recurrent NAFLD is similar to that of the native liver. Liver biochemistries remain non-specific and up to one-third of patients with recurrent NASH may have normal liver enzymes [156]. While radiologic imaging may detect the presence of steatosis, it cannot differentiate from other causes of fatty liver or determine severity. Thus, liver biopsy may be useful to confirm the diagnosis, determine extent of disease, and exclude other causes. Unfortunately, similar to NAFLD of the native liver, there is no pharmacotherapy proven to be efficacious and safe for the treatment of NAFLD recurrence. The mainstay of treatment focuses on modification of those conditions associated with the metabolic syndrome. This includes weight loss through lifestyle modifications, as well as strict medical management of diabetes and dyslipidemia. It is anticipated that current promising treatments for NAFLD in the native liver will translate into treatments for recurrent NAFLD, but thus far data are lacking on the prevention and treatment of recurrent disease.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the sixth most common solid organ malignancy, and the third leading cause of cancer-related mortality worldwide [163]. For the most part, HCC almost exclusively occurs in the setting of underlying cirrhosis and is most commonly associated with HCV infection in the United States and HBV infection worldwide. It is projected that the incidence of HCC

will continue to rise in the United States over the ensuing decades as a consequence of the high prevalence of HCV and NASH [164]. In recent years, significant advances have been made in the treatment of HCC, including development of locoregional ablation therapy and use of chemotherapeutic agents like sorafenib; however, surgical treatment remains the mainstay of curative therapy for HCC. Eighty percent of patients presenting with HCC are unresectable due to extent of tumor burden or underlying poor functional hepatic reserve in the presence of cirrhosis [165]. Therefore, liver transplantation is the optimal surgical treatment for most patients, providing complete tumor excision as well as removal of the diseased liver.

Initial transplantation for HCC was performed in the setting of large, unresectable, and advanced-staged HCC, resulting in very disappointing outcomes with poor patient survival rates due to high HCC recurrence rates [166,167]. The landmark study by Mazzafero et al. [168] in 1996 demonstrated much improved survival for patients transplanted with early HCC and defined specific patient selection criteria to optimize transplant outcomes. These criteria, now known as the Milan criteria, include the presence of a single lesion $\leq$5 cm or up to three lesions all $\leq$3 cm, no macrovascular invasion, and no extrahepatic spread. Adhering to these criteria resulted in similar outcomes to other benign indications for OLT, with a 5-year patient survival rate of 70% and a HCC recurrence rate of 8%. The positive impact of the Milan criteria was confirmed in an analysis of the UNOS database that included 985 patients transplanted for HCC between 1987 and 2001. In this study, Yoo et al. [167] demonstrated a dramatic improvement in post-OLT patient survival following the adoption of the Milan criteria (5-year survival: pre-Milan criteria 25% versus post-Milan 61%; $P = 0.001$). More recently, some transplant centers have challenged the Milan criteria as being too restrictive and advocated for expansion of the criteria to enable more patients to undergo OLT, without incurring an increased risk of HCC recurrence or decreased patient survival. The most established of these expanded criteria comes from the University of San Francisco (UCFS criteria), which allow transplant for a solitary HCC up to 6.5 cm in diameter or up to three nodules, none larger that 4.5 cm with a cumulative diameter up to 8 cm [169]. The reported 5-year patient survival rate using this model was 75%, with an HCC recurrence rate of 11%. Still, the use of expanded criteria has not been universally accepted and remains a debated topic that warrants more prospective investigation.

The reported incidence of recurrent HCC post-OLT has been variable, ranging from 6% to 56%, most likely the result of differences in patient selection criteria for transplantation [170–173]. HCC recurrence after OLT most commonly involves the liver graft, although initial presentation with extrahepatic recurrence has also been reported in 10–43% of cases [174–181]. The extrahepatic sites of HCC recurrence most commonly involve the lungs and bone, but also can be found in abdominal lymph nodes, adrenal glands, and peritoneum. Mechanisms that may attribute to HCC recurrence include the presence of microscopic malignant foci in lymph nodes or distant organs at the time of transplant, as well as hematogenous or peritoneal tumor seeding during transplantation. While the risk of recurrence appears to be highest within the first 2 years post-OLT, late recurrence beyond 5 years has also been reported [174–181].

Although the incidence of HCC recurrence may vary between transplant centers, several recipient and tumor-specific prognostic factors for tumor recurrence have been identified. The most powerful predictor of HCC recurrence is the presence of macro- or microvascular invasion which portend a poor prognosis [182,183]. Tumor size greater than 5 cm, total number of lesions, and bilobar disease are also associated with increased risk of recurrence and are likely surrogates of microvascular invasion [184–186]. Similarly, poor tumor differentiation is a predictor of microvascular invasion, and an independent risk factor for tumor recurrence; however, assessment of tumor grade requires percutananous liver biopsy, which is not without the risk of biopsy tract tumor seeding which can occur in up to 3% of cases [187–190]. The value of determining tumor histology warrants further investigation, as it has been suggested that poor differentiation is a better predictor of recurrence than being within or outside Milan criteria [191]. There have been a number of studies that have attempted to identify surrogate molecular markers for microvascular invasion and recurrence without the risk of tumor seeding. The most widely used marker is serum alfa-fetoprotein (AFP), which has been associated with HCC recurrence; however, its use is limited as 30–40% of HCC patients have normal AFP levels [192]. Numerous other molecular biomarkers targeting cell proliferation, cell adhesion and extracellular matrix, angiogenesis, cell surface markers, and transcription factors are being investigated.

Locoregional ablation therapy has been proposed in order to slow progression of HCC while awaiting OLT to avoid drop-out (i.e. removal from the transplant waiting list due to progression beyond transplant criteria). For this purpose, intention to treat analyses suggest that such bridge therapy is effective for patients with anticipated transplant waiting times longer than 6 months, but there are differing results on whether locoregional therapy results in decreased recurrence and improved survival after OLT [193–197]. Locoregional therapy may also be used for down-staging of tumors initially found outside Milan criteria, thereby allowing OLT. There is evidence that down-sizing tumor burden to within Milan criteria can be done successfully without incurring an increased risk of HCC recurrence. Furthermore, tumor response to down-staging may be a useful predictor of post-OLT outcomes and assist with patient selection for transplantation [193,194,198]. Historically, adjuvant chemotherapy has not been proven to be beneficial for the treatment of HCC pre- or post-OLT; however, the multikinase inhibitor, sorafenib, has recently demonstrated a significant improvement in overall survival for patients with advanced HCC [199]. Whether an agent such as sorafenib has the ability to reduce the risk of HCC recurrence post-OLT and in what setting needs to be addressed with further investigation.

Immunosuppression is commonly associated with an increased risk of malignancy post-OLT, and it may be an influential factor in HCC recurrence and tumor progression. Vivarelli et al. [200] observed a direct relationship between higher cumulative doses of cyclosporine during the first year post-OLT and a lower rate of 5-year HCC recurrence-free survival. This observation was confirmed in a retrospective analysis of 70 patients transplanted for HCC, which found post-OLT HCC recurrence was statistically associated with higher cyclosporine levels [201]. A subsequent publication by the same group identified higher doses of both cyclosporine and tacrolimus as independent risk factors for HCC recurrence after OLT [202]. Similarly, steroids may also have an adverse role in the recurrence of HCC, and it has been suggested that early withdrawal of steroids post-OLT may decrease the risk of disease recurrence [209]. M-TOR (mammalian target of rapamycin) inhibitors have drawn considerable interest in the setting of HCC. In addition to potent immunosuppression activity, m-TOR inhibitors have demonstrated a potential anticancer effect related to

impairment of vascular endothelial growth factor (VEGF) production. Sirolimus was shown to induce cell cycle arrest and blocked proliferation of an HCC cell line [203]. In a matched case-controlled study, the 3-year recurrence-free survival was 30% higher for patients maintained on sirolimus post-OLT compared with those on standard treatment tacrolimus [204]. Further promising clinical data were presented by Toso et al. [205] who analyzed the Scientific Registry of Transplant Recipients in the United States to determine whether the type of immunosuppression used influenced survival after OLT in HCC patients. The authors concluded that sirolimus-based maintenance therapy was associated with improved survival. Despite these findings, there is no definitive link between one specific immunosuppression regimen and recurrent HCC following OLT; however, available data would suggest minimizing calcineurin inhibitor dosage or switching to an m-TOR inhibitor is beneficial for patients at high risk of HCC recurrence.

There are very few published data to help guide the most efficacious and cost-effective strategy to monitor for HCC recurrence after OLT, and surveillance protocols vary among transplant centers [206]. Once identified, the reported 5-year survival rate for recurrent metastatic HCC following OLT is 13–35% [175–181]. Unfortunately, limited treatment options for HCC recurrence are currently available, as many patients present with established disseminated disease. Although the data are limited, aggressive surgical intervention may be considered in a select group of patients with localized recurrence. Regallia et al. [175] first reported on a small group of post-transplant patients who underwent surgical resection for isolated recurrence in the liver, lung, bone, and skin. These patients had a 4-year survival rate of 57%, compared with 14% for patients with unresectable disease who did not undergo surgical intervention. A more recent study from Mount Sinai Medical Center reported a similar advantage, with patients who underwent radical surgical resection for localized HCC recurrence having an improved survival [181]. By multivariate analysis, surgical treatment was independently associated with prolonged survival, in addition to the absence of bony metastasis as well as time to recurrence of >1 year post-OLT. Based on experience in the pretransplant HCC population, other potential treatments for HCC recurrence following liver transplantation include use of locoregional ablation therapy or chemotherapy with agents such as sorafenib; however, sufficient data are lacking in the post-OLT setting to draw any firm conclusions. Finally, radiation therapy can be utilized as a treatment option for symptomatic palliation of extrahepatic disease, primarily bone metastasis [207,208]. Reducing the incidence of HCC recurrence and its associated mortality after liver transplantation requires careful consideration of pretransplant factors and post-transplantation immunosuppression, as well as the use of a multidisciplinary approach to patient care that includes hepatology, transplant surgery, radiology, and oncology.

Conclusions

Liver transplantation remains the mainstay for the treatment of most end-stage liver diseases. Unlike other transplanted organs, where rejection is the fundamental problem influencing survival, the liver is much more influenced by recurrent viral and autoimmune diseases. Clinicians must therefore remain constantly aware of the risk of disease recurrence, and familiarize themselves with the diagnostic maneuvers and treatment options available to accurately diagnose and manage these myriad diseases. Immune management should go hand in hand with use of viral specific therapies to achieve optimal results and minimize the need for retransplantation.

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CHAPTER 79

Recurrent Disease after Heart Transplantation

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Introduction

The risk of recurrent disease following cardiac transplantation generally relates to the etiology of the underlying condition for which transplantation was carried out. Fortunately, for the vast majority of heart transplant recipients this risk is limited. Dilated cardiomyopathy and coronary artery disease are the two most common indications for heart transplantation. The majority of cases of dilated cardiomyopathy are idiopathic, post-viral, or familial, and these conditions do not affect the post-transplant course. In a small portion of cardiomyopathy cases the etiology is infiltrative and, for some of these (particularly myocarditis, sarcoid, or Chagas disease), there is a finite risk of recurrence. The most common cause of allograft dysfunction in the long term and a principal cause for limiting graft survival after cardiac transplantation is the development of what may be considered a form of recurrent disease, cardiac allograft vasculopathy (CAV), a process with many similarities to native non-transplant atherosclerosis. Approximately half of all heart transplants are performed for end-stage disease due to coronary artery disease. However, the immune system has a critical role in the development of CAV and the process thus is a manifestation of chronic allograft rejection.

In cardiac transplantation, mortality in the first year is predominantly dictated by increased risk of rejection and infection associated with the concomitant need for augmented immunosuppression (see Chapter 105). However, after the first year, a principal determinant limiting long-term allograft survival is the development of CAV. This phenomenon was first noted in experimental animal models of cardiac transplantation, which demonstrated a time-dependent development of coronary intimal thickening with histologic features similar to atherosclerosis. CAV was noted clinically in early transplants when histologic evaluation of coronary arteries of transplanted hearts revealed extensive disease in vessels that had appeared normal angiographically at the time of transplantation. Despite the introduction of cyclosporine in the 1980s, which had a significant impact in reducing allograft rejection rates and improving survival, there appeared to be no corresponding decrease in the prevalence of CAV [1].

Registry data suggest a constant annual mortality rate of 4% per year after the first year (Figure 79.1). In the 3 years after transplant, acute rejection is responsible for up to 9% of deaths [2]. After 3 years, the incidence of death due to acute rejection declines significantly. Up to 20% of deaths after the first year are attributed to non-specific allograft failure for which a precise cause has not been identified. These cases likely represent undiagnosed allograft rejection or sequelae of recurrent or low-grade rejection such as small vessel CAV or restrictive physiology, and in many cases the processes leading to graft failure remain poorly understood. After the first year following transplantation, allograft failure may result from antibody-mediated rejection and CAV. Approximately 10% of deaths between 1 and 3 years after transplant are attributable to CAV, with the prevalence increasing thereafter to 20% at 3 years, 30% at 5 years, and 45% at 8 years after transplant. The impact of newer immunosuppressive regimens has been limited on CAV, with only a small decrease of approximately 2–4 percentage points in the cumulative incidence of CAV in patients who underwent transplant between 2001 and June 2009 compared with those between April 1994 and 2000 (Figure 79.1).

Pathology

Non-transplant atherosclerosis is characterized by a chronic inflammatory process affecting the vessel wall with accumulation of macrophages, T cells, and smooth muscle cells. Ross et al. [3] postulated that the process represented a response to injury of the endothelium. The process is believed to be reflected in CAV, although the initial injury to the endothelium is believed to be as a result of a number of factors attributable to transplantation including ischemia–reperfusion injury, the immune response, donor factors, and traditional risk factors for atherosclerosis.

The pathology of CAV differs somewhat from non-transplant atherosclerosis in many other ways (see Chapter 83) (Table 79.1). In CAV, the disease tends to be more diffuse, with concentric narrowing of vessels including frequent involvement of not only the large- and medium-sized vessels, but also the microvasculature [4]. The concentric and diffuse nature of CAV generally limits its detection by angiography [4], and is better appreciated by intravascular ultrasound (IVUS). However, many patients may have the more typical focal lesions seen in non-transplant atherosclerosis, although the lipid component may be highly variable and calcification appears late if at all [5]. The correlation between coronary calcification and CAV has been debated. Early studies have suggested an association between coronary calcium score assessed by electron-beam computed tomography and severity of disease noted by coronary angiography or IVUS [6,7]. More recent data suggest coronary calcification may not correlate with the severity of CAV. In a recent study [8], 4 of 11 patients with severe CAV by coronary

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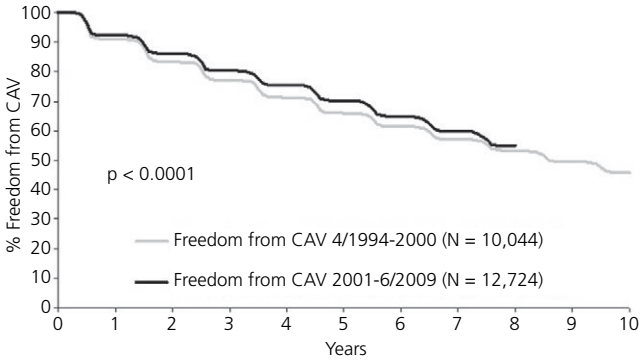

Figure 79.1. Freedom from cardiac allograft vasculopathy for adult heart recipients (Transplants: April 1994–June 2009). Reproduced from [2] Stehlik et al., *Journal of Heart and Lung Transplantation* 2011;30:1078–1094 with permission from Elsevier. Copyright © 2011, Elsevier.

Table 79.1. Typical features of non-transplant atherosclerosis and cardiac allograft vasculopathy

Non-transplant atherosclerosis	Cardiac allograft vasculopathy
Mostly epicardial vessels affected	Panvascular disease including microvasculature
Slow progression	Rapid progression
Lesions eccentric	Lesions concentric
Generally lipid-rich	Generally lipid-poor
Calcification common	Calcification sparse
Compensatory dilatation (Glagov phenomenon)	Arteries constrict

angiography had a calcium score of 0. The diagnostic accuracy of the test was less than 60%. The authors postulate that coronary calcification seen in patients with CAV may represent pre-existing disease or de novo traditional coronary atherosclerosis.

The concentric intimal thickening is comprised predominantly of smooth muscle cells and extracellular matrix, and the internal and external elastic laminae are generally intact. The disease thus not only affects the intima, but the media and adventitia frequently also undergo fibrous infiltration. Consequently, compensatory remodeling of the artery is attenuated (the Glagov phenomenon seen in non-transplant atherosclerosis) and the artery may even undergo constriction [9]. Generally, the contribution to luminal loss tends to occur from intimal hyperplasia in the first year and then by constrictive remodeling thereafter [10,11].

Immune mechanisms contributing to CAV

Adaptive immunity

As a highly vascular organ with significant endothelial exposure to recipient blood, the transplanted heart represents a major antigenic stimulus, and the principal determinant of the recipient immune response is the recognition of foreign major histocompatibility antigens. Antigenic recognition (covered in depth in Chapter 5) by circulating host T lymphocytes and dendritic cells results in a robust immune response that leads to production of a variety of cytokines including interleukins, γ-interferon (INF-γ), and tumor necrosis factor α (TNF-α). These cytokines subsequently allow development of effector mechanisms including cytotoxic T cells, infiltrating macrophages, and antibody production (covered in depth in Chapters 5–7). Although this immune response is mainly responsible for allograft dysfunction seen in acute rejection, it likely has an equally important role in the development of CAV, a process that is a recognized component of most syndromes of chronic rejection seen in some form not only in the transplanted heart, but also in renal, lung, and liver allografts. Indeed, a substantial amount of the fibrotic degeneration seen in other allografts likely relates to some extent to ischemic atrophy downstream of vessels with progressive vasculopathy. In transplanted lungs chronic rejection presents as obliterative bronchiolitis and in liver allografts the equivalent process is known as vanishing bile duct syndrome.

Direct allorecognition, with recognition of foreign human leukocyte antigen (HLA) antigens on donor cells by recipient dendritic cells, is predominantly responsible for acute rejection. In contrast, indirect allorecognition, when donor antigens are internalized, processed, and presented as peptides by host dendritic cells, likely contributes to chronic rejection and the development of CAV [12,13]. Severe CAV is positively correlated with degree of HLA mismatch and production of donor-specific antibodies (see Chapters 6 and 36) [14]. Both symptomatic and asymptomatic antibody-mediated rejection predispose to the development of CAV.

Conventional immunosuppressive therapy is mainly targeted at T cells to prevent acute cellular rejection, and the use of calcineurin inhibitors (particularly tacrolimus) and mycophenolate mofetil (MMF) has been associated with a significant decline in rejection rates in the recent era [15]. However, these agents have had a modest effect on the development of CAV in heart transplant recipients [2]. While the agents are effective at suppressing the production of many Th-1 cytokines and limiting T-cell activation in general, the processes that lead to antibody production appear to be less affected [16]. Following solid organ transplantation, a significant portion of patients continue to make antibodies to the allograft. Anti-HLA antibody production in recipients has been associated with a higher mortality [17] and CAV [18,19]. Thus, alloimmune insults distinguish CAV from general progressive atherosclerotic vascular disease, and likely contribute to the accelerated course of CAV.

Production of non-HLA antibodies likely also contributes to the development of CAV (see Chapter 36). These antibodies do not appear to contribute to the development of native cardiomyopathies. The presence of circulating anti-endothelial antibodies has been shown to correlate with CAV [20]. The development of antibodies to the intermediate filament vimentin, a protein characteristic but not restricted to endothelial cells, has been particularly associated as an independent risk factor for the development of CAV [21]. Vimentin is diffusely expressed in the intima and media of normal and diseased coronary arteries. Therefore, injury early following transplantation, for example by ischemia and reperfusion, may lead to release of vimentin into the circulation. The protein may then be taken up by antigen-presenting cells (APCs) and presented as an autoantigen, as it is not normally exposed to the immune system. Interestingly, these antibodies do not seem to mediate complement-mediated cytotoxity to endothelial cells in vitro, a process associated with hyperacute rejection. Anti-vimentin antibodies may therefore exert a more subtle form of low-grade damage in a process consistent with the chronic progressive course of CAV development. Also, transplant patients on tacrolimus compared with cyclosporine, and MMF compared with azathioprine appear to develop fewer anti-vimentin antibodies [22,23].

In addition to vimentin, other antibodies against various antigens of the endothelial cells, such as angiotensin II type 1 receptor, and anti-major histocompatibility complex class I chain-related A (MICA) or B, have been found in solid organ transplant recipients

and associated with poor graft outcome [24,25]. MICA antigens are HLA-related polymorphic glycoproteins expressed on the surface of human endothelial cells, epithelial cells, and fibroblasts. The expression of MICA is induced by stress. These proteins act as ligands for the activating receptor NKG2D on natural killer (NK) cells and $CD8^+$ T cells. The observation that MICA is expressed on endothelial cells but not on lymphocytes may explain some cases of graft loss in patients with a negative cross-match to donor lymphocytes.

The presence of anti-MICA antibodies and MICA expression in endomyocardial biopsies of the allograft has been correlated with acute heart allograft rejection [26]. Antibody production against MICA antigens can induce complement-dependent cytotoxicity [27]. MICA antibody production in heart-transplant recipients has been associated with both acute rejection and the development of CAV [28].

Innate immunity

Although conventional immunosuppressive agents are able to efficiently target the adaptive immune system, these agents largely spare the innate immune system. An intact innate immune response may contribute both to stimulation of the adaptive response and to the future development of CAV (see Chapter 7). Perhaps the best-studied components of the innate immune system are the toll-like receptors (TLRs), a family of transmembrane proteins expressed on a variety of cells including epithelial cells, dendritic cells, macrophages, and T and B lymphocytes. TLRs, some of the most conserved components of the immune system throughout evolution, bind to conserved ligands on microbial pathogens such as lipopolysaccharide on Gram-negative bacteria. However, TLRs are also thought to be activated both by exogenous and by endogenous ligands [29]. Ischemia–reperfusion injury following donor heart engraftment leads to release of a number of endogenous ligands, including products of direct tissue injury such as the extracellular matrix polysaccharide hyaluronan. Hyaluronan has been demonstrated to activate dendritic cells predominantly by binding to TLR [30]. Other components of ischemia–reperfusion injury that may lead to activation of the innate immune system include heat shock protein 70 [31] and allograft inflammatory factor-1 (AIF-1). Activation of TLRs initiates an inflammatory cascade, which likely contributes to both acute allograft rejection and the development of CAV. In heart transplant recipients, expression profiles of AIF-1, TLR-2, and interleukin-18 were correlated with biopsy-proven allograft rejection in both peripheral blood and biopsy tissue [32]. Transplant recipients with endothelial dysfunction – as measured by compromised coronary flow reserve – demonstrate increased mRNA transcript levels for TLR-4, surface expression of TLR-4, and increased expression of downstream components of TLR signaling such as B7-1, interleukin-12, and TNF-α compared with controls [33].

Endothelial activation

Endothelial cells likely have a key role in mediating both acute and chronic rejection [34]. Endothelial injury and dysfunction appears to be the sentinel event leading to the development of CAV [35] as the endothelium forms the initial interface between donor and the host circulating lymphocytes and maintains a barrier to prevent entry of inflammatory cells into the interstitium. Endothelial cells are also highly responsive to cytokines [36], and express Class II antigens particularly in the microvasculature [37,38] and coronary arteries. They also act as APCs [39,40]. In vitro, endothelial cells from human coronary arteries are able to stimulate allogeneic T cells [41].

Endothelial cell hyperpermeability is a proposed mechanism of increased lipid infiltration into the vessel walls of allografts [42], contributing to the development of CAV. Vascular endothelial growth factor (VEGF) is a potent inducer of vascular permeability and its expression is up-regulated in human heart allografts, particularly after acute rejection [43]. Its presence has therefore been associated with the development of CAV [44,45].

Allograft vascular endothelial cells express HLA Class II antigens and adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM) [46,47]. The expression of these molecules on endothelium reflects ongoing endothelial activation. These molecules are not typically expressed on endothelium of normal hearts.

Although expression of adhesion molecules on endothelium is also a feature of non-transplant atherosclerosis, the induced expression of HLA antigens appears to be unique to the transplant endothelium. A consequence of endothelial activation is that co-culture of these cells in vitro with $CD4^+$ T cells results in their proliferation and production of interleukin-2 [46]. Chronically activated donor endothelium may therefore provide a sustained stimulus to recipient lymphocytes to maintain a chronic immunologic response. Allografts expressing ICAM-1 and HLA-DR on vascular endothelium have been shown to develop transplant vasculopathy [48] but there is also evidence that a certain polymorphism of ICAM-1 detected in donor hearts may protect from the development of CAV [49] and this may relate to an allelic change that renders decreased ICAM-1 binding to B cells.

Endothelial activation may be caused by a number of factors, and likely distinguishes the phenotype of accelerated CAV from native atherosclerosis (Figure 79.2). Cytokines released from inflammatory cells following an episode of acute rejection may precipitate endothelial activation, and development of CAV has been correlated with the frequency of acute allograft rejection episodes [50]. Other triggers of endothelial activation include injury by endothelial-specific T lymphocytes or generation of recipient antibodies, such as anti-vimentin antibodies, to donor endothelium. Ischemia–reperfusion injury at the time of transplantation also causes endothelial activation. Human endothelial cells subjected to hypoxia experimentally do show up-regulation of ICAM-1 [51]. In animal models, the induction of adhesion molecules on allograft endothelium prior to transplantation has also been shown to be associated with the subsequent development of CAV [52]. In humans, the assessment of circulating soluble ICAM-1 following transplantation has been suggested to be a marker for the subsequent development of CAV [53]. IFN-γ has a central role as it activates macrophages, augments inflammatory cell recruitment, and amplifies immune responses [54]. Even in the setting of ongoing acute rejection, the absence of IFN-γ prevents development of CAV [55].

Integrins and chemokines

There is evidence that integrins and chemokines also contribute to transplant vasculopathy. Studies imply a correlation between sustained expression of the vitronectin receptor (integrin α-V,β-3) and progression of CAV by IVUS [56]. Myocardial ischemic injury at the time of transplant is associated with up-regulation of α-V,β-3, tissue factor, and activation of the matrix metalloproteinase system [57]. Interestingly, the mode of donor death may also determine integrin regulation. Intracranial bleeding as a cause of donor death

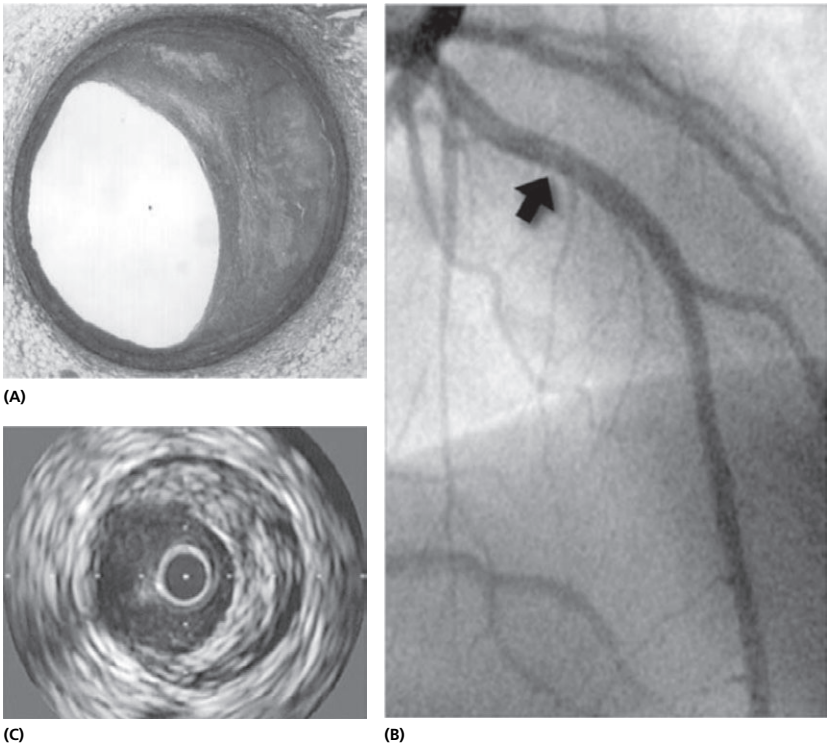

Figure 79.2. Concentric or eccentric subintimal proliferation in CAV seen histologically (A) are underestimated in lesion severity angiographically (B) but are better appreciated by IVUS (C). Reproduced with permission from [219]: Patel JK, Kobashigawa JA. Cardiac allograft vasculopathy. In: Ahsan N, ed. *Chronic Allograft Failure: Natural History, Pathogenesis, Diagnosis and Management*. Austin: Landes Bioscience, 2008:65 (Figure 3).

has been associated with the development of CAV [58] after heart transplant and this appears to correlate with systemic activation of α-V,β-3 [59]. Cytomegalovirus (CMV) infection, strongly associated with transplant vasculopathy, may involve an angiogenic response that results from endothelial proliferation and migration. This CMV-induced angiogenic response may occur as a result of viral binding to and signaling through the beta 1 and beta 3 integrins and the epidermal growth factor receptor, via their ability to activate the phosphatidylinositol 3-kinase and the mitogen-activated protein kinase signaling pathways [60].

Besides facilitating endothelial cell interactions, lymphocyte integrins can also act as costimulatory molecules during antigen presentation. In experimental models, blockade of ICAM-1 and leukocyte function associated antigen-1 (LFA-1) interactions induces allograft tolerance [61], and significantly abrogates CAV development [62].

Expression of the chemokine RANTES, a chemokine that selectively chemoattracts T lymphocytes, NK cells, monocytes, and eosinophils, has been shown to be expressed in human CAV [63]. In an animal model, sustained RANTES production was required for both monocyte recruitment and the development of intimal thickening and this required the presence of $CD4^+$ cells [64]. In humans, the presence of elevated peripheral blood levels of the CCR3 chemokine ITAC/CXCL11 is associated with severe CAV [65]. In this study, the CCR3 chemokine ligand ITAC/CXCL11 localized on the endothelial surface of CAV lesions, and underlying infiltration of inflammatory mononuclear cells expressed CCR3. In a mouse model, blockade of chemokine receptors CCR1 and CCR5 attenuated development of CAV [66]. In a rat model, there is evidence to suggest that the protective effect of statins may occur partly through modulation of chemokines and their receptors [67]. Both low and high doses of simvastatin significantly decreased the CAV score, inhibited recruitment of T lymphocytes and macrophages, reduced levels of intragraft monocyte chemoattractant protein 1 (MCP-1), RANTES, and interferon-inducible protein 10 (IP-10) and down-regulated expression of chemokine receptors CCR2 and CCR5.

A typical feature of a CAV lesion is the subintimal proliferation of smooth muscle cells. Initially, these were thought to be derived from the donor. However, in gender-mismatched cardiac allografts, Y-chromosome in situ hybridization has revealed the presence of recipient-derived smooth muscle cells, suggesting an important role for host-derived stem cells in the development of CAV [68,69].

Coagulopathy

One of the distinguishing features of CAV is the diffuse nature of the disease, affecting not only the arterial network, but also the capillary network and the venous system [70], suggesting that this is truly a panvascular disease. Cardiac microvasculature is normally highly resistant to thrombosis. However, in the first month following cardiac transplantation, endomyocardial biopsies begin to show evidence of microvascular fibrin deposition and this subsequently significantly correlates with the development of CAV [71]. Microvascular thrombosis also results in acute myocardial cell damage from ischemia. Troponins, sensitive markers for myocardial damage, are indeed elevated in transplant patients with evidence of microvascular fibrin deposition on endomyocardial biopsies [72]. Furthermore, patients with persistently elevated troponins in the first year had a significantly higher likelihood of developing graft failure or severe CAV. In the first month following cardiac transplantation, troponins are elevated, presumably due to the ischemia–reperfusion injury occurring at the time of transplantation. However, in some patients, troponins remain persistently elevated in the following months and this appears to correlate with ongoing microvascular thrombosis presumably relating to a more persistent thrombogenic insult such as allograft rejection. Time-dependent

factors may contribute to microvascular injury, with ischemia–reperfusion injury being important early following transplantation, and development of antibodies or cytokine-dependent endothelial cell activation being more important later on. The early events may serve to propagate chronic injury, for example by release of endothelial cell antigens such as vimentin during ischemia–reperfusion injury to which a humoral response is subsequently mounted.

The risk for vascular thrombosis depends upon the balance between procoagulant factors and endogenous fibrinolytic activity. A decrease in fibrinolytic activity would prevent removal of fibrin and, in a prothrombotic microvasculature, would facilitate further production of thrombin and fibrin. Both increased plasma fibrinogen concentration and decreased plasma fibrinolytic activity have been associated with increased arterial intimal thickening in cardiac transplant patients [47,73]. An increased incidence of angiographic coronary artery disease in transplant patients with lower levels of tissue plasminogen activator (t-PA) on endomyocardial biopsies has been observed [47]. These patients developed earlier and more aggressive disease than those patients with normal t-PA levels within the arteriolar microvasculature. Circulating levels of t-PA and plasminogen activator inhibitor-1 (PAI-1) also correlate with development of CAV [74].

Development of CAV may also relate to abnormalities of the coagulation system following transplantation. Microvascular antithrombin depletion early after transplantation has been associated with the subsequent development of CAV [75]. Grafts with recovery of antithrombin expression appear to be less prone to CAV. The mechanisms leading to change in antithrombin expression are unclear but may include cytokine release after rejection or changes in the expression or availability of molecules that bind antithrombin, such as heparan sulfate proteoglycan molecules [76]. Ischemia and reperfusion may lead to release of enzymes from recruited neutrophils including neutrophil elastase and heparanase. Decreased antithrombin binding due to resulting loss of heparan sulfate would then lead to generation of thrombin, a process that would lead to further loss of heparan sulfate proteoglycan, thereby sustaining a prothrombogenic milieu within the microvasculature.

Influence of non-immune factors in the development of CAV

Although the alloimmune response is the predominant factor that determines graft outcome both in the short term and in the long term, a number of other non-immune determinants contribute to the development of CAV. Temporally, the immune response may be important in the early phases of CAV, while non-immune factors may dictate the severity of CAV late after transplantation [77].

Donor-related issues

Profound physiologic derangements accompany cadaveric organ procurement. Catastrophic central nervous system injury is often accompanied by periods of labile blood pressure and the need for prolonged inotropic support is not infrequent (see Chapter 20). Circulating neuroendocrine hormonal levels may be increased while levels of other hormones may decrease [78]. Inflammatory markers including cytokines and adhesion molecules may be up-regulated leading to apoptosis and cardiac dysfunction [79]. Organ procurement and transport contribute to ischemic injury, and further perturbations take place following engraftment and reperfusion. Spontaneous or atraumatic intracranial bleeding occurs in about 40% of brain-dead donors and is associated with poor short-term outcomes and development of CAV at 5 years [80].

Endothelial injury during ischemia–reperfusion may produce changes critical to the subsequent development of CAV in the ensuing years [81]. Early changes include endothelial cell desquamation and retraction allowing increased permeability. Hypoxia can induce a number of endothelial genes including VEGF, basic fibroblast growth factor (b-FGF), and platelet-derived growth factor (PDGF) [82–84]. PDGF and b-FGF induce intimal smooth muscle proliferation and migration and VEGF stimulates endothelial proliferation and enhances vascular permeability [85]. Angiotensin II is also a potent mitogen for smooth muscle cell proliferation and, experimentally, its inhibition has been shown to be effective in inhibiting the development of CAV [86,87]. Angiotensin-converting enzyme (ACE) expression is increased during vascular injury and, experimentally, its inhibition has been shown to ameliorate CAV. ACE inhibitors may therefore also be of some potential value as preventive agents [86].

In a large multi-institutional analysis with long-term follow-up [88], risk factors for fatal CAV included older donor age, male donor, younger recipient age, earlier date of transplant, ischemic etiology, history of recipient cigarette use, history of gouty arthritis, black recipient, and positive donor CMV serology. In an earlier study [89], risk factors identified for the earlier onset of CAV included many that are common to native atherosclerosis including older donor age, donor male sex, donor hypertension, recipient male sex, and recipient black race. The actuarial incidence of severe coronary artery disease was 9% at 5 years, and was associated with a particularly high mortality. In a multicenter retrospective analysis of cardiovascular risk factors in heart transplant recipients, hypercholesterolemia and diabetes mellitus were associated with nonfatal major adverse cardiac outcomes, whereas high creatinine and body mass index were associated with graft loss [90]. Interestingly, in this study no co-variates were identified to be significant for CAV. Studies have suggested an increased risk of CAV progression with donor-transmitted coronary disease [91,92], and the presence of native coronary disease predicts a 3–5 times greater relative risk for cardiac events including myocardial infarction, heart failure, and sudden death [93,94]. However, other studies suggest that CAV is multifactorial and that atherosclerotic plaque in the donor heart may not necessarily progress to CAV [95]. Using serial IVUS measurements, Li et al. [96] demonstrated that pre-existing donor atherosclerotic lesions do not accelerate the development of CAV either at the site of pre-existing donor atherosclerosis or elsewhere within the same artery.

Hyperlipidemia

Ischemic cardiomyopathy is a common cause of end-stage heart disease and about half of all heart transplants are performed for patients with this diagnosis. The majority of these patients have a history of hyperlipidemia as a major contributor to their disease, and after transplant hyperlipidemia is a widely observed phenomenon [97]. A number of factors contribute to post-transplant hyperlipidemia. Steroids increase apolipoprotein B production and also contribute to post-transplant obesity. Cyclosporine increases hepatic lipase activity and decreases lipoprotein lipase activity [98], resulting in impaired very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) clearances. The use of tacrolimus has been associated with a minimization of lipid abnormalities [99,100].

Hyperlipidemia is a well-established risk factor for non-transplant atherosclerosis. Both clinical and experimental observations suggest that it is an important contributor to CAV [101–103]. In one study, elevated lipid values 6 months following transplantation had a strong predictive value for the development of CAV at 3 years [102]. In another study, post-transplant elevation of LDL at 1 year was the only predictor for the development or progression CAV by IVUS [104]. In early experimental studies, rabbits fed a high cholesterol diet following cardiac transplantation develop accelerated CAV [105]. Hyperlipidemia likely compounds the effect of endothelial activation observed after transplantation. Greater intimal thickening, more intimal angiogenesis, and a greater accumulation of T cells is seen in transplanted vasculature compared to native vessels in animals exposed to the same level of hyperlipidemia [106,107]. The net effect of immunosuppression on CAV, however, may depend upon its ability to suppress the allogeneic immune response and prevent smooth muscle proliferation regardless of the effect of the agent on the lipid profile so that in the case of proliferation signal inhibitors, their effect on attenuating CAV development occurs despite a significant elevation in serum lipids with the use of these agents [108].

Interestingly, one class of drugs of particular benefit in cardiac transplantation appears to be effective not only at significantly reducing hyperlipidemia, but also decreasing the alloimmune response. Treatment initiated within 2 weeks of transplantation with a hydroxymethylglutaryl co-enzyme A (HMG Co-A) reductase inhibitor (statin) is associated not only with decreased development of coronary intimal thickening, but also a lower frequency of hemodynamically compromising rejection episodes and improved survival [109]. These agents appear to have an immunosuppressive effect that may be at least as important in cardiac transplantation as their lipid-lowering activity. Statins appear to have pleiotropic effects. They have been shown to decrease cytokine production and improve coronary endothelial function in patients [110], inhibit IFN-γ induced MHC class II expression in human vascular endothelial cells [111], decrease cytotoxicity of NK cells [109,112], decrease post-transplant hypercoagulability by inhibiting monocyte tissue factor [113], and modulate chemokine and chemokine-receptor function (see earlier).

Hypertension

Hypertension is a well-established risk factor for non-transplant coronary atherosclerosis. Hypertension is common following heart transplantation, and is attributed to the use of corticosteroids, frequent associated weight gain, and calcineurin inhibitors. Prevalence of hypertension increases after transplant from 74% at 1 year to 98% at 10 years [114]. Studies confirming the link between post-transplant hypertension and CAV have been sparse [115], with most studies being unable to confirm an association [89,90,116].

However, there are data to suggest a therapeutic benefit with regard to decreased CAV with the use of antihypertensive agents. In one study [117], patients randomized to diltiazem demonstrated a significant decrease in coronary artery luminal diameter by **quantitative coronary angiography** (QCA) compared with baseline at 1 and 5 years. At 5 years there was also a significant difference in freedom from both death and CAV. In a rat heterotopic transplant model, amlodipine was shown to significantly decrease the development of allograft vasculopathy [118]. In vitro, calcium channel blockers have been shown to stabilize endothelial cell function, inhibit platelet aggregation, and decrease the release of platelet-derived growth factors [119].

One study demonstrated that the use of calcium channel blockers or ACE inhibitors was associated with a significant decrease in intimal thickening at 1 year by IVUS [120]; the benefit was seen with the use of calcium channel blockers either alone or in combination with ACE inhibitors. As recipients were treated with any calcium channel blocker or ACE inhibitor, it was determined that there was a class effect. Another study suggested that the benefit of calcium channel blockers and ACE inhibitors was synergistic on the development of transplant vasculopathy as determined by IVUS at 1 year [121].

In an experimental transplant model, the angiotensin receptor blocker losartan was noted to be superior to enalapril in preventing allograft vasculopathy [122]. The effect of angiotensin receptor blocker on attenuating neointimal proliferation of smooth muscle cells may be due to decreased differentiation of peripheral mononuclear cells into a smooth-muscle phenotype [123] or involve inhibition of peroxisome proliferator-activated receptor gamma (PPAR-γ) [124].

Diabetes

More than 30% of adult heart recipients are diabetic 1 year after transplantation [114]. Predominant factors contributing to the development of new-onset diabetes after transplantation include increased pretransplant body mass index [125] and immunosuppressive therapy, corticosteroids and calcineurin inhibitors in particular [126]. In a retrospective analysis, pretransplant glucose intolerance, family history of diabetes, and the need for insulin more than 24 hours after transplantation was associated with post-transplant diabetes [127]. Corticosteroids are associated with the greatest risk of developing new-onset diabetes after transplantation. Higher doses of prednisolone also have been found to be a risk factor for the development of new-onset diabetes in heart transplant recipients [127]. Tacrolimus appears to be more diabetogenic than cyclosporine [126,128]. However, glycemic control, as determined by hemoglobin A1c measurement, was found to be a more important factor associated with the severity of CAV than cumulative doses of diabetogenic immunosuppressive agents in one study [129]. In an analysis of 20,000 heart transplant recipients in the United Network for Organ Sharing (UNOS) database, diabetes was associated with an increased risk of CAV [130].

Most heart transplant recipients are markedly insulin-resistant and demonstrate many of the features of compensatory hyperinsulinemia, including the atherogenic and proinflammatory profiles [131,132]. Insulin resistance and a proinflammatory state are associated with the development of CAV despite the use of statin therapy [133]. The use of immunosuppressive drugs and factors such as post-transplant obesity are major contributors to insulin resistance syndrome, which appears to correlate with the development of CAV [134].

Cytomegalovirus

Human CMV is ubiquitous, with a wide global distribution (see Chapter 94). Exposure to CMV increases with age such that by the age of 60, most have evidence of prior exposure. Fortunately, in the majority with a well-developed competent immune system, CMV poses little threat, with most infections causing only mild subclinical disease. However, in immune-compromised patients – for example, patients with AIDS, cancer patients undergoing chemotherapy, and organ transplant recipients – CMV infection can be life-threatening, leading to systemic viremia, hepatitis,

pneumonitis, retinitis, colitis, and other manifestations. In heart transplant recipients, CMV infection has been associated with development of CAV. Several clinical studies have shown an association between evidence of CMV infection and subsequent development of CAV [135–139]. CMV infection occurs in 25–50% of cardiac transplant recipients. CMV infection increases with the use of anti-lymphocyte induction therapy and with acute rejection episodes [1]. In earlier studies, the 5-year survival rate was about 70% in patients without CMV compared with about 30% in those with CMV infection [136].

The mechanisms by which CMV infection contributes to CAV are unclear. Endomyocardial biopsy studies demonstrate that CMV disease in heart transplant recipients have a greater degree of severe angiographically demonstrable coronary artery disease, intimal thickening, vascular smooth muscle cell proliferation, and significant perivascular inflammation when compared with patients without CMV disease [140]. CMV infection after transplant is associated with chronic endothelial dysfunction [141,142] through a pathway that results in impaired endothelial nitric oxide production [143].

In animal studies, in rat aortic allograft models, CMV infection at the time of transplantation results in neointimal proliferation [144]. Endothelial cell infection leads to up-regulation of adhesion molecules on both infected and non-infected cells and may be mediated by a paracrine effect from interleukin-1 beta production [145]. In co-culture experiments, CMV infection of T cells induces production of IFN-γ and TNF-α, which, through a paracrine effect, are then able induce expression of Class I and II antigens, VCAM-1 and ICAM-1. CMV infection may also contribute to neointimal proliferation by inhibition of apoptosis of smooth muscle cells [146].

Clinical assessment

Due to cardiac denervation, symptoms resulting from CAV may not occur until the disease is very advanced and beyond therapeutic intervention. Most programs therefore use a surveillance strategy to monitor the development of CAV. Although invasive strategies with coronary angiography and IVUS have been traditionally used, non-invasive methods have become increasingly popular in recent years. Non-invasive strategies have typically involved stress testing, which is limited because these techniques depend upon the presence of hemodynamically significant lesions. Dobutamine stress echocardiography (DSE) has been used for detection of CAV with variable sensitivity and specificity and is predictive of adverse cardiac events [147,148]. The method may be limited by the presence of resting wall motion abnormalities in cardiac allografts and abnormalities in certain parameters such as Doppler tissue imaging, which may not be attributable to CAV [149,150]. However, quantitative techniques and use of myocardial contrast [151,152] appear to improve the ability of DSE to detect CAV even in the absence of significant angiographically detectable disease [153].

Myocardial perfusion imaging (MPI) has the advantage of detecting perfusion associated with both large epicardial vessels and the microvasculature. MPI reliably identifies patients at risk for subsequent cardiac events in cases of CAV, with a high negative predictive value [154–156]. Measurement of quantitative myocardial blood flow and reserve by positron emission tomography (PET) may be particularly useful in the determination of microvascular allograft vasculopathy as a marker for early disease and to monitor disease progression [157].

Ultrafast computerized tomography (CT) has been used to detect coronary calcification and may be useful in the detection of CAV [158], but still does not provide detailed information about the vessel lumen or wall. CT angiography (CTA) has become an increasingly useful tool in the non-invasive assessment of coronary disease. Development in the field with 64-slice, dual source, and ECG gating technologies has allowed detailed luminal and extraluminal evaluation of coronaries with significant decrease in radiation exposure. Diagnostic accuracy for detection of CAV is comparable to IVUS [159], and CTA may also allow detailed evaluation of plaque composition [160]. Serial CTA follow-up may be a reasonable alternative to conventional invasive coronary angiography in many patients [161].

Magnetic resonance imaging (MRI) is a very promising technology for evaluation of cardiac structure and function. In heart transplantation, it may be useful in the evaluation of acute allograft rejection, allowing simultaneous assessment of cardiac function and early contrast enhancement and myocardial edema as non-invasive correlates for biopsy proven rejection [162]. ^{31}P magnetic resonance chemical shift imaging with measurement of phosphocreatine to adenosine triphosphate ratio has been shown to predict angiographic CAV [163]. Quantification of myocardial strain and perfusion reserve by MRI has also been shown to correlate with CAV [164] and may be particularly useful for assessment of microvascular disease.

Invasive methods have traditionally been the standard for assessment of CAV. Coronary angiography requires the ability to compare normal segments of the vessel with diseased segments. However, the panvascular nature of CAV often results in underestimation of disease because of the absence of a reference segment in which the normal diameter of the vessel can be assessed. The presence of minimal luminal irregularities on angiography often suggests the presence of early disease and is therefore commented upon. Comparison with prior studies may help determine progression of disease but requires the use of comparable angiographic views and magnification at each study for precise evaluation. This methodology also allows use of computer-assisted QCA, improving the sensitivity of detection of CAV. However, QCA still does not allow evaluation of the vessel wall and may miss early disease where compensatory dilatation of the vessel may occur to preserve luminal area. Interestingly, coronary angiography has allowed some estimation of the presence of microvascular disease. Distal pruning on angiography frequently suggests diffuse small vessel involvement. The presence of coronary collaterals with myocardial blush has been interpreted as a correlate for microvascular disease [165]. Transplant patients generally have microcirculatory aberrations as determined by Thrombolysis in Myocardial Infarction (TIMI) myocardial perfusion grade, and severity appears to correspond to both epicardial disease and survival [166]. More recent quantitative assessment of angiographic myocardial blush has been shown to be predictive of survival in transplant recipients and may help with the identification of early CAV in patients with impaired perfusion reserve but without angiographically evident atherosclerosis [167].

While coronary angiography is the gold standard for the assessment of coronary artery disease, conventional coronary angiography generally underestimates the extent of disease because lesions are frequently concentric due to subintimal cellular proliferation. In contrast, IVUS allows direct imaging of the vessel wall to determine the extent of intimal and medial disease (Figure 79.2). Quantitative assessment of intimal and medial area by planimetry allows assessment of even early plaque burden

not appreciable angiographically. Sequential images are usually obtained by automated catheter pull-back to determine the extent of disease along a vessel wall. Several studies have confirmed that IVUS is more sensitive than angiography in detecting CAV [168–170]. Serial IVUS assessment allows determination of the percentage change in atheroma volume, with considerable statistical power to detect small changes. The technique is therefore routinely used to assess efficacy of newer treatment regimens in reducing the development of CAV. IVUS studies suggest that the most rapid rate of progression of intimal thickening occurs during the first year, followed by slow but inexorable progression over time [171]. The progressive development of intimal thickening as recognized by IVUS is predictive of development of angiographically apparent CAV. Rapidly progressive CAV, defined as an increase of $\geq$0.5 mm in maximal intimal thickness within the first year after heart transplant, is associated with a significantly increased risk of all-cause death, myocardial infarction, and the subsequent development of angiographically severe CAV [171,172]. The performance of IVUS at 1 and 12 months after heart transplant is therefore increasingly used to identify patients at high risk for future cardiovascular events, and may aid in allowing therapeutic adjustment of immunosuppression. More recently, IVUS techniques have been developed to allow assessment of plaque composition using radio frequency analysis [173,174]. This approach, known as virtual histology, is a promising development which may allow not only quantification of plaque burden but also determination of changes in plaque composition which may be of prognostic value [173,175].

Despite these promising developments, IVUS has several limitations: it is highly invasive, requires anticoagulation, use of expensive single-use catheters, and evaluation is mainly limited to the major epicardial vessels. Catheter-induced coronary vasospasm is also a particular concern in heart transplant recipients due to persistent endothelial dysfunction.

Determination of coronary flow reserve with intracoronary Doppler flow measurement may be useful in assessing CAV, although the clinical importance of this information has yet to be determined and non-invasive methods using PET myocardial perfusion imaging or echocardiography may provide similar information more easily. Intracoronary flow velocities are determined using a Doppler transducer mounted on a guide wire. Pharmacologic intervention with adenosine or dipyridamole allows measure of maximal coronary flow and calculation of flow reserve. Coronary flow reserve has been shown to be reduced in patients with CAV and deteriorates with increasing time after transplantation [176,177]. Measurement of coronary flow reserve reflects changes in the microvasculature as well as the epicardial vessels and it correlates with plaque burden assessed by IVUS [178].

Unfortunately, the definitions of CAV are diverse, and there are no uniform international standards for the nomenclature of this entity. However, a consensus document commissioned by the International Society of Heart and Lung Transplantation Board has been published, which is based on best evidence and clinical consensus derived from critical analysis of available information pertaining to angiography, IVUS, microvascular function, cardiac allograft histology, circulating immune markers, non-invasive imaging tests, and gene-based and protein-based biomarkers [179]. This document represents a working formulation for an international nomenclature of CAV, similar to the development of the system for adjudication of cardiac allograft rejection by histology (Table 79.2).

Table 79.2. Recommended nomenclature for cardiac allograft vasculopathy

ISHLT CAV_0 (Not significant): No detectable angiographic lesion
ISHLT CAV_1 (Mild): Angiographic left main (LM) <50%, or primary vessel with maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction
ISHLT CAV_2 (Moderate): Angiographic LM <50%; a single primary vessel $\geq$70%, or isolated branch stenosis $\geq$70% in branches of two systems, without allograft dysfunction
ISHLT CAV_3 (Severe): Angiographic LM $\geq$50%, or two or more primary vessels $\geq$70% stenosis, or isolated branch stenosis $\geq$70% in all three systems; or ISHLT CAV1 or CAV2 with allograft dysfunction (defined as LVEF $\leq$45% usually in the presence of regional wall motion abnormalities) or evidence of significant restrictive physiology (which is common but not specific; see text for definitions)

Definitions
(a) A **primary vessel** denotes the proximal and middle 33% of the left anterior descending artery, the left circumflex, the ramus and the dominant or co-dominant right coronary artery with the posterior descending and posterolateral branches
(b) A **secondary branch vessel** includes the distal 33% of the primary vessels or any segment within a large septal perforator, diagonals and obtuse marginal branches or any portion of a non-dominant right coronary artery
(c) **Restrictive cardiac allograft physiology** is defined as symptomatic heart failure with echocardiographic E to A velocity ratio >2 (>1.5 in children), shortened isovolumetric relaxation time (<60 ms), shortened deceleration time (<150 ms), or restrictive hemodynamic values (right atrial pressure >12 mmHg, pulmonary capillary wedge pressure >25 mmHg, cardiac index <2 L/min/m^2)
ISHLT, International Society for Heart and Lung Transplantation.
Reprinted from [179] Mehra MR, Crespo-Leiro MG, Dipchand A, et al. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy. *Journal of Heart and Lung Transplantation.* 2010;29:717–727 with permission from Elsevier. Copyright © 2010, Elsevier.

Pharmacologic therapy

The impact of immunosuppressive agents on the development of CAV is complex (see Chapters 98–102). The indirect effects of long-term steroid therapy on lipid metabolism have already been described. Cyclosporine further enhances this effect although substitution of cyclosporine for tacrolimus appears to attenuate the rise in serum lipids [100]. The number and severity of episodes of acute rejection contributes to CAV [50,77] and even asymptomatic antibody- mediated rejection appears to be a risk factor for the development of allograft vasculopathy [180]. More effective immunosuppressive regimens may therefore have a beneficial impact on the development of allograft vasculopathy.

Although tacrolimus has been shown to be superior to cyclosporine in preventing chronic rejection on liver transplantation [181], data in cardiac transplant patients remain limited. This is despite demonstrated improvements with tacrolimus in hypertension and lipid profiles in comparison to cyclosporine [100], although rates of diabetes are demonstrably higher with tacrolimus. Five-year results of a randomized study comparing tacrolimus with microemulsion cyclosporine revealed comparable outcomes with respect to the development of CAV [182].

MMF, an inhibitor of the de novo pathway for purine biosynthesis, has been shown to be more effective at reducing cardiac allograft rejection and post-transplant mortality [183]. It may also be more effective at reducing allograft vasculopathy [184]. This effect may be related at least in part to its ability to reduce B-cell responses, as patients treated with MMF developed lower antivimentin titers that correlated with the lower incidence of CAV by IVUS. In comparison to azathioprine, MMF has been shown by IVUS to reduce intimal thickness [185], although rates of CMV infection, a risk factor for CAV, appear to be increased with the use of MMF [186].

The availability of proliferation signal inhibitors (PSI) sirolimus and everolimus represents a major advance in the management of CAV. These agents are a class of anti-proliferative agents shown to inhibit smooth muscle cell proliferation in vitro and in experimental animal models of vascular injury, a key component in the development of CAV. The agents are clinically effective in reducing in-stent restenosis in native coronary artery disease [187,188]. In a randomized clinical trial involving 136 patients [189], the use of sirolimus in de novo heart transplantation was associated with decreased development of CAV at 2 years compared to patients receiving azathioprine. In a subsequent sentinel multicenter study [190], over 600 patients were randomized to everolimus or azathioprine within 72 hours of transplantation. IVUS demonstrated that the average increase in maximal intimal thickness at 12 months was significantly smaller with everolimus than azathioprine. This finding was significant as it has been noted that an increase in maximal intimal thickness at 1 year is associated with higher post-transplant mortality at 5 years [172].

Treatment

Clinically apparent disease is associated with a poor prognosis and therefore prevention is an important strategy in addressing transplant vasculopathy. In addition to the use of anti-proliferative immunosuppressants, agents used in the treatment and prevention of native coronary disease are also utilized in CAV. Aspirin is widely used because of its widely established use in non-transplant coronary disease and may have an important role in preventing thrombosis in the setting of persistent endothelial dysfunction and prothrombotic milieu seen in CAV. Control of hypertension and hyperlipidemia has already been discussed. The use of statins is particularly important as it also helps prevent allograft rejection. In contrast to native atherosclerosis, vitamin C and E supplementation may be beneficial in decreasing CAV [191].

Once clinically apparent disease is apparent, a number of approaches are available to relieve ischemia. For focal disease, percutaneous coronary intervention (PCI) with balloon angioplasty has been successful, although restenosis is particularly common in the transplant setting [192], especially with the use of cutting balloon techniques [193]. Stenting has helped to address this problem and the use of drug-eluting stents has significantly attenuated restenosis rates [194,195]. No studies are available to show whether PCI alters the prognosis of CAV and because many patients with significant disease are asymptomatic, intervention often presents a dilemma. Furthermore, patients who develop in-stent restenosis after PCI appear to develop accelerated disease in the non-intervened vessels and may be at higher risk for myocardial infarction or death [196], suggesting that a heightened immune response contributes to progression of CAV as a panvascular phenomenon [197].

Patients with multi-vessel focal disease with adequate distal target vessels may be candidates for coronary artery bypass surgery (CABG). Efficacy is difficult to determine as relatively small numbers have been reported, reflecting the many patients who do not have adequate targets and the preferential use of PCI. Surgical revascularization appears to be a safe option with reasonable outcomes for the select patients with CAV with suitable targets [198,199].

Retransplantation may be the only consideration for many patients with advanced CAV not amenable to PCI or CABG. Survival rates reported after retransplantation have been consistently lower than those after primary transplantation. In one, the 1-year actuarial survival rate of those patients who underwent retransplantation specifically for CAV approached 1-year survival rates following primary transplants [200]. This study also showed that patients having a second heart transplant do not have an increased risk for development of CAV in the second donor heart. Recent data suggest that patients undergoing retransplantation for CAV or chronic graft failure appear to have acceptable long-term outcomes with a median survival over 10 years [201].

Cardiac transplantation for infiltrative cardiomyopathies and risk of recurrence

A number of infiltrative diseases may lead to end-stage heart disease with a requirement for transplantation. These include cardiac sarcoidosis, myocarditis (giant-cell, lymphocytic, eosinophilic, or viral), autoimmune diseases such as lupus and amyloidosis. In many cases, the diagnosis is only made upon histology of the explanted heart. For most conditions, post-transplant outcome is comparable with those patients undergoing heart transplantation for other conditions despite documented cases of recurrence of disease noted on endomyocardial biopsies [202].

Cardiac sarcoidosis

There are a few case reports of recurrent cardiac sarcoidosis in the transplanted heart, with many occurring several years after transplantation [203–207]. A case series of four patients transplanted for sarcoid heart disease has been reported, with no evidence of post-transplant recurrence [208]. Although data are sparse, the risk of recurrence of cardiac sarcoid appears to be significantly lower than the risk of recurrence of pulmonary sarcoidosis after lung transplantation, where the recurrence rate is as high as 50%. Recurrence of disease following cardiac transplantation is treated with augmentation of immunosuppression, particularly corticosteroids.

Giant-cell myocarditis

Giant-cell myocarditis causes irreversible fulminant left ventricular dysfunction with associated conduction abnormalities and congestive failure. Response to immunosuppressive therapy is poor [209] and cardiac transplantation is the only viable treatment option. The histologic hallmarks of giant-cell myocarditis include a polymorphous inflammatory response with numerous multinucleated giant cells and extensive myocyte necrosis in a geographic pattern. In the Giant Cell Myocarditis Registry, of 38 patients receiving a heart transplant for giant-cell myocarditis, there were nine recurrences of disease in the allograft. Due to the high risk of recurrence, concern has been expressed that recurrence of giant-cell myocarditis in the allograft might be a contraindication for cardiac transplantation. In one series of five patients from the Cleveland Clinic [210], one patient developed recurrence early after transplant in conjunction with an episode of cellular rejection with subsequent resolution with treatment. Despite the increased rate of recurrence, patients with giant-cell myocarditis are considered for transplantation as without this option mortality remains high. Interestingly, while the condition appears to respond poorly to immunosuppressive treatment, its recurrence post-transplant appears to be amenable to augmentation of immunosuppressive therapy. This might be the result of detection of the disease at an earlier stage than in the native heart, or the immunosuppression milieu in the allograft. The favorable response to therapy suggests that the likelihood of

recurrence of giant-cell myocarditis should not be considered a barrier to transplantation.

Chagas disease

Chagas disease, caused by the parasite *Trypanosoma cruzi*, is an important cause of cardiac disease in endemic areas of Latin America. It is now being diagnosed in non-endemic areas because of immigration and is a more frequent cause of presentation with end-stage heart disease in the United States. There is also a significant reservoir of the parasite in raccoons in the United States, although autochthonous transmission is rare [211]. It is also becoming an issue of concern as a transmissible organism during organ donation, requiring establishment of appropriate screening strategies in potential donors. This is further complicated as serology may be unreliable and accurate diagnosis depends on tissue samples, histology, or polymerase chain reaction (PCR) assays. Typical cardiac manifestations of Chagas disease include dilated cardiomyopathy, congestive heart failure, arrhythmias, cardioembolism, and stroke.

Chagas disease is the third leading indication for cardiac transplantation in Brazil, representing 15–20% of cases [212]. The recurrence rate of Chagas disease is around 40% [212,213], although there is some evidence that in the most recent era, with minimization of immunosuppression, close surveillance, and initiation of appropriate therapy, rates are much lower [212]. In an analysis of 107 heart transplants for Chagas disease between 1985 and 2010 in Sao Paulo, Brazil [212], the hospital mortality was 17.7% (n = 19) and causes of death were infection (n = 6; 31.5%), graft dysfunction (n = 6; 31.5%), rejection (n = 4; 21.1%), sudden cardiac arrest (n = 2; 10.5%) or ABO incompatibility (n = 1; 5.3%). Late mortality occurred in 27 (25.2%) patients. The main causes of death were rejection (n = 6; 22.2%), infection (n = 6; 22.2%), lymphoma (n = 4; 14.8%), sarcoma (n = 2; 7.4%), pericarditis (n = 2; 7.4%), and reactivation of Chagas disease in the central nervous system (n = 1; 7.1%). Early experience was associated with higher rates of malignancy attributed to higher immunosupression [214]. Improvement in survival and a decrease in reactivation rate has been associated with lower immunosuppression, particularly steroid weaning [212] and avoidance or minimization of MMF [215,216]. Patients with a history of rejection and malignancy are at higher risk of reactivation [216]. Current strategies for monitoring for reactivation rely on PCR testing of blood and this technique is also useful for monitoring treatment efficacy [217]. Episodes of parasitemia, myocarditis, or Chagas disease reactivation are treated with benznidazole in a 5 mg/kg/day dosage for 60 days. Nifurtimox is another option for reactivation and high-dose allopurinol has been used based on an anecdotal report [214,218].

Conclusions

CAV is an important complication that limits the long-term survival of cardiac allografts. The disease has many similar features to native non-transplant coronary atherosclerosis, although there are also several unique features in both pathology and distribution. Development of CAV requires several contributing factors including risk factors associated with conventional atherosclerosis, ischemia–reperfusion injury at the time of transplant, host alloimmune response, hemostatic factors, and the modulating effect of post-transplant medications. Clinical presentation of CAV is frequently atypical due to surgical denervation. Diagnosis and monitoring of disease depends mostly on invasive techniques, although recent advances in non-invasive techniques may decrease the need for routine invasive evaluation in the future. Given the relatively poor prognosis of CAV, prevention remains an important strategy. A greater understanding of its pathology has offered more promising immunosuppressive regimens such as PSI. A greater awareness of the role of antibody-mediated rejection in the development of CAV may allow the development of strategies particularly focused on abrogating the humoral response. In the meantime, patients with established symptomatic disease have conventional revascularization techniques available to them for palliation, and retransplantation may be a consideration for select patients.

For the relatively few who undergo cardiac transplantation for an infiltrative process, the risk of recurrent disease appears to be greatest for Chagas disease and giant-cell myocarditis. Close surveillance for recurrence of disease and prompt therapy appears to allow satisfactory outcomes in these patients.

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CHAPTER 80

Recurrent Disease after Lung Transplantation

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Introduction

Lung transplantation has become the definitive therapy for many different types of advanced lung disease. Although advances in surgical technique (see Chapter 59) and donor organ preservation have led to improvement in early post-transplant survival, infections (see Chapters 93 and 94) and bronchiolitis obliterans syndrome (BOS; see Chapter 72) have remained major impediments to long-term survival. Therefore, a great deal of attention and research has centered on these allograft-related complications.

However, little consideration has been given to the potential for recurrence of native lung disease as an etiology of allograft dysfunction following lung transplantation. Recurrence has been reported for a number of lung diseases following lung transplantation, resulting in a spectrum of outcomes ranging from incidental biopsy findings to graft failure and death (Box 80.1). In this chapter, we survey the evidence supporting the recurrence of native lung disease following lung transplantation, examine the variable impact of recurrent native disease on allograft function, explore the pathogenesis of recurrent disease, and discuss the potential implications of recurrent disease on the decision to perform transplantation.

Sarcoidosis

Sarcoidosis is a multi-organ granulomatous disease of unclear etiology. The development of sarcoidosis most likely begins with presentation of an unknown antigen by macrophages or dendritic cells to naïve T lymphocytes (primarily $CD4^+$), resulting in a polarized Th1 immune response. This ultimately leads to the formation of granulomas, characterized by epithelioid histiocytes and multinucleated giant cells mixed with $CD4^+$ lymphocytes. The diagnosis of sarcoidosis requires the exclusion of a number of alternative diagnoses, and generally requires a combination of clinical, radiographic, and/or histologic findings involving multiple organ systems [1].

The lung is the most commonly affected organ in patients with sarcoidosis [2,3]. Granulomas can occur in the parenchyma as well as the airways, and therefore can lead to both restrictive and obstructive lung physiology [4,5]. Severity of pulmonary disease can range from isolated radiographic findings in the absence of symptoms to end-stage lung disease requiring lung transplantation. Therapy for pulmonary sarcoidosis currently includes the stepwise use of corticosteroids [6,7], steroid-sparing agents (methotrexate, azathioprine, leflunomide, mycophenolate) [8–11], and biologic agents capable of blocking tumor necrosis factor (infliximab) [12,13].

Lung transplantation has become the definitive therapy for patients with severe refractory lung disease secondary to sarcoidosis. Between 1995 and 2010, a total of 783 total lung transplant procedures (236 single lung transplant and 547 double lung transplant) were performed for patients with sarcoidosis, representing 2.6% of all indications for transplant. The survival half-life following lung transplantation for sarcoidosis is 5.3 years, compared to 7.4 years for cystic fibrosis, 6.3 years for alpha-1 antitrypsin deficiency, 5.3 years for chronic obstructive pulmonary disease (COPD), 4.5 years for idiopathic pulmonary fibrosis, and 4.9 years for idiopathic pulmonary arterial hypertension [14].

Sarcoidosis is the most common native lung disease to recur in the lung allograft, with a reported incidence of 30–80% following lung transplantation [15–22]. Although several early reports described the findings of non-caseating granulomas on biopsy specimens from patients following lung transplantation for sarcoidosis [18–20], none of the patients in these reports had symptoms or radiographic findings consistent with sarcoidosis. Interestingly, one of the reports described a patient with recurrence of sarcoidosis granulomas in the transplanted allograft, as well as recurrence in a second contralateral allograft following retransplantation for obliterative bronchiolitis [20].

Martinez et al. [21] published the first case report of a lung transplant recipient with symptoms and computed tomography (CT) scan findings, in addition to biopsy pathology, consistent with a diagnosis of sarcoidosis. The patient in their report presented with dyspnea and fatigue 13 months following lung transplantation, and a high resolution computed tomography (HRCT) scan demonstrated upper lobe micronodular infiltration. A subsequent bronchoscopic lung biopsy revealed non-caseating granulomas. The patient's symptoms and radiographic abnormalities resolved following treatment with oral corticosteroids, but the finding of non-caseating granulomas persisted in biopsies performed 3, 6, and 9 months later.

Since then several reports have described a total of 31 cases of recurrent sarcoidosis following lung transplantation. Milman et al. [17] published the long-term outcomes of seven patients receiving single lung allografts for pulmonary sarcoidosis. These patients were followed for a median of 31 months post-transplant (range 12–136 months). Only one of the patients had radiographic findings consistent with pulmonary sarcoidosis. Three patients had

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Box 80.1. Reports of recurrent native lung disease following lung transplantation

- Sarcoidosis [15–22]
- Lymphangioleiomyomatosis [47–53]
- Langerhans cell histiocytosis [79–81]
- Bronchioloalveolar carcinoma [100–105]
- Emphysema secondary to alpha-1 antitrypsin deficiency [140,141]
- Pulmonary alveolar proteinosis [122,123]
- Desquamative interstitial pneumonia [148–150]
- Giant cell interstitial pneumonia [153]
- Diffuse panbronchiolitis [155]
- Pulmonary capillary hemangiomatosis [160,161]
- Williams–Campbell syndrome [164]

pathologic evidence of sarcoidosis in the allograft, revealed in biopsies performed at 2, 5, and 6 months post-transplant, respectively. Ionescu et al. [15] described a series of eight patients with recurrent sarcoidosis following lung transplantation. Histologic evidence of sarcoid granulomas was identified during the first 6 months in two cases, between 6 and 12 months in two other cases, and between 1 and 2 years in the other four cases.

The cumulative findings in these reports suggest that recurrence of sarcoidosis is most often diagnosed by the incidental finding of non-caseating granulomas on transbronchial biopsies. Clinical signs and symptoms are often absent and, when present, are generally less severe than in primary disease. Radiographic findings are uncommon, but may include lymphadenopathy, solitary or military nodules, and diffuse reticulonodular infiltrates [23,24]. Graft dysfunction related to the appearance of granulomas is rare. It is possible that the mild form of recurrent disease, compared to the original native disease, may be a result of the effects of post-transplant immunosuppressive therapy. Interestingly, acute rejection has been reported at a much higher rate in the presence of recurrent sarcoidosis [15,16,18,21,24]. Based on this observation, it has been proposed that rejection and sarcoidosis may share some common immunologic pathways [17,21,25].

It is not completely known if recurrent sarcoid granulomas in the transplanted lung is derived from recipient or donor cells, or both. Donor-acquired sarcoidosis has been proposed, in which sarcoidosis in the lung allograft is thought to originate from donors with undiagnosed sarcoidosis [16]. However, there are few data supporting the likelihood that significant sarcoidosis could develop in "normal" recipients following transplantation of organs from donors with known sarcoidosis. Heatly et al. [26] reported the transplant of a lung allograft from a donor with pulmonary sarcoidosis (unknown at the time of transplant) into a recipient with primary pulmonary hypertension and no known history of sarcoidosis. During a follow-up period of 16 months post-transplant, there was no evidence of histologic, radiologic, or clinical evidence of pulmonary sarcoidosis involving the transplanted lung. Ionescu et al. [15] performed polymerase chain reaction (PCR) DNA analysis of lung samples from the native lung, allograft lung, and recurrent sarcoid granuloma (in the allograft) in three lung transplant recipients with recurrent sarcoidosis, and demonstrated an increase in the percentage of recipient DNA in the epithelioid cell clusters within the granulomas. Milman et al. [22] used fluorescence in situ hybridization (FISH) to analyze immune cells in recurrent granulomas from the allograft of a female lung transplant recipient with sarcoidosis who received an allograft from a male donor, and demonstrated that the immune cells in the granulomas were X-chromosome positive and Y-chromosome negative. These results suggest that the recurrent granulomas originate in the recipient, and likely result from the infusion of recipient macrophages into the allograft, rather than a response of donor histiocytes to a recipient antigen.

Lymphangioleiomyomatosis

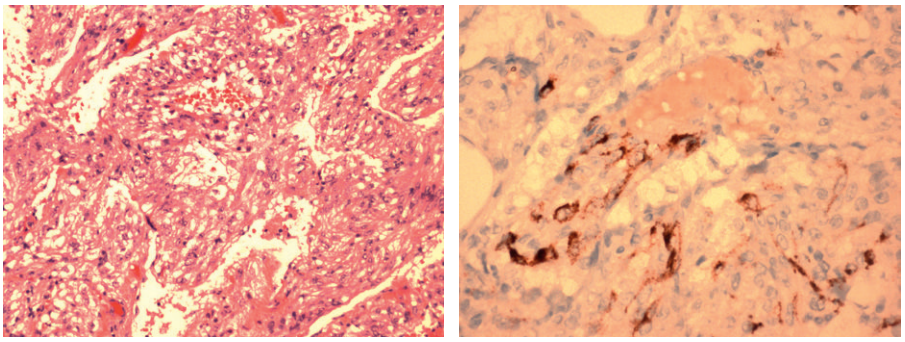
Lymphangioleiomyomatosis (LAM) is a rare idiopathic disease primarily affecting women of reproductive age, although it has been reported to develop in a small percentage of men in association with tuberous sclerosis [27–29]. LAM is characterized by smooth muscle proliferation and thin-walled cyst formation in the lungs, kidneys, liver, spleen, uterus, ovaries, and retroperitoneal lymphatics. The lung is the most commonly affected organ, and dysfunction results from smooth muscle proliferation and cyst formation in the lung parenchyma, airways, vessels, and lymphatics [30]. Symptoms include progressive dyspnea, pneumothoraces from ruptured cysts, chylothoraces from occluded lymphatics, and pulmonary hemorrhage. HRCT scan demonstrates evenly distributed thin-walled cysts of varying sizes [31]. Histology is characterized by cysts surrounded by proliferating smooth muscle-like spindle cells, which can be identified by immunostaining for melanoma-related marker (HMB-45) [32].

LAM has a very slow and progressive natural history, with an approximately 70% 10-year survival rate [30]. Therapeutic strategies have revolved around preventing further proliferation of the smooth muscle-like cells, in the past primarily involving surgical or medical reduction of estrogen production [27,28,33–35]. More recently, the discovery of tuberous sclerosis genes *TSC1* and *TSC2* in LAM have led to the successful use of the mammalian target of rapamycin (mTOR) inhibitor, sirolimus, in the treatment of LAM [36–42].

Despite advances in the understanding and treatment of LAM, lung transplantation remains the only definitive therapy. A total of 308 lung transplant procedures (101 single, 207 double) were performed for patients with LAM between 1995 and 2010, representing 1% of all lung transplants performed [14]. The actuarial survival rates following lung transplantation for LAM based on published US and European data have been reported as 79–86% at 1 year, 73–76% at 3 years, and 65% at 5 years [43,44]. Postoperative LAM-related complications include significant pleural adhesions and bleeding, chylothorax, and pneumothorax of the native lung [43,45,46].

Recurrence of LAM has been demonstrated in the allograft following lung transplantation in at least seven published reports [47–53]. Early reports of LAM recurrence were primarily based on findings at autopsy. However, as LAM is generally characterized by an obstructive pattern of pulmonary function testing, recurrence following lung transplantation may be misdiagnosed as BOS.

O'Brien et al. [48] reported the first case of recurrent LAM following lung transplantation in a 38-year-old woman who underwent right single lung transplantation for pulmonary LAM. One month following lung transplant surgery, the patient's lung function began to decline, and an open-lung biopsy revealed evidence of moderate acute cellular rejection and lymphocytic bronchiolitis. There was no evidence of LAM on the biopsy, or on subsequent transbronchial biopsies. Despite intensification of immunosuppressive therapy, the patient's lung function did not significantly improve, and the patient eventually died 18 months later from invasive aspergillosis. The autopsy did not reveal evidence of acute



(A) (B)

Figure 80.1. The lung shows replacement of the normal alveolar interstitium by muscle-like spindle cells and slit-like lymphatic spaces (A). An HMB-45 immunostain highlights the infiltrating spindle cells. The findings are diagnostic of lymphangioleiomyomatosis (B) H&E, original magnification x250. Courtesy of Dr. Richard Kradin.

rejection or obliterative bronchiolitis, but did demonstrate nodular proliferation of spindle cells blended with smooth muscle, consistent with a histologic diagnosis of LAM. These findings suggested that LAM may have had a significant role in the earlier loss of allograft function.

The challenge of clinically differentiating LAM from BOS was also highlighted in a report by Bittman et al. [50,51]. The authors described a 31-year-old woman who underwent right single lung transplantation for pulmonary LAM, and developed a significant decline in lung function 1 year following transplant. An open lung biopsy revealed bronchiolitis obliterans, but at that time no histologic evidence of recurrent LAM was reported. During the next several months, her lung function continued to decline, presumably from progressive obliterative bronchiolitis. She presented 3 years post-transplant with a pneumothorax on the side of the right allograft, and died of related complications. As expected, the postmortem examination revealed histologic evidence of significant bronchiolitis obliterans, but also demonstrated proliferation of smooth muscle cells that stained strongly positive for HMB-45. Moreover, the original open lung biopsy was reanalyzed with immunohistochemical staining, revealing evidence of smooth muscle proliferation with positive HMB-45 staining. These histologic findings, along with the clinical sequela of a pneumothorax, highlight the potential clinical significance of recurrent LAM in this patient.

Recurrent LAM has also been described following living-donor lobar transplantation. Chen et al. [52,53] described a 20-year-old woman with pulmonary and abdominopelvic LAM who underwent living-donor lobar transplantation, and subsequently developed recurrent chylous pleural effusions and pneumothoraces within the first year post-transplant. HRCT findings demonstrated emphysematous changes and pulmonary infiltrates, and a transbronchial biopsy of the left lung revealed histologic evidence of LAM. Sirolimus therapy was substituted for azathioprine, and continued along with tacrolimus. The patient's symptoms, HRCT findings, and pulmonary function testing subsequently improved, and remained stable during the next 2 years.

We have recently performed a bilateral retransplantation for a patient with recurrent LAM [54]. The patient is a 34-year-old woman who had pulmonary and abdominopelvic LAM and initially underwent living-donor lobar transplantation. HRCT performed 6 years later first detected the appearance of multiple bilateral lung cysts, consistent with a diagnosis of recurrent LAM. Her lung function gradually deteriorated, presumably from a combination of recurrent LAM and bronchiolitis obliterans syndrome, and 9 years post-transplant she eventually underwent bilateral lung retransplantation. Histologic analysis of her explanted lobar allografts revealed muscle-like spindle cells and slit-like lymphatic spaces, and HMB-45 immunostain confirmed the infiltrating spindle cells, consistent with a diagnosis of LAM (Figure 80.1A,B). Evidence of patchy obliterative bronchiolitis was also seen, consistent with concomitant chronic rejection. Sirolimus was initiated approximately 5 months post-transplant to prevent recurrence of LAM in the new allografts. Although recurrent pulmonary LAM has not subsequently been detected in a 2-year follow-up period, a significant increase in hepatic and renal LAM lesions occurred during this interval.

The origin of recurrent LAM lesions in the lung allograft has been debated. Early reports utilizing non-isotopic in situ hybridization (NISH) with probes of the human Y and X chromosomes suggested that the cells were of donor origin [46,50]. However, it was then discovered that recipient LAM cells were often tightly associated with cells of donor origin, resulting in misinterpretation of the NISH signals. Subsequent analysis using simultaneous NISH and immunohistochemical staining revealed that the LAM cells actually originated from the recipient, not the donor [51]. Karbowniczek et al. [47] utilized microsatellite marker fingerprinting and *TSC2* mutational analysis to demonstrate that recurrent LAM cells in an allograft from a patient receiving a single lung transplant for LAM originated from the native lung and lymph nodes. These findings supported the hypothesis that LAM cells may actually migrate or metastasize either from the native lung and lymphatics, or from abdominopelvic foci.

The recognition that LAM can recur in the lung allograft, coupled with the common current practice of routine allograft surveillance with transbronchial biopsies, has created an opportunity for early therapeutic intervention. The discovery of *TSC2* mutation in LAM has invoked a significant role for the mTOR pathway in the pathogenesis of LAM. This finding has led to the use of sirolimus, an inhibitor of the mTOR pathway, in the treatment of LAM cell proliferation [36–42]. Chen et al. [52,53] reported the successful use of sirolimus in treating chylous effusions and declining lung function in a patient with recurrent LAM following lung transplantation. Three other reports demonstrated that initiation of sirolimus therapy resulted in significant improvement in lesions associated with retroperitoneal and recurrent pulmonary LAM in

lung transplant recipients [55–57]. We have successfully utilized sirolimus to prevent pulmonary recurrence of LAM in a patient following retransplantation for recurrent LAM in the original allografts [54]. However, progression of hepatic and renal LAM occurred despite sirolimus therapy.

Pulmonary Langerhans cell histiocytosis

Pulmonary Langerhans cell histiocytosis (PLCH) is a rare disease characterized by the infiltration and accumulation of Langerhans cells, bone-marrow derived dendritic cells, within the alveolar airspaces and interstitium [58]. Although Langerhans cell histiocytosis (LCH) can effect extrapulmonary organs such as bone, skin, and pituitary gland (particularly in children), most afflicted adults have isolated pulmonary disease [59–61]. PLCH is very strongly associated with cigarette smoking; 90% of patients with PLCH are active smokers at the time of diagnosis [59,60,62,63]. Patients with PLCH may present with mild dyspnea or cough or, in some instances, spontaneous pneumothoraces. Many patients are asymptomatic and are diagnosed by incidental findings on a chest radiograph. HRCT scan of the chest reveals the characteristic finding of a combination of cysts and nodules in predominantly upper- and mid-lung zone distribution [64–66]. Transbronchial biopsies have a very low sensitivity in the diagnosis of PCLH, and therefore surgical biopsy is generally required to make a diagnosis [67]. Histologic analysis reveals lesions with LCH cells, readily identified by characteristic kidney bean-shaped nuclei, immunohistochemical staining for CD1a, and the presence of Birbeck granules (cytoplasmic inclusion bodies) evident on electron microscopy [68–70].

The clinical course of PLCH is quite variable. Some patients spontaneously improve without any therapy, while others develop progressive disease despite potent immunosuppressive therapy. In light of the strong association between PLCH and cigarette smoking, smoking cessation is considered to be an essential intervention. Indeed, smoking cessation alone has been shown to be effective at reducing the disease manifestations in several published reports, although worsening of disease despite this behavioral modification has also been described [71–73]. Corticosteroid therapy is reserved for symptomatic patients with predominantly inflammatory lesions, although few data exist in the literature supporting evidence of a clear benefit [60,74,75]. Although potent systemic chemotherapeutic cytotoxic drugs such as cyclophosphamide, methotrexate, vinblastine, and etoposide have been utilized in the treatment of multisystem LCH, the role of these agents in the treatment of PLCH is unclear [76–78].

Lung transplantation has become the final therapy for a small number of patients with PLCH who have developed progressive refractory respiratory failure, primarily from severe cystic lung destruction and/or severe pulmonary hypertension [58,79–81]. Although many transplant centers have limited experience in the lung transplantation of patients with PLCH, there are few published data concerning short and long-term post-transplant outcomes. Dauriat et al. [58] conducted a retrospective multicenter study in France, including 39 patients who underwent lung transplantation (15 single-lung transplant, 15 double-lung transplant, 9 heart–lung transplant) for end-stage PLCH. All but two of the patients were current or former cigarette smokers. Most of the patients had moderate to severe preoperative pulmonary hypertension, and approximately one third had extrapulmonary involvement of LCH. The post-transplant survival rates for the patients in this study are 76.9% at 1 year, 63.6% at 2 years, 57.2% at 5 years, and 53.7% at 10 years. Although survival data for more common indications for transplant at these centers were not presented, these survival rates are comparable to data reported in the International Society for Heart and Lung Transplantation Registry during this same period [82]. In addition, there were no differences in survival rates related to the type of transplant procedure performed (i.e. single, double, heart–lung). The incidences of acute cellular rejection and bronchiolitis obliterans syndrome reported for this cohort were also similar to accepted rates in the general lung transplant population.

Clinical and radiographic evidence of PLCH, with or without concomitant histologic confirmation, has been reported following lung transplantation [79–81]. Gabbay et al. [79] described the first case of a patient with symptomatic recurrent PLCH following lung transplantation, a 32-year old-man with a history of diabetes insipidus (presumably from pituitary LCH) and progressive pulmonary failure. A surveillance lung biopsy performed at 24 and 30 months post-bilateral lung transplant revealed infiltration of Langerhans cells, interstitial fibrosis, and aggregates of Langerhans cells around terminal bronchioles. During this same period, the patient developed dyspnea and a progressively restrictive pattern of pulmonary function testing, and HRCT demonstrated findings consistent with interstitial fibrosis. The patient had been a lifelong non-smoker prior to transplant, and did not smoke after transplant. Interestingly, during this same period the patient's diabetes insipidus worsened, requiring increased therapy with nasal desmopressin. Following addition of cyclophosphamide to his maintenance immunosuppression of cyclosporine and prednisolone, his symptoms, lung function, and diabetes insipidus all improved. In this same report, the authors also mentioned four other patients who had undergone transplantation for PLCH at their center; only one of those patients demonstrated findings of Langerhans cells on biopsy.

Habib et al. [81] reported a case of recurrent PLCH in a 27-year-old Asian male who underwent bilateral lung transplantation. The patient smoked cigarettes preoperatively, but did not resume smoking following transplant. Approximately 2 years post-transplant, his respiratory function deteriorated, accompanied by HRCT findings of widespread cystic changes in the mid and lower lung zones of both lungs. This uncharacteristic distribution of disease in the lower lung fields (as opposed to the characteristic upper lung zone predominance normally associated with LCH) was also noted in the native lungs of this patient. Histologic analysis of a transbronchial biopsy specimen from the right lower lobe revealed clusters of interstitial, peribronchiolar, and perivascular Langerhans cells. Chemotherapy consisting of doxorubicin, vincristine, and etoposide was not tolerated, and the addition of cyclophosphamide to the maintenance immunosuppression regimen was ineffective.

Etienne et al. [80] related their single-center experience regarding recurrence of PLCH following lung transplantation, reporting the outcomes of seven patients who underwent lung transplantation for PLCH (six single lung, one heart–lung). Five of the patients did not demonstrate evidence of recurrence, and were alive and well at time intervals ranging from 15 to 90 months. Two patients developed recurrence of LCH in their lung allografts, in each case highlighted by radiologic, histologic, and spirometric abnormalities. Both patients had resumed smoking cigarettes, at 3 and 6 months post-transplant, respectively.

The largest single report of recurrence of PLCH following lung transplantation was described in the French multicenter study previously mentioned [58]. In this study, eight of the 39 patients

transplanted for PLCH (20.5%) developed recurrence of disease, although two of these patients were the same patients reported previously by Etienne et al. [80]. Except for these two patients, only one other patient in the cohort had resumed smoking post-transplant. Recurrence was notably more common in patients with extrapulmonary involvement than those with isolated pulmonary disease (41.7% versus 11.1%).

Although these reports were limited to a total of 10 patients with recurrent PLCH in the lung allograft(s), the findings highlight some interesting observations regarding the pathogenesis of LCH recurrence following lung transplantation. First, only 3 of the 10 patients with recurrence had resumed smoking following transplant. Therefore, it does not appear that smoking alone is responsible for disease recurrence. As previously discussed, there is a very strong association between smoking and the development of pretransplant PLCH. It has been proposed that tobacco injures the bronchiolar epithelium resulting in modification of specific cell surface antigens, recruitment of Langerhans cells, and an increase in the presentation of antigens by Langerhans cells to $CD4^+$ T lymphocytes [59,83]. One could argue that the relatively low rate of PLCH recurrence following lung transplantation may be in large part due to the low incidence of smoking recidivism in this subgroup of patients. Second, extrapulmonary involvement of LCH appears to be an important risk factor for the development of recurrence post-transplant. This observation suggests that recurrence of disease possibly results from the repopulation of the donor allograft by recipient Langerhans cells. It is conceivable that this process may be augmented by bronchiolar epithelial injury from tobacco and/or other environmental stimuli (i.e. respiratory viruses).

Despite the possibility of recurrence of PLCH following lung transplantation, the incidence of clinically significant disease is relatively low. Lung transplantation should therefore remain a viable therapeutic option for patients with refractory progressive lung failure secondary to PLCH, particularly for those patients with significant secondary pulmonary hypertension. Patients should be counseled repeatedly regarding abstinence from smoking following lung transplantation. Surveillance of the lung allograft should include pulmonary function testing, HRCT, and transbronchial biopsy. Surgical lung biopsy should be performed in cases of suspected recurrence if transbronchial biopsy is non-diagnostic. Patients with extrapulmonary LCH should be monitored even more closely for evidence of recurrent disease.

Bronchioloalveolar carcinoma

Bronchioloalveolar carcinoma (BAC) is a rare form of pulmonary carcinoma characterized by growth of well-differentiated neoplastic cells along the walls of alveoli without destruction or invasion of the pulmonary architecture, or lymphatic or systemic metastases [84,85]. BAC therefore represents an in situ carcinoma, although tumors with BAC features and areas of invasion are more common, classified as adenocarcinomas of mixed subtype [84,86,87]. BAC represent only 5% of all non-small cell lung cancers, but as many as 20% of all non-small cell lung cancers are adenocarcinomas with mixed BAC subtype [86,87]. BAC most commonly presents as a solitary nodule, often discovered incidentally on a chest radiograph or HRCT. BAC can also present as multiple nodules or diffuse parenchymal disease, although the latter form usually has features of the mixed adenocarcinoma and BAC type. The nodules of BAC most commonly appear as ground glass opacities on HRCT, although areas of consolidation are also frequently observed [88–92]. Treatment of isolated BAC tumors usually involves sublobar or lobar surgical resection, and multifocal BAC usually requires sublobar resection of all foci, with or without systemic chemotherapy [85,93–96]. The discovery that epidermal growth factor receptor (EGFR) mutations are much more common in BAC than in other lung cancers has led to the use of EGFR-tyrosine kinase inhibitors (TKI) in the treatment of patients with advanced or recurrent BAC [97–99].

Lung transplantation has become a therapeutic modality for a small number of patients with recurrent or unresectable BAC, particularly those with diffuse pneumonic disease [100]. BAC has been considered a controversial indication for lung transplantation because of the significant risk for recurrence [101–105]. The most significant experience in performing lung transplantation for patients with BAC exists at the University of Alabama Lung Transplant Program. Garver et al. [104] from that center initially reported a small series of patients who received lung transplantation for treatment of advanced BAC. Four of seven patients who received single or double lung transplantation developed recurrence of BAC in the allograft(s), although two of these patients enjoyed initial recurrence-free periods of 39 and 48 months. The same group subsequently reported the results of a study to determine whether lung transplantation would provide curative therapy for patients with BAC [100]. Eight patients with confirmed BAC underwent lung transplantation, although it is unclear whether any of these patients were also included in the original report. Six of the eight patients had recurrence of BAC after transplantation, with the time of recurrence ranging from 12 to 75 months post-transplant. Two of the six patients had localized recurrence; one of these patients underwent left lower lobectomy and right middle and lower lobe wedge resections, and the other patient required a left lung pneumonectomy. Four of the six cases of recurrence involved a diffuse pattern of recurrent disease. Two of these four patients died of pulmonary failure, a third underwent retransplantation and died shortly after, and the fourth was still alive at 39 months post-transplant with recurrent disease. Despite the very high rate of recurrence in this small cohort, the 5-year survival rate was 52%, a rate similar to the total 5-year survival rate at the authors' transplant center. However, based on the high recurrence rate of 75% in the original cohort, the authors decided to terminate the study.

The origin of recurrent BAC tumors in the lung allograft has been a topic for debate. Most reports of multifocal BAC support a monoclonal origin of tumor followed by spread via intra-alveolar, lymphatic, or aerosolization routes [101,106,107]. In the cohort of patients reported by the University of Alabama group, analysis of the recurrent tumors in the allografts of three of the patients revealed similar radiographic and histologic characteristics when compared with the original tumors. Subsequent PCR amplification of microsatellite DNA showed similar fragments in the recurrent tumor compared with the original tumor in all three patients [103,104]. These findings support the hypothesis that recurrent BAC after transplantation originates from recipient neoplastic cells. This hypothesis was further supported in a published case report of a transplant recipient with BAC, in which the authors reported the results of molecular fingerprinting on DNA extracted from original and recurrent tumors [101]. Analysis of 15 highly polymorphic loci revealed that the neoplastic cells were similar in the primary and recurrent tumors.

Lymphatic spread has been the mechanism most commonly proposed for the recurrence of BAC following lung transplantation. However, because most of the cases of recurrent BAC following

lung transplantation have not demonstrated evidence of lymph node metastases, either at the time of transplant or at the time of resection of recurrent tumors, this explanation does not seem plausible. It is therefore possible that contamination of the allograft may occur as a result of aerosolization of neoplastic cells from the native lungs. In light of this possibility it has been proposed that meticulous surgical technique and airway irrigation have a vital role in preventing recurrence of BAC following transplantation [101,102]. Despite these advances in the understanding of BAC and peri/post-transplant surgical management of patients with BAC, lung transplantation remains a controversial therapeutic modality for this indication.

Pulmonary alveolar proteinosis

Pulmonary alveolar proteinosis (PAP) is an uncommon lung disease characterized by the accumulation of surfactant protein in the alveolar spaces. The pathogenesis of PAP remains poorly understood, although significant advances in potential therapies have occurred during the past decade. PAP likely involves a combination of abnormal production of surfactant by type II pneumocytes, and impaired clearance of surfactant by alveolar macrophages [108–112]. PAP generally occurs as one of three different types of disorders:

1 A congenital group of diseases resulting from mutations in the genes expressing surfactant proteins and/or granulocyte–macrophage colony-stimulating factor (GM-CSF);
2 In association with certain chemical or mineral dust exposures, or in the setting of certain hematological malignancies and immunodeficiency disorders; or
3 Idiopathic, or primary PAP, with no obvious etiology.

This latter idiopathic form is the most common presentation, accounting for almost 90% of all cases of PAP [111,113]. Whole-lung lavage is generally the only effective therapeutic modality, indicated primarily for the treatment of the idiopathic form of PAP [114–116]. GM-CSF is believed to have a significant role in the pathogenesis of PAP, and autoantibodies to GM-CSF have been discovered in most adult patients with the idiopathic form. These findings have led to the use of exogenous GM-CSF as a therapeutic approach to PAP, but the reported efficacy of this approach has been variable [116–120].

Lung transplantation rarely has been performed for progressive lung failure secondary to PAP [121–125]. Recurrence of PAP in the allograft following lung transplantation has been described [122,123]. Parker et al. [123] reported a case of recurrent PAP in a 44-year-old woman 3 years following bilateral lung transplantation for PAP. At that time she presented with fatigue and dyspnea, and chest radiograph revealed new infiltrates. Histologic analysis of transbronchial biopsies revealed alveolar air spaces filled with eosinophilic dense flocculent material, along with the presence of scattered foamy macrophages and cholesterol clefts. Electron microscopy confirmed the presence of surfactant accumulation.

Santamaria et al. [122] subsequently described fatal recurrent PAP in a child who had undergone heart–lung transplantation for PAP secondary to lysinuric protein intolerance (LPI). The patient was a 3-year-old boy who had previously been diagnosed with LPI, a rare genetic disorder caused by the defective plasma membrane transport of cationic amino acids. Pulmonary manifestations, primarily characterized by severe PAP, began more than 1 year prior to transplant. Attempts at therapy with whole lung lavage and GM-CSF were unsuccessful, and the child eventually underwent successful heart–lung transplantation. Eighteen months after transplantation, he developed severe dyspnea and hypoxemia, presumed to be secondary to a severe viral pneumonia. However, subsequent transbronchial biopsy revealed the presence of PAP in the allograft, with no evidence of infection or rejection. Neither therapy with whole-lung lavage nor GM-CSF therapy led to any improvement, and the child eventually died 26 months after transplantation.

The pathogenesis of recurrent PAP following lung transplantation is poorly understood. The presence of circulating antibodies directed against GM-CSF has been proposed as a potential mechanism in the development of PAP, and could be an explanation for recurrence in the allograft. A second hypothesis is that PAP results from a defect in bone marrow-derived monocytes, such as a dysfunctional GM-CSF receptor. In this scenario, lung transplantation may not be beneficial because immature alveolar macrophages, unable to clear surfactant effectively, would continue to be ineffective following transplantation [122,126–128].

Previous published reports describing the development of de novo PAP in lung transplant recipients have provided some insight into the mechanism behind recurrence of native PAP [129,130]. Yousem et al. [129] described three patients who developed PAP following lung or heart–lung transplant, despite lacking evidence of PAP prior to transplant. The authors hypothesized that repeated allograft injury resulting from primary graft injury, rejection, and infections, in addition to the use of chronic corticosteroid therapy, may have resulted in excessive surfactant production in the alveolar spaces. Gal et al. [130] identified a patient who developed de novo PAP 66 days after undergoing single lung transplantation for pulmonary fibrosis. The diagnosis of PAP was based on the persistent finding of amorphous, acellular, finely granular material in bronchoalveolar lavage (BAL) fluid. The patient died 35 months later from progressive BOS and cytomegalovirus pneumonitis. Autopsy examination of both lungs confirmed the presence of PAP in the native lung, but not the allograft. The authors suggested that immunosuppressive medications might have impaired alveolar macrophage function, and ability to clear surfactant. They also concluded that careful examination of BAL fluid might be the most sensitive method in diagnosing PAP following lung transplantation.

Emphysema secondary to alpha-1 antitrypsin deficiency

Alpha-1 antitrypsin deficiency (ATD) is a genetic disorder that effects almost 3.4 million individuals worldwide, and may account for up to 2% of all cases of COPD in the United States [131,132]. The most frequently affected organs are the liver and lung. Liver disease, which often progresses to cirrhosis, occurs most likely as a result of the accumulation of an altered form of alpha-1 antitrypsin protein [133]. Lung disease, primarily in the form of panlobular emphysema beginning in the fourth or fifth decade, is a result of a reduction in circulating alpha-1 antitrypsin protein. Emphysema likely results from a protease–antiprotease imbalance, leading to unopposed proteolytic enzyme activity. An increase in inflammatory conditions also seems to be an important co-factor, and cigarette smoking probably plays the most important part in the ultimate development of lung disease [134,135]. Treatment of alpha-1 ATD has focused primarily on alpha-1 antitrypsin replacement therapy, in the form of intravenous human plasma-derived therapy, inhalation therapy, recombinant therapy, or synthetic elastase inhibition [136–139].

Lung transplantation has been a successful treatment modality for patients with advanced emphysema secondary to alpha-1 ATD. Almost 2000 lung transplant procedures (728 single, 1225 double) have been performed for patients with alpha-1 ATD since 1995, representing approximately 6.5% of all transplants [14]. Patients undergoing lung transplantation for alpha-1 ATD have a post-transplant survival half-life of 6.3 years, second only to patients with cystic fibrosis (7.4 years) [14].

Indeed, lung transplantation addresses the emphysematous damage to the native lungs. However, it does not treat the etiology of the lung disease, the systemic deficiency in alpha-1 antitrypsin. It would therefore seem likely that recurrent emphysema following lung transplantation would be quite common. However, there were no published reports of recurrent emphysema secondary to alpha-1 ATD until 2002, when Glanville et al. [140] reported two patients with postmortem histologic findings of alpha-1 ATD in the lung allografts. Neither of these patients was suspected of having recurrent disease prior to the autopsy findings.

Mal et al. [141] subsequently published a report of recurrent emphysema in the allograft of a patient with alpha-1 ATD 10 years following single lung transplantation. At that time a chest CT revealed findings of emphysema in the allograft, and during the next 2 years the patient had a progressive deterioration in lung function. Interestingly, the patient had experienced a number of episodes of acute rejection in the first 18 months post-transplant, and had resumed smoking cigarettes approximately 3 years prior to the CT findings of emphysema. The authors hypothesized that the initial inflammation associated with repeated acute rejection, coupled with the resumption of smoking, may have triggered the recurrence of the emphysema. In addition, a high level of elastase activity was found in the BAL fluid and serum of the patient. Prior studies have demonstrated that high levels of unopposed elastase activity are present in BAL fluid obtained from lung transplant recipients during periods of significant inflammation associated with allograft rejection or infection [142,143].

In light of the potential role for increased unopposed elastase activity in lung transplant recipients with alpha-1 ATD, it has been proposed that supplementation of alpha-1 antitrypsin following lung transplantation may be beneficial [142–145]. As suggested by Mal et al. [141], replacement therapy with alpha-1 antitrypsin may be particularly helpful in patients with BOS. BOS is associated with an increased concentration of neutrophils in the airways and parenchyma, potentially resulting in a significant increase in elastase-mediated injury. Many lung transplant programs have adopted a practice of intravenous replacement of alpha-1 antitrypsin for patients with signs of BOS. Some centers even initiate alpha-1 antitrypsin replacement therapy for all alpha-1 ATD patients shortly after transplant surgery, although at present there is little conclusive evidence to support this practice.

Other lung diseases recurring after transplant

There have been a number of published case reports describing recurrence of native lung disease in the pulmonary allograft, in most cases involving very uncommon indications for lung transplant.

Desquamative interstitial pneumonia

Desquamative interstitial pneumonia (DIP) is a rare interstitial pneumonia characterized by the accumulation of large numbers of macrophages in the alveolar spaces, likely in response to injury to the alveolar epithelium. DIP has a strong association with cigarette smoking, but other types of drugs and inhaled agents have also been linked to the development of disease. Although most patients with DIP improve with immunosuppressive treatment, a small number develop irreversible pulmonary fibrosis and require lung transplantation [146,147].

Recurrence of DIP in the allograft following lung transplantation has been reported [148,149]. King et al. [148] described a 50-year-old woman with DIP who underwent a left single lung transplant. One month following transplant the patient developed progressive cough and dyspnea in the absence of radiographic findings, and a thoracoscopic lung biopsy demonstrated evidence of DIP. Despite repeated therapy with high dose corticosteroids, her condition continued to deteriorate, and she eventually died of respiratory failure. Autopsy findings confirmed the presence of diffuse DIP in the allograft. Verleden et al. [149,150] subsequently published a second case of recurrent DIP following lung transplantation. In this case, however, DIP did not recur in the allograft until 1 year post-transplant, confirmed by the characteristic histologic findings noted on transbronchial biopsy. In contrast to the refractory course (ultimately leading to death) reported by King et al. [148], the patient in this report completely recovered following a prolonged course of increased corticosteroids.

Giant cell interstitial pneumonia

Giant cell interstitial pneumonia (GIP) is a rare form of interstitial pneumonia associated with exposure to certain hard metals, characterized by the alveolar accumulation of mineral particles and enlarged multinucleated macrophages [151,152]. Frost et al. [153] reported a case of recurrence of GIP in the allograft of a patient who had undergone single-lung transplantation for GIP secondary to cobalt exposure. The interesting observation in this case was that the patient did not have any further exposure to cobalt following lung transplantation to explain the recurrence of disease. Furthermore, eventual autopsy analysis of the allograft revealed characteristic findings of GIP, but no evidence of inorganic particles. The authors hypothesized an autoimmune etiology for the recurrent disease. It is conceivable that inorganic particles in the existing native lung or from an extrapulmonary source may have served as a stimulus for the autoimmune response.

Diffuse panbronchiolitis

Diffuse panbronchiolitis is an uncommon complex genetic lung disease almost exclusively affecting East Asians, with a strong association with Class I HLA-B54 and HLA-A11. It is characterized by chronic inflammation in respiratory bronchioles, and chronic bacterial infections in the airways and sinuses. Pulmonary function testing reveals a pattern of airway obstruction and hyperinflation. Histologic analysis of lung tissue reveals lymphocytes and foamy macrophages around respiratory bronchioles. The use of macrolide therapy has dramatically improved the prognosis of patients with diffuse panbronchiolitis, although the mechanism of action is poorly understood. Some patients do not respond to therapy and progress to lung failure, rarely necessitating consideration for lung transplantation [154].

Only one case of recurrence of diffuse panbronchiolitis has been reported following lung transplantation. Baz et al. [155] described a patient who developed recurrence of diffuse panbronchiolitis 10 weeks after bilateral lung transplantation, resulting in a very rapid deterioration in allograft function. Therapy with erythromycin was

initiated, and allograft function significantly improved within a few weeks. Although this was an isolated single report of a rare indication for lung transplantation, the findings may support the use of macrolide therapy in patients following lung transplantation for diffuse panbronchiolitis.

Pulmonary capillary hemangiomatosis

Pulmonary capillary hemangiomatosis (PCH) is a rare etiology of pulmonary hypertension characterized by uncontrolled capillary proliferation in the pulmonary interstitium and vascular and alveolar walls. HRCT findings include centrilobular nodular opacities, septal lines, pleural effusions, and lymphadenopathy, although similar findings occur with pulmonary veno-occlusive disease. PCH is a rapidly progressive disease, and lung transplantation remains the only treatment [156–159].

Few published data exist concerning lung transplantation for PCH, but recurrence of disease has been described. The first report of PCH following lung transplantation occurred in a patient 3 months after undergoing bilateral lung transplantation for another indication (emphysema) [160]. The authors hypothesized that the de novo occurrence of PCH resulted from uncontrolled angiogenesis as a result of an infectious or inflammatory trigger.

Lee et al. [161] subsequently reported the only published case of recurrence of native PCH following lung transplantation. The authors described a patient who developed clinical and histologic findings consistent with recurrent PCH 6 months following bilateral lung transplantation for an original diagnosis of PCH. Interestingly, her early postoperative course had been complicated by recurrent acute cellular rejection, bronchiolitis obliterans, and organizing pneumonia. The patient died 8 months after transplant, and autopsy analysis revealed extensive bilateral findings consistent with PCH with associated veno-occlusive disease. The authors postulated that the recurrence of PCH resulted from recurrent early allograft injury, leading to the recruitment to the lung of circulating endothelial cells with a genetic predisposition to abnormal proliferation.

Williams–Campbell syndrome

Williams–Campbell syndrome is a very rare disorder affecting children, characterized by a deficiency of cartilage in the subsegmental bronchi resulting in airway collapse and bronchiectasis. The clinical course is quite variable; some children rapidly progress to respiratory failure, and others survive into adulthood suffering from recurrent infections and airway obstruction [162,163]. Although there is no known treatment, there has been only one report in the literature involving lung transplantation for this disorder. Palmer et al. [164] described a 28-year-old male who underwent bilateral lung transplantation for respiratory failure secondary to Williams–Campbell syndrome. Although the patient initially did well, his post-transplant course was complicated by severe proximal bronchomalacia of the mainstem bronchi, leading to recurrent infections and death 1 year following transplantation. The authors postulated that although the cartilage deficiency in this syndrome typically involves distal airways, there may have been a more subtle decrease in cartilage in the proximal airways, leaving them susceptible to the effects of post-transplant airway ischemia. Impaired bronchial perfusion post-transplant has been linked to the development of bronchomalacia associated with obliterative bronchiolitis [165]. Based on the airway complications encountered in this case, the authors recommended that bilateral lung transplantation not be considered for this indication, but suggested that perhaps *en bloc* bilateral lung transplantation may be a viable option.

Conclusions

Recurrence of native lung disease has been reported for several types of native lung disease following lung transplantation (Box 80.1). Sarcoidosis is the most common etiology for recurrent disease following transplantation, with a rate of recurrence as high as 80%. However, most of the cases of recurrence are limited to incidental biopsy findings, with little obvious impact on graft function. This mild nature of recurrent disease compared with the primary native disease may in large part be related to the use of post-transplant immunosuppressive agents. BAC commonly recurs following lung transplantation, with a reported recurrence rate as high as 75%. Despite the significant amount of morbidity and surgical intervention resulting from recurrence, post-transplant survival outcomes for patients with BAC are comparable to other indications. LAM and PLCH may also commonly recur following transplant, and, as a result of the obstructive physiology associated with these diseases, may often be misdiagnosed as BOS. Alveolar proteinosis and DIP are very rare indications for transplant, but may have a high incidence and potential for recurrence. Recurrence of emphysema from alpha-1 antitrypsin deficiency may be underappreciated, but may become more prevalent as recipients survive for longer post-transplant periods.

The potential for recurrence of native lung disease highlights the importance and utility of post-transplant allograft surveillance. Routine chest radiography and HRCT may identify specific findings consistent with recurrent disease, particularly in the cases of BAC, PLCH, and LAM. Radiographic patterns of recurrent disease most often mimic those of the native pretransplant findings. Pulmonary function testing may detect an obstructive defect characteristic of diseases such as sarcoidosis, LAM, PLCH, and emphysema, although these diagnoses may be difficult to distinguish from allograft rejection or infection based on pulmonary function testing alone. Transbronchial or surgical biopsies remain the gold standard for confirmation of recurrent disease, highlighting another benefit for the practice of post-transplant surveillance lung biopsies.

The pathogenesis of most types of native lung disease recurrence following transplantation remains poorly understood. In cases of sarcoidosis and LAM, in situ hybridization and immunohistochemical studies have demonstrated that recurrent disease likely results from infiltration of recipient immune cells into the allograft. The association between extrapulmonary involvement of LCH and recurrence of PLCH following transplant also supports a possible mechanism of metastases and infiltration of recipient immune cells (Langerhans cells). Recurrent BAC possibly results from aerosolization of neoplastic cells from the native lungs, perhaps at the time of transplant. In the cases of recurrent PAP, DIP, and GIP, few published data exist regarding the mechanism of recurrence, although it has been proposed for both diseases that abnormal recipient immune cells (monocytes/macrophages) may have a significant role. A similar mechanism may occur in the recurrence of PCH involving recruitment of recipient circulating aberrant endothelial cells. Recurrent emphysema from alpha-1 antitrypsin deficiency likely results from a lack of the protective effect of a deficient proteinase in the face of ongoing inflammation in the allograft (i.e. secondary to BOS).

The recognition that native lung diseases may recur following transplant affords the opportunity for using prophylactic and/or

early treatment interventions. mTOR inhibitor drugs may be effective at preventing or treating recurrence of LAM following transplant. Augmentation of immunosuppression or systemic chemotherapy may be effective in treating recurrent PLCH and sarcoidosis. Replacement therapy with alpha-1 antitrypsin may help to prevent the onset or progression of recurrent emphysema. The incidence and impact of smoking recidivism following lung transplantation has not been as well-studied as alcohol recidivism following liver transplantation. However, abstinence from, or cessation of, cigarette smoking may play a significant part in preventing recurrence of PLCH, DIP, and alpha-1 antitrypsin deficiency-related emphysema. Precise transplant surgical technique and careful irrigation of native airways may help prevent aerosolization and contamination of the allograft with neoplastic cells.

Given the potential for recurrence of certain native lung diseases following lung transplantation, one may question whether transplant should be considered as a therapeutic option for these indications. Although in some of these diseases the rates of recurrence may be quite high, with a few exceptions, the incidence of significant graft dysfunction and morbidity secondary to recurrence is relatively low. BAC represents the most important exception, as recurrence is quite common and necessitates radical, often recurrent, surgical intervention. In light of this likelihood, BAC remains a controversial indication for lung transplant. It is important to again point out, however, that despite the morbidity associated with recurrence of BAC, post-transplant survival rates have been comparatively good.

In conclusion, native lung disease may recur following lung transplantation, with variable impact on allograft function. Recurrence of lung disease has been most often described for less common indications for lung transplantation. In most cases, the possibility of recurrence should not preclude transplant as a therapeutic modality. However, the transplant recipient and transplant physician or surgeon should be aware of the potential for, and clinical signs of, recurrent disease. Specific prophylactic behavioral and medical therapies and early treatment interventions should be implemented in the appropriate clinical settings.

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CHAPTER 81

Histopathological Syndromes of Kidney Allograft Rejection and Recurrent Disease

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Introduction

Renal transplantation has been conducted with increasing success for nearly five decades [1]. Since the first successful renal transplant in 1954 [2], immunosuppression has become progressively more effective in preventing and treating acute rejection episodes, particularly those occurring less than 1 year post-transplantation. However, challenges still exist in treating late cellular and antibody-mediated rejection (AMR), as well as in preventing chronic rejection and recurrent disease [1].

Renal biopsies offer a useful diagnostic aid for clinicians, often leading to changes in the treatment plan in up to approximately 40% of patients. Renal biopsies lead to a decrease in immunosuppression levels in around 20% of patients [3–5]. Most renal allograft biopsies involve the analysis of routine light microscopic findings; however, ancillary techniques (i.e. fluorescence and electron microscopy (EM)) are crucial to renal allograft biopsy analysis, and new tools such as molecular assessments are rapidly emerging. Renal biopsies are important at all time points in the life of an allograft: before transplantation, when pretransplant donor biopsies are employed in triaging available organs; early after transplantation, when allograft rejection is important; and late after transplantation, when considerations include chronic rejection, recurrent and/or de novo disease, and infection [3]. This chapter provides the reader with an understanding of the major types of graft rejection, other relevant disorders in renal allografts, and the types of de novo and recurrent disease that can occur.

Graft rejection

Before the establishment of the Banff classification in 1991 by Dr. Kim Solez and others, no unified diagnostic criteria for diagnosing rejection were available; diagnoses were rendered based on local experience and individual pathologists' judgment [6–12]. Since the early 1990s [9], consensus criteria have been developed and constantly refined by panels of experts, initially for renal allograft pathology and then for the pathology of other allograft organs [9]. Renal allograft rejection can typically be attributed to either cellular- or antibody-mediated (humoral) rejection mechanisms, or a combination of the two [5,13].

Acute rejection refers to a relatively rapid deterioration of renal function due to immunologic injury on the allograft, typically within days. Although the term "acute" is used, an episode of acute rejection can occur from hours to years after transplantation, but typically evolves over a shorter period of time [14]. In contrast, chronic rejection refers to a slow deterioration of renal function through smoldering injury to the renal allograft, typically evolving and occurring over the space of months to years [14]. Furthermore, the term "chronic" is used when respective morphologic changes of structural remodeling in the allograft are seen (e.g. intimal arterial fibrosis or glomerular basement double contours). While these processes differ in regard to their typical clinical characteristics, their diagnosis relies on examination of an allograft biopsy.

As defined in the Banff 1997 criteria, an "adequate" biopsy is one with at least 10 glomeruli and two arteries, and a "minimal" sample is one with seven glomeruli and one artery. At least two cores are recommended. Alternatively, it is suggested that at least two separate areas of cortex be on the same core. For evaluation of renal allograft biopsies, it is best if there are seven slides containing multiple sequential 3–4 μm sections stained with the following stains: three with hematoxylin and eosin, three with periodic acid–Schiff (PAS) or silver stains, and one with a trichrome stain [7,15]. Furthermore, since C4d staining is currently part of the Banff classification, C4d is typically performed using fluorescence or immunohistochemistry [8]. Biopsies are scored according to histologic criteria, with pathologic lesions scored semi-quantitatively in all morphologic compartments of the kidney, and, following the thresholds of certain criteria, cases are fitted into consensus Banff diagnoses (Tables 81.1 and 81.2). It should be noted that variation in histologic grading can be substantial, showing limitations in interobserver agreement and providing opportunities for future refinements and technical improvements in the process [16]. As the effector mechanisms involved in rejection are active in other immune-mediated renal diseases, some clinical correlation of the histologic findings may at times be required.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

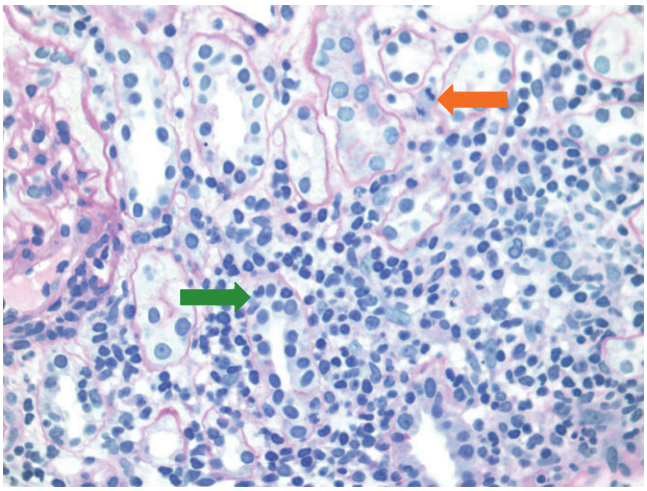


Figure 81.1. Interstitial inflammation and tubulitis. Interstitial inflammation with an "activated" infiltrate displays conspicuous mitotic activity (orange arrow) and tubulitis (green arrow) in acute cellular rejection, type 1.

Cell-mediated rejection

Cell-mediated rejection (CMR), under most circumstances, is conducted by effector T cells originating from the lymphoid organs and infiltrating the allograft, leading to an inflammatory response (covered in detail in Chapter 5). Thus, the term "cell-mediated rejection" typically refers to T-cell-mediated rejection (TCMR). The T cells are thought to be reactive against donor alloantigen [14,17]. The interstitium is permeated by this cellular infiltrate, eventually leading to tubulitis lesions (i.e., the infiltration of T cells between tubular epithelial cells), an early hallmark of many rejection episodes (Figure 81.1) [5,18]. Most of the cells in the interstitium are composed of $CD4^+$ and $CD8^+$ T cells and $CD68^+$ monocyte–macrophages. Eosinophils may also be present, possibly indicative of a worse outcome [13,19]. Tubules may also display increased HLA-DR expression [20]. However, although tubulitis is frequently considered fairly specific for TCMR, recent data suggest that tubulitis is the consequence rather than the cause of epithelial injury [21]. Furthermore, tubulitis can be observed in other disease of renal allograft and also in native kidneys.

Acute tubulointerstitial rejection consists of interstitial edema with a mononuclear infiltrate accompanied by tubulitis lesions. For a diagnosis fulfilling the criteria for tubulointerstitial acute CMR under Banff criteria (Table 81.1), the interstitial infiltrate and tubulitis are given an "i" and "t" score respectively (as shown in Table 81.2). Acute CMR, type IA, consists of renal allografts displaying tubulitis reaching a threshold of "moderate" severity (t2, or 5–10 inflammatory cells/tubular cross-section) when accompanied by a "significant" inflammatory infiltrate (i2 or i3, >25% of the non-scarred cortical parenchyma affected). If the severity of tubulointerstitial rejection process is greater, consisting of more intense tubulitis (t3), then a designation of acute CMR, type IB, can be made, which has been shown to be associated with a worse prognosis. Such more severe tubulointerstitial acute CMR is primarily characterized by multifocal rupture–destruction of tubular basement membranes, putatively indicating irreversible destruction of nephrons [8,22].

In the category of tubulointerstitial rejection, a "gray zone" exists in which either only tubulitis (t1, 2, or 3) is present without a substantial interstitial infiltrate (having only i0 or i1) or an interstitial infiltrate is accompanied by only mild tubulitis (t1). Such cases are termed "borderline" or "suspicious" for acute CMR [8]. The interpretation of such lesions is controversial and often depends on the center or the study protocol. Some centers interpret these borderline cases as rejection while others do not [5]. A recent study using gene expression analysis with borderline cases revealed that one third of these cases present with a molecular phenotype identical to "true" TCMR cases, while two thirds were similar to cases showing no evidence of rejection [23]. Past studies have indicated that conservative therapy may be appropriate in some of these cases [24]. Therefore, ultimately it is hoped that further research and guidelines will clarify the significance of the borderline category and ultimately contribute to the elimination of this ambiguous gray zone [23].

Plasma cell-rich forms of rejection may be present, displaying marked numbers of interstitial plasma cells. In addition, some cases may have prominent edema. In the past these forms of rejection have often been associated with a more aggressive course and may portend a worse prognosis. However, most of these cases were described prior to robust antibody testing and screening for polyomavirus, and thus may actually represent other underlying entities like AMR and polyomavirus nephritis (PVAN). In some of these cases C4d deposition in peritubular capillaries (PTCs) may be detected; however, the AMR component in many of these cases may be attenuated, late in their course, and co-exist with the cellular component. Also, PVAN frequently presents a plasma cell-rich interstitial infiltrate, edema, and tubulitis and thus represents a challenging differential diagnosis. When present, plasma cell-rich infiltrates must also be distinguished from post-transplant lymphoproliferative disorder (PTLD) by staining for kappa and lambda light chains and, with this testing, for potential clonality of the plasma cells [25–27].

In addition, vessels may be infiltrated by inflammatory cells, leading to undermining of the endothelium, a process variably referred to as endothelialitis, vascular rejection or, more accurately, endarteritis (Figure 81.2) [5]. If mild to moderate endarteritis is present (designated v1), a rejection episode is designated acute CMR, type IIA under Banff criteria. If severe (v2) arteritis is present, a designation of acute CMR, type IIB can be rendered [8]. These cases have been shown to be associated with a worse prognosis than Banff type IIA cases [28]. Cases with transmural arteritis and/or arterial fibrinoid change (considered v3) are given a designation of acute CMR, type III; however, it is generally recognized that such lesions may often have an antibody-mediated component [5,8]. It is important to note that the term "vascular rejection" is also frequently used in the context of pure AMR particularly in the xenograft literature. Studies have indicated a worse prognosis for cases with endarteritis, but it must be noted that most of these studies were carried out prior to sensitive antibody testing [29].

Data presented at the 2011 Banff meeting indicated that cases with "isolated v-lesions" (i.e. endarteritis in the absence of significant tubulointerstitial inflammation or capillaritis) might actually represent a heterogeneous group of disease processes. Results from a retrospective multicenter case cohort study were presented. After the exclusion of AMR cases, two additional types of isolated v-lesion cases were identified: those associated with TCMR and those with delayed graft function. Thus, a more differentiated diagnostic and tailored therapeutic approach toward cases with endarteritis seems warranted [30].

Table 81.1. Banff classification for renal allograft pathology: diagnostic categories summarized with updates

Category	Features
1	**Normal**
2	**Ab-mediated changes (± Cat. 3, 4, 5, 6)**
	Acute/active ABMR; all three features must be present for diagnosis[a,b]
	1 Histologic evidence of acute tissue injury, including one or more of the following:
	• Microvascular inflammation (g > 0[c] and/or ptc > 0)
	• Intimal or transmural arteritis (v > 0)[d]
	• Acute thrombotic microangiopathy, in the absence of any other cause
	• Acute tubular injury, in the absence of any other apparent cause
	2 Evidence of current–recent antibody interaction with vascular endothelium, including at least one of the following:
	• Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)*
	• At least moderate microvascular inflammation ([g + ptc] > 2)[e]
	• Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated[f]
	3 Serologic evidence of donor-specific antibodies (HLA or other antigens)
	Chronic, active ABMR; all three features must be present for diagnosis[a,g]
	1 Morphologic evidence of chronic tissue injury, including one or more of the following:
	• Transplant glomerulopathy (cg > 0)[h], if no evidence of chronic TMA
	• Severe peritubular capillary basement membrane multilayering (requires EM)[i]
	• Arterial intimal fibrosis of new onset, excluding other causes[j]
	2 Evidence of current–recent antibody interaction with vascular endothelium, including at least one of the following:
	• Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)*
	• At least moderate microvascular inflammation ([g + ptc] > 2)[e]
	• Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated[f]
	3 Serologic evidence of donor-specific antibodies (HLA or other antigens)
	C4d staining without evidence of rejection; all three features must be present for diagnosis[j]
	1 Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)*
	2 g = 0, ptc = 0, cg = 0 (by LM and by EM if available), v = 0; no TMA, no peritubular capillary basement membrane multilayering, no acute tubular injury (in the absence of another apparent cause for this)
	3 No acute cell-mediated rejection (Banff 97 type 1A or greater) or borderline changes
3	**Borderline changes: "suspicious" for acute T-cell-mediated rejection (± Cat. 2, 5, 6)**
	No arteritis but present are tubulitis (t1, t2, or t3) with minor interstitial infiltration (i0 or i1) or interstitial infiltration (i2, i3) with mild tubulitis (t1)
4	**T-cell-mediated rejection (± Cat. 2, 5, 6)**
	• Acute TCMR
	IA. Significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2)
	IB. Significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3)
	IIA. Mild to moderate intimal arteritis (v1)
	IIB. Severe intimal arteritis comprising >25% of the luminal area (v2)
	III. "Transmural" arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle with accompanying lymphocytic inflammation
	• Chronic active T-cell-mediated rejection
	"Chronic allograft arteriopathy" (arterial intimal fibrosis with mononuclear infiltration of fibrosis, formation of neointima)
5	**Interstitial fibrosis and tubular atrophy (IFTA), no evidence of specific etiology**
	(Graded according to IFTA; may also have non-specific vascular and glomerular sclerosis)
	I. Mild IFTA (<25% of cortical area)
	II. Moderate IFTA (25–50% of cortical area)
	III. Severe IFTA/loss (>25% of cortical area)
6	**Other**
	Changes not due to rejection – acute or chronic – ay include cg or cv lesions, ± Cat. 2, 3, 4, 5

Source: Sis et al. 2010 [8] and Haas et al. 2014 [36]. Adapted with permission from John Wiley and Sons.

Ab, antibody; ABMR, antibody-mediated rejection; C4d+, C4d deposition in peritubular capillary endothelium according to Table 81.2; Cat., category; EM, electron microscopy; cg, Banff chronic transplant glomerulopathy score; g, Banff glomerulitis score; i, Banff interstitial inflammation score; IFTA, interstitial fibrosis and tubular atrophy; ptc, Banff peritubular capillaritis score; TCMR, T-cell-mediated rejection, ± may coincide with; v, Banff arteritis score.

*In lieu of C4d deposition, ABMR can be diagnosed if either at least moderate microvascular inflammation is present ([g+ptc] ≥ 2]) or if there is a valid gene transcript measurement of increased endothelial injury.

[a]For all ABMR diagnoses, it should be specified whether the lesion is C4d-positive (C4d2 or C4d3 by IF on frozen sections; C4d > 0 by IHC on paraffin sections) or without evident C4d deposition (C4d0 or C4d1 by IF on frozen sections; C4d0 by IHC on paraffin sections.

[b]These lesions may be clinically acute, smoldering, or subclinical. Biopsies showing two of the three features, except those with donor-specific antibodies and C4d without histologic abnormalities potentially related to ABMR or TCMR (C4d staining without evidence of rejection; see footnote k, below) may be designated as "suspicious" for acute/active ABMR.

[c]Recurrent/de novo glomerulonephritis should be excluded.

[d]Arterial lesions may be indicative of ABMR, TCMR, or mixed ABMR/TCMR. "v" lesions are only scored in arteries having a continuous media with two or more smooth muscle layers.

[e]In the presence acute T-cell-mediated rejection, borderline infiltrates, or evidence of infection, ptc ≥2 alone is not sufficient to define moderate microvascular inflammation and g must be ≥1.

[f]At present the only validated molecular marker meeting this criterion is ENDAT expression, and this has only been validated in a single center (University of Alberta). The use of ENDAT expression at other centers or other test(s) of gene expression within the biopsy as evidence of ABMR must first undergo independent validation as was done for ENDAT expression by Sis et al. [8].

[g]Lesions of chronic, active ABMR can range from primarily active lesions with early transplant glomerulopathy (TG) evident only by electron microscopy (cg 1a) to those with advanced TG and other chronic changes in addition to active microvascular inflammation. In the absence of evidence of current/recent antibody interaction with the endothelium (those features in section 2), the term active should be omitted; in such cases donor-specific antibodies may be present at the time of biopsy or at any previous time post-transplantation.

[h]Includes glomerular basement membrane (GBM) duplication by electron microscopy only (cg1a) or GBM double contours by light microscopy.

[i]≥Seven layers in one cortical peritubular capillary and ≥5 in two additional capillaries, avoiding portions cut tangentially.

[j]While leukocytes within the fibrotic intima favor chronic rejection, these are seen with chronic TCMR as well as chronic ABMR, and are therefore helpful only if there is no history of TCMR. An elastic stain may be helpful as absence of elastic lamellae is more typical of chronic rejection and multiple elastic lamellae are most typical of arteriosclerosis, although these findings are not definitive.

[k]The clinical significance of these findings may be quite different in grafts exposed to anti-blood-group antibodies (ABO-incompatible allografts), where they do not appear to be injurious to the graft and may represent accommodation, and anti-HLA antibodies where more clinical outcome data are needed.

Table 81.2. Banff scores based on quantitative criteria (data from [7] and [36])

Lesions	Abbreviation	Banff score			
		0	**1**	**2**	**3**
Interstitial inflammation[a]	**i**	Interstitial mononuclear inflammatory cells in <10% of non-scarred cortex	Interstitial mononuclear inflammatory cells in 10–25% of non-scarred cortex	Interstitial mononuclear inflammatory cells in 26–50% of non-scarred cortex	Interstitial mononuclear inflammatory cells in >50% of non-scarred cortex
Tubulitis[b]	**t**	No tubulitis	1–4 mononuclear inflammatory cells/tubular cross-section	5–10 mononuclear inflammatory cells/tubular cross-section	>10 mononuclear inflammatory cells/tubular cross-section or two areas of tubular basement membrane destruction accompanied by i2/i3 inflammation and t2 tubulitis elsewhere in biopsy
Intimal arteritis[c]	**v**	No arteritis	Subendothelial mononuclear inflammatory cells involving <25% of luminal area	Subendothelial mononuclear cells involving >25% of luminal area, no necrosis	Transmural inflammation and/or arterial fibrinoid necrosis with mononuclear cells
Glomerulitis[d]	**g**	No glomerulitis	Mononuclear cells in <25% of glomeruli	Mononuclear cells in 26–75% of glomeruli	Mononuclear cells in >75% of glomeruli
Peritubular capillaritis[e]	**ptc**	Absent or <10% of cortical PTCs with inflammatory cells	3–4 luminal inflammatory cells in ≥10% of cortical PTCs	5–10 luminal inflammatory cells in ≥10% of cortical PTCs	>10 luminal inflammatory cells in ≥10% of cortical PTCs
Transplant glomerulopathy[f]	**cg**	Double contours in <10% of capillary loops in most severely affected glomerulus	Double contours in 10–25% of capillary loops in most severely affected glomerulus	Double contours in 26–50% of capillary loops in most severely affected glomerulus	Double contours in >50% of capillary loops in most severely affected glomerulus
Mesangial matrix increase[g]	**mm**	No mesangial matrix increase	Present in up to 25% of non-sclerotic glomeruli	Present in 26–50% of non-sclerotic glomeruli	Present in >50% of non-sclerotic glomeruli
Interstitial fibrosis	**ci**	Interstitial fibrosis in 0–5% of cortex	Interstitial fibrosis in 6–25% of cortex	Interstitial fibrosis in 26–50% of cortex	Interstitial fibrosis in >50% of cortex
Tubular atrophy	**ct**	No tubular atrophy	Tubular atrophy in up to 25% of cortical tubules	Tubular atrophy in 26–50% of cortical tubules	Tubular atrophy in >50% of cortical tubules
Arterial fibrous intimal thickening[h]	**cv**	No arterial fibrous intimal thickening	Arterial fibrous intimal thickening with 1–25% luminal narrowing	Arterial fibrous intimal thickening with 26–50% luminal narrowing	Arterial fibrous intimal thickening with >50% luminal narrowing
Arteriolar hyalinosis[i]	**ah**	No arteriolar hyalinosis	Mild–moderate hyalinosis in at least one arteriole	Moderate–severe hyalinosis in more than one arteriole	Severe hyalinosis in many arterioles
Peritubular capillary basement membrane multilayering*	**ptcml**	1–2 basement membrane layers in PTCs assessed by electron microscopy	3–4 basement membrane layers in PTCs assessed by electron microscopy	5–6 basement membrane layers in PTCs assessed by electron microscopy	>6 basement membrane layers in PTCs assessed by electron microscopy
Total Interstitial inflammation[j]	**ti**	No or trivial (<10% of parenchyma)	10–25% of cortical parenchyma	26–50% of cortical parenchyma	>50% of cortical parenchyma
Arteriolar hyaline thickening[k]	**aah**	No lesions typical of CNI arteriolopathy	1 arteriole, not circumferential	>1 arteriole, not circumferential	Any number of arterioles, circumferential
C4d in PTCs by Immunofluorescence (IF)[k]	**C4d (IF)**	0% of biopsy area, considered Negative	1 < 10% of biopsy area with linear, circumferential stain in PTC, considered Minimal/Negative	10–50% of biopsy area with linear, circumferential stain in PTC, considered Focal Unknown	>50% of biopsy area with linear, circumferential stain in PTC, considered Diffuse Positive
C4d in PTCs by Immunohistochemistry (IHC)[k]	**C4d (IHC)**	0% of biopsy area with linear, circumferential stain in PTC, considered Negative	1 < 10% of biopsy area with linear, circumferential stain in PTC, considered Minimal/Unknown	10–50% of biopsy area with linear, circumferential stain in PTC, considered Focal Positive	>50% of biopsy area with linear, circumferential stain in PTC, considered Diffuse Positive

[a] Note if "remarkable" number of eosinophils, neutrophils, plasma cells and denote with asterisk.
[b] Applies to tubules no more than mildly atrophic.
[c] Note number of arteries present and number affected; indicate infarction and/or interstitial hemorrhage with asterisk.
[d] Complete or partial occlusion of ≥1 glomerular capillary by leukocyte infiltration and endothelial cell enlargement.
[e] Comment on composition (mononuclear cells and neutrophils) and extent (focal ≤50%; diffuse >50%).
[f] This refers to most severely affected glomerulus; note number and percent sclerotic. Also, cg1a denotes no GBM double contours by light microscopy but GBM double contours by electron microcopy (EM) with endothelial swelling and/or subendothelial electron lucent widening, and cg1b can denote ≥1 double contours in ≥1 non-sclerotic glomerulus, confirmed by EM if available].
[g] This refers to at least a moderate increase, which is an increase by an average of >2 mesangial cells in the mesangial interspace between adjacent capillaries in at least two adjacent capillaries.
[h] This is characterized by features of chronic rejection (fibrointimal thickening/neointima formation ± breach of internal elastic lamina or presence of occasional mononuclear or foam cells, ± breaks in elastic lamina).
[i] Indicate arteriolitis with an asterisk.
[j] Alternate scoring for interstitial inflammation not always used diagnostically.
[k] Alternate scoring for hyaline arteriolar thickening (not always used diagnostically) due to calcineurin inhibitors (CNI).

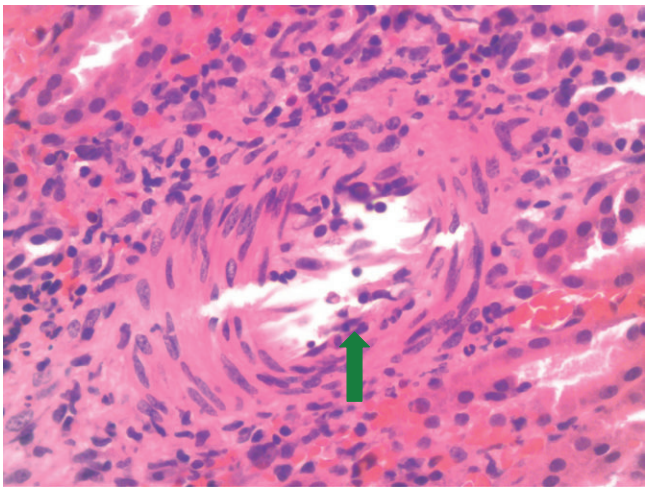


Figure 81.2. Arteritis. With endarteritis in a case of acute cellular rejection, type 2, mononuclear cells can be observed underlying the endothelium (green arrow).

Interstitial fibrosis and tubular atrophy (IFTA) is the non-specific final common pathway in both chronic CMR and AMR as well as in other forms of graft deterioration [31]. However, the only accepted diagnostic criteria for chronic CMR under Banff criteria is chronic allograft arteriopathy, consisting of arterial intimal fibrosis with mononuclear infiltration of fibrosis and formation of a neo-intima [8]. With IFTA, the renal tubules become smaller, acquire wrinkled thickened tubular basement membranes, and are infiltrated by inflammatory cells. Inflammation in areas of IFTA is currently not considered for the Banff i score by convention; however, recent studies have shown the importance of inflammation in areas of fibrosis, suggesting a role in chronic rejection and ultimate graft deterioration, at least as a robust prognostic marker [32–34]. A scoring system has been proposed to score total interstitial inflammation [the ti-score] and is currently under review for its interobserver reproducibility and clinical utility [11,35]. To determine the importance of inflammation in sclerotic areas and the ti score, a Banff working group on T-cell CMR was formed to assess these issues as well as the significance of "borderline" infiltrates [36].

After treatment, there is a decrease in the inflammation associated with acute CMR, in particular after early CMR with less IFTA. However, up to approximately 50% of biopsies taken 1–2 months after acute CMR show continued inflammation. In these biopsies, inflammation primarily remains in areas of fibrosis. Inflammatory cells may remain in the intima in some cases, sometimes embedded within a loose matrix, possibly representing a precursor to transplant arteriopathy [13]. This observation stresses the difficulties with the discrimination between injury-related inflammation and infiltrates mediating acute rejection.

Antibody-mediated rejection

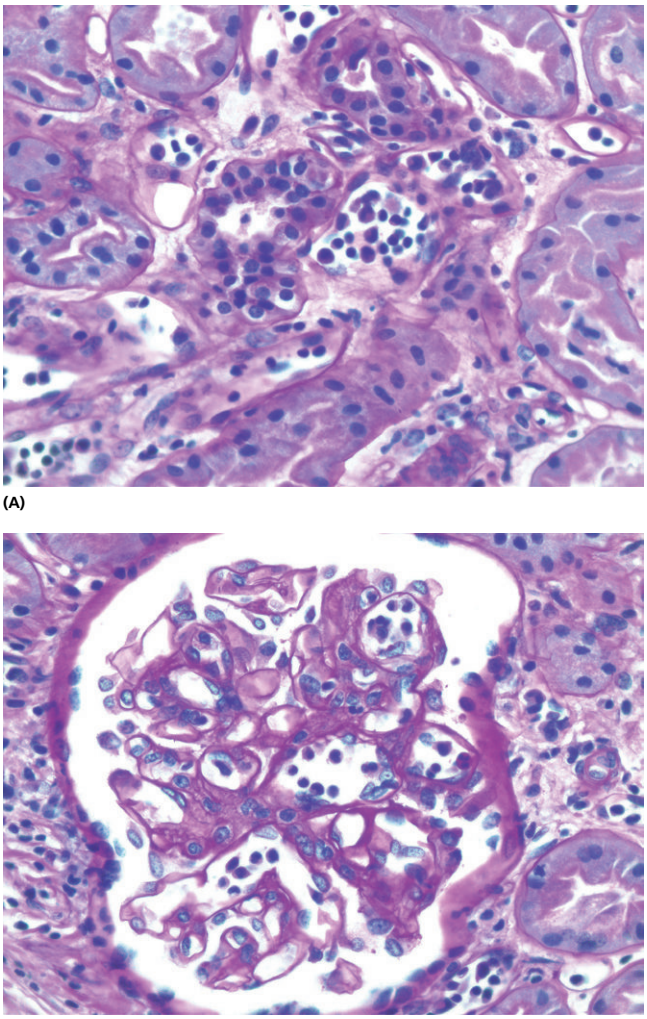
With the exception of hyperacute rejection mediated by pre-existing unrecognized donor-specific antibodies (DSAs) [37,38], for much of the five decades since the first kidney transplant, T cells were thought to be the major determinant in graft rejection. However, over approximately two decades it has become clear that much graft injury occurs through antibody-mediated (humoral) rejection, primarily directed against donor HLA [14]. Consequently, in 2001, AMR was introduced as a diagnostic category into the Banff classification [6]. AMR contributes to both early and, in particular, to late graft loss [39,40]. Antibody causes injury through the action of complement, producing tissue injury and coagulation and the recruitment of neutrophils and macrophages, culminating in endothelial injury. Edema results from the release of histamine from mast cells. Endothelial cell adhesion molecules (e.g. E-selectin, intracellular adhesion molecule 1, and vascular adhesion molecule 1) are up-regulated through the action of complement. Eventually, the membrane attack complex, C5b-9, leads to endothelial cell lysis. This process is complex and may not always be dependent on complement as AMR mediated by natural killer (NK) cells independent of complement has been described [3,14,41,42].

Clinically, two AMR phenotypes can be identified: phenotype 1 occurring in sensitized patients with pre-existing DSA early post-transplantation after incomplete desensitization; and phenotype 2 observed late post-transplantation after de novo development of DSA, frequently in the setting of non-compliance. Morphologically, acute AMR has three main patterns:

1. Type/Grade I, an acute tubular necrosis-like (ATN) minimal inflammation pattern;
2. Type/Grade II, a pattern containing PTC and/or glomerular inflammation (= microcirculation inflammation) (Figure 81.3), sometimes accompanied by thrombi; and
3. Type/Grade III, vascular fibrinoid necrosis (v3).

According to current Banff consensus criteria, in addition to one of these morphologic patterns of injury, two other criteria are required for the diagnosis of AMR: the identification of a DSA and the presence of the complement split product component C4d in the endothelium of PTCs (Figure 81.4) [8]. Ultrastructurally, peritubular and glomerular capillary endothelium shows signs of injury with cell enlargement, loss of fenestrations, detachment from basement membranes, lysis, and apoptosis [43].

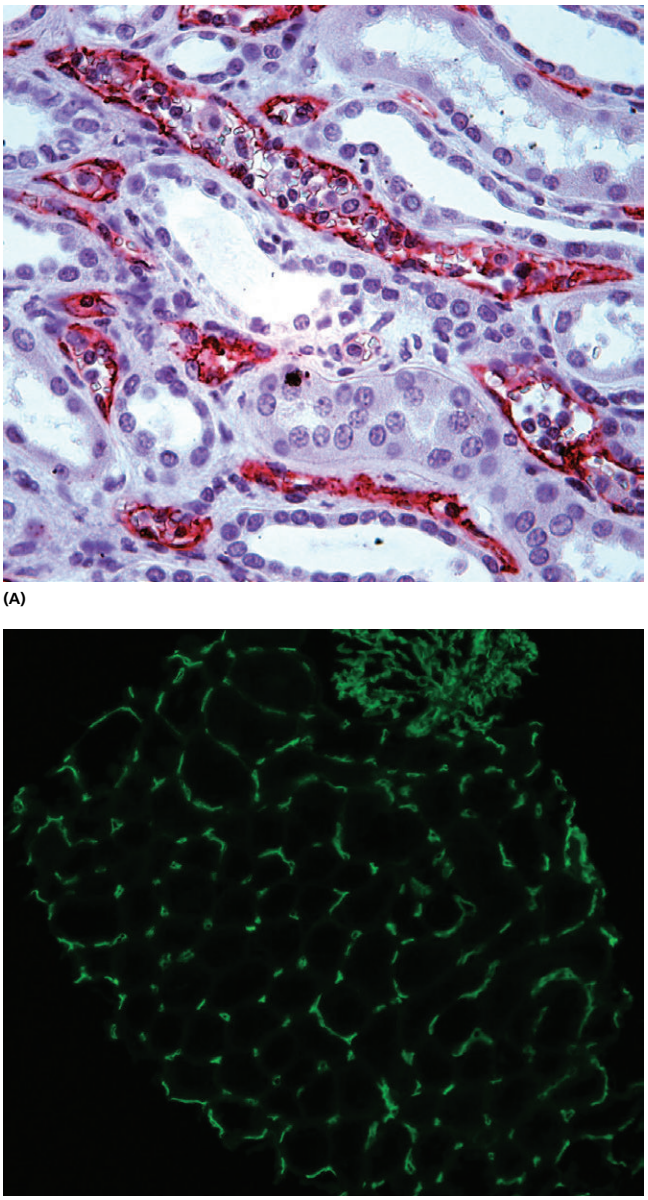
Chronic antibody-mediated rejection (CAMR) is characterized by structural remodeling primarily in the microcirculation of the kidney, but also in larger arteries (intimal hyperplasia) with secondary IFTA and eventual decline in renal allograft function. Clinically, CAMR may be accompanied by proteinuria [13,44]. In the microcirculation, renal glomerular basement membranes undergo reduplication, developing "double contours" in a process termed transplant glomerulopathy [44]. Under Banff criteria, for a diagnosis of CAMR there must be morphologic signs of chronic remodeling, C4d in PTC endothelium, and a documented DSA [8]. Morphologic signs of chronic antibody-mediated injury accepted as evidence of CAMR include: glomerular double contours (Figure 81.5), PTC basement membrane multilayering, IFTA, and fibrous thickening in arteries [8]. At least two of four of these morphologic signs of injury are typically recommended for a CAMR diagnosis [5]. Glomerular and PTC basement membrane multilamination are considered to be the most valuable evidence in the diagnosis of chronic renal allograft rejection [45]. In addition, as in acute AMR, there is typically glomerulitis and peritubular capillaritis present in most biopsies with CAMR. These findings can be interpreted as signs of ongoing disease activity and thus progression. Glomerular inflammatory cells are mostly composed of $CD68^+$ monocytes with fewer numbers of $CD3^+$ T cells, and occasionally neutrophils. PTCs contain C4d in CAMR, and C4d staining may also be seen as a non-specific finding in glomeruli with double contours. It is likely that CAMR occurs in multiple stages, a concept that has been demonstrated in non-human primate models [46]. It has been postulated that CAMR is initiated by the production of DSA, followed by deposition of C4d in PTCs,



(A)

(B)

Figure 81.3. Acute antibody-mediated rejection. In acute antibody-mediated rejection both (A) peritubular capillaritis and (B) glomerulitis can be seen.



(A)

(B)

Figure 81.4. C4d staining. (A) C4d stain in acute antibody-mediated rejection demonstrates a linear, circumferential staining of the peritubular capillary endothelium using both (A) immunohistochemistry (with a red chromogen) and (B) immunofluorescence (with a green fluorophore).

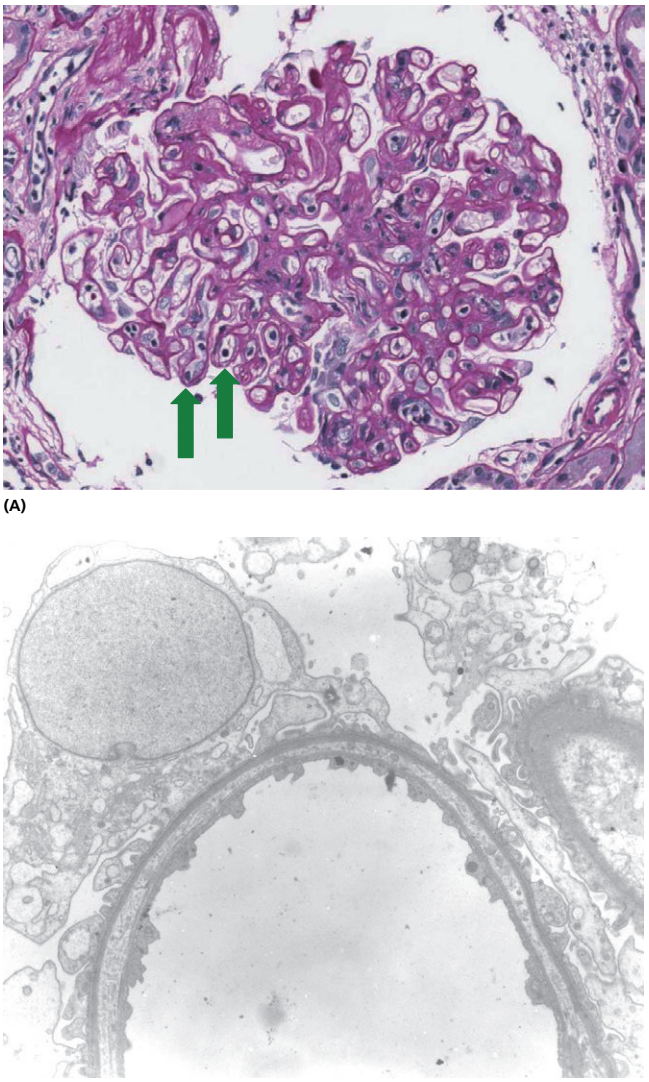
and subsequent acute and then chronic morphologic signs of microcirculation injury, eventually leading to graft dysfunction. C4d and DSA may be intermittently present throughout this process. These stages can occur within months or be spread over years in humans, mostly depending on the compliance and immunosuppressive status of the patient [13,47].

In regards to AMR, attention has recently been drawn to C4d-negative AMR. These cases have DSA and morphologic evidence of antibody-mediated injury but lack C4d positivity in PTC endothelium. Negativity for C4d in AMR can be explained by various mechanisms: complement independent antibody-mediated injury; lack of sensitivity and reproducibility of the staining methods; arbitrary criteria for defining "positivity"; or time-dependent degradation of C4d deposits in the microcirculation. Molecular studies identified a subset of cases with DSA and morphologic features of antibody-mediated injury showing increased expression of endothelial cell associated transcripts as a sign of endothelial cell activation and stress [48]. These data suggest that 50–60% of AMR cases are missed by current Banff criteria due to C4d-negativity. AMR in the absence of C4d deposition has now been added to the Banff diagnostic armamentarium after data was gathered by a topic-specific Banff Working Group [30,36,49].

AMR may co-exist with CMR, in particular in non-compliant patients [13]. The presence of a substantial interstitial inflammatory infiltrate and tubulitis tends to favor acute CMR. Mononuclear tubulitis is found in 30–80% of cases of acute AMR and is thought to represent a concurrent TCMR component [5,50–52], and HLA-DR expression is also thought to denote cases with concurrent T cell and AMR [5,53].

Accommodation

A peculiar scenario related to AMR has been termed accommodation. Accommodation refers to the presence of PTC C4d deposition



(A)

(B)

Figure 81.5. Transplant glomerulopathy. Transplant glomerulopathy in chronic antibody-mediated rejection can be demonstrated with both (A) light microscopy, in which glomerular basement membrane duplication can be appreciated (green arrows), and (B) electron microscopy, in which the glomerular basement duplication is more evident.

in the absence of other evidence of antibody-mediated injury, and is thought to represent a process of endothelial cell adaptation to antibody and complement over time. In these cases, DSA may be detectable; however, there are no morphologic signs of tissue injury. Signs of acute or chronic TCMR or AMR are absent; more specifically, there is no ATN-like minimal inflammation, no glomerulitis (g0), no chronic transplant glomerulopathy (cg0), no peritubular capillaritis (ptc0), and no PTC basement membrane multilamination (<5 layers by EM). These cases are considered to be "C4d deposition without evidence of active rejection" under current Banff criteria and are considered indeterminate if there are simultaneous borderline changes [8]. Accommodation is often described in the setting of ABO-incompatible allografts. The long-term significance of these relatively rare cases is still under investigation [13,54,55].

Atypical rejection syndromes

Novel immunosuppression regimens can lead to novel patterns of rejection. For example, alemtuzumab (CAMPATH-1H) leads to pronounced lymphocyte depletion [56–58]. Acute CMR with a predominance of monocytes (i.e. an acute monocytic rejection) has been described with lymphocyte depletion with alemtuzumab. In these cases, much of the interstitial infiltrate accounting for the rejection stains for CD68, which correlates with the renal dysfunction and tubular stress, measured with HLA-DR staining. However, in these cases, T cells did not correlate with renal dysfunction or HLA-DR staining [56].

Studies recently have included protocols for simultaneous bone marrow and kidney transplantation in attempts to induce tolerance to the transplanted organ. In such patients, HLA-mismatched renal transplants have been conducted, and maintenance immunosuppression has been withdrawn in some of the patients with relative preservation of renal function [59]. In many of these patients, a capillary leak or engraftment syndrome has been observed around 10 days after a simultaneous kidney/bone marrow transplant preceded by a non-myeloablative conditioning regimen. In this "engraftment syndrome," PTCs are congested with mononuclear cells and red blood cells, accompanied by acute tubular injury (ATI). Immunohistochemistry shows that the cells are mostly $CD68^{+}MPO^{+}$ mononuclear cells and $CD3^{+}CD8^{+}$ T cells, the latter with a high proliferation index ($Ki67^{+}$). Fluorescence in situ hybridization (FISH) showed that the cells in PTCs are derived from the recipient, correlating with chimerism studies showing that this is the time period at which there is a decline in circulating donor cells and recovery of recipient circulating cells. EM shows PTC endothelium injury in these cases [59,60]. Others have performed combined kidney and bone marrow transplants without this phenomenon, and the etiology of the syndrome remains undefined [61].

In sensitized patients bearing a high risk for AMR despite desensitization, inhibition of the complement cascade has been tested as a treatment option. Early biopsies from patients treated with eculizumab were diffusely C4d-positive but did not show morphologic signs of AMR including lack of endothelial cell activation by EM. The fact that complement component C5 as the target of eculizumab is downstream of C4d explains the diffuse positivity for C4d, while the lack of respective pathology suggests that eculizumab simultaneously protects the endothelium. However, a few patients developed AMR despite eculizumab, in particular chronic AMR with transplant glomerulopathy (TG) [62]. This highlights the fact that complement inhibition alone does not prevent the development of chronic antibody-mediated microcirculation injury. Furthermore, these observations also illustrate the limitations in the diagnostic reliability of C4d and DSA in this setting and emphasize the need for the refinement of the respective diagnostic criteria.

Other important disorders observed in allografts

A variety of disorders should be considered in the differential diagnosis of rejection (Box 81.1), including a variety of infections, drug effects, and anatomic problems [7]. Acute tubular injury/necrosis (ATI/ATN) is also a common finding, as discussed later [7].

Infection should be excluded before a diagnosis of acute rejection is made [7]. Pyelonephritis may be present in allografts. Pyelonephritis typically has a more prominent neutrophilic component to the inflammatory infiltrate, sometimes displaying neutrophilic

Box 81.1. Non-rejection differential diagnostic considerations

- Anatomic
  - Vascular
    - Vascular anastomotic defect
    - Thrombosis
    - Pretransplant acute endothelial injury
  - Urinary
    - Obstruction/reflux
    - Urine leak
- Infection
  - Polyomavirus nephritis
  - Cytomegalovirus
  - Adenovirus
- Drug
  - Calcineurin inhibitor effect
  - Acute interstitial nephritis from drug effect as well as other causes
- Post-transplant lymphoproliferative disorder
- Acute tubular injury/necrosis from variety of causes, including some of the above
- Papillary necrosis
- Non-specific
  - Focal interstitial inflammation without tubulitis
  - Reactive vascular changes
  - Venulitis
- Subcapsular injury or "healing in"
- Recurrent/de novo glomerular disease

Adapted from [7] Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. Kidney International. 1999;55:713–723 by permission from Macmillan Publishers © copyright 1999.

casts (Figure 81.8). Clinical correlation with urinalysis and urine culture may be useful in such cases [5]. In addition, a variety of viral infections may be present in renal allografts, some of which are discussed later [7].

Drug effects may cause changes in renal allografts. For example, the calcineurin inhibitor immunosuppressive agents are important in this regard (Figure 81.6), as discussed later. Acute interstitial nephritis may be present in renal allografts and may often be drug-induced.

Urinary tract anatomic problems can be present in renal allografts, examples of which include obstruction and/or reflux and urine leak. In spite of the fact that surgical techniques have improved over the past decades, leading to a lower incidence of technical complications, some anatomic problems still can be caused by problems with surgical technique, including misplacement of ureteral sutures, insufficient ureteral length, and ureteral or renal pelvis laceration. Most ureteral complications occurring after the first week are related to ureteral ischemic injury. In some of these circumstances, the dysfunctional anastomosis may lead to reflux and/or leakage [5,63–66]. The related morphologic features in transplant biopsies are identical to those in native kidneys with similar problems. Focal dilatation of tubules with cast formation and occasional rupture of the tubular basement membrane can be seen, which is then accompanied by a focal inflammatory response.

Vascular problems may also be present, including anastomotic defects leading to renal artery stenosis [7,67–70]. Vascular problems are caused by intimal injury during procurement, anastomotic stenosis, excessive trauma, and renal vein narrowing, compression, or twisting. These problems may be complicated by a hypercoagulable state from disorders such as factor V Leiden disorder, antiphospholipid antibody disorder, and nephrotic syndrome [13].

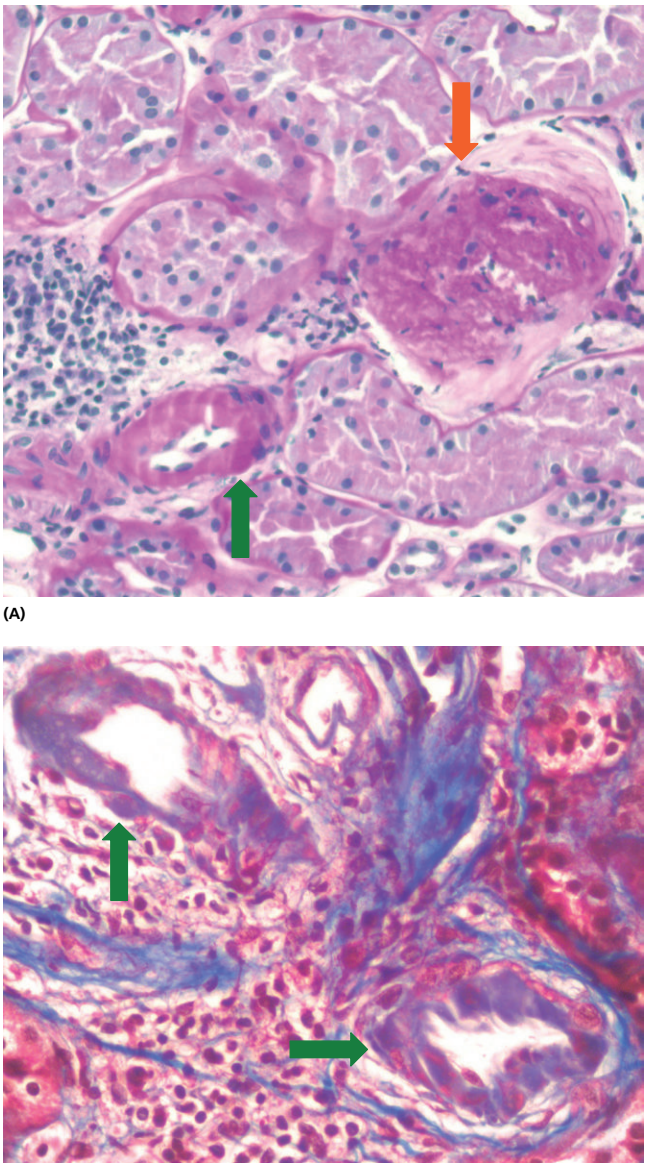


Figure 81.6. Arteriolar hyalinosis. Arteriolar hyalinosis of the type associated with chronic calcineurin inhibitor toxicity can be readily observed on both (A) PAS and (B) trichrome stains (green arrows). The hyalinosis focally has a nodular quality. A sclerotic glomerulus is also present (orange arrow).

Transplant renal artery stenosis may be caused by atherosclerosis, intimal flaps, kinking, and chronic rejection later in the life of the allograft; the arterial stenosis may result in refractory hypertension due to increased renin production [71–73]. Vascular thrombosis may cause renal dysfunction and injury to the renal parenchyma [7,71–73]. Lymphoceles may also be present as a result of incomplete lymphatic anastomosis, leading to mild inflammation that may fulfill the criteria for "borderline/suspicious" for acute CMR and be erroneously attributed to CMR [74–76].

Non-specific histologic changes may be present and must be distinguished from rejection. Venulitis is not considered to be pathognomonic for rejection in kidney allografts. Furthermore, the subcapsular region often contains IFTA, inflammation, and

glomerulosclerosis; however, findings in subcapsular region must be interpreted with caution because subcapsular injury or "healing in" is a rather common non-specific finding in renal allografts, which is not considered pathognomonic of rejection [5,7].

Acute tubular injury

The epithelium of the nephron is the crucial determinant of kidney function and outcome [77–79]. However, the fact that biopsies from acutely injured non-functioning kidneys frequently show "normal" histology or non-specific injury feature like loss of brush border, epithelial necrosis, and cellular degeneration highlights the limited ability of light microscopy to assess, in particular, the early potentially reversible stages of epithelial injury. Furthermore, the continuous process of nephron dedifferentiation from acute reversible injury to chronic irreversible atrophy represents a morphologic challenge to determine the exact turning-point when an injured tubule becomes atrophic. Therefore, significant research efforts are underway for identifying in situ injury markers enhancing light microscopy in assessing epithelial injury (e.g. Kim-1, vimentin, and integrin-beta 6) [80,81]. Elegant molecular, mechanistic, and imaging data are available demonstrating that epithelial cells show a highly stereotyped response to any kind of injury [82,83]. This includes the re-expression of embryonic and developmental pathways; and with this, epithelial cells assume a more mesenchymal phenotype as a sign of dedifferentiation, which often represents a transient state [84,85]. The expression of the mesenchymal phenotype frequently co-localizes with increased inflammation and matrix production in the adjacent interstitium. However, there is no convincing evidence, at least in kidney allografts, that the source of fibrosis is a migrated dedifferentiated epithelial cell, but rather an adjacent residential interstitial cell (i.e. there is no evidence for true epithelial-to-mesenchymal-transition (EMT)). Rather, this process may simply represent an epithelial-to-mesenchymal phenotype (EMP) [86]. Thus, in situ markers for epithelial injury will be clinically very useful in kidneys with early stages of injury as a reflection of potentially reversible injury and in kidneys with later stages as a reflection of epithelial dedifferentiation and atrophy.

Polyomavirus nephritis

Current drug regimens can lead to such profound immunosuppression that viral infection can be a problem. Clinically, these patients can present with features of renal dysfunction identical to those seen in rejection. The polyomavirus BK virus is a particular problem in this regard, accounting for around 80% of cases of PVAN. The polyomavirus JC virus, which may account for up to almost 20% of cases, can also cause PVAN; however, cases caused by JC virus are typically milder. PVAN results in a tubulointerstitial nephritis and preferentially infects the renal tubular epithelium, producing nuclear inclusions in many cases (Figure 81.7). The inflammatory infiltrate often has numerous plasma cells. Plasma cell tubulitis may sometimes be seen [13]. Immune complex deposits can be seen along tubular basement membranes. Eventually IFTA results in advanced stages, which are associated with an inferior prognosis [13,87–89]. It has been postulated that acute CMR and polyomavirus may co-exist; however, the differential diagnosis of these two entities is difficult [90].

Immunohistochemistry, typically directed against the polyomavirus SV40 large T antigen, can be used to detect virally infected cells and thus confirm the diagnosis. Although some morphologic changes in the way of tubulointerstitial inflammation are typically present in polyomavirus nephritis [91], a low threshold for performing the SV40 stain is recommended given the threat of polyomavirus nephritis/nephropathy, particularly when mild inflammatory infiltrates or a "borderline" pattern are seen [87,92]. Virally infected cells may also be detected with in situ hybridization or EM, which can display paracrystalline arrays of viral particles [13,87–89].

Adenovirus

Adenovirus may infect renal allografts. Infections are very rare (<1% of transplants), typically presenting in the 1–3 months after transplant, although the condition may be under-diagnosed. Patients may be febrile and may present with hematuria from hemorrhagic cystitis. Histologically, tubular necrosis may be appreciated (Figure 81.7); and granulomatous inflammation may be present. Tubular epithelial cells may appear smudgy and contain basophilic intranuclear inclusions. Immunohistochemistry can be helpful in diagnosing the virally infected cells [13,93–95].

Cytomegalovirus

Cytomegalovirus (CMV) infection, although frequent in solid organ recipients, may rarely be seen as nephritis in renal allografts. Patients are often profoundly immunocompromised and typically present with flu-like symptoms and renal dysfunction. Allograft CMV infections have become less common with prophylaxis using drugs such as ganciclovir. CMV inclusions may be seen very rarely in the tubular epithelium and also in glomerular capillary and/or PTC endothelial cells (Figure 81.7). This may be accompanied by interstitial inflammation. Special stains, such as immunohistochemistry for CMV, are available to aid in the identification of infected cells [13,96–98].

Calcineurin inhibitor toxicity

Calcineurin inihibitors (e.g. cyclosporine and tacrolimus) are instrumental in preventing rejection in renal allografts [17]; however, calcineurin inhibitors can cause both acute and chronic nephrotoxicity. Acute toxicity can be associated with tubular injury necrosis with isometric vacuolization of the proximal straight tubules. Afferent arterioles can display smooth muscle cell necrosis with eventual hyalinization. Uncommonly, thrombotic microangiopathy (TMA) can develop. Chronically, the damage to smooth muscle cells caused by calcineurin inhibitor toxicity leads to more extensive hyaline deposits, which often have a beaded appearance and protrude into the adventitia (Figure 81.6). The interstitium may also display striped fibrosis and tubular atrophy [99].

However, none of these histologic features are specific for calcineurin inhibitor toxicity, and can be observed frequently in kidneys from patients who have never received calcineurin inhibitors [100]. Thus, the actual contribution of calcineurin inhibitors to renal allograft loss as well as a reliable risk–benefit analysis of calcineurin inhibitors is still a matter of ongoing debate.

Post-transplant lymphproliferative disease

PTLD is a disorder in lymphoid proliferation in the post-transplantation setting, typically due to the profound immunosuppression and associated Epstein–Barr virus (EBV) reactivation. PTLD may be seen in both solid organ or bone marrow transplant patients. Eventually, frank lymphoma may result, leading to a reported mortality of 40–60%. Most (85–90%) cases of PTLD are of B-cell origin, expressing CD20. Many cases are driven by EBV, and EBV seronegativity prior to transplantation is a risk factor for the development of PTLD. Morphologically, most of these cases are

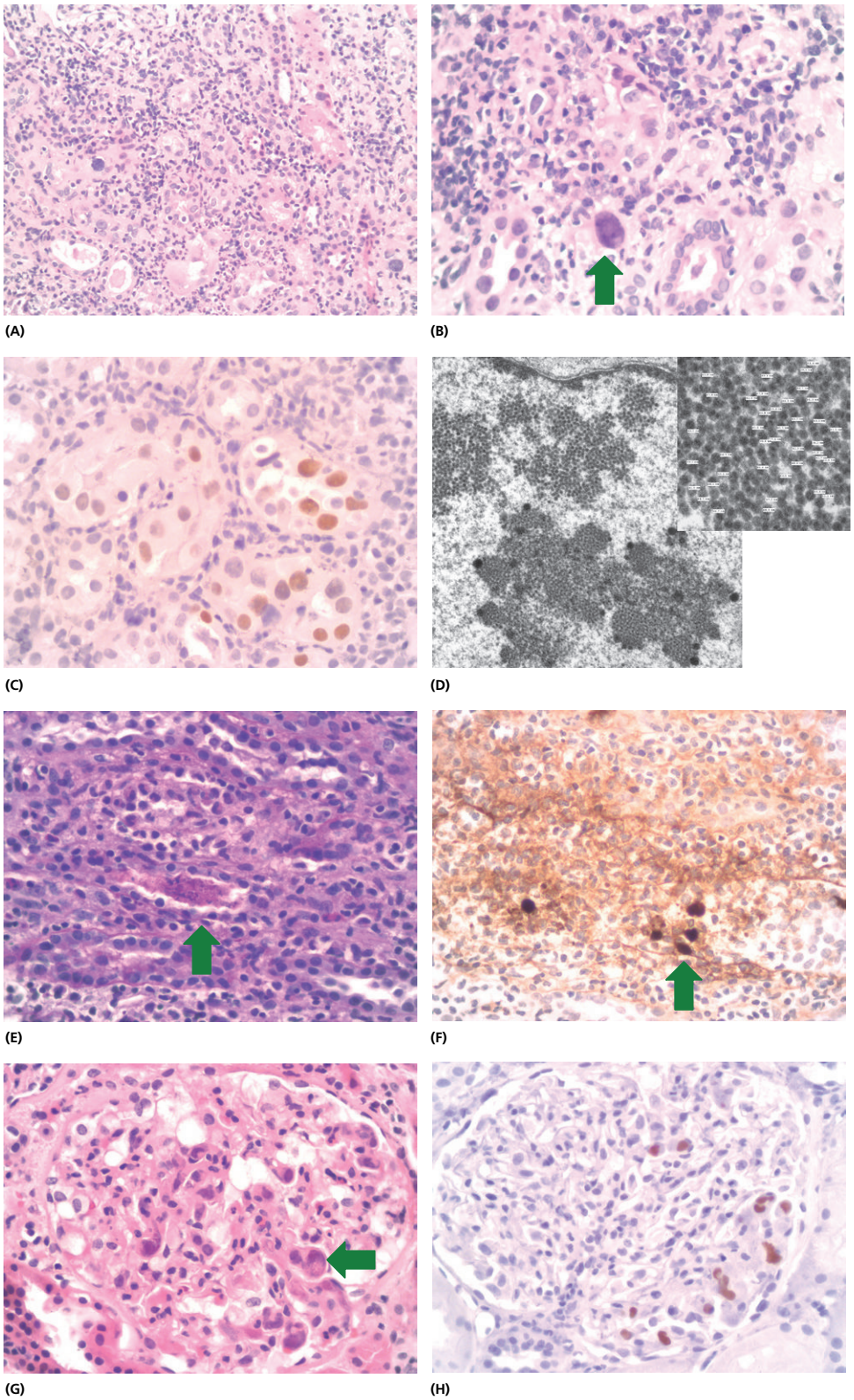


Figure 81.7. Viral nephritis. In polyomavirus nephritis, there is often (A) a dense lymphoplasmacytic infiltrate; and (B) markedly enlarged tubular epithelial cells can be seen (arrow). (C) SV40 immunohistochemistry can be used to confirm that the cells are infected by polyomavirus, and (D) the polyomavirus virions can be seen on electron microscopy measuring approximately 40–50 nm (inset). (E) In adenovirus nephritis, necrotic material can often be appreciated in the renal tubules (arrow); and (F) adenovirus immunohistochemistry can confirm the presence of adenovirus (arrow). (G) Cytomegalovirus viral inclusions may sometimes be seen in glomeruli (arrow), which can be confirmed with positivity staining using cytomegalovirus immunohistochemistry (H).

composed of "activated" mononuclear cells with enlarged nuclei, prominent nucleoli, and frequently evident mitotic activity. Immunohistochemistry may be useful in looking for the presence of B- or T-cell antigens and the EBV-associated antigens (such as the EBV-latent membrane protein), and in situ hybridization maybe useful in looking for EBV-encoded RNA (EBER). Classification of PTLD has been established by individuals working under the auspices of the World Health Organization (WHO), and PTLD under this WHO classification fits into four major categories: early lesions; polymorphic PTLD (plasmacytic hyperplasia and infectious mononucleosis-like PTLD); monomorphic PTLD (B, T, and NK cell); and classic Hodgkin lymphoma [13,101]. PTLD is treated with a reduction in immunosuppression, antiviral drugs, anti-CD20 (rituximab, if of B-cell phenotype), chemotherapy, and radiation.

Recurrent and de novo disease

With successes in the treatment of acute graft rejection, allografts have lasted longer and recurrent disease has become an important contributor to renal allograft deterioration (Table 81.3). Detailed investigation has indicated that the majority of cases have an identifiable cause of renal allograft deterioration, consisting of both alloimmune and non-alloimmune mechanisms. Glomerular pathology includes recurrent disease in a substantial number of patients [102]. Clinically relevant proteinuria may eventually be present in approximately 40% of kidney transplant recipients with the most common cause being CAMR; however, rejection-associated causes are closely followed by post-transplant glomerulonephritis [103,104].

Studies have indicated that recurrent renal disease may be the third leading cause of graft failure in patients with glomerulonephritis. Important examples of renal disease that have a prominent impact on graft survival include recurrent focal segmental glomerulosclerosis (FSGS) [105–107], atypical hemolytic uremic syndrome (HUS) [103], lupus nephritis [108], and membranoproliferative glomerulonephritis (MPGN) [103,109,110]. Other noteworthy diseases that may recur after transplantation include membranous glomerulonephritis (MGN), antiglomerular basement membrane (GBM) disease, antineutrophil cytoplasmic antibody (ANCA) [13,111], and post-infectious glomerulonephritis [112].

In general, recurrent and de novo diseases present with identical morphologic features and clinical symptoms, as in native kidneys. The C4d stain routinely performed in allograft biopsies can serve as a screening test in this regard. Granular C4d deposition by immunofluorescence and immunohistochemistry (particularly along the glomerular basement membrane) raises the possibility of de novo or recurrent glomerulonephritis. Mesangial C4d is a normal finding by immunofluorescence [13]. Some diseases recur relatively fast; however, others such as diabetic glomerulopathy may take years to manifest recurrent disease [13,111].

Immunofluorescence and EM are crucial in making a diagnosis of recurrent disease (Figure 81.8). Features of recurrent disease may be superimposed upon acute or chronic allograft rejection or drug toxicity, and by this represent a differential diagnostic challenge. Recurrent disease should be distinguished from de novo disease; thus, knowledge of the primary disease is important in recognizing and interpreting relevant pathologies in renal allografts because the morphologic features are identical in recurrent and de novo diseases. However, this distinction is frequently impossible as only a minority of renal allograft recipients undergo biopsies of their native kidney disease. In general, recurrent disease is favored if there is an early onset (<6 months).

Primary FSGS may recur rapidly (within minutes) after transplantation, resulting in profound proteinuria, extensive podocyte foot process effacement often in a minimal change-like disease picture, and eventually producing segmental sclerosing lesions [105,106]. Recurrence is five times more likely in children than in adults [13]. Some cohorts have shown the same histologic pattern

Table 81.3. Recurrent diseases (data from [102,103])

Disease	Recurrence rate (% of recipients)	5–10 year graft loss (% of recipients)	Comments
Disease primarily affecting glomeruli			
FSGS	20–40	20	Typically accompanied by proteinuria and may display collapsing features
MPGN, type I	20–33	High	
MPGN, type II (DDD)	67–100	34–66	
MGN	10–30	50	IgG4 is the dominant subclass compared to IgG1 in de novo MGN
IgA nephropathy/HSP	7–30	3–16	Crescentic GN may be present
Lupus nephritis	Up to 30	<5	Podocytopathy or FSGS may be present
Anti-GBM disease	<5	Rare	
Pauci-immune crescentic GN (ANCA associated)	0–20	10	
Fibrillary GN	50	20	
Immunotactoid glomerulopathy	Rare	Uncertain	
Systemic/metabolic disease			
Diabetic nephropathy	>50	5	
HUS (Idiopathic D-)	33–82	90	
Amyloidosis, AL type	10–30	35	
Amyloidosis, AA type	<10	5	
MIDD	70–85	>50	
Fabry disease	Low	Rare	Enzyme replacement therapy has decreased incidence
Primary hyperoxaluria	90–100	80–100	
Cystinosis	Rare	0	
Sickle cell nephropathy	Rare	Not available	

ANCA, antineutrophil cytoplasmic antibody; DDD, dense deposit disease; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; GN, glomerulonephritis; HSP, Henoch–Schönlein purpura; HUS, hemolytic uremic syndrome; MGN, membranous glomerulonephritis; MIDD, monoclonal immunoglobulin deposition disease; MPGN, membranoproliferative glomerulonephritis.

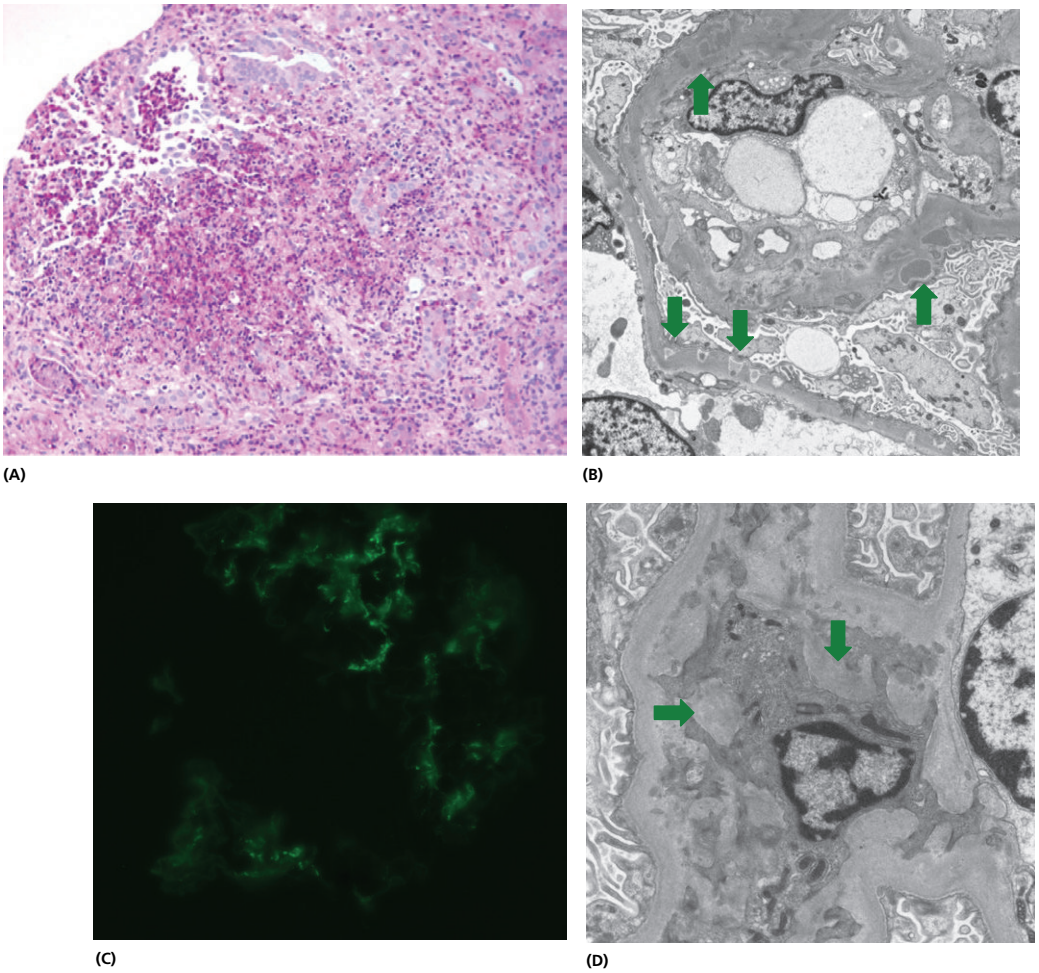


Figure 81.8. Examples of non-rejection disease, including recurrent/de novo glomerulonephritis. (A) In transplant pyelonephritis, there is often a dense inflammatory infiltrate composed of a predominance of neutrophils. (B) Electron microscopy in de novo membranous glomerulonephritis demonstrates numerous subepithelial immune-type deposits (arrows). In a case of recurrent IgA nephropathy (C) IgA immunofluorescence shows a granular mesangial staining pattern; and (D) electron microscopy shows electron dense immune-type deposits (arrows) closely apposed to reactive-appearing mesangial cells.

according to the Columbia classification [113] of FSGS in the FSGS recurrence [105]; however, other studies have failed to show this fidelity [106]. Attempts have been made to treat some cases with intense plasma exchange, displaying promising results in some [13,103,107,114].

FSGS may also occur as a de novo disease. It may sometimes have a collapsing pattern with glomerular capillary loop collapse and overlying podocyte hypertrophy. De novo FSGS is also associated with proteinuria. The collapsing pattern has been attributed to severe vascular disease and calcineurin inhibitor toxicity. The de novo and collapsing patterns have 5-year graft survival rates of approximately 60% and 50%, respectively [13,115–117]. FSGS is also seen frequently as a secondary lesion to various glomerular pathologies and hypertension occurring in allograft recipients.

Glomerular basement double contours in transplants (i.e. transplant glomerulopathy) most often represents a manifestation of CAMR. In approximately 30–40% of the cases with glomerular basement double contour, no C4d and DSA can be detected at time of biopsy [118]. In these cases, recurrent or de novo glomerular disease has to be taken into consideration. The most relevant entities in this regard are TMA and MPGN, in particular in the setting of underlying viral hepatitis [119,120]. MPGN typically has immunoglobulin and C3 deposits seen by immunofluorescence, prominent electron-dense subendothelial deposits on EM, and negative C4d in PTCs [13].

MGN recurs in about 30% of cases with severe proteinuria being present as early as 1 week after transplantation [13,121]. In MGN, glomerular basement membrane thickening, vacuolization, or spike formation may be present, together with the characteristic EM and immunofluorescence findings of MGN (Figure 81.8) [13]. Some MGN in allografts may represent a peculiar result of humoral rejection and may have concurrent C4d deposition [13,122–125].

In recurrent lupus nephritis, mesangial hypercellularity or sclerosis may be present, and crescent formation and fibrinoid necrosis may be seen. Segmental sclerosis or prominent podocyte injury may sometimes be present [13,108].

IgA nephropathy/Henoch–Schönlein purpura (HSP) is a recurrent disease that may manifest in the allograft. As in native kidneys with IgA nephropathy, there may be varying degrees of mesangial hypercellularity and crescent formation, and characteristic fluorescent microscopy and EM changes can be demonstrated (Figure 81.8) [13,126–128].

Serologic investigation may be important in the investigation of recurrent ANCA or anti-GBM-mediated disease. Serum and/or urine protein electrophoresis may be important in diagnosing recurrent or de novo immunoglobulin deposition diseases or amyloidosis [13].

An anti-GBM type disease may develop in allografts transplanted into recipients with Alport syndrome as their original disease. In this disorder, antibodies develop against the α-3, α-4, or α-5 chains of collagen type IV of the kidney allograft because the recipient was never exposed to these collagens before. Patients with Alport syndrome with relatively large deletions of *COL4A5* are thought to be more susceptible to anti-GBM disease development. Patients with Alport syndrome with X-linked disease targeting the NC1 domain of *COL4A5*, and other patients with Alport syndrome have autosomal recessive disease targeting the NC1 domain of either *COL4A3* or *COL4A4*. About 3–5% of patients with Alport syndrome develop de novo anti-GBM disease after kidney transplantation; approximately 75% of cases occur in the first year after transplant, and about 90% of grafts fail within months after onset. This anti-GBM disease is manifested by hematuria. Microscopically, there is cellular crescent formation and/or fibrinoid necrosis in >80% of glomeruli. By immunofluorescence, strong IgG staining along the GBM is seen. Plasmapheresis is typically the treatment of choice; immunosuppressive agents such as cyclophosphamide, high-dose corticosteroids, antithymocyte globulin, and mycophenolate mofetil have also been used [13,129–135].

Some metabolic and other familial diseases may recur in renal transplants. For primary hyperoxaluria, liver transplantation may be curative and make the need for kidney transplantation unnecessary. Excretion of oxalate may stimulate disease recurrence [13]. Fabry disease has a decreased recurrence rate with the development of enzyme replacement therapy. In cystinosis, macrophages containing cystine crystals may accumulate in the interstitium or glomerular mesangium; cysteine may also deposit in other organs [13]. Familial, atypical, or non-shiga toxin HUS has a high recurrence rate for complement factor H and I mutations; 20% recurrence for CD46 mutation [13,136,137]. Sickle cell nephropathy may also recur in renal transplants [13,138].

Future directions in biopsy assessment

Improved biopsy assessment can be expected to result from new developments in molecular and image analysis techniques. Significant progress has been made in applying "omics" technologies to allograft biopsies [77,78]. The molecular phenotype of renal allograft biopsies is already widely defined and understood. The next step will be further validation of its clinical utility (i.e. the identification of those areas and disease where molecular assessments are superior to histopathology). In addition, further refinement of turn-around times, development of reporting formats for high-dimensional "omics" data, and reduced costs are necessary to make molecular biopsy assessment a clinically feasible adjunct to histopathology. Thus, a new Banff working group has been formed to validate molecular markers and develop consensus guidelines for their use [36].

In addition, the advent of digital microscopy (i.e. whole slide scanning) offers significant potential for further improvements in assessment of biopsy results. Digital files of scanned histologic biopsy sections offer unprecedented detail and richness of image information, which allows significant information to be extracted by computer-based image analysis techniques. For example, assessment of interstitial fibrosis will very likely be automated in the next couple of years. Studies over the prior decade have demonstrated the utility of such an approach. Several applications require accurate measurement of interstitial fibrosis [139–161], including research focused on therapeutic inhibition of interstitial fibrosis and comparison of renal allograft protocol biopsies [162–165]. Furthermore, the combination of multicolor immunofluorescence techniques with digital microscopy and tailored image analysis algorithms will allow for quantitative and reproducible assessment of inter-related proteins representative of relevant immunopathologic pathways operating in the biopsy.

Conclusions

Kidney graft rejection and recurrent disease are important to recognize in renal allografts. In particular, AMR and glomerular disease are relevant because they represent the two major causes for late renal allograft loss [39,40,102]. In 80% of all kidney allografts with dysfunction and IFTA in the biopsy, an underlying disease process causing the chronic tissue injury can be identified [102]. It is important to expect the biopsy to reveal a specific diagnosis. Non-specific terms trying to summarize numerous pathomechanisms into one "pseudo-entity" like chronic allograft nephropathy (CAN) are obsolete [12]. Common histologic features like IFTA and interstitial inflammation are mostly non-specific epiphenomena of an underlying specific disease. The detailed understanding of the morphologic features and their integral interpretation with corresponding serologic, laboratory, and clinical features is crucial for making appropriate therapeutic decisions. Recent developments in assessing molecular phenotypes from allograft biopsies have shed important insights into the pathogenesis of some changes in renal allografts; and it is anticipated that molecular assays will continue to make even greater advances in our evaluation and understanding of these changes, complementing histology [36,77–79], and possibly eventually alleviating the need to perform some biopsies.

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Statement of competing financial interests

The authors have no relevant competing financial interests to disclose.

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CHAPTER 82

Histopathological Syndromes of Liver Allograft Rejection and Recurrent Disease

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Introduction

Liver transplantation is utilized to treat a broad spectrum of end-stage liver diseases. Hepatitic C virus (HCV), alcohol, and non-alcoholic fatty liver disease are leading indications in North America, Europe, and South America, whereas in Asia, hepatitis B virus (HBV)-induced cirrhosis accounts for the majority of transplants.

This chapter focuses on unique aspects of common diseases modified by transplantation as well as on conditions unique to allografts, such as rejection, small-for-size syndrome, and preservation–reperfusion injury. We employ tabular presentations for easier reference, including comprehensive tables prepared by the Banff Working Groups on acute and chronic rejection and late allograft dysfunction. The chapter complements Chapters 78 and 70, which deal with the clinical management of recurrent diseases of the liver and allograft rejection, respectively.

Deceased donor biopsy evaluation

Tissue for frozen section should be representative and obtained fresh, preferably in the presence of the pathologist who should also grossly inspect the organ. We routinely obtain three tissue samples if the gross appearance is uniform: two 2.0-cm 16-gauge needle cores, one each from the right and left lobes, and one 2.0-cm^2 subcapsular right lobe wedge biopsy. Cores are used to stage fibrosis; a wedge can be additionally helpful for evaluating arterial or arteriolar disease and steatosis.

Pitfalls to avoid during sample preparation include air-drying or storage in physiologic saline, which can cause hepatocytes to appear shrunken or necrotic. Also, absorbent substrates can leach fat out of the tissue resulting in underestimation of steatosis. Biopsies should be transported immediately to the frozen section room on a paper towel moistened with preservation solution or in a plastic specimen container.

Difficulty in sectioning should alert the pathologist to the possibility of steatosis. Recognition of hepatocytes in various stages of injury can be enhanced by staining sections for increasing lengths of time in eosin, which renders necrotic hepatocytes hypereosinophilic, also often allowing recognition of early karyorrhexis.

The pathologist should request additional information if biopsy findings do not correlate with known history or events. Donor biopsy evaluation is only one laboratory test; the pathologist is unable to predict organ function after transplantation based on donor frozen section evaluation in the absence of significant histopathologic findings.

Biopsy findings that usually disqualify organs include diffuse hepatocyte necrosis, severe macrovesicular steatosis (defined later), advanced intrahepatic atherosclerosis, and unequivocal bridging fibrosis [1–4]. Polarization microscopy offers a quick and accurate estimate of liver fibrosis without trichrome stain and represents a useful diagnostic adjunct.

Macrovesicular steatosis is typically defined as large fat globules (usually >1/2 nuclear diameter) associated with peripheral nuclear displacement, whereas small vacuolar or **microvesicular steatosis** is defined as multiple small fat globules (<1/2 nuclear diameter) associated with a centrally placed nucleus. Macrovesicular steatosis (>30%) increases susceptibility to preservation–reperfusion injury, impairs regeneration, and is associated with decreased graft survival [5–7]. Microvesicular steatosisis often occurs after a short period of warm ischemia and usually does not adversely affect outcome. However, one study associated "high grade" microvesicular steatosis with delayed graft function [8]. In our opinion, the severity of macrovesicular steatosis can be *roughly estimated* on hematoxylin and eosin (H&E) stained slides alone.

The interpretation algorithm at our institution is as follows. Mild donor macrovesicular steatosis (<10%) does not influence the decision-making process. Livers with moderate macrovesicular steatosis (10–30%) are usually still used, but other factors are also taken into consideration in the decision-making process (e.g. extended criteria donor (ECD) characteristics). Livers with >30%, or severe steatosis, are used only under special circumstances, such as when cold ischemic time is kept to a minimum (usually <8–9 hours) and there are very few or no other ECD risk factors. The outcome in such situation can be comparable to non-steatotic donor livers [9,10].

Necrosis in donor biopsies has negatively impacted recipient outcome in some [11,12], but not all studies [8,13]. In our experience, the liver is usually disqualified if >10% (roughly estimated) of hepatocytes are necrotic and the necrosis diffusely involves both the wedge and needle cores; the assessment should not be based on isolated subcapsular necrosis and should be correlated with donor liver injury test profiles.

HCV-postive donor livers at our institution undergo frozen section biopsy analysis. Those without bridging fibrosis (<3/6; Ishak scale) are offered to potential recipients after informed

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consent. Other groups report one stage lower fibrosis cutoff (<2/6) [14].

A variety of neoplastic, infectious, and metabolic diseases have been inadvertently transferred from donors to recipients [15]. Examples that *might* be detectable by pathologists include various cancers, amyloidosis, hemochromatosis, fungal, viral, and parasitic diseases [15]. Metabolic diseases, such as familial amyloid polyneuropathy [16], oxalosis [17], and possibly α_1-antitrypsin deficiency [18] can be intentionally or unintentionally transferred with the donor organ in "domino" transplants.

Living donor biopsy evaluation

Living donor liver operations currently account for ≤5% of North American and European transplants, but for the vast majority of operations in Asia [19]. As major liver resection entails risk (mortality roughly of 2:700 [20], some centers routinely biopsy potential living donors to further minimize risk [21–24]; 20–50% of potential living donor biopsies show usually mild abnormalities.

The most common pathology and also the most common reason for donor disqualification is macrovesicular steatosis of variable severity in 14–53% [21–26]. Most programs limit macrovesicular steatosis in living donors to <30% because this level does not appear to adversely impact the course of either donor or recipient [23]. Other programs limit macrovesicular steatosis to <10% or 20% [26,27].

Other biopsy findings include low-grade chronic hepatitis of undetermined etiology, granulomas, and a variety other unexpected findings (e.g. unexpected early stage primary biliary cirrhosis (PBC)) [24]. Mild periportal hepatocellular iron deposits are present in ~17% of mostly male potential donors. This subclinical finding does not preclude transplantation. Unexplained portal tract eosinophilia can also occur and did not adversely affect the postoperative donor or recipient clinical course in two cases [24].

Determination of causes of graft dysfunction after transplantation (Table 82.1)

Post-transplant allograft needle biopsies

Allograft biopsy monitoring is used: (1) to determine the cause of dysfunction, if present; (2) assess the effect of therapy and/or progression of disease; (3) document the immunologic and architectural status to help guide immunosuppression therapy (IS). For native livers, the American Association for the Study of Liver Disease has recommended two passes with a 16-gauge needle for adequate assessment of fibrosis due to sampling error inherent with small biopsies (<20 mm long); additionally, those containing <11 portal tracts might not be representative (reviewed in [28]). Similar guidelines should be followed for liver allograft biopsies, particularly those obtained late after transplantation where assessment of fibrosis is important.

Most histopathologic studies can be completed on routinely processed formalin-fixed paraffin-embedded sections. A clinical differential that includes antibody-mediated rejection (AMR) optimally includes fresh frozen tissue for immunofluorescent staining for immunoglobulin and complement, but less sensitive formalin-fixed paraffin embedded samples can also be used for C4d staining [29,30]. Most frequently utilized special stains, ordered only on indication after review of the H&E slides, include trichrome, iron, and copper to detect chronic cholestasis. Cytokeratins 7 or 19 can help to localize bile ducts and ductular metaplasia of periportal hepatocytes [31] in cases with suspected ductopenia and chronic rejection.

It is often best to first complete the slide review and then correlate findings with the clinical history and laboratory results to generate a differential diagnosis. We also routinely compare current findings with findings in previous biopsies. This greatly assists with the interpretation, assessment of therapeutic intervention, and disease progression. Re-review of all liver allograft biopsies at a weekly clinicopathologic conference is an essential quality assessment tool that provides feedback to clinical physicians and pathologists.

Failed allograft evaluations

Gross examination of failed allografts should follow a standardized approach [32] with special attention paid to anastomotic sites: biliary, hepatic artery, portal and hepatic veins. At times this will require assistance of the operative surgeon to explain the surgical anatomy. Routine tissue sampling should include: (1) anastomoses, if present; (2) superficial and deep sections of the right and left lobes; (3) at least one deep hilar section with cross-sections of medium-sized bile ducts and arteries; and (4) any grossly obvious defects.

Preservation–reperfusion injury or primary non-function, vascular thrombosis, and patient death are the leading causes of allograft failure within the first few weeks after transplantation [33,34]. Very few allografts fail because of acute cellular rejection (ACR) or AMR [35,36]. Recurrent disease, delayed manifestations of technical complications, such as vascular thrombosis or biliary sludge syndrome, and patient death are most commonly responsible for late (>1 year) graft failures [37,38]. Chronic rejection is uncommon as a cause of graft failure [37,39]. Recurrent HCV-induced cirrhosis is a leading cause for allograft failure that challenges organ allocation algorithms [40].

Special considerations in reduced size and living-related liver allografts

Normal liver structure and function is dependent on optimal portal venous and hepatic artery inflow and adequate venous outflow and biliary drainage. Transplanting only a portion of the liver necessarily compromises at least one of these conduits, especially near the cut edge of the residual liver. It is therefore important for the pathologist to be aware of the technical details of the operation and the exact origin of the post-transplant biopsy because of non-representative and potentially misleading local alterations related to sampling site. For example, infarcted parenchyma or high grade biliary or venous outflow obstruction changes can be seen in biopsies obtained near the cut surface in otherwise well recipients with normal or near-normal liver injury tests.

Early after transplantation. reduced size and living donor allografts typically undergo robust regeneration and might be more susceptible to damage from needle biopsies [41] and AMR [42]. Late after transplantation, portal venopathy, low-grade ductular reactions, and nodular regenerative hyperplasia changes are fairly common in reduced size and living donor liver allografts [43].

Preservation–reperfusion injury

Preservation–reperfusion injury refers to manifestations of donor organ damage that result from the cumulative effects of agonal donor events (warm ischemia), cold ischemia, warm sanguineous reperfusion in the recipient, and perioperative events [44,45]. Donation after circulatory death (DCD) donors merit special mention because these organs all suffer an extended warm

Table 82.1. Approximate timing of common allograft syndromes (data from [260] and [156])

Syndrome	Clinical associations/observations	Peak time period
"Preservation" reperfusion injury	Long cold (>12 h) or warm (>120 min) ischemic time; older (>60 yr), hemodynamically unstable, DCD, "re-do" of anastomoses; poor bile production; prolonged cholestatic phase predisposes to biliary sludge syndrome	Recognized primarily in post-reperfusion biopsies and biopsies obtained within the first several weeks after OLTx. Changes can persist for several months depending on the severity of the initial injury
AMR	ABO-incompatible donor; high titer (>1 : 32) lymphocytotoxic cross-match DSA; presents with persistently low platelet counts and complement levels during first few weeks after transplantation	First few weeks to months after transplantation; later onset less common and not well-defined
Acute cellular rejection	Younger "healthier" female, and inadequately immunosuppressed recipients, long cold ischemic times, and those with disorders of dysregulated immunity (e.g. PSC, AIH, PBC)	Peak dependent on IS regimen; usually 3–40 days; later onset usually associated with inadequate IS
Chronic rejection	Usually occurs in inadequately immunosuppressed patients (e.g. infections, tumors, PTLD) and patients have a history of moderate or severe or persistent acute rejection episodes or are non-compliant	Bimodal distribution; early peak during first year and later increase in non-compliant and inadequately immunosuppressed patients
Hepatic artery thrombosis	Suboptimal anastomosis; pediatric/small caliber vessels; donor and/or recipient atherosclerosis; suboptimal or difficult arterial anastomosis; large difference in vessel caliber across anastomosis; hypercoagulopathy; suboptimal arterial flow (vasospasm from small-for-size syndrome	Bimodal distribution; early peak 0–4 weeks and later peak between 18 and 36 months (see text)
Biliary tract obstruction or stricturing	Arterial insufficiency or thrombosis; long cold ischemia, DCD, difficult biliary anastomosis; AMR; original disease of PSC	Variable, but timing can be used to determine etiology: <6 months usually mechanical, preservation–reperfusion injury (ischemic cholangiopathy) or AMR; >6 months recurrent disease, mechanical
Venous outflow obstruction	Difficult "piggyback" hepatic vein reconstruction; cardiac failure	Usually during the first several months
"Opportunistic" viral (CMV, EBV, adenovirus, etc.) and fungal infections (see text)	Seropositive donors to seronegative recipients (often pediatric); over-IS	0–8 weeks, much less common thereafter except for EBV-related PTLDs and other EBV-related tumors (see text)
Recurrent or new onset of viral hepatitis (e.g. HBV, HCV, HEV).	Original disease HBV, HCV, or acquired HEV-induced hepatitis in patients with contact with animals or culinary inconsistencies	Usually first becomes apparent 4–6 weeks after transplantation and persists thereafter; earlier onset (within 2 weeks) in aggressive cases
Recurrent AIH, PBC, and PSC	Original disease of AIH, PBC, or PSC (see text for risk factors)	Usually more than 6 months after transplantation; incidence of recurrence increases with time after transplantation
Alcohol abuse	Psychiatric co-morbidity/social instability; non-compliance with treatment protocols; γ-GTP : ALP ratio >1.4 (see text for risk factors)	Usually >6 months
NASH	Original disease NASH or cryptogenic cirrhosis; persistent or worsening risk factors for NASH in general population	Usually >3–4 weeks and increases with time if risk factors persist

AIH, autoimmune hepatitis; ALP, alkaline phosphatase; AMR, antibody-mediated rejection; CMV, cytomegalovirus; DCD, donation after circulatory death; DSA, donor-specific antibody; EBV, Epstein–Barr virus; γ-GTP, gamma-glutamyl transpeptidase; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HLA, human leukocyte antigen; HSV, herpes simplex virus; IS, immunosuppression; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; PTLD, post-transplant lymphoproliferative disorder; VZ, varicella-zoster virus.

ischemic insult. Even in the best of hands, considerable blood component sludging and subsequent damage occurs in the peribiliary plexus, which in turn predisposes to ischemic cholangiopathy [46–48].

Clinical presentation

Early signs of preservation–reperfusion injury include poor bile production and persistent elevation of serum lactate. This is usually associated with marked (>2500 IU/mL) elevations of serum alanine aminotransferase (ALT) and aspartate transaminase (AST) during the first few days after transplantation [3], followed by rapid ALT/AST normalization during the first week and a prolonged "cholestatic phase" characterized by persistent elevation of total bilirubin and gamma-glutamyl transpeptidase (γ-GTP). Recovery is accompanied by a gradual resolution of abnormal liver injury tests, but such grafts are also at risk for developing ischemic cholangiopathy and the biliary sludge syndrome [49,50].

Histopathologic findings

Post-reperfusion needle biopsies obtained within several hours of revascularization can reliably gauge the extent of preservation–reperfusion injury [3,51]. Mild damage is common and shows microvesicular steatosis, hepatocellular cytoaggregation (i.e. detachment of individual hepatocytes and "rounding up" of cytoplasm), and hepatocellular swelling [3,32]. Severe injury is characterized by zonal or confluent coagulative necrosis, which can be periportal, bridging, and combined with severe neutrophilic inflammation [3,51].

Mild injury is usually followed by hepatocellular mitoses, nuclear enlargement, and thickened trabeculae. Continuing injury manifests as typically co-existent mild centrilobular hepatocellular swelling and hepatocanalicular cholestasis, which often persist for several weeks. Severe injury is usually accompanied by marked centrilobular hepatocellular swelling and hepatocanalicular and cholangiolar cholestasis, which may persist for 1–2 months [3,32]. Cholangiolar proliferation is usually triggered by periportal and confluent bridging necrosis with reticulin framework collapse [3,32]. With recovery, normal architecture can be restored but risk for ischemic cholangiopathy remains.

Preservation–reperfusion injury of livers with >20% macrovesicular steatosis causes death of some fat-containing hepatocytes with lipid release into sinusoids. These fat droplets coalesce into larger globules, triggering local fibrin deposition, neutrophilia, congestion, and local sinusoidal obstruction [1]. If recovery occurs, the

large fat globules become surrounded by macrophages and eventually resolve over several weeks.

As hepatocytes require only 4–6 hours to undergo the entire apoptotic cycle, recognition of hepatocyte apoptosis or coagulative necrosis in a biopsy obtained more than several days after transplantation should arouse the suspicion of another, usually ischemic, insult [52].

Differential diagnosis

Biliary obstruction and pancreatitis, sepsis, AMR, and cholestatic hepatitis can produce histopathologic changes resembling preservation–reperfusion injury. Detailed donor information helps to determine the likely source of injury [35]. Preservation–reperfusion injury and operative technical difficulties most often prove to be the cause of liver injury early after transplantation.

Examination of true bile ducts and cholangioles provide useful clues in distinguishing between preservation–reperfusion injury and biliary tract obstruction or stricturing. The latter etiology usually causes at least some periductal lamellar edema surrounding true bile ducts or produces stellate-shaped septal bile duct lumens often containing intraluminal or intramural neutrophils. Preservation–reperfusion injury, in contrast, does not usually show "true" bile duct changes. Instead, neutrophils surround interface zone cholangioles. Centrilobular hepatocanalicular and cholangiolar cholestasis and intralobular neutrophil clusters are common to both disorders.

ACR superimposed on preservation injury is recognized by "typical rejection-type infiltrate" in portal and perivenular regions: blastic and smaller lymphocytes and especially eosinophils, which are an excellent marker of an early emerging rejection reaction, as is convincing lymphocytic cholangitis, and lymphocytic central perivenulitis.

Cholestatic hepatitis can be difficult to distinguish from preservation injury in the absence of clinical context. Cholestatic hepatitis has only been reported in patients infected with HBV or HCV and is distinctly unusual before 3–4 weeks after transplantation. Cholestatic hepatitis usually worsens with time unless the patient is specifically treated with decreased IS and/or antiviral therapy, whereas the trend is toward gradual improvement with preservation–reperfusion injury.

Portal hyperperfusion or "small-for-size" syndrome

Pathophysiology

Excessive venous inflow can injure portal venous and periportal sinusoidal endothelium [43,53] and contribute to allograft dysfunction referred to as the portal hyperperfusion (PHP) or small-for-size graft syndrome (SFSS). Increased portal venous flow causes adenosine washout and arterial constriction. This, in turn, predisposes to arterial thrombosis and ischemic cholangitis.

Liver regeneration [43] is dependent on portal blood and too little inflow can impair regeneration and cause graft steatosis [54,55]. Failure of liver regeneration is generally not a major problem unless also impeded by suboptimal hepatic venous drainage [56].

Clinical presentation

Dahm et al. [57] defined PHP/SFSS as at least two of the following complications on three consecutive days within the first few weeks after transplantation: elevated bilirubin (>100 μmol/L), INR >2, and grade 3–4 encephalopathy after exclusion of technical, immunologic, or infectious complications. PHP/SFSS probably also contributes to so-called technical complications, found with increased frequency in reduced size allografts [43].

Histopathologic findings

Denudation of portal vein and periportal sinusoidal endothelium may occur as early as 5 min following transplantation [43,53,58–60]. In severe cases, microvascular rupture at portal vein–sinusoidal junctions can cause portal stromal hemorrhage that can dissect into hepatic parenchyma [61,62]. Reparative changes occur if the allograft survives the initial crisis: endothelial cell hypertrophy, subendothelial edema, and in-growth of myofibroblasts and endothelial cells lead to fibrointimal hyperplasia/intimal thickening and luminal obliteration or recanalization of thrombi. Venous findings are uncommonly seen in biopsies. Instead, one often observes nonspecific centrilobular cholestasis, centrilobular hepatocyte steatosis, or hepatocyte atrophy with sinusoidal dilatation, and/or centrilobular hepatocyte necrosis and low-grade ductular reaction.

Hilar sections of failed allografts frequently show traumatic injury to larger portal vein endothelium, focal fibrointimal hyperplasia of vein branches, evidence of arterial vasospasm, and, in some cases, ischemic cholangitis, particularly if the hepatic artery has thrombosed [43]. Eventually, if the graft recovers, portal hypertension and ascites resolve over several weeks. This is accompanied by restoration of normal architecture, except that some grafts eventually develop significant nodular regenerative hyperplasia resulting from portal venopathy [43].

Differential diagnosis

Arterial thrombosis, stricturing, sepsis, hypotension, biliary tract obstruction and stricturing and ischemic cholangitis can cause histopathologic changes similar to PHP/SFSS. Preservation injury might also be a differential diagnostic consideration, but living donor grafts are usually not affected significantly. Biliary tract obstruction or stricturing alone is usually not accompanied by either portal tract connective tissue hemorrhage or centrilobular hepatocyte ischemic changes, or significant nodular regenerative hyperplasia.

Vascular complications

Hepatic artery thrombosis

Pathophysiology

Hepatic artery thrombosis (HAT) is the most frequent major vascular complication and usually occurs in 2–20% of transplants within 30 days of transplantation [63,64]. Because the hepatic artery supplies the extrahepatic and intrahepatic bile ducts, hilar and portal tract connective tissue, and hilar lymph nodes, these structures are preferentially damaged. The injury pattern is expressed in the generic phrase "ischemic cholangitis" [32,49] or "ischemic cholangiopathy" [50], which refers to ischemic damage to the biliary tree manifest as frank necrosis, poor wound/anastomotic healing, biliary leaks, and cholangitic abscesses, resulting in the biliary sludge syndrome [65].

Clinical presentation

Most early HAT manifests clinically as fever, leukocytosis, severe elevations in liver injury tests, and septic shock [63,64]. Late HAT can be asymptomatic or present insidiously with cholangitis, relapsing fevers, and sepsis related to hepatic infarcts, abscesses, ischemic cholangiopathy, and subsequent impaired bile flow

[63,64]. Ultrasonography can be used to screen hepatic arterial flow status, but angiography is the most reliable method of establishing the diagnosis with certainty.

Histopathologic findings

Hepatic artery thrombosis can be diagnosed on peripheral core needle biopsies, but not reliably because needle biopsies sample the subcapsular parenchyma, which is variably affected, leading to sampling issues [32,66]. Structures most commonly and reliably susceptible to ischemic injury, such as perihilar tissue and large bile ducts, are not routinely sampled.

Peripheral core needle biopsies can show centrilobular coagulative necrosis or marked centrilobular hepatocyte swelling and biliary tract complications, such as cholangiolar proliferation, acute cholangiolitis, and obstruction or stricturing. The biopsy might even be unremarkable, either because the patient has developed vascular collaterals or because biliary sludging secondary to hilar bile duct necrosis has not yet developed in the periphery. HAT can occasionally present as spotty acidophilic necrosis of hepatocytes or "ischemic hepatitis", mimicking acute viral hepatitis. Chronic suboptimal arterial flow can cause centrilobular hepatocellular atrophy and sinusoidal widening.

Failed allografts with HAT often show necrosis of hilar or perihilar bile ducts with bile leakage into surrounding connective tissue, biliary sludge, biliary abscesses seeded with fungi and bacteria, infarction of hepatic hilar lymph nodes, and patchy parenchymal infarction.

Differential diagnosis

As HAT or narrowing can mimic almost every liver allograft syndrome, a high index of suspicion should be maintained. Ischemic necrosis, often centrilobular, most reliably points toward HAT, but suboptimal flow can also result in centrilobular hepatocyte swelling, biliary tract obstruction and cholangitis. Chronic suboptimal arterial flow can cause biliary epithelial cell senescence changes that resemble chronic rejection. "Ischemic hepatitis" (spotty hepatocyte apoptosis) can be virtually indistinguishable from the lobular phase of acute viral hepatitis. The relationship between arterial thrombosis and biliary tract complications is so common that examination of hepatic arterial patency should be routinely considered when biliary tract complications are encountered.

Portal vein thrombosis

Pathophysiology

Portal vein complications are much less frequent than arterial complications and involve ~1–2% of recipients [67]. Long-surviving liver allografts with recurrent disease or cirrhosis are also are susceptible to portal vein thrombosis, as with any cirrhotic liver [67].

Clinical presentation

Portal vein thrombosis in a non-cirrhotic allograft can cause widespread necrosis and patients present with bleeding varices, fulminant hepatic failure, and portal hypertension with massive ascites and edema [67]. If obstruction is incomplete, small infarcts can develop or the liver can become seeded by intestinal bacteria resulting in relapsing fever.

Histopathologic findings

Complete portal venous obstruction early after transplantation often causes massive coagulative necrosis. Suboptimal portal vein flow because of strictures, kinks, or persistent collateral circulation can cause periportal or midzonal hepatocyte atrophy, coagulative necrosis, unexplained zonal or panlobular steatosis, nodular regenerative hyperplasia [68], or hepatic steatosis. Bacterial or fungal infection of a partial portal vein thrombus can result in miliary seeding of the liver with small abscesses.

Differential diagnosis

Suboptimal portal vein blood flow can be difficult to distinguish from suboptimal hepatic venous drainage. Linear zones of ischemic necrosis and hepatocellular atrophy favor the former, whereas red blood cell congestion within central veins and centrilobular sinusoids and obliterative central venopathy favors the latter. Ultrasonography and angiography are necessary to characterize the cause of the vascular abnormality. Cases of suboptimal portal vein flow presenting with intrahepatic steatosis need to be distinguished from recurrent or de novo steatosis or steatohepatitis.

Hepatic vein and vena caval complications

Complications involving the hepatic venous outflow are relatively uncommon. Significant stenosis or thrombosis of the outflow tract is mostly always associated with either significant clinical or histopathologic manifestations.

Clinical presentation

Severe stenosis or thrombosis presents as the Budd–Chiari syndrome including hepatic enlargement, tenderness, ascites, and edema; less severe stenosis might initially result only in histopathologic manifestations or an increase in portal vein–vena cava pressure gradient.

Histopathologic findings

Congestion and hemorrhage involving the hepatic venules and surrounding perivenular sinusoids are the most reliable histopathologic correlates of suboptimal venous drainage. Bland centrilobular hepatocyte necrosis and dropout are also usually present. Chronic changes can include nodular regenerative hyperplasia, perivenular fibrosis, and central vein occlusion, particularly if outflow obstruction is severe or of long duration, and venocentric cirrhosis. Chronic changes can also be accompanied by a prominent ductular reaction at the interface and perivenular areas, which can make it difficult to recognize architectural landmarks and to exclude biliary obstruction.

Differential diagnosis

If any of the above-mentioned changes are *not* accompanied by significant inflammation, a mechanical or outflow obstruction cause should be suspected. Conversely, if centrilobular changes are accompanied by significant lymphocytic, histiocytic or lymphoplasmacytic inflammation, an immunologically mediated cause of injury, such as acute or chronic rejection, autoimmune hepatitis (AIH), or drug-induced liver injury (less likely) should be suspected. However, perivenular inflammation can be transient in some cases of "immune-mediated" centrilobular injury and can present at a later stage when it is indistinguishable from mechanical causes of venous outflow obstruction [69]. Review of the clinical history and previous biopsies can help determine the underlying cause.

Bile duct complications

The biliary tree continues to be the "Achilles' heel" of liver transplantation with complications occurring in ~20% of recipients – an even higher incidence is observed in reduced-size allografts. The

distal-most portion of the extrahepatic donor bile duct is particularly vulnerable to ischemia and the peribiliary plexus vulnerable to both preservation–reperfusion injury and operative injury [70].

Strictures are the most common complication; they are categorized according to time after transplantation (early or late) and location (anastomotic, non-anastomotic, or intrahepatic) [71–75]. Intrahepatic strictures are further categorized into hilar or peripheral. Early (<1 year) non-anastomotic strictures are often associated with preservation-related injury [74,76] and usually occur in perihilar bile ducts. Late (>1 year) non-anastomotic strictures are usually peripheral and associated with immunologic risk factors, such as primary sclerosing cholangitis (PSC) as the original disease.

Histopathologic findings

Allograft biliary tract complications are histopathologically identical to those seen in native livers. Portal and periductal edema, predominantly neutrophilic portal inflammation, intraepithelial and intraluminal neutrophils within true bile ducts, an interface ductular reaction of varying severity, centrilobular hepatocanalicular cholestasis, and small clusters of neutrophils throughout the lobules are the typical findings of stricturing or obstructive cholangiopathy. Chronic biliary tract strictures, or intermittent obstruction, are often associated with mixed or predominantly chronic portal inflammation, biliary epithelial cell senescence changes, and low-grade ductopenia involving the small bile ducts. More than 1 year after transplantation, in addition to the classic features described above, biliary strictures are a relatively common cause of portal eosinophilia.

Biliary and vascular fistulas are recognized by red blood cells in lumens of bile ducts or bile concretions in blood vessels. Occasionally, inordinate elevations of serum bilirubin, beyond normal pathophysiologic ranges, can also be seen with a biliary-vascular fistula. In contrast, **periductal** hemorrhage surrounding small interlobular bile ducts is an inconsequential finding in asymptomatic patients when a biopsy is obtained within a day or so after transhepatic cholangiography [77].

Differential diagnosis

Within the first few weeks biliary obstruction or cholangitis can be difficult to distinguish from preservation–reperfusion injury and ACR, particularly if the patient was treated with increased IS before the biopsy was obtained.

Features that favor obstruction or stricturing over ACR early after transplantation include neutrophilic-predominant portal infiltrate, periductal edema, retention of the normal nuclear : cytoplasmic ratio and biliary epithelial cells, and an absence of perivenular mononuclear inflammation. Acute rejection is favored when mixed portal infiltrate comprised of blastic and small lymphocytes, plasma cells, and eosinophils; lymphocytic cholangitis, and increased nuclear : cytoplasmic ratio in biliary epithelial cells, and perivenular inflammation are seen. Portal eosinophilia in early acute rejection can be quite striking, especially in patients treated with steroid-sparing IS [78].

Late-onset and chronic biliary tract complications can occasionally present with predominantly mononuclear portal inflammation, and biliary epithelial senescence and low-grade ductopenia can develop [79]; they can also cause isolated portal eosinophilia, mimicking acute and chronic rejection, viral hepatitis, and recurrent disorders such as PSC.

Features that favor biliary strictures or recurrent PSC over chronic rejection include a history of biliary tract complications or PSC, periductal lamellar edema, stellate portal expansion, portal neutrophilia, a ductular reaction, and deposition of copper or copper-associated protein in periportal hepatocytes. Features that favor acute or chronic rejection include a previous history of rejection or inadequate IS, lymphoplasmacytic portal inflammation, small portal tracts, absence of a ductular reaction, and active central perivenulitis and perivenular fibrosis.

Additional findings in failed allografts can also be used to distinguish between biliary strictures or recurrent PSC and chronic rejection [80]. Chronically rejected livers are usually of normal or slightly increased weight, whereas those with obstructive cholangiopathy are usually significantly enlarged. In the hilar nodes, obstructive cholangiopathy usually causes bile-pigmented sinus histiocytosis, whereas chronically rejected nodes are usually atrophic or fibrotic. Perihilar arteries are either normal or show mild and focal eccentric fibrointimal hyperplasia, whereas foam cell arteriopathy and significant concentric fibrointimal hyperplasia are typical of chronic rejection. In obstructive cholangiopathy, extrahepatic and large intrahepatic ducts often show focal ulceration, periductal lymphoplasmacytic inflammation, fibrosis, whereas in chronic rejection ulceration is unusual. When the infiltrate in either a periphery needle biopsy or explant perihilar section of a large bile duct shows plasma cell-rich infiltrates, the possibility of recurrent or de novo IgG4 sclerosing disease should be considered [81].

Cholangitis favors a biliary tract complication whereas cholangiolitis and lobular disarray favor chronic hepatitis. The cholestatic liver injury tests profile favors obstructive cholangiopathy unless the patient has a cholestatic variant of viral hepatitis. Deposits of copper or copper-associated protein in periportal hepatocytes are not seen in chronic rejection alone.

Rejection

Antibody-mediated rejection

Crossing ABO blood group barriers in recipients with high titer isoagglutinins causes predictable and severe liver allograft injury [82] and is generally avoided in most North American programs because it leads to a high incidence (~60%) of significant AMR and graft failure [83]. ABO-incompatible liver allografts are still utilized in Asian programs where the donor pool is more limited. Plasmapheresis, vigorous anti-rejection, and microcirculatory protective therapy are needed to achieve reasonable results under these circumstances [84,85]. Even so, ABO-I recipients are still at risk for late complications such as ischemic cholangitis [83,86].

Clinical presentation

The most dramatic form of AMR has largely been eliminated by avoiding ABO-I transplants without pretreatment [84,85]. When it does occur, hyperacute rejection begins immediately after reperfusion and evolves over a period of hours to days, rather than minutes to hours, as in other allograft types [83]. Initial signs of dysfunction usually develop in the operating room after complete revascularization recognized by uneven reperfusion, swelling, dusky appearance, and cessation of bile flow in a liver that initially produced bile. These signs are often accompanied by coagulopathy [82]. Precipitous allograft failure is distinctly unusual.

A more common presentation is an increase or persistent elevation of serum bilirubin and ALT/AST during the first few days to weeks. This is often accompanied by persistence of the positive cross-match and/or donor-specific antibody (DSA), unexplained and often refractory thrombocytopenia, and low serum

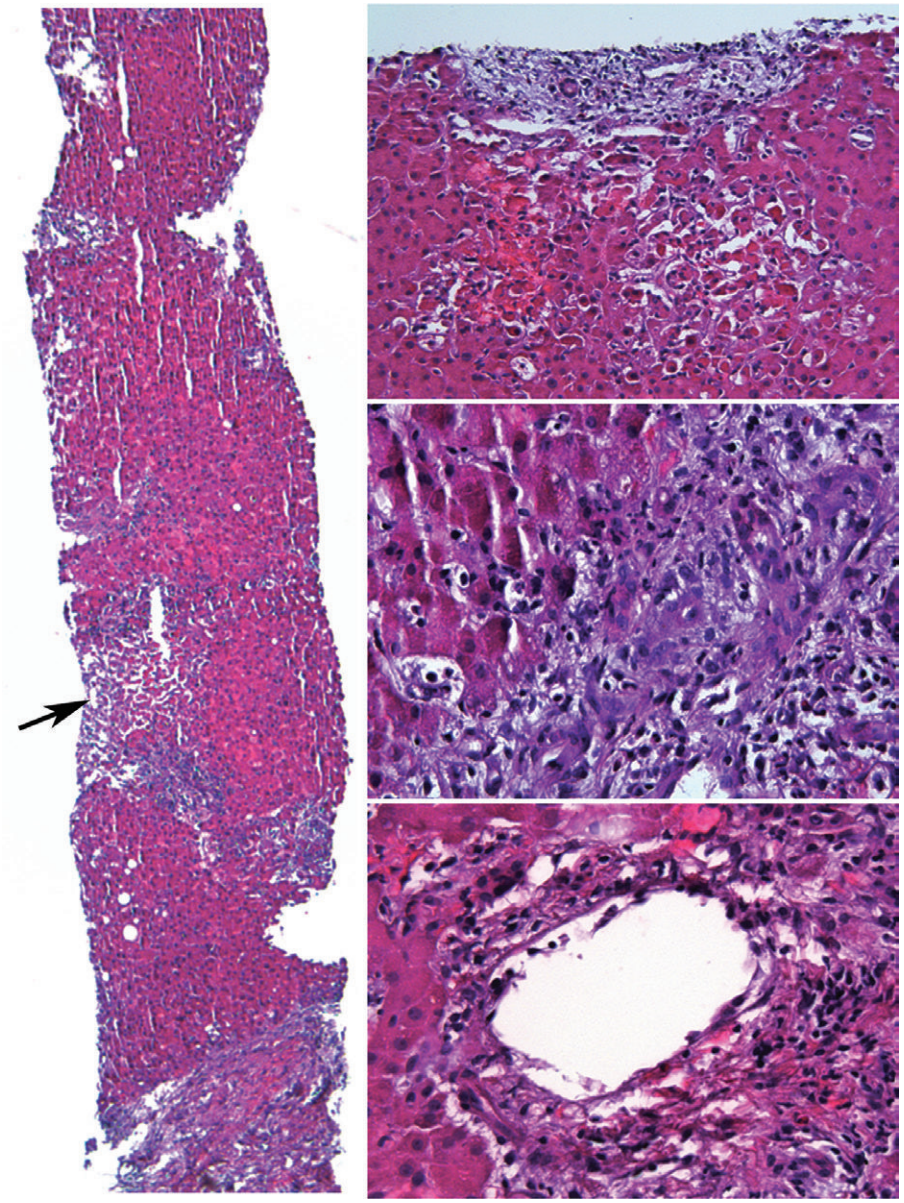

Figure 82.1. Acute antibody-mediated rejection (AMR). This biopsy was obtained from an ABO incompatible liver allograft 8 days after transplantation. The biopsy showed patchy areas of coagulative necrosis (arrow), which is illustrated at higher magnification in the right upper inset. Note also the mild mixed portal inflammation with neutrophils (right middle inset) and marked endothelial/hypertrophy reactivity of the terminal venule (right lower inset). AMR of an ABO-incompatible liver allograft tend to cause substantially more damage than lymphocytotoxic antibodies.

complement [30,87,88]. In severe cases, hepatic angiograms show segmental narrowing indicative of arterial vasospasm [83].

Histopathologic findings

Histopathologic manifestations of AMR are variable, with isoagglutinins usually causing more injury than anti-HLA or lymphocytotoxic antibodies (Figures 82.1 and 82.2).

High titer isoagglutinins (>1:64) [82,89,90] often cause prominent red blood cell and focal neutrophil sludging in the sinusoids, platelet-fibrin thrombi in periportal sinusoids, portal and central veins. Acidophilic hepatocyte necrosis can be seen within 2–6 hours after reperfusion in untreated recipients. This is often quickly followed portal or periportal edema, necrosis, focal hemorrhage, and C4d deposits in portal stroma [89] and diffuse endothelial C4d deposits in portal veins, capillaries, and sinusoids [35,89]. Confluent coagulative necrosis, prominent sinusoidal and venous congestion, and edema and hemorrhage into the portal tract connective tissue begin to appear within 1–5 days in peripheral core needle biopsies (Figure 82.1) [35,82,89]. Portal veins often show fibrin deposition. Medium-sized arteries are not often sampled in needle biopsies, but when present frequently show endothelial cell hypertrophy and evidence of arterial vasospasm, such as mural myocyte vacuolization, wrinkling of the elastic lamina, and thickening of the wall with narrowing of the lumen. Neutrophilic and necrotizing arteritis is only occasionally observed. Portal neutrophilia, cholangiolar proliferation, and

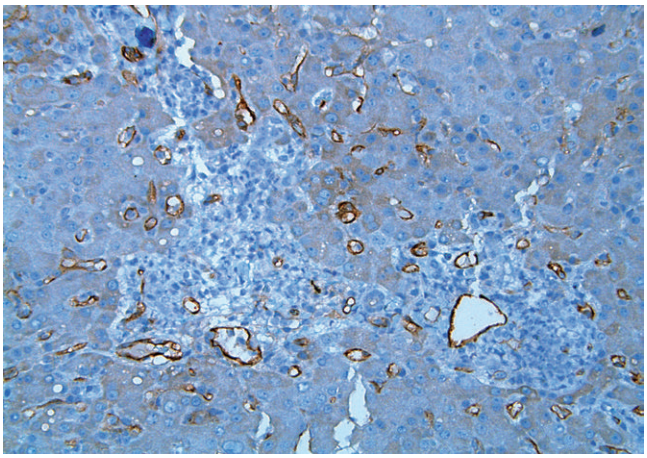

Figure 82.2. C4d staining of the same biopsy as illustrated in Figure 82.1. Note the strong C4d staining in the capillaries and venules of the portal tract as well as in the sinusoidal endothelium. C4d staining can be used to detect complement deposition in formalin-fixed paraffin-embedded tissue samples.

small areas of confluent hepatic necrosis usually begin to appear at 2–3 days. If untreated, progressive hemorrhagic infarction can occur in ABO-incompatible organs over a relatively short period of time (1–2 weeks).

ABO-I allografts that fail because of AMR are often grossly enlarged, cyanotic, and mottled with areas of geographic necrosis. Capsular ruptures, hepatic artery or portal vein thrombosis can be seen in extreme cases. Changes in the hilum–perihilar region can be particularly helpful in establishing a diagnosis of AMR. Included are congestion and leukocyte margination in the peribiliary vascular plexus, partially organized thrombi in arterial branches, focal mural necrosis of large septal bile ducts, and inflammatory and/or necrotizing arteritis. Late sequela of AMR include biliary sludge and stricturing with obstructive cholangiopathy, and obliterative arteriopathy and loss of small bile ducts, or chronic rejection [82,84,86,91,92].

Reperfusion biopsies from patients with high titer (>1:32) lymphocytotoxic antibodies show platelet aggregates in the portal or central veins more often than cross-match negative controls [2,87]. Spotty acidophilic necrosis of hepatocytes and centrilobular hepatocellular swelling, accompanied by cholangiolar proliferation and hepatocanalicular cholestasis, often appear during the first week after transplantation in those with high titer. Inflammatory or necrotizing arteritis is rarely present, but nearly diagnostic when found. The histopathologic changes closely resemble those seen in preservation–reperfusion injury. Clinicopathologic correlation with exclusion of other causes of dysfunction and staining for C4d and other immune deposits is needed to confirm the diagnosis (Figure 82.2) [29,30,35,93].

An AMR diagnosis in liver allograft should be based on all of the following:

1 Careful biopsy review showing changes consistent with a pattern of AMR injury;
2 Clinicopathologic correlation with exclusion of other insults;
3 Serologic evidence of DSA; and
4 Evidence of strong and diffuse antibody and/or complement (C4d) deposition within the injured allograft [35,82,87].

The latter is defined as strong portal vein and capillary and usually periportal sinusoidal endothelial staining involving the majority of portal tracts.

Compared with other allografts, however, a diagnosis of isolated liver allograft AMR because of anti-HLA antibodies is difficult to establish with certainty. Difficulties are encountered in establishing the diagnosis because:

1 Liver allografts are large, able to absorb high antibody loads, especially anti-class I HLA [94], and are resistant to AMR-related damage [3,83,87,95,96];
2 Immune deposits pointing toward an underlying injury cause are: (a) ephemeral [82,87]; (b) can be associated with other insults (see below); and (c) are more easily detected in frozen sections [97]; and
3 Clinicopathologic similarities exist between AMR and preservation injury, sepsis, and biliary or vascular complications [82,87].

Intrahepatic immune deposits are ephemeral in AMR, even when using the more sensitive technique of immunofluorescence on frozen tissue [30,82,87]. In severe cases, selective deposits of IgG, and/or IgM, C3, and C4 are usually detectable diffusely along the sinusoids and in perihilar arteries, portal veins, and peribiliary plexus [30,82,87]. Thereafter, immune deposits become patchy in distribution and may be difficult to distinguish from background staining unless antibody titers are quite high. Regardless, if AMR is suspected, saving frozen tissue for immunofluorescence testing can facilitate the diagnosis.

Interpretation and practical utility of C4d staining in liver allografts is more challenging than in kidney allografts [89,98–105]. This literature was recently reviewed by Bellamy [29]. Normal livers and liver allograft biopsies are usually C4d$^-$. Portal venous, arterial, and portal capillary and sinusoidal endothelial C4d has been detected in cross-match (XM) positive (XM+) recipients more often than XM negative (XM−) controls [103] and in those who developed isolated AMR [97,105]. Endothelial cell C4d staining might be most specific for AMR, but "portal C4d stromal" staining has also been described in ABO-incompatible AMR [89], ACR [103], and chronic rejection [93,106].

As in other allografts, C4d deposits are also often accompanied by ACR [35,89,93,98–105] and in some studies are directly proportional to the Banff grade [89,98–105]. Necrotic and steatotic hepatocytes can show non-specific C4d staining.

Portal vein and capillary C4d deposits can also be detected in other circumstances, including biliary obstruction [98], recurrent HBV infection [99], recurrent HCV infection [101], and de novo AIH [107]. C4d staining has also been described in portal venous and capillary, sinusoidal, central vein, and arterial endothelial cells, in lymphoid nodules, and in periductal and portal stromal cells in *native* pediatric livers with HBV, HCV, AIH, and overlap syndromes between AIH and PSC [108]. Endothelial C4d deposits in non-rejection-related allograft disorders are reportedly less widespread than in severe ACR or AMR. Similar to kidney and heart allografts, liver C4d deposits have also been associated with macrophage and plasma cell infiltrates [100].

Differential diagnosis

AMR should be suspected in a female liver allograft recipient with high titer, DSA, or lymphocytotoxic antibodies, who received a liver with a short cold ischemic time, but shows persistence of the antibodies after transplantation and develops graft dysfunction, refractory and otherwise unexplained thrombocytopenia, and

circulating low complement levels within the first few weeks after transplantation [30,35,87,88].

AMR should be favored over preservation–reperfusion injury when the following histopathologic findings are detected: (1) neutrophils, macrophages, and, to a lesser extent, lymphocytes, marginating on the luminal aspect and beneath the portal and central veins, along with marked and diffuse endothelial cell hypertrophy/reactivity; (2) edema, eosinophilia and microvasculitis, and (3) diffuse C4d staining in the microvasculature [35]. Portal or periportal edema, necrosis, and hemorrhage should raise the possibility of ABO incompatibility.

Precipitous allograft failure from severe AMR is currently rare, except in ABO-I livers. When it does occur, however, it can be difficult to distinguish from hemorrhagic liver necrosis caused by hypotension and poor perfusion, sepsis, or vascular thrombosis. Unless unequivocal evidence of AMR is detected, such as inflammatory or necrotizing arteritis and/or diffuse immunoglobulin and complement and C4d deposition combined with serologic evidence of antidonor antibodies, the cause of allograft failure can be extremely difficult to determine with certainty. More commonly changes diagnostic of AMR are not present and the diagnosis requires a thorough clinicopathologic correlation.

Acute (cellular or T cell-mediated) rejection

Acute (cellular) rejection is defined as "inflammation of the allograft, elicited by a genetic disparity between the donor and recipient, primarily affecting interlobular bile ducts and vascular endothelia, including portal and hepatic veins and occasionally the hepatic artery and its branches" [109]. Early acute rejection episodes rarely lead to allograft failure or permanent damage [39,110], whereas episodes occurring late after transplantation more often produce permanent allograft damage, possibly because there is a delay in treatment [111–114].

Clinical presentation

ACR usually presents clinically between 5 and 30 days after transplantation. Currently, ACR affects about 30% of liver transplant recipients [111]. Earlier presentations can be seen in presensitized patients or in those who receive less than optimal baseline IS. Later presentations can be seen in recipients treated with lymphocyte-depleting antibodies or in those with inadequate maintenance IS. Clinical findings are often absent early when tissue damage is mild. Later or with severe acute rejection, fever and allograft enlargement, cyanosis, and tenderness can occur. Early after transplantation when biliary T-tubes are in place, decreased bile flow and thin and pale bile are commonly seen. Ascites can occasionally develop because of increased intrahepatic hydrostatic pressure [115].

Histopathologic findings and grading

ACR is characterized by the following:

1 Predominantly mononuclear but mixed portal inflammation containing blastic or activated lymphocytes, neutrophils, and eosinophils;
2 Subendothelial inflammation of portal and/or terminal hepatic venules; and
3 Bile duct inflammation and damage [115,116].

Minimal diagnostic criteria needed to establish the diagnosis of acute rejection include at least two of the above histopathologic findings. The diagnosis is strengthened if greater than 50% of the ducts or terminal hepatic veins are damaged or if unequivocal endotheliitis of portal or terminal hepatic vein branches can be identified. Histopathologic evidence of severe injury, which is used for histopathologic grading, includes perivenular inflammation, centrilobular necrosis, arteritis, and inflammatory, usually central-to-central, bridging inflammation and necrosis [110,115].

Acute "rejection-type" infiltrates, described above, are encountered mostly in portal tracts (Figures 82.3 and 82.4) and occasionally

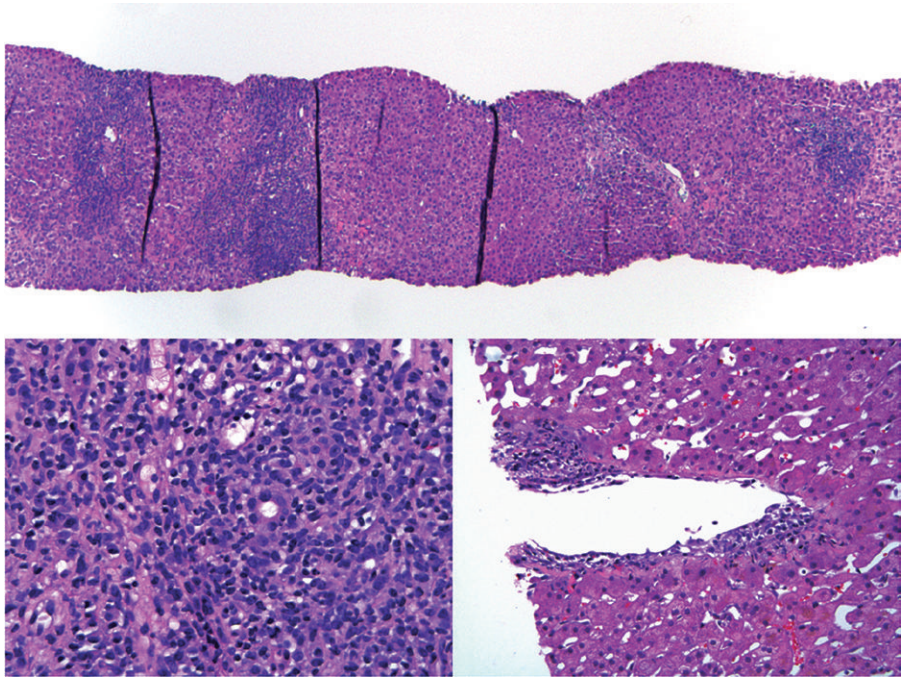

Figure 82.3. This biopsy shows histopathologic features of moderate acute T cell-mediated (cellular) rejection. As shown in this photomicrograph, most of the portal tracts show intense "rejection-type" portal infiltrate with bile duct inflammation and damage (left inset). The right inset illustrates classic "endothelialitiis," or infiltration of inflammatory cells into the subendothelial space.

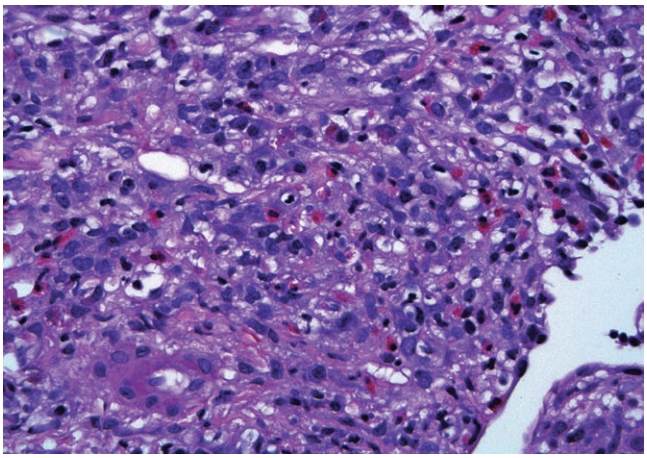

Figure 82.4. This photomicrograph illustrates the pleomorphic, typical, "rejection-type" infiltrate comprised of blastic and small lymphocytes eosinophils and neutrophils. This biopsy is the same biopsy as illustrated in Figure 82.3.

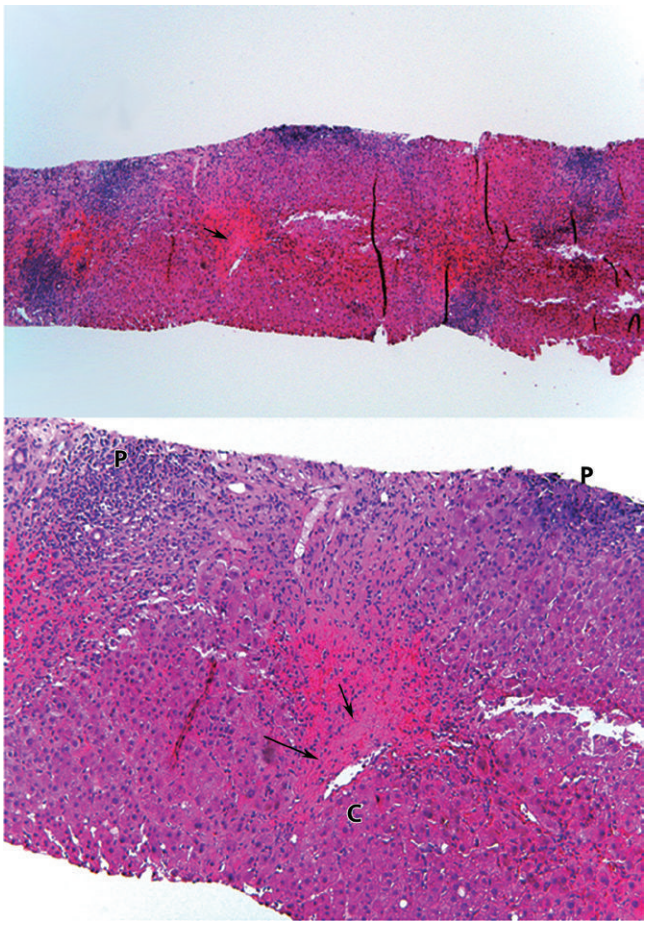

Figure 82.5. This example of severe acute T cell-mediated (cellular) rejection shows noticeable expansion of all of the portal tracts by a significant "rejection-type" infiltrate. There is also prominent central perivenulitis with widespread perivenular hepatocyte necrosis and dropout (arrows), which is also illustrated at higher magnification (bottom) (P, portal tract; C, central vein).

also around central veins. "Endotheliitis" or "endothelialitis" refers to lymphocytes underneath the portal and central vein endothelium (Figure 82.3). This characteristic feature of acute rejection can also occur with other causes of allograft dysfunction [117]. Immunophenotypic analysis of acute rejection infiltrates usually shows a predominance of T lymphocytes, as expected, and $CD8^+$ cells often surround and invade damaged bile ducts [118,119]. B cells usually comprise a minor fraction of the infiltrates. Macrophages and other leukocytes are also present and can predominate in severe acute rejection [118–120]. Routine immunophenotypic analysis of graft infiltrating lymphocytes is not useful for establishing the diagnosis of acute rejection, except when attempting to distinguish acute rejection (T-cell predominant) from a post-transplant lymphoproliferative disorder (B-cell predominant; see also Differential diagnosis).

Lymphocytic cholangitis involving small bile ducts (<30 μm diameter) is an important diagnostic feature. Lymphocytes are found inside the ductal basement membrane in association with evidence of biliary epithelial cell injury and response to injury. Included are paranuclear vacuolization, apoptotic bodies, and increased nuclear:cytoplasmic ratio, mitoses, and nucleoli. Cytoplasmic eosinophilia and multinucleation usually signal senescence-related changes and chronic injury [79]. Breaks in the basement membrane signify severe bile duct damage. Portal and peribiliary granulomas are not features of either acute or chronic rejection.

Inflammatory or necrotizing arteritis is a defining feature of severe acute rejection, but is rarely detected in needle biopsies and is poorly reproducible [121] and therefore is not generally included in grading schema. The interface zone is usually relatively unremarkable in typical early mild and moderate ACR. In severe acute rejection, portal inflammatory infiltrates spill over into the periportal sinusoids [113].

Rejection-type inflammation can also be seen in the connective tissue and perivenular sinusoids surrounding terminal hepatic venules – "central perivenulitis" [113], in up to 30% of acute rejection episodes. It is more frequent late (>100 days) after transplantation. Severe ACR is diagnosed only when typical portal changes of acute rejection are accompanied by perivenular inflammation with zonal centrilobular congestion, hemorrhage, and hepatocyte necrosis and dropout (Figure 82.5).

The Banff classification for acute and chronic rejection was constructed on data generated by recognized experts in liver transplant pathology, hepatology, and surgery from many of the major hepatic transplant centers in North America, Europe, and Asia (Tables 82.2 and 82.3) [116]. It is widely utilized [122], simple and easy to apply, reproducible [123], scientifically correct, and shown to have prognostic significance in prospective [110] and retrospective [124] studies.

The Banff classification includes descriptive grades of indeterminate, mild, moderate, and severe (Table 82.2), and a semiquantitative rejection activity index (RAI) (Table 82.3) [116]: a remnant of the European grading system [125] and conceptual equivalent of the hepatitis activity index [126]. RAI semiquantitatively scores the prevalence and severity of three separate histopathologic features on a scale of 0–3: portal inflammation, bile duct damage, and subendothelial inflammation. The individual components are then added together for a total RAI score.

There is a direct correlation between the total RAI score and descriptive rejection grade and an increased risk of persistent or

Table 82.2. Banff grading of acute liver allograft rejection (data from [261])

Global assessment	Criteria
Indeterminate	Portal rejection-type (see text) inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute reject (see text)
Mild	Rejection infiltrate in a minority of the triads, that is generally mild, and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all of the triads
Severe	As above for moderate, with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

NOTE: Global assessment of rejection grade is made on review of the entire biopsy, and only after the diagnosis of rejection has been established. It is inappropriate to provide a "rejection grade" when the diagnosis of rejection is uncertain.

Table 82.3. Acute rejection activity index (RAI) (data from [261])

Category	Criteria	Score
Portal inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils, and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile duct inflammation damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear:cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity, and cytoplasmic vacuolization of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal luminal disruption	3
Venous endothelial inflammation	Subendothelial lymphocytic infiltration involving some, but not the majority of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

Note: Total RAI score = sum of all component scores for portal inflammation, bile duct inflammation/damage, and venous endothelial inflammation.

recurrent acute rejection, chronic rejection, and graft failure [110]. The usual ranges of RAI scores are indeterminate (1–2), mild (3–4), moderate (5–6), and severe acute rejection (>6). The maximum possible total RAI score is 9, but biopsies rarely achieve this score [110]. Instead, most episodes are mild, have a total RAI <6, respond to increased IS, and do not lead to significant fibrosis, bile duct loss, or arteriopathy in subsequent or follow-up biopsies [110].

Additional IS given before a biopsy specimen is obtained can make histopathologic interpretation more difficult because resolution of some characteristic findings, such as subendothelial infiltration of veins, can occur within 24 h. Treatment before biopsy can also contribute to centrilobular hepatocyte swelling and hepatocanalicular cholestasis, causing further confusion. In general, 7–10 days are usually required for rejection-related changes to completely resolve after therapy.

Box 82.1. Banff descriptive grading of rejection presenting primarily in perivenular regions

Descriptor	Findings
Minimal/indeterminate	Perivenular inflammation involving a minority of terminal hepatic veins with patchy perivenular hepatocyte loss without confluent perivenular necrosis
Mild	As above, but involving the majority of terminal hepatic veins
Moderate	As above, with at least focal confluent perivenular hepatocyte dropout and mild moderate inflammation, but without bridging necrosis
Severe	As above, with confluent perivenular hepatocyte dropout and inflammation involving a majority of hepatic venules with central-to-central bridging necrosis

Late acute rejection (>100 days) shares many features of early acute rejection. However, slightly atypical features can also be seen (reviewed in [113]): fewer blastic lymphocytes, more necro-inflammatory type interface activity, less venous subendothelial inflammation, a higher incidence of perivenular inflammation, and slightly more lobular activity, which cause these biopsies to more closely resemble chronic hepatitis (reviewed in [113]).

Late acute rejection can also present as exclusively or predominantly perivenular inflammation and hepatocyte dropout with minimal or no portal tract changes (isolated central perivenulitis) [127–129]. These cases can later evolve into typical chronic rejection with ductopenia and perivenular fibrosis. In such cases, subendothelial inflammation of portal or central veins is not a required finding. Perivenular fibrosis and a Budd–Chiari or a veno-occlusive-like clinical syndrome can develop as a consequence of the severe perivenular injury [69,130]. The Banff Working Group proposed the descriptors in Box 82.1 for grading this reaction 8 [113]:

"Minimal" and "mild" cases, as described in Box 82.1, may resolve spontaneously [129]. More severe perivenular changes probably warrant more aggressive treatment, but no prospective studies on the effect of therapy have been carried out.

Differential diagnosis

ACR should be distinguished from preservation injury and obstructive cholangiopathy and cholangitis during the first several months after transplantation, discussed earlier.

Distinguishing acute rejection from recurrent HBV, HCV, and AIH can be especially difficult. The distinction can be achieved by closely examining the severity and prevalence of the bile duct damage, interface activity, lobular changes, and perivenular inflammation and hepatocyte dropout [131]. Features that favor ACR include inflammatory bile duct damage and perivenular inflammation involving the majority of the ducts and central veins, respectively; and low-grade or absent lobular and interface necro-inflammatory activity. Conversely, recurrent or new-onset viral or AIH is favored when the interface and lobular necro-inflammatory activity predominate over bile duct damage and perivenular changes.

Allograft AIH (reviewed in [132]) resembles viral hepatitis in most respects, except AIH more often shows conspicuous plasma cells as a component of the infiltrate and more commonly shows aggressive interface activity. Some allograft recipients with AIH also show prominent plasma cell-rich central perivenulitis involving the

majority of central veins, as with AIH in native livers. In such cases, severe interface activity, plasma cell predominance in the infiltrate, and relatively mild bile duct damage favor AIH over acute rejection.

Chronic rejection

Chronic rejection (CR) has been defined as immunologic injury that usually evolves from severe or persistent acute rejection and results in potentially irreversible damage to bile ducts, arteries, and veins (reviewed in [133]). "Chronic" implies a time parameter, but none is strictly intended [115] because it often occurs within several months and allograft failure can occur within the first year after transplantation (reviewed in [133]). Classically defined CR currently affects about 3–5% liver allograft recipients by 5 years after transplantation, which is a dramatic decrease from 15–20% in the 1980s [133]. CR does not appear to increase with time after transplantation, unless one includes idiopathic post-transplant hepatitis (IPTH) in this category.

However, CR still occurs and can be an important cause of late liver allograft dysfunction and failure, particularly if one includes patients with IPTH. CR is seen mostly in non-compliant patients, HCV-positive recipients treated with drugs such as α-interferon [134,135], and recipients who have IS lowered because of side-effects [136].

Clinical presentation

CR usually occurs in patients with a history of significant acute rejection, who develop progressive cholestasis and an increase in canalicular enzymes unresponsive to anti-rejection treatment [115]. Standard liver injury tests usually show preferential elevation of γ-GTP and alkaline phosphatase [115,137,138]. Persistent elevation of ALT and total bilirubin usually mark transition from acute to chronic rejection and can presage allograft failure [127,136,139]. Clinical symptoms, if present, resemble those of acute rejection until dysfunction becomes significant enough to cause jaundice. Biliary sludging, biliary strictures, hepatic infarcts, and loss of hepatic synthetic function, clinically manifest as coagulopathy and malnutrition, are other late findings that may presage allograft failure [115]. Hepatic angiograms often shows pruning of the intrahepatic arteries with poor peripheral filling and segmental narrowing [115,140].

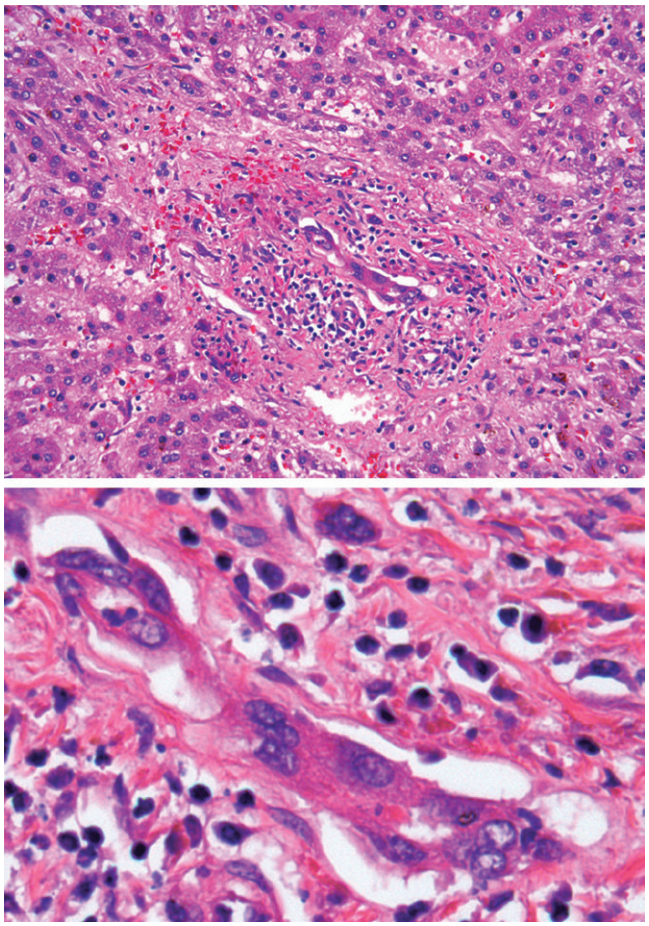

Figure 82.6. Early chronic rejection is characterized by duct damage with biliary epithelial senescence changes involving the majority of bile ducts. If mononuclear portal inflammation is present, it is usually concentrated around the small ducts as seen in this biopsy. If duct loss is present, it involves less than 50% of the portal tracts. The biliary epithelial senescence changes, which are illustrated at higher magnification (bottom), include eosinophilic transformation of the cytoplasm, an increased nuclear : cytoplasmic ratio, and uneven nuclear spacing.

Histopathologic findings and staging

Chronic rejection primarily affects portal tracts and perivenular regions and is divided into "early" (Figure 82.6) and "late" stages by the Banff classification (Table 82.4) [133]. Early CR is characterized by mild portal inflammation with lymphocytic cholangitis, biliary epithelial cell senescence changes involving a majority of small bile ducts, and variable small duct loss usually involving <50% of portal tracts (Figure 82.6). Compared with acute rejection, chronic rejection usually has less inflammation, fewer eosinophils, and an inflammatory infiltrate comprised primarily of lymphocytes, plasma cells, and mast cells [141].

Recognition of biliary epithelial cell senescence changes is critical to the diagnosis of early chronic rejection [79]: nuclear enlargement, hyperchromasia, and uneven spacing resembling cytologic dysplasia; syncytia formation; eosinophilic transformation of the cytoplasm; and bile ducts only partially lined by biliary epithelial cells (Figure 82.6). Immunohistochemical markers of senescence, such as p16 [142], $p21^{WAF1/Cip1}$, without co-existent Ki-67 expression, become up-regulated [79]. Down-regulation of epithelial junctional proteins combined with up-regulation of mesenchymal proteins can also occur, perhaps in response to injury [143]). Late stage chronic rejection is characterized by bile duct loss involving >50% of portal tracts; arteriolar loss can also be seen.

Crawford et al. [144] defined a portal tract as "a focus within the parenchyma containing connective tissue (by Masson's trichrome stain) and at least two luminal structures embedded in the connective tissue mesenchyme, each with a continuous connective tissue circumference." In normal livers, bile ducts and hepatic artery branches are detectable in 93 ± 6% and 91 ± 7% of portal tracts, respectively [144]. Lower figures were cited by others using larger tissue samples [145]. Using 2 standard deviations from the normal as a cutoff, bile duct loss is considered present when <80% of portal tracts contain bile ducts; arterial loss is considered present when <77% of the portal tracts contain hepatic artery branches. A similar approach has recently been advocated using the concept of bile duct–artery parallelism: ductopenia was defined by at least one unpaired artery in >10% of all portal tracts or two unpaired arteries in different portal tracts [146]. Unpaired arteries were defined as

Table 82.4. Features of early and late chronic liver allograft rejection (Reproduced from [133] with permission from John Wiley and Sons. Copyright © 2000 American Association for the Study of Liver Diseases)

Structure	Early chronic rejection	Late chronic rejection
Small bile ducts (<60 μm)	Bile duct loss in <50% of portal tracts Degenerative change involving a majority of ducts: eosinophilic transformation of the cytoplasm; nuclear hyperchromasia; uneven nuclear spacing; ducts only partially lined by biliary epithelial cells	Loss in ≥50% of portal tracts Degenerative changes in remaining bile ducts
Terminal hepatic venules and zone 3 hepatocytes	Intimal/luminal inflammation Lytic zone 3 necrosis and inflammation Mild perivenular fibrosis	Focal obliteration Variable inflammation Severe perivenular fibrosis, defined as central-to-central bridging fibrosis
Portal tract hepatic arterioles	Occasional loss involving <25% of portal tracts	Loss involving >25% of portal tracts
Other	So-called "transitional"* hepatitis with spotty necrosis of hepatocytes	Sinusoidal foam cell accumulation; marked cholestasis
Large perihilar hepatic artery branches	Intimal inflammation, focal foam cell deposition without luminal compromise	Luminal narrowing by subintimal foam cells Fibrointimal proliferation
Large perihilar bile ducts	Inflammation damage and focal foam cell deposition	Mural fibrosis

* "Transitional" hepatitis. Mild lobular disarray and spotty acidophilic necrosis of hepatocytes that can occur during evolution or transition from early to late stages of chronic rejection [151].

arteries without an accompanying bile duct within a radius of 10 hepatic artery diameters from the edge of the artery.

However, late chronic rejection can cause both bile duct and arterial loss [145,147], which can present difficulties when trying to apply these algorithms. Portal tract recognition should be based primarily on the location of the putative structure – cholestasis in chronic rejection is centrilobular.

A ductular reaction at the interface zone is unusual in chronic rejection, unless the liver is recovering from chronic rejection [139,148,149]. Cytokeratins 19 and 7 can be used to help substantiate bile duct loss and, with the latter stain, detect ductular metaplasia of periportal hepatocytes [31]. When a ductular reaction is easily noticeable by routine light microscopy, however, it signals either regrowth of bile ducts [139,148,149] or an underlying biliary stricture. The latter can be substantiated by periportal hepatocyte copper deposits, which are not seen in chronic rejection.

Early chronic rejection in terminal hepatic venules and surrounding perivenular parenchyma is characterized by subendothelial and/or perivenular mononuclear inflammation (Figure 82.6). The perivenular infiltrate usually consists of lymphocytes, pigment-laden macrophages, and plasma cells [133,150], often accompanied by perivenular hepatocyte dropout and mild perivenular fibrosis [133]. Spotty acidophilic hepatocytes, or "transitional hepatitis," can be seen during evolution from early to late chronic rejection [151].

Perivenular changes in late chronic rejection are characterized by severe (bridging) perivenular fibrosis with at least focal central-to-central or central-to-portal bridging and occasional obliteration of terminal hepatic venules [133]. Well-developed cirrhosis, primarily attributable to chronic rejection, is unusual until the very late stages when venous obliteration leads to areas of parenchymal extinction and venocentric cirrhosis [152]. True "regenerative" nodules are uncommon. Perhaps this is because a combination of venopathy and obliterative arteriopathy blunts any regenerative response [153]. Perivenular hepatocyte ballooning and dropout, centrilobular hepatocanalicular cholestasis, nodular regenerative hyperplasia changes, and intrasinusoidal foam cell clusters are other common findings in late chronic rejection.

A final diagnosis of classic chronic rejection should be based on a combination of the clinical, radiologic, laboratory, and histopathologic findings. In a biopsy specimen, minimal diagnostic criteria for chronic rejection are the following:

1 Senescent changes, affecting the majority of the bile ducts, with or without bile duct loss;
2 Convincing foam cell obliterative arteriopathy; or
3 Bile duct loss affecting greater than 50% of the portal tracts [94].

A diagnosis of chronic rejection is much easier to establish in an explanted **failed allograft** because characteristic diagnostic findings can be directly observed in the first, second, and third-order branches of the hepatic arterial tree in and around the liver hilum. Obliterative arteriopathy is usually seen in at least some perihilar arteries, except in cases characterized by duct loss or perivenular fibrosis alone. Accumulation of foamy macrophages usually first occurs in the intima, which triggers proliferation of intimal, and migration of medial donor-derived myofibroblasts. This eventually causes intimal thickening/luminal narrowing and medial thinning as arteries attempt to dilate and compensate for reduced arterial flow. Eventually, however, even compensatory mechanisms fail and the entire wall can be completely replaced by foam cells or the artery can undergo thrombosis causing necrosis of large bile ducts and ischemic cholangiopathy.

Foamy macrophages can also be seen around bile ducts and veins in the connective tissue. Large perihilar bile ducts can show focal sloughing of epithelium, papillary hyperplasia, mural fibrosis, and acute and chronic inflammation.

Staging of CR assumes that the diagnosis has already been correctly established [133]. **Early CR** implies that a significant potential for recovery exists if the source or cause of immunologic can be controlled or reversed. **Late CR** implies that the potential for recovery is limited and retransplantation should be considered, if otherwise clinically indicated. It is not well established that all patients proceed sequentially in an orderly fashion from early to late CR. Some patients appear to persist in the acute or early stage for months or years, while others rapidly develop severe fibrosis and late changes within the first year after transplantation or within weeks or months after the first onset. Some show predominantly or exclusively either bile duct loss or arteriopathy alone, but usually both features occur together [133].

The important practical implication of CR staging is that the biopsy findings do not absolutely define a point of no return. Instead they provide information about the likelihood of reversal, which should be correlated with other clinical and laboratory parameters, such as persistently elevated serum bilirubin

>20 mg/dL, progressive decline in synthetic function, superimposed hepatic artery thrombosis, and bile duct necrosis or biliary sludging.

Differential diagnosis

In needle biopsy specimens, a CR diagnosis is primarily based on damage and loss of small bile ducts and perivenular fibrosis. Arteries with pathognomonic changes are rarely present in needle biopsy specimens [133]. Duct injury and ductopenia can also occur because of non-rejection-related complications, such as obstructive cholangiopathy, hepatic artery stricturing or thrombosis, "cholangitic" drug-induced liver injury, and cytomegalovirus (CMV) infection. Perivenular fibrosis can also be caused by suboptimal hepatic venous drainage and other causes of perivenular injury. Therefore, a diagnosis of CR based on biliary epithelial senescence or loss or perivenular fibrosis alone should first exclude other non-rejection-related causes of ductal injury and loss or perivenular fibrosis.

CR can be very difficult to distinguish from biliary tract obstruction or stricturing, including recurrent PSC [80]. Features that favor obstructive cholangiopathy include the following:

1 Bile duct loss in some portal tracts accompanied by a ductular reaction in others;
2 Neutrophil clusters within lobules;
3 Bile infarcts;
4 Deposition of copper or copper-associated protein in periportal hepatocytes; and
5 Hepatocanalicular cholestasis out of proportion to the prevalence of ductopenia (<50%).

Features that favor CR include: (1) bile duct changes combined with central perivenulitis and/or fibrosis; and (2) absence of changes typical of recurrent PSC (described earlier). Cholangiography and/or angiography may be required in some case to distinguish between CR and biliary obstruction. Such studies usually show "pruning" and poor peripheral filling in CR.

Isolated ductopenia involving less than 50% of portal tracts can be seen without significant elevations of liver injury tests. Whether these uncommon cases are an early phase of CR is uncertain. Isolated perivenular fibrosis can be caused by mechanical outflow obstruction, drug-induced liver injury, and all causes of veno-occlusive disease and Budd–Chiari syndrome in native livers.

The safest approach to the diagnosis of CR in any setting is to review prior biopsies and closely correlate the histopathologic findings with the clinical course. The usual scenario is a history of severe or unresolved acute rejection preceding the development of histologic findings interpreted as CR.

Bacterial and fungal infections

Stress and tissue damage from the transplant operation combined with high IS needed to prevent acute rejection within the first 2 months after transplantation places the recipient at highest risk for serious "opportunistic" fungal and viral infections. After 6 months post-transplant, more typical bacterial infections occur. Fever, anastomotic or wound dehiscence, retransplantation, persistent abdominal pain, and vascular thrombosis are clinical situations, signs and symptoms that should further arouse suspicion of a serious infection.

Bacterial and fungal infections often arise in non-viable tissue; therefore, most necrotic tissue should be routinely subjected to special stains for bacteria and fungi. Co-existent acute and chronic inflammation and granulomas are always good surrogate markers of infection but might not appear because of heavy IS. Histopathologic manifestations of deep fungal and bacterial infections are familiar to most pathologists and beyond the scope of this chapter.

"Opportunistic" hepatitis viruses

Cytomegalovirus hepatitis

Clinical presentation

Any organ system can be involved depending on the extent of viral dissemination, but the most common signs and symptoms of active CMV infection or disease arise from gastrointestinal involvement and include fever, diarrhea and gastrointestinal ulcers, leukopenia, and low-grade hepatitis with modestly elevated liver injury tests [154,155]. Respiratory insufficiency, pneumonia, and retinitis are signs of severe disseminated disease. CMV can also occasionally cause a syndrome that mimics Epstein–Barr virus (EBV) associated post-transplant lymphoproliferative disorder (PTLD) with lymphadenopathy, fever, and atypical lymphocytosis (reviewed in [156]).

Histopathologic findings

CMV hepatitis has become rare because of serologic monitoring, prophylactic and pre-emptive antiviral therapy [154,155]. CMV hepatitis, when it occurs, is usually characterized by spotty lobular necrosis, Kupffer cell hypertrophy, mild lobular disarray, and patchy lobular inflammation (described later). Inclusions tend to be limited to patients who are over-immunosuppressed and not adequately monitored or treated. Any cell type can be infected. Diagnostic features include large eosinophilic intranuclear inclusions surrounded by a clear halo accompanied by small basophilic or amphophilic cytoplasmic inclusions. CMV alone does not cause submassive or massive necrosis [156].

Infected cells are often surrounded by neutrophilic microabscesses or clusters of macrophages and lymphocytes – "microgranulomas." CMV hepatitis can also be associated with mild lymphoplasmacytic portal inflammation showing bile duct infiltration and damage that can superficially resemble acute or early CR. In fact, bile duct loss and CR have been associated with persistent allograft CMV infection (reviewed in [154,155]).

Because of medical therapy, "fragmented" nuclear CMV inclusions are seen, which are difficult to recognize without immunostain or in situ hybridization to detect viral antigens or nucleic acid, respectively. Rapidly dividing tissues such as young granulation tissue, proliferating cholangioles, edges of infarcts, abscesses, or other intraparenchymal defects are fertile soil for CMV growth [32]. .

Differential diagnosis

Subtle clues raising the possibility of CMV hepatitis that enable distinction from early acute HBV or HCV include less lobular disarray and hepatocyte swelling with CMV. In addition, microabscesses or microgranulomas are not generally seen with the early or lobular phase of HBV or HCV. A definitive diagnosis of CMV hepatitis requires either characteristic inclusions, which are rarely present, or specific detection of viral antigens or viral nucleic acids.

CMV hepatitis can superficially resemble EBV hepatitis because each has mild lymphoplasmacytic portal and lobular inflammation and both occasionally contain blastic and atypical lymphocytes. In such cases, EBV hepatitis usually demonstrates more lymphoplasmacytic cytologic atypia, whereas CMV hepatitis usually causes more intralobular inflammation (e.g. microgranulomas and

microabscesses). Deeper sections, staining for EBV and CMV viral antigens and in situ hybridization for EBV nucleic acids, are usually required to make the distinction.

Herpes simplex and varicella-zoster viral hepatitis

Histopathologic findings

Recognition and prompt reporting of herpes simplex virus (HSV) and varicella-zoster (VZ) hepatitis on needle biopsy evaluation is essential because effective therapy is available and, without treatment, infection can be rapidly fatal. Two patterns of HSV hepatitis have been described: localized and diffuse [157]. Both cause characteristic circumscribed areas of coagulative-type necrosis showing no respect for lobular architecture (reviewed in [156,157]). The center of the necrotic zones is occupied by hepatocyte "ghosts," intermixed with neutrophils and nuclear debris, whereas more viable hepatocytes rimming the periphery usually contain recognizable HSV or VZ inclusions, if present. Infected cells are usually slightly enlarged and contain "smudgy" ground glass nuclei or characteristic Cowdry type A eosinophilic inclusions. Multinucleate cells are occasionally present, but, not infrequently, diagnostic inclusions of HSV or VZ will be absent on the H&E slides.

In our experience, antibody preparations used to detect HSV I and II show can show cross-reactivity with each other and with VZ, making it difficult to distinguish among these viruses. Antibodies used to detect VZ precursor and mature glycoprotein may be more discriminating in this circumstance.

Differential diagnosis

If a histopathologic diagnosis of HSV or VZ virus hepatitis is considered on H&E sections, regardless of whether the diagnosis is confirmed, the clinical physician should be immediately notified [158]. This will prompt effective antiviral therapy that can be discontinued if the diagnosis is not confirmed on immunostaining. Necrotic lesions of HSV and VZ hepatitis are distinguished from infarcts by examining viable cells at the edge of the necrotic lesions for characteristic viral inclusions. Frequently, however, only cells with smudged nuclear chromatin are seen at this location. It is our policy to "over-diagnose" HSV hepatitis on H&E sections followed by HSV II and II and VZ immunostains to confirm or exclude the diagnosis.

The histopathologic differential diagnosis of CMV and HSV hepatitis has been discussed earlier.

Human herpes virus-6

Human herpes virus-6 (HHV-6), a member of the β-Herpesviridae subfamily of human herpes viruses, is a ubiquitous virus. Reactivation infection occurs in 15–80% of liver allograft recipients, usually during the first 2–8 weeks after transplantation, often precipitated by heavy IS [159].

Indirect effects attributed to HHV-6 include exacerbation of CMV disease, increased severity of HCV recurrence, increased risk of other opportunistic infections, allograft dysfunction, and ACR [159].

Liver allograft biopsy shows variable findings: moderate lymphocytic portal and patchy lobular inflammation, microabscesses [160,161], and syncytial giant cell hepatitis [162]. ACR has been seen at time of disease [160,161]. Consistent with the known biology of the virus, immunoperoxidase stains for HHV-6 viral antigens decorated mononuclear inflammatory cells [160] and hepatocyte syncytial giant cells in a single case report [162].

Human herpes virus-8

Human herpes virus-8 (HHV-8) is a lymphotropic gammaherpes virus family member exerting a pathogenic role in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma [163–165].

Primary infection might be more severe and can result in variable manifestations, including liver dysfunction, multiorgan failure, multicentric Castleman's disease, and Kaposi's sarcoma [163–165]. Liver biopsy findings are typical for acute viral hepatitis: lobular disarray, ballooning, hepatocyte swelling and apoptosis, and variable ductular reaction. When multicentric Castleman's disease, Kaposi's sarcoma, or an unexplained acute hepatitis is recognized, one should include HHV-6 and HHV-8 in the differential diagnosis.

Epstein–Barr virus

EBV infection "immortalizes" B lymphocytes and lies dormant in B lymphocytes and possibly some epithelial cells [166–168]. Potent immunosuppression increases the risk of various EBV disease manifestations ranging from self-limited mononucleosis-like syndromes to aggressive clonal post-transplant lymphoproliferations and overt lymphomas [166–168]. These are currently all subsumed under the generic heading of PTLD.

Histopathologic findings

"Typical" EBV hepatitis manifests as mild portal lymphoplasmacytic inflammation combined with sinusoidal lymphocytosis comprised of small or mildly atypical lymphocytes. "Lining-up" of lymphocytes in the sinusoids should suggest an EBV-related disorder, as in native livers. Lobular changes include focal hepatocellular swelling, mild acidophilic necrosis of hepatocytes, and mild lobular disarray and regenerative activity.

In the liver, PTLDs usually present with cytologically atypical lymphoplasmacytic portal infiltrates. In early or polymorphic lesions, atypical cells are usually intermixed with small and blastic lymphocytes, plasmacytoid lymphocytes, and plasma cells. In monomorphic lesions atypical cells predominate, and involvement manifests as map-like enlargement of portal tracts because of sheets of atypical immunoblastic cells that obscure normal portal architectural landmarks. Some cases are accompanied by significant hepatic necrosis. Hodgkin's-like lymphoma PTLDs with classic Reed–Sternberg cells can also occur and be associated with bile duct loss, as in native livers. Subendothelial localization of lymphocytes in portal or central veins can mimic acute rejection.

Diagnosis of any EBV-related disorder is confirmed by in situ hybridization for EBV RNA (EBER sequence), which should be liberally employed to evaluate any suspicious infiltrate. Our routine workup also includes immunohistochemical stains or in situ hybridization for kappa and lambda light chains, and CD20 to determine possible responsiveness to anti-CD20 antibodies [168]. Occasionally, we also examine EBV antigen expression, and if enough fresh tissue is available, a portion is also submitted for flow cytometry and molecular analyses, which enable a more detailed phenotypic characterization and immunoglobulin gene rearrangements studies.

Differential diagnosis

Features that favor acute rejection over EBV-related disorders include pleomorphic "rejection-type" portal and/or perivenular inflammatory infiltrates, including conspicuous eosinophils; and severe and prevalent bile duct damage that is proportional to the

severity of inflammation. Features favoring EBV over acute rejection include relatively monomorphic portal infiltrates consisting primarily of activated and immunoblastic mononuclear cells, many of which show features of plasmacytic differentiation and some of which show atypical cytologic features; and patchy or mild inflammatory bile duct damage less than would be expected based on the severity of the portal infiltrate.

HCV and EBV hepatitis can both show sinusoidal lymphocytosis, but EBV-related disorders usually contain at least occasional atypical cells. In contrast, lymphocytes associated with HCV hepatitis are usually small, round, and inactive-appearing and form nodular aggregates in the portal tracts [169–172]. Clinical suspicion and increased peripheral blood EBV levels also point toward the correct diagnosis.

In most cases, the final diagnosis of any EBV-related disorder is heavily dependent on in situ hybridization for EBV RNA (EBER), but results should be interpreted with caution [173] because rare EBER-positive cells are not uncommon even in lymphoid tissues from the general population. In our opinion, however, clustering of EBER-positive cells into aggregates, or the presence of EBER-positive cells in tissues showing other histopathologic features of EBV-associated disease, is indicative of enhanced EBV replication and such patients are at increased risk of developing EBV-related disease.

Adenoviral hepatitis

Pathophysiology

Adenoviral-related disease after liver transplantation is largely restricted to pediatric recipients with primary infections [174,175], although occasional cases have been reported in adults [176,177].

Histopathologic findings

Considerable experience is required to diagnose adenoviral hepatitis on H&E sections because the disease is uncommon and typical nuclear inclusions are difficult to recognize with certainty. Typical cases show"pox-like" granulomas consisting of macrophages with or without neutrophils spread randomly throughout the parenchyma; in other cases granulomas surround small map-like areas of necrosis [174–176]. Typical adenoviral inclusions are usually found in nuclei of viable hepatocytes near the edge of the necrotic zones or granulomas. Infected cells show a "smudgy" appearance with crowding of chromatin towards the nuclear membrane, which makes the nucleus look like a "baked muffin." Immunohistochemical staining is usually required to confirm the diagnosis and given 52 serotypes it seems prudent to employ antibodies reactive with the hexon protein common to all serotypes of adenovirus (e.g. MAB805, blend of clones 20/11 and 2/6; CHEMICON International Inc., Temecula, California, USA) [177].

Differential diagnosis

On H&E sections, characteristic inclusions can be used to distinguish adenoviral hepatitis from other causes of focal hepatic necrosis and hepatic granulomas such as HSV and VZ, infarcts, and deep fungal or mycobacterial infections. HSV/VZ and CMV hepatitis can also have granulomatoid infiltrates and focal necrosis, respectively. However, adenovirus usually causes less necrosis than either HSV or VZ hepatitis. Granulomas associated with adenovirus are much larger than "micro-granulomas" typical of CMV hepatitis and multinucleated giant cells are rare in adenoviral hepatitis. CMV hepatitis, in contrast, causes cytomegaly and produces eosinophilic intranuclear inclusions, surrounded by a clear halo, and basophilic or amphophilic small cytoplasmic inclusions. Adenovirus does not cause cytomegaly, the nucleus assumes a "smudgy-appearance," and cytoplasmic inclusions are not seen.

Ultimately, however, immunostaining or in situ hybridization for adenoviral antigens is usually needed to confirm the diagnosis, often in conjunction with stains to exclude HSV/VZ, and CMV viral antigens and/or nucleic acids.

Considerations involved in the evaluation of late liver allograft dysfunction and protocol biopsies

A broad spectrum of insults can cause dysfunction and many show overlapping clinical, serologic, and histopathologic features. These issues were addressed by the Banff Working Group for liver allograft pathology who constructed a consensus document to help guide interpretation of such biopsies (Tables 82.5 and 82.6) [113].

Leading causes of late liver allograft dysfunction include recurrence of original disease and obstructive cholangiopathy [137,178]; acute and/or chronic rejection was detected in only 4–38% of late biopsies. Most (~75%) biopsies from recipients surviving more than 1 year with abnormal liver tests or symptoms show significant abnormalities [113,137,178], usually attributable to recurrent disease or biliary strictures, some of which are clinically occult. About 25% biopsies from long-surviving asymptomatic recipients with normal liver tests also show significant abnormalities, particularly if the original disease is one that commonly recurs (e.g. HCV, PBC, and AIH [113,137,178]).

Minor histopathologic abnormalities occur in about 60–70% of symptomatic patients even in the absence of recurrent disease with (near-) normal liver tests. Common minor abnormalities include portal venopathy, nodular regenerative hyperplasia, thickening and hyalinization of small hepatic artery branches, and "non-specific" portal and lobular inflammation. The significance of these unexplained findings is currently under scrutiny.

Many late post-transplant biopsies show portal-based mononuclear inflammation with variable necro-inflammatory-type interface activity, even in patients without recurrence of chronic necro-inflammatory diseases [113]). Even after extensive analysis, the underlying cause of the inflammation may not always be apparent. Rendering a definitive diagnosis, therefore, particularly during the early stages of a disorder, might not be possible. In such cases, using "features suggestive of early" emphasizes a tentative diagnosis [113].

Laboratory tests used to establish a diagnosis before transplantation may not have the same significance after transplantation [113]. Antimitochondrial antibodies (AMA) and antinuclear antibodies (ANA) often transiently disappear, only to reappear at lower titers after transplantation in patients with PBC or AIH – even without histopathologic evidence of recurrent disease. Patients without AIH before transplantation can develop autoantibodies either as a complication of otherwise typical rejection [179–181], or in association with new-onset AIH [113,132].

Recurrent diseases and diseases induced by transplantation

Recurrent original disease is the most common and significant cause of late liver allograft injury and dysfunction. This is covered in detail in Chapter 78 and includes infectious diseases (e.g. viral hepatitis A, B, C, D, E), disease of dysregulated immunity (AIH,

Table 82.5. Histopathologic features most commonly detected with various causes of late liver allograft dysfunction* (Reproduced from [113] with permission from John Wiley and Sons. Copyright © 2006 American Association for the Study of Liver Diseases)

Histopathologic features	AIH†	Acute rejection	Chronic rejection	Chronic viral hepatitis types B and C	PBC	PSC/BD strictures
Distribution, severity and composition of portal inflammation	Usually diffuse predominantly mononuclear of varying intensity Often prominent plasma cell component	Usually diffuse, variable intensity Mixed "rejection-type" (see text) infiltrate	Patchy, usually minimal or mild lymphoplasmacytic	Patchy, variable intensity; predominantly mononuclear; nodular aggregates	Noticeably patchy and variable intensity; predominantly mononuclear; nodular aggregates and granulomas	Usually patchy to diffuse depending on stage; mild neutrophilic, eosinophilic, or occasionally mononuclear predominant
Presence and type of interface activity	Usually prominent and defining feature: necro-inflammatory-type; often plasma-cell-rich	Focally present and mild necro-inflammatory type	Minimal to absent	Variable, usually not prominent: necro-inflammatory and (ductular-type)	Important feature later in disease development: ductular and necro-inflammatory-type with copper deposition	Prominent and defining feature: ductular-type with portal and periportal edema
Bile duct inflammation and damage	Variable; if present involves a minority of bile ducts	Present and usually involves the majority of bile ducts	Focal ongoing lymphocytic bile duct damage; inflammation wanes with duct loss	Variable; if present involves a minority of bile ducts	Granulomatous or focally severe lymphocytic cholangitis is diagnostic in proper setting	Periductal lamellar edema, "fibrous cholangitis," acute cholangitis, multiple intraportal ductal profiles
Biliary epithelial senescence changes and small bile loss	Absent or involves only a minority of ducts/portal tracts, but may be focally severe	Absent or involves only a minority of ducts	Senescence/atrophy/atypia involve the majority of remaining ducts (see text)	Absent or involves only a minority of ducts	Small bile duct loss associated with ductular reaction	Small bile duct loss associated with ductular reaction
Perivenular mononuclear inflammation and/or hepatocyte dropout	Variable, can involve the majority of perivenular regions, similar to rejection (see text); may be plasma-cell-rich	Variable, if defining feature should involve the majority of perivenular regions; may also show subendothelial inflammation of vein (see text)	Usually present, but variable	Variable, but generally mild, if present involves a minority of perivenular regions	Variable, but generally mild, if present involves a minority of perivenular regions	Absent
Lobular findings and necro-inflammatory activity	Variable severity; rosettes may be present and/or prominent	Variable, if present, concentrated in perivenular regions	Variable; if present, concentrated in perivenular regions	Disarray variable Variable severity necro-inflammatory activity	Mild disarray, parenchymal granulomas Periportal copper deposition and cholatestasis are late features	Disarray unusual Neutrophils clusters ± Cholestasis
Pattern of fibrosis during progression toward cirrhosis	Usually macronodular, post-hepatitic pattern	Rare	Uncommon, if present usually a venocentric pattern; may evolve to biliary pattern over time	Usually macronodular, hepatitic pattern; may be micronodular (see text)	Biliary pattern	Biliary pattern

AIH, autoimmune hepatitis; BD, bile duct; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.
*The histopathologic findings in this table should be combined with clinical, serological, radiographic, and important exclusionary criteria listed in Table 82.2 to arrive at a final diagnosis.
†The same findings apply to recurrent and de novo AIH.

PBC, PSC, and overlap syndromes), malignancies (hepatocellular and cholangiocarcinomas), toxic insults, such as alcohol abuse and drug-induced liver injury and hepatic-based metabolic diseases such as α_1-antitrypsin deficiency and Wilson's disease and extrahepatic metabolic disorders (e.g. metabolic syndrome, hemochromatosis, Gaucher's disease).

Numerous other uncommon diseases of uncertain etiology (-ies) also can recur after liver transplantation including sarcoidosis [182,183], idiopathic granulomatous hepatitis [137], post-infantile giant cell hepatitis [184], and Budd–Chiari syndrome [185–187]. Liver transplantation can transmit diseases such as familial amyloidosis, polyneuropathy [16], and oxalosis [17] when these genetically diseased, but phenotypically normal, livers are used as "domino" transplants.

Hepatitis virus infections

Hepatitis A

Several case reports show that hepatitis A virus (HAV) can persist or recur after transplantation, as determined by detection of genomic HAV RNA by reverse transcription polymerase chain reaction (RT-PCR) in liver tissue, serum, and stool at the time of transient graft dysfunction and in one case of apparent HAV-induced allograft failure [188,189]. Biopsy findings showed changes generally attributable to hepatitis: variable hepatocyte apoptosis, degenerative changes of hepatocytes with cholestasis, mild portal inflammation with variable bile duct damage, and ductular cholestasis [188,189]. However, HAV RNA can be detected in liver tissue of patients with otherwise typical acute or chronic rejection [188–191]. The diagnosis, therefore, of recurrent or persistent HAV after

Table 82.6. Inclusionary and exclusionary criteria for the diagnosis of recurrent and new-onset chronic necro-inflammatory diseases after liver transplantation and timing of first onset and pattern of liver test elevations* (data from [113]and [260])

Diagnosis	Original disease	Serology/molecular testing†	Timing and liver injury test profile‡	Important exclusionary criteria
Recurrent AIH	AIH	Autoantibodies (ANA, ASMA, ALKM) usually in high titers (>1:80); raised serum IgG	>6 months Hepatocellular	Acute and chronic rejection, HBV, HCV, HEV infection, as determined by third generation ELISA assay and/or by serum or tissue PCR
Plasma Cell Hepatitis (De novo AIH)	Other than AIH	Same as above	>6 months Hepatocellular	Same as above
Recurrent HBV or HCV	HBV- or HCV-induced cirrhosis	HBV or HCV infection using standard, third generation serologic criteria and/or positive molecular testing for HBV or HCV nucleic acids	Usually 6–8 weeks, but as early as 10 days Usually hepatocellular; but may be cholestatic	Acute and chronic rejection AIH
Recurrent PBC	PBC	Positive AMA, but little additional benefit because AMA remains elevated in the majority of patients after transplantation	>1 year Cholestatic	Biliary tract obstruction/strictures
Recurrent PSC	PSC	NA	Usually >1 year Cholestatic	HA thrombosis/stenosis, chronic (ductopenic) rejection, abnormal surgical anatomy, anastomotic strictures alone, non-anastomotic strictures occurring < 90 days after OLTx, and ABO incompatibility
Acute rejection	NA (see text for risk factors)	NA	Any time Usually hepatocellular; may be mixed if superimposed on chronic rejection	Inadequate IS usually, but not always present (see text) Important exclusions: biliary tract obstruction/strictures, HBV, HCV, AIH
Chronic rejection	NA (see text for risk factors)	NA	Any time, but usually <1 year Cholestatic; rarely hepatocellular in veno-occlusive variant (see text)	Inadequate IS usually, but not always present (see text) Important exclusions: biliary tract obstruction/strictures, HBV, HCV, AIH
Idiopathic post-transplant hepatitis	Non-viral and non-AIH	Negative testing for HBV, HCV, and HEV infection and autoantibodies	>1 year Usually hepatocellular	Acute and chronic rejection, all other causes of chronic hepatitis, and biliary tract obstruction/strictures reasonably excluded. All attempts should be made to determine a cause before establishing this diagnosis

AIH, autoimmune hepatitis; ALKM, anti-liver–kidney microsomal antibodies; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; ASMA, anti-smooth muscle antibodies; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; IS, immunosuppression; PBC, primary biliary cirrhosis; PCR, polymerase chain reaction; PSC, primary sclerosing cholangitis.

*See Table 82.5 for compatible histopathologic findings.

†Timing = usual timing of first onset;

‡Sustained elevation for more than 1 month: hepatocellular = ALT and/or AST > ALP and/or GGTP; cholestatic = ALP and/or GGTP > AST and/or ALT.

transplantation should be based on complete clinicopathologic and serologic correlation.

Hepatitis B and Delta

Clinical presentation

Untreated recipients, largely of historical interest, show that HBV reinfection of the allograft occurs immediately after transplantation. Clinical manifestations first become obvious about 6–8 weeks as otherwise unexplained elevations of ALT and AST [192,193]. More significant hepatitis is accompanied by nausea, vomiting, jaundice, and, in rare cases, fulminant hepatic failure. In the liver transplant setting, recipients treated with too much IS can develop severe disease because of fibrosing cholestatic hepatitis. In contrast, rapid tapering or withdrawal of IS should also be avoided in HBV-positive recipients with evidence of active viral replication. This maneuver can "re-arm" the immune system and cause severe immunologically mediated liver injury and fulminant hepatic failure.

Histopathologic findings

The histopathologic manifestations of untreated HBV infection of hepatic allografts are similar to that seen in native livers, except for the rare occurrence of the fibrosing cholestatic variant [193,194], which has largely been eliminated because of effective therapy.

HBV-related disease typically begins with acute hepatitis usually recognizable within 4–6 weeks after transplantation. A small percentage of untreated HBV-positive recipients will develop confluent/bridging, and even submassive necrosis, especially if IS is rapidly reduced [192]. Delta antigen can be detected by immunohistochemical staining [195].

Cirrhosis can develop rapidly within 12–18 months after transplantation in untreated recipients [192,193]. Lobular findings in the chronic phase are usually much less conspicuous than during the acute phase and include ground glass hepatocytes or hepatocytes with sanded nuclei that stain positively for hepatitis B surface and core antigen, respectively, mild disarray and low grade necroinflammatory activity.

Massive HBV replication because of over-immunosuppression and major histocompatibility complex (MHC) non-identity between liver and recipient can lead to the development of fibrosing cholestatic hepatitis; it can also occur with the emergence of viral mutants [193,196]. Findings include marked hepatocyte swelling, lobular disarray, and cholestasis combined with prominent ductular and fibrotic-type interface activity often with minimal or mild portal and lobular inflammation. Swollen and degenerating hepatocytes usually show massive hepatocellular expression of hepatitis B core and/or surface antigen, suggesting that HBV is directly cytopathic under these circumstances [193,197].

Delta agent co-infection of HBV-positive recipients usually results in a lower incidence of recurrent disease compared to HBV-positive/delta-negative recipients, particularly in pharmacologically

treated recipients [198]. Effects of HDV on HBV-related pathology in untreated recipients would be expected to be similar to that seen in the general population [199].

Differential diagnosis

Acute HBV should be distinguished from other causes of acute hepatitis, such as CMV, HCV, and EBV and other conditions such as "ischemic" hepatitis and portal and so-called "transitional" hepatitis that accompanies the transition from acute to chronic rejection. The most reliable method of distinguishing acute HBV hepatitis from the other insults is a review of the clinical, histopathologic, immunohistochemical, and serologic profile.

Chronic HBV hepatitis needs to be distinguished from other causes of chronic hepatitis such as HCV, AIH, and drug-induced liver injury. Detection of ground glass hepatocytes or viral antigens or nucleic acid in the blood or tissues, in the absence of other causes, favors chronic HBV. Detection of viral antigens or nucleic acids in the blood or tissues combined with histologic features of active lobular hepatitis, in the absence of other causes, favors recurrent HBV, but does not exclude other causes [193].

Features used to distinguish between acute rejection and acute or chronic hepatitis have already been discussed (see Acute rejection, Differential diagnosis).

Hepatitis C virus

Liver damage from recurrent HCV is mediated via a combination of viral replication and immune-mediated damage: either can damage the liver [200]. Rapid tapering of IS is associated with more rapidly progressive recurrent HCV [201–203], probably related to "re-arming" of the immune system at a time of high HCV replication. This triggers a more aggressive immune-mediated hepatitis [201–203], as noted decades ago for HBV-induced chronic liver disease. Slower tapering of IS, particularly in long-surviving recipients, seems to be more advisable [201–203].

In keeping with fibrogenesis models [200], any insult in addition to HCV (e.g. co-existent steatosis, high viral replication and oxidative stress, iron deposits, or co-existent damage from preservation–reperfusion injury, biliary structuring, or acute rejection) will accelerate disease progression. This concept also explains why use of extended criteria donors in HCV-positive recipients has offset the recent improvements in medical management of HCV leading to no net improvement in long-term outcomes [200,204,205].

Clinical presentation

The clinical presentation of HCV hepatitis is similar to that seen in the general population. Acute hepatitis, which develops in most recipients, is often asymptomatic, recognized primarily by elevation of ALT and AST to 4–8 times baseline levels [200]. This usually becomes apparent 3–6 weeks after transplantation; earlier onset within 10–14 days can be associated with more aggressive disease.

Fibrosing cholestatic hepatitis (FCH) HCV is associated with "over IS" and characterized clinically by malaise, jaundice, and marked and preferential elevations of bilirubin, alkaline phosphatase, and γ-GTP.

Histopathology

Usual variant. The usual appearance and evolution of HCV in liver allografts is similar to that seen in native livers. In allografts, however, the acute phase generally shows less portal and lobular inflammation; the chronic phase less often shows nodular aggregates of portal-based lymphocytes; and there is more ductular-type interface activity [200,206].

Focal inflammatory bile duct damage can be seen, but is neither severe nor widespread, involving a minority of bile ducts, and duct loss is not seen. Inflammation in and around the central veins can be seen in a minority of lobules. However, central perivenulitis is neither severe nor widespread in recurrent HCV.

Plasma cell-rich "autoimmune" variant of HCV. Recurrent chronic HCV can present with aggressive plasma-cell-rich interface and perivenular necro-inflammatory activity resembling AIH (Figure 82.7) [132,200,207]. More studies are needed to determine whether this represents an "autoimmune variant of HCV," acute rejection, actual AIH, or a combination of these possibilities [132,200,207].

Immunosuppressive therapy can lessen the severity of liver damage in patients with plasma cell-rich HCV in the general population [208] and in allograft recipients with plasma-cell-rich interface and perivenular necro-inflammatory activity [207,209]. However, this can occur at the expense of enhancing HCV replication and impeding eventual HCV clearance [208].

Perivenular necro-inflammatory activity involving the majority of central veins, plasma-cell-rich or not, is a feature of acute rejection and AIH, but not of recurrent HCV. Central perivenulitis is usually responsive to increased IS, regardless of whether the patient is HCV-positive, and regardless of whether the patient is an allograft recipient [132,200,207].

Fibrosing cholestatic hepatitis. FCH HCV usually occurs within the first year after transplantation in the context of over-IS. It is typically characterized by homogeneous viral quasi-species and massive HCV RNA levels in the peripheral circulation (usually >30–50 million IU/mL). The most common features of FCH include cholestasis, hepatocyte ballooning degeneration, fibrosis (periportal, portal, and bridging), and ductular reaction [210]. Spotty apoptosis/necrosis and mild mixed or even neutrophilic-predominant portal inflammation are also often seen. FCH HCV occurs as a spectrum of severity: mild cases show only mild hepatocyte swelling, slightly more mononuclear portal inflammation, and only a low grade or minimal ductular reaction.

Diagnosing co-existent conditions in the context of recurrent HCV

Establishing a histopathologic diagnosis of acute or chronic rejection in the context of recurrent HCV is often difficult. The most frequent mistake is to over-diagnose ACR, leading to unnecessary increases in IS [131,200]. Features attributable to acute and chronic rejection include the following:

1 Mononuclear inflammatory bile duct damage and/or biliary epithelial senescence, respectively; and
2 Central perivenulitis and fibrosis, involving the majority of bile ducts or terminal hepatic veins, respectively.

Features attributable to recurrent or new-onset HCV include lobular necro-inflammatory activity and necro-inflammatory and ductular type interface activity. The key to establishing a final diagnosis is to determine which constellation of findings predominates.

Most clinically significant rejection episodes occurring in the context of recurrent HCV are graded as "moderate" according to the Banff criteria [116]. Biliary epithelial cell senescence involving the majority of bile ducts leads to a diagnosis of CR, which is often associated with a reduction in IS or treatment with an immune

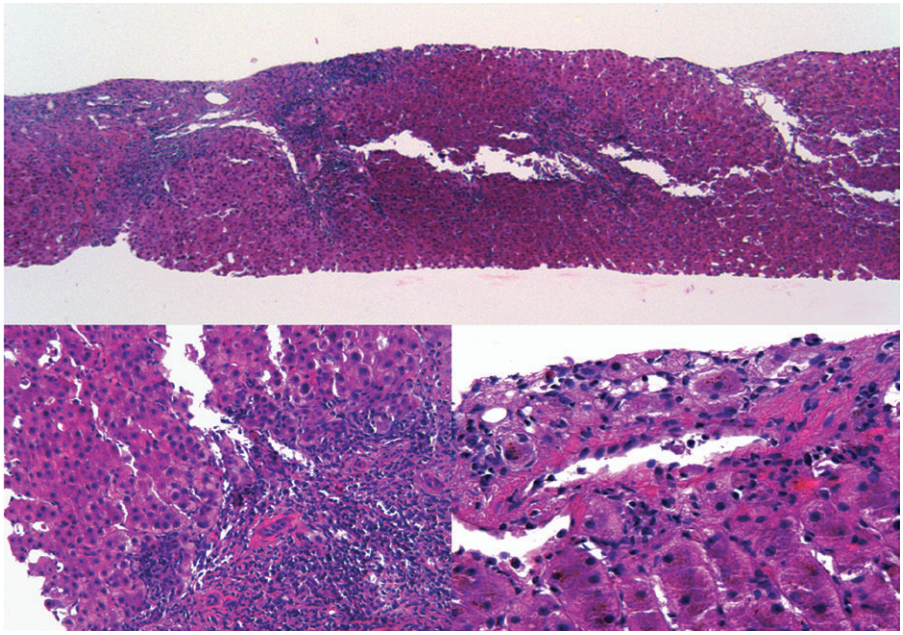

Figure 82.7. Recurrent HCV appears usually similar to that seen in native livers. Some cases of recurrent HCV, however, show "autoimmune" features such as plasma-cell-rich portal inflammation, aggressive interface activity (bottom left), and plasma cell-rich perivenular inflammation (bottom right). This biopsy was obtained about 26 months after transplantation from a female recipient who was HCV RNA-positive, but also had serologic evidence of co-existent autoimmunity. It is difficult to distinguish between acute cellular rejection and autoimmunity in such cases. Both will respond to increased immunosuppressant, but also usually show an increase in HCV RNA levels.

stimulator like α-interferon [200,211]. When either acute or chronic rejection is the predominant process the changes should be obvious. In addition, clinically significant rejection episodes occurring in the context of recurrent HCV are usually, but not invariably, associated with relatively low peripheral blood HCV RNA levels (<1–5 million IU/mL), suggesting that immunologic mechanisms associated with rejection also contribute to viral clearing [131,205].

Differential diagnosis

The differential diagnosis for acute and chronic HCV includes acute and chronic rejection, recurrent non-HCV hepatitis (e.g. HBV, CMV, EBV), and recurrent or new-onset AIH, PBC, and PSC, and biliary tract obstruction or stricturing [200,206]. C4d deposits and HCV protein and nucleic acid expression have been employed to assist with the differential diagnosis with limited success [200,206]. It is important to recognize other causes of injury occurring in the context of recurrent HCV. HCV is an uncommon cause of allograft dysfunction during the first several weeks after transplantation except for rare cases that begin as early as 10–14 days. Most cases occur 3–8 weeks after transplantation. Most acute rejection episodes, in contrast, occur within the first 30 days, with a median of 8 days [212].

Distinguishing HCV from other causes of chronic hepatitis also requires evaluation of the complete clinical, biochemical, and serologic profile. Viral antigen and/or nucleic acid detection, ground glass cells, or sanded nuclei distinguish HBV from HCV. Confluent necrosis is rarely, if ever, seen in HCV, alone. Recurrent or de novo AIH is recognized by sheets of plasma cells in the portal and perivenular inflammation with confluent perivenular necrosis, especially when combined with autoantibodies and hypergammaglobulinemia. In contrast, low-grade periportal and mid-zonal steatosis and portal lymphoid aggregates favor recurrent HCV.

FCH HCV can be difficult to distinguish from bile duct obstruction and hepatic artery thrombosis. Portal edema and portal or periportal neutrophilia are common in duct obstruction and acute cholangitis. A prominent ductular reaction and acute cholangiolitis without portal edema is more characteristic of non-obstructive cholestatic hepatitis. Lobular disarray and marked hepatocellular swelling are usual for viral hepatitis, but uncommon in duct obstruction alone.

Hepatitis E virus

Hepatitis E (HEV) is a single-stranded non-enveloped hepatotropic RNA virus endemic in Southern Asia and Africa. HEV infection in liver recipients has been associated with acute and chronic hepatitis [213–220]. Some cases spontaneously resolve, but up to 60% of chronically infected liver allograft recipients (defined as HCV RNA+ in the stool or serum for 6 months) develop chronic hepatitis and 15% of those progress toward cirrhosis [217]. Importantly, lowering IS can lead to resolution of infection and favorably influence chronic hepatitis [217], which is of potential importance for drug minimization trials.

Histopathologic findings and differential diagnosis

Similar to HBV and HCV, acute and chronic phases are described [213,215,218,220]. The acute phase is characterized by predominantly lobular inflammation and spotty hepatocyte necrosis and apoptosis. Portal tracts show mild to moderate expansion by inflammation composed mainly of lymphocytes and mild necroinflammatory-type interface activity [213].

Biopsies from patients with chronic infection show typical chronic viral hepatitis, characterized by variable lymphocytic and lymphoplasmacytic portal infiltrates with variable necroinflammatory-type interface activity and progressive fibrosis [213,

215,218,220]. The same criteria used to distinguish chronic HBV or HCV from acute and chronic rejection can be employed for HEV.

Disorders of dysregulated immunity

Disorders of immune regulation commonly recur after liver transplantation; the approximate incidence is about 25% by 5 years. The rate of progression of recurrent disease, however, might be less rapid than the same disease before transplantation [221,222]. Long-term graft and patient survival has not yet been significantly influenced by recurrent disease. However, there appears to be a progressive increase in the incidence of recurrent disease with time. Therefore, it is likely that diseases will begin to negatively impact long-term morbidity and mortality [222].

Primary biliary cirrhosis

Clinical presentation

Programs that conduct protocol biopsies most often detect recurrent disease from asymptomatic patients who often have (near-) normal liver injury test profiles [223]. In the large Mayo experience, only 12% of patients with recurrent PBC reported disease-related symptoms, with fatigue and pruritus most common [223]. In programs where biopsies are done by indication, recurrent PBC is usually first suspected because of increased alkaline phosphatase and γ-GTP as part of routine serologic monitoring.

Recurrent PBC can progress after liver transplantation, but retransplantation for recurrent PBC is rare and long-term patient and graft survival results are excellent [223]. AMA are of little additional benefit in establishing the diagnosis because AMA transiently decline shortly after transplantation and then recur in the vast majority, even without recurrent disease.

Histopathologic findings

Histopathologic diagnosis of recurrent PBC is based on the same clinicopathologic criteria as before transplantation. Diagnostic lesions include non-infectious non-caseating granulomatous bile duct damage or severe lymphocytic cholangitis producing breaks in the ductal basement membranes – referred to as florid duct lesions [223]. Patchy dense portal plasmacytic infiltrates at 1 year after transplantation presage later development of full-blown disease [224].

Patients often present with patchy mononuclear portal inflammation with focal lymphocytic cholangitis accompanied by portal lymphoid nodules and prominent ductular type interface activity resulting in a "biliary" gestalt. This refers to a ductular reaction at the interface zone, periportal "clearing" or edema, cholatestasis, accumulation of copper or copper-associated pigment in periportal hepatocytes, and patchy small bile duct loss. Such cases are "strongly suggestive" of recurrent PBC if they occur in proper context, which includes an original disease of PBC with no other reasonable explanation for the biliary pathology and preferential elevation of the γ-GTP and alkaline phosphatase.

The diagnosis of recurrent PBC is less certain in biopsies without significant lymphocytic cholangitis or a biliary gestalt. For example, "possible" recurrent PBC might first present as unexplained chronic hepatitis [137,225] because sampling problems may have missed the bile duct damage or the patient is presenting with an overlap syndrome with AIH [225], or as AIH alone [226].

As in native livers, lobular findings in recurrent PBC are usually mild.

Differential diagnosis

The differential diagnoses for recurrent PBC includes acute and chronic rejection, chronic obstructive cholangiopathy, chronic viral, autoimmune, or idiopathic hepatitis, and drug-induced liver injury. These same disorders can also co-exist with recurrent PBC. Other causes of granulomatous cholangitis, such as fungal or acid-fast bacterial infections and HCV, should be reasonably excluded.

A "biliary gestalt," described previously, is one of the most helpful constellations of findings that can be used to distinguish biliary tract pathology from other causes of dysfunction. Neither acute nor chronic rejection shows a significant ductular reaction biliary fibrosis or cirrhosis. Rejection-associated portal inflammation and lymphocytic cholangitis usually involve the majority of portal tracts and preferentially involves small bile ducts ($<20\,\mu m$ in smallest diameter). PBC-associated portal inflammation and lymphocytic cholangitis, in contrast, is typically patchy and preferentially involves medium-sized bile ducts ($>40–50\,\mu m$ in shortest diameter).

Patients with PBC can also develop de novo AIH or an overlap syndrome after liver transplantation [226]. Clinical, serologic, and histopathologic criteria used to establish the diagnosis of AIH and overlap syndrome before transplantation can also be used after transplantation, but are more difficult to apply.

Recurrent PBC can be quite difficult to distinguish from obstructive cholangiopathy because both produce a biliary gestalt. Histopathologic features that favor obstructive cholangiopathy over recurrent PBC include the following:

1. Edema and/or neutrophilic inflammation in and around the true bile ducts in the middle of the portal connective tissue;
2. Centrilobular hepatocanalicular cholestasis;
3. Bile infarcts; and
4. Intralobular neutrophil clusters.

In some cases, cholangiography may still be required to exclude a mechanical problem.

PBC can be difficult to distinguish from chronic viral and AIH, particularly because recurrent HCV can show a prominent ductular reaction. In such cases, careful examination of the bile ducts for evidence of significant lymphocytic or granulomatous duct damage and small bile duct loss can be helpful. Portal granulomas have been reported with recurrent chronic HCV [227]. However, this observation is uncommon and the granulomas rarely cause noticeable or severe ductal damage. Finally, copper and copper-associated protein stains can be used to distinguish between ductular reactions attributable to chronic cholestasis and those related to other insults.

Recurrent and new-onset "autoimmune" hepatitis

Establishing a diagnosis of recurrent or de novo AIH after transplantation is even more difficult than before transplantation. This is because: (1) serologic abnormalities either persist or transiently decline and then reappear without association to disease recurrence; and (2) histopathologic evidence of liver injury from other causes of allograft injury can mimic AIH [228,229]. The Banff Working Group advocated relatively strict criteria [113] to establish the diagnosis AIH after transplantation, but more study is needed.

Histopathology and differential diagnosis

Native liver, de novo, and recurrent AIH are indistinguishable and characterized in the typical case by aggressive, often plasma cell-rich necro-inflammatory-type interface and variable perivenular necro-inflammatory activity. Some patients with recurrent AIH present with plasma cell-rich central perivenulitis (see Acute

rejection). Distinguishing between centrilobular-based acute rejection and AIH can be problematic in such cases. A high percentage of plasma cells (>30%) favors AIH, whereas inflammatory bile duct damage or biliary epithelial senescence changes involving the majority of ducts favors rejection.

Patients who develop de novo bile salt export pump (BSEP) antibodies present histologically with cholestasis, hepatocyte multinucleation/giant cells, prominent interface ductular reaction, and fibrosis, which can lead to allograft failure. Aggressive plasma cell-rich interface or perivenular necro-inflammatory activity or inflammatory bile duct damage has *not* been described [230–232].

Once a "hepatitic" pattern of injury is established, examination of the clinicopathologic and serologic profiles are needed to: (1) support an autoimmune etiology (e.g. anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), liver-kidney microsomal (LKM), serum gamma globulins); and (2) reasonably exclude other causes of a chronic hepatitis, such as HBV, HCV, HEV, PBC, as well as obstructive cholangiopathy [178,228,229].

Distinguishing AIH from conventional acute and chronic rejection uses the same criteria as those used to distinguish acute and chronic rejection from viral hepatitis (see Hepatitis C virus). Distinguishing AIH from obstructive cholangiopathy and PBC was discussed in the sections on biliary tract complications and PBC, respectively.

Recurrent primary sclerosing cholangitis

The early stage of recurrent PSC usually first comes to clinical attention more than 6–9 months after transplantation because of selective elevation of alkaline phosphatase and γ-GTP. Non-anastomotic intrahepatic biliary strictures that develop *before* 90 days after transplantation are usually not attributable to recurrent disease and therefore other causes should be sought.

Histopathologic findings

Histopathologic features of recurrent PSC are identical to those described for native livers and cannot be reliably distinguished from other causes of biliary tract obstruction or stricturing in a needle biopsy. Findings typical of early stage include mild non-specific acute and chronic "pericholangitis" and variable low-grade interface ductular reaction. A "biliary" gestalt appears as the disease becomes well-developed. Included are irregular stellate fibrous expansion of most portal tracts accompanied by variable portal edema, periductal lamellar edema, stellate-shaped lumen of septal bile ducts, intraepithelial or intraluminal neutrophils within bile ducts, fibrous cholangitis, focal small bile duct loss, pigmented macrophages in portal connective tissue, ductular-type interface activity surrounded by edema, and periportal deposition of golden pigment and copper and copper-associated protein. As is typical for biliary diseases, the spatial relationship between the expanded portal tracts and the central veins remains intact until well-developed cirrhosis appears.

Lobular findings in early recurrent PSC include variable cholestasis, lobular neutrophil clusters, and mild nodular regenerative hyperplasia changes. Later stage are characterized by the development of biliary cirrhosis, cholestasis, intralobular foam cell clusters, marked deposition of copper and copper-associated protein, and Mallory's hyaline at the edge of nodules.

Differential diagnosis

Histopathologic recognition of the "biliary" gestalt, preferential elevation of γ-GTP and alkaline phosphatase points toward "biliary" pathology. However, extensive clinicopathologic and radiographic correlation are needed to determine whether recurrent PSC or one of the many other causes of biliary tract pathology is responsible for the changes observed. This distinction is not possible based on peripheral core needle biopsy findings alone. Instead, recurrent PSC should be diagnosed only after a complete analysis of clinical, histopathologic, and radiographic findings, including important exclusionary criteria.

Metabolic diseases and toxic insults

Jaffe [233] devised an algorithm to separate metabolic diseases into three categories for the purpose of understanding the impact of liver transplantation on the disease process and the possibility of recurrent disease (Table 82.7). As more medical therapies appear

Table 82.7. Summary of metabolic disease treated by liver transplantation classified according the system of Jaffe et al. [233] (see text)

Liver is the site of the primary metabolic defect and liver is usually diseased (see text)	Liver is the site of primary metabolic defect, but liver is usually normal or near-normal	Site of primary metabolic defect is probably extrahepatic and liver transplantation decreases morbidity and mortality associated with liver disease
α_1-Antitrypsin deficiency [234,262]	Branched chain amino acid deficiencies (reviewed in [234])	Cystinosis [233]. Does not generally cause liver disease; one patient developed intrahepatic crystal deposits in liver with perivenular fibrosis and recurrent disease in the allograft [233]
Bile acid synthesis defects [234]	Crigler–Najjar syndrome [263,264]	Cystic fibrosis; cures liver disease, and if liver transplant is performed early, lung function can improve [234]
Carbohydrate metabolism defects (reviewed in [234])	FAP [265]. Mild liver abnormalities: amyloid deposits in portal tracts and nerve trunks; use of FAP-affected liver is controversial	Porphyria (reviewed in [266,267])
Familial intrahepatic cholestasis syndromes (reviewed in [234])	Familial hypercholesterolemia [233]	Hemochromatosis or (inadvertent transplantation of donor with hemochromatosis) (reviewed in [268])
Glycogen storage disease, types I and Ib, III, IV [234,269,270]	Hemophilia A and B [233]	Niemann–Pick disease [233,234]
Hemochromatosis, neonatal (reviewed in [234])	Oxaluria, type I [233,234]	Sea-blue histiocyte syndrome [271]
Mitochondrial defects, limited to liver (reviewed in [234])	Urea cycle enzyme deficiencies [233,234])	
Tyrosinemia [234,272]		
Wilson's disease [234,273]		

FAP, familial amyloid polyneuropathy.

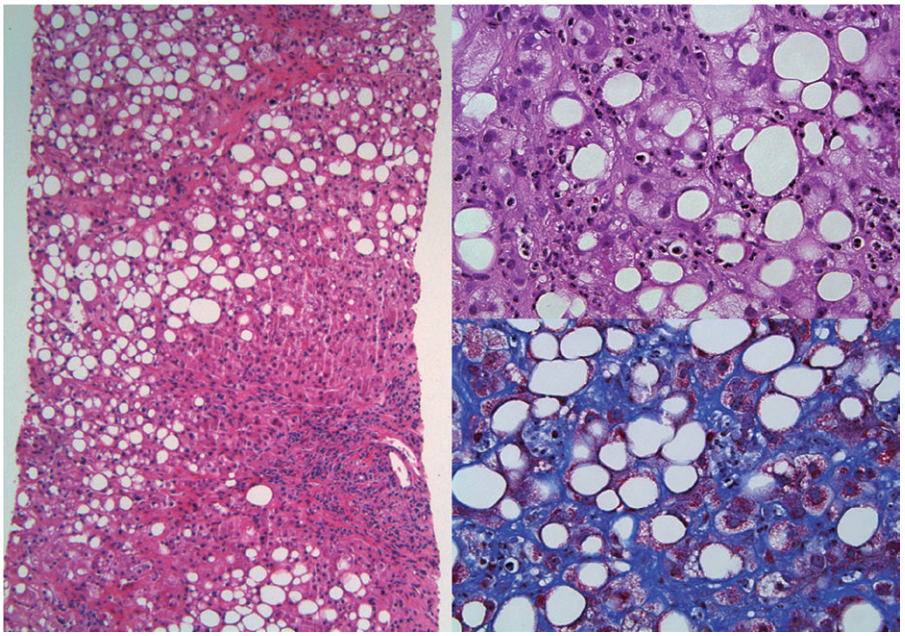

Figure 82.8. Recurrent alcoholic liver injury is a relatively uncommon cause of significant allograft injury. Although it is difficult to distinguish alcoholic from non-alcoholic fatty liver disease, the strict perivenular distribution of steatosis (left) with so-called "foamy" degeneration of hepatocytes (right upper inset) and neutrophilic inflammation are more typical of recurrent alcohol use. This can lead to subsinusoidal fibrosis (right lower inset).

the need for liver replacement therapy is decreasing, but is still effective when required [234]. Patients in the first group who failed medical or other surgical therapy, if available, are prime candidates for liver transplantation because the cirrhotic liver is replaced by a genetically and structurally normal one that generally cures the disease. Some familial intrahepatic cholestasis syndromes, however, recur after transplantation because of de novo antibodies that develop in response to mutated non-inherited proteins that are introduced with the donor organ (see AIH section) [230–232].

The liver is structurally normal or near-normal in the second group. The liver allograft is vulnerable to recurrent disease in the third group because the metabolic disorder persists after transplantation. Improved survival and/or quality of life issues, however, justify liver replacement [233].

Recurrent alcoholic liver disease

Clinical presentation

Problems with recidivism are usually detected because of elevation of liver injury test results that are routinely obtained in liver transplant populations [235], missed medical appointments [236], inappropriate social behavior [137], and non-compliance with IS (reviewed in [235,237]). A high γ-GTP : alkaline phosphatase ratio might provide biochemical evidence of alcohol recurrence [137,235], but blood alcohol levels are more definitive proof of relapse. A small minority of patients experience a rapid downhill course after transplantation because of recidivism and recurrent steatohepatitis [238–240]. However, most alcoholics do not relapse badly enough to cause significant alcoholic liver disease in the allograft.

Histopathologic findings and differential diagnosis

The histopathologic findings in alcoholic allograft liver disease are identical to those seen in native livers and will not be discussed in detail here. The most common histopathologic presentation is small and large droplet steatosis involving primarily centrilobular hepatocytes. The zonal distribution pattern of the steatosis is usually distinctive (Figure 82.8). More significant abuse can lead to so-called "foamy" degeneration of centrilobular hepatocytes, followed by fully developed "alcoholic hepatitis" with Mallory's hyaline, and ballooning degeneration of hepatocytes, with associated lobular neutrophilic inflammation.

For differential diagnosis, see the section on Non-alcoholic steatohepatitis.

Recurrent non-alcoholic steatohepatitis

Clinical presentation

Non-alcoholic fatty liver disease (NAFLD) is currently the third leading indication for liver transplantation in the United States, but is on trajectory to become the most common indication [241]. Those who undergo liver replacement for NAFLD are likely to be older, female, and have a higher body mass index.

A significant proportion of patients who underwent liver transplantation for cryptogenic cirrhosis will also develop NAFLD after transplantation [242–245]. Presumably, these patients also had undetected NAFLD before transplantation, but the histopathologic features were not obvious in the explant cirrhotic liver.

Recurrent NAFLD is usually first detected on protocol or indicated in allograft biopsies in asymptomatic patients. Many patients have multiple risk factors for NAFLD. A minority of otherwise healthy recipients develops unexplained NALFD after transplantation; in these patients metabolic testing for insulin resistance and a search for more esoteric causes is warranted.

Histopathology and differential diagnosis

A detailed description of the histopathology of hepatic steatosis and steatohepatitis are known to most pathologists and appear similar

to native livers. Readers, therefore, are referred elsewhere for an excellent review [246].

Determining the underlying cause of steatohepatitis in allografts can be particularly difficult in a minority of allografts. As in native livers, steatohepatitis is seen most commonly with morbid obesity, diabetes, insulin resistance, and alcohol abuse. However, it can also be associated with a number of other causes, and reference to standard medical texts is recommended. In the case of allografts, abnormalities of hepatic blood flow are an important cause of hepatic steatosis and steatohepatitis especially when it first appears within a few months after transplantation.

A thorough clinicopathologic correlation is needed to substantiate a suspicion of alcohol relapse or metabolic abnormalities leading to NAFLD. Awareness of the original disease(s), detailed clinical history, including current alcohol use, blood alcohol levels, and the ratio of γ-GTP : alkaline phosphatase can be used to distinguish between these possibilities.

Idiopathic post-transplant hepatitis

Pathophysiology and clinical presentation

Idiopathic post-transplant hepatitis is a diagnostic term coined by Hubscher [247] to describe chronic hepatitis changes on allograft biopsy (i.e. mononuclear portal inflammation with variable interface activity) without clinical or serologic evidence of viral hepatitis infection, autoimmunity, or drug-induced liver injury (reviewed in [113,248]). According to the definition, features used to diagnose acute rejection, such as bile duct damage and venous endothelial inflammation, are neither severe nor widespread [113,248].

As with other idiopathic disease categories, the expectation is that the diagnosis of IPTH will gradually dwindle as the underlying etiology is discovered: cases reassigned an etiology of chronic HEV infection, discussed above, is one example. Another small subgroup probably represents recurrent AIH, although strict criteria for AIH might not always be met [247]. The underlying cause of chronic hepatitis still cannot be determined in substantial percentage of cases. More study is clearly needed on this topic, but current thinking suggests that a substantial percentage of IPTH cases represent a variant of late-onset acute rejection [113,178,248,249].

Histopathology and differential diagnosis

IPTH is characterized by chronic portal inflammation, variable interface and lobular necro-inflammatory activity, but without prominent or prevalent bile duct damage or central perivenulitis. Prevalent lymphocytic cholangitis and central perivenulitis are more suggestive of an active rejection reaction. Significant plasma cell inflammation comprising >30% of the infiltrates is uncommon and suggests recurrent or de novo autoimmune hepatitis. Other causes of chronic hepatitis, such as HBV, HCV, HEV, and recurrent or de novo AIH should be excluded using clinical, serologic, and molecular diagnostic parameters.

The differential diagnosis for IPTH is the same as that for HBV or HCV infection or AIH (see earlier).

Considerations in immunosuppression optimization protocols

Clinical presentation

Evaluation of allograft biopsies has an important role in IS minimization or optimization, including complete withdrawal of IS in a small number of highly selected recipients [113,178,248,250]. This is a unique aspect of liver transplantation compared to other organs because: (1) liver allografts are more "tolerogenic" than other solid organ allografts [111]; and (2) if rejection does occur during or after weaning, it is usually rapidly reversible with current IS regimens without significant fibrosis or loss of function [111,113,178,248,250].

Histopathology and differential diagnosis

Pre-weaning biopsies are usually considered mandatory in IS minimization trials [248]. Biopsy findings associated with successful weaning include: (1) less portal inflammation [251,252]; (2) fewer $CD3^+$ and $CD8^+$ lymphocytes, but more $CD45RO^+$ lymphocytes within the lobules [6,252], more portal fibrosis in HCV-positive recipients [253], and less prevalent microvascular C4d deposits [251].

Follow-up biopsies are usually obtained during or after IS weaning, triggered by an elevation in liver tests [248,250]. However, liver test elevations are non-specific and weaning of IS can worsen recurrence of the underlying original disease [248,250]. γ-GTP elevation might be the most specific and sensitive indication of ACR after weaning [254,255].

The routine histopathologic appearances of ACR during weaning from IS most often resembles "classic" ACR, but atypical or incomplete presentations are common [113,248,250] and can include: (1) less prevalent and severe inflammatory bile duct damage; (2) more prevalent and severe interface and lobular necro-inflammatory activity; and (3) less portal venous subendothelial inflammation in the former. Therefore, biopsies might resemble low-grade chronic hepatitis [248,250], making differential diagnosis difficult, especially as interface hepatitis can also be seen after weaning in patients with underlying AIH or PBC, probably as a manifestation of recurrent disease.

Early and rapid weaning of IS from HCV-positive [203] and some HCV-negative recipients [256] treated with lymphocyte-depleting antibodies can "rearm" the immune system (immune reconstitution syndrome) [203], which manifests as aggressive hepatitis with rapid progression of fibrosis [203].

Development of progressive rejection-related fibrosis is of particular concern [113,248]. To monitor for this complication, the Banff Working Group encouraged adequate (two passes with a 16-gauge needle >20 mm and >11 portal tracts [28]) protocol biopsies in patients who do not develop symptoms or biochemical evidence of liver injury at 1, 3, 5, and 10 years after major decreases or total withdrawal of IS.

Findings in follow-up biopsies that should elicit concern for close follow-up include any noticeably increased inflammation, biliary epithelial cell damage, fibrosis, perivenular necro-inflammatory activity, or obliterative arteriopathy.

Long-term changes not readily explained by recurrent disease

Some histopathologic changes in long-surviving allografts cannot be attributed to recurrence of a specific disease and might represent adverse side-effects of medications or the effects of long-term engraftment and abnormal graft physiology [113,137,178]. Included are portal venopathy and nodular regenerative hyperplasia; thickening and hyalinization of small hepatic artery branches [137,257], subsinusoidal fibrosis, and "non-specific" portal and lobular inflammation [113,137,178]. Older livers placed into younger recipients

continue to age at the same or an accelerated rate (unpublished observation) and do not appear to experience any rejuvenation by being placed into a young body [258]. If nodular regenerative hyperplasia changes are detected early (<4 years) after transplantation progression to portal hypertension can occur [259]. As long-term survival after liver replacement is becoming increasingly common, more studies on these issues are needed.

Conclusions

Histopathologic evaluation of the liver is among the most diverse in transplantation, and the pathologist should be considered an integral part of the clinical liver transplant team. Indeed, real-time histopathologic assessment is required to inform the clinical management of the transplant recipient from organ acceptance through the life of the allograft, incuding retransplantation when necessary. The types of injuries commonly seen post-transplant include preservation–reperfusion injury small-for-size syndrome and rejection, and these give way to recurrent disease entities and viral hepatitis with time. Alterations in immune management also lead to alterations in hepatic histology. In general, changes in allograft status should prompt a biopsy, which in turn should prompt a critical assessment by a pathologist with specific experience in the spectrum of transplant-related disease.

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CHAPTER 83

Histopathological Syndromes of Heart Allograft Rejection and Recurrent Disease

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Introduction

Orthotopic heart transplantation is an effective therapy for end-stage heart disease of coronary and non-coronary etiology. Survival has continued to increase over time. The most common indications for cardiac transplantation in the adult have not changed in the last three decades: non-ischemic cardiomyopathies (53.3%) and ischemic disease (37.7%) [1]. In the pediatric age group, congenital heart disease is the most common indication for recipients <1 year of age. while cardiomyopathies account for more than 50% of the diagnosis in patients 1 year or older [2]. The current 10-year survival rate after cardiac transplantation is 50% and better in high-volume centers [1,2].

In this chapter, we focus on the technical, morphologic, and interpretive aspects of the anatomic pathology of the graft. We discuss the evolution and current status of the International Society for Heart and Lung Transplantation Working Formulations of 1990, 2005, and 2011 (ISHLT-WF-1990, ISHLT-WF-2005, and ISHLT-WF-2011) for the pathologic evaluation of transplant biopsies. This is followed by some comments about the pitfalls and caveats likely to be found, as well as controversial issues in the diagnosis of rejection in heart allografts. The chapter complements Chapters 71 and 79, which detail the clinical diagnosis and management of heart allograft rejection and recurrent disease, respectively.

Endomyocardial biopsy

The endomyocardial biopsy (EMB) remains the gold standard diagnostic tool for rejection surveillance in the heart transplant patient. It has a high sensitivity and specificity for the diagnosis of acute cellular rejection [3,4]. No other current modality of cardiac imaging, serum markers, or molecular diagnostics have higher sensitivity and specificity with a very short turnaround time to replace the performance of surveillance biopsies in the post-transplantation care and management of these patients [5]. While molecular testing has been developed for diagnosis, the biopsy is, at present, far more accessible for decision-making in real time.

Timing of the biopsy. In an ideal situation, a baseline donor biopsy should be obtained at the time of the transplant to provide assessment of the donor organ and rule out myocarditis, ischemic injury, or other pathologic changes. In most heart transplant centers, surveillance protocol biopsies are performed once a week for the first month, every 2 weeks for the second month, and every 4–8 weeks from the third to the twelfth month.

Procurement of the tissue. Bioptomes are used from either the jugular or femoral vein to sample the right ventricular septal wall. Bioptomes are available in different sizes (Figure 83.1), so the size of the piece of tissue retrieved will differ slightly. The common sizes used are 7 F (French) and 9 F in adults, and 3 F, 5 F, and 7 F in pediatric patients [6]. The cutting "jaws" procure adequate samples of subendocardial myocardium, usually from the interventricular septum.

Minimum number of tissue samples. As acute cellular rejection is not uniformly distributed in the heart, it is important to take multiple samples during the biopsy procedure. Pomerance and Stovin [7] showed that if three biopsy pieces taken show no rejection, there is a 5% and 0% chance of missing a mild and moderate–severe rejection, respectively. However, if four pieces are examined, the false-negative rate of mild rejection is further reduced to 2% [8]. Other investigators have emphasized that the extent of infiltration is also important, given that if at least three of the four fragments are graded separately as mild rejection, with a calculated frequency of 13.57% of the time, the probability of missing moderate–severe rejection is as high as 25% [9].

The ISHLT-WF-1990 required four pieces of tissue examined and optional procurement of one piece of tissue for freezing and one for electron microscopy [10]. In comparison, the ISHLT-WF-2005 formulation calls for the examination of at least three pieces, and preferably four (or more) of myocardium, 50% of which must be evaluable myocardium and not biopsy site or scar [11]. Specimens that do not meet these criteria should be diagnosed as "inadequate biopsy" [10]. The revised working formulation does not require a separate piece of frozen tissue or tissue routinely fixed for electron microscopy [11].

Pathologic evaluation of the biopsy. Optimal preservation of the tissue for diagnostic analysis can easily be done in the heart biopsy suite. The tissue should be fixed immediately in the desired fixative which has been allowed to reach room temperature of 25°C. Cold fixative enhances contraction band artifact. The tissue should not be allowed to sit on filter paper, gauze, or any other surface

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

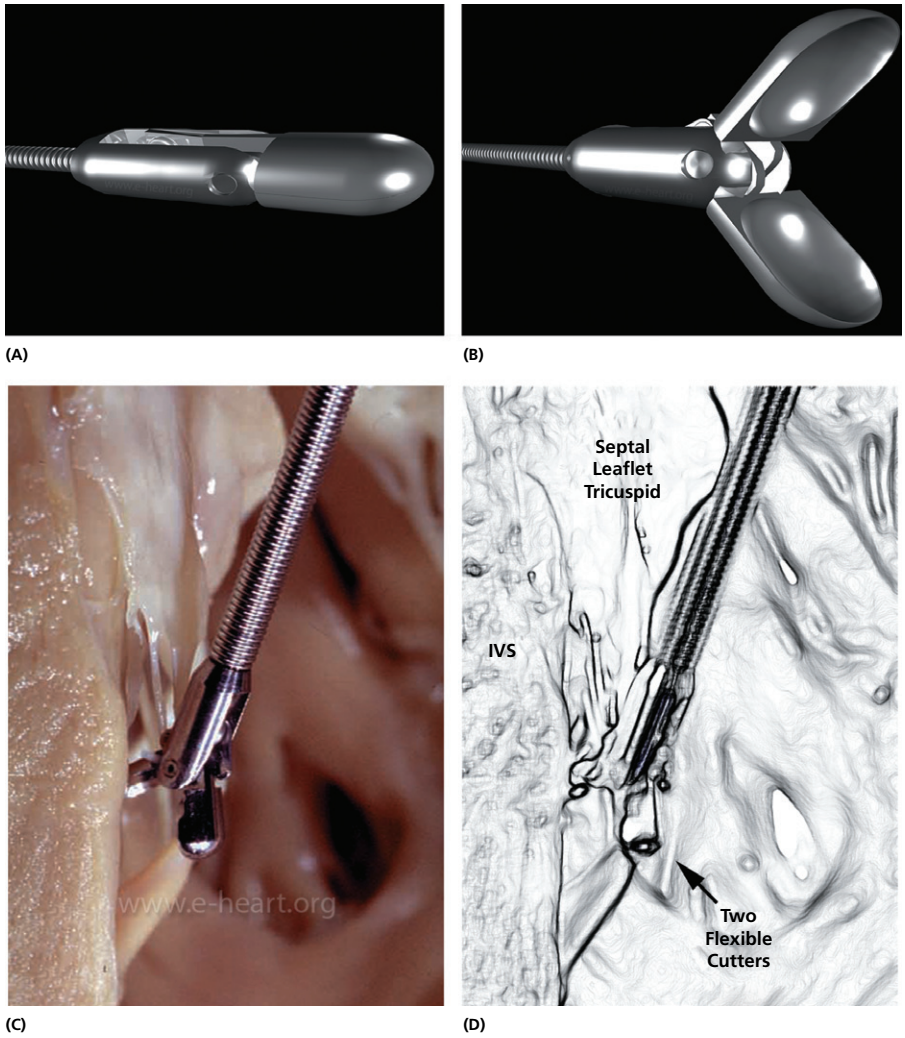


Figure 83.1. The bioptome (A,B) is the instrument used to procure endomyocardial biopsies. In North America, most biopsies are procured from the right side of the heart via transjugular approach. The target is the interventricular septal myocardium (C,D). The two spoon-shaped cutters provide adequate myocardial samples with minimal artifact.

impregnated with saline for a long period of time. Saline is a very poor solution to preserve the morphology of myocardium and readily creates artifacts. Other fixatives or preservatives that maintain tissue antigens in a better state for immunohistochemistry or immunofluorescence studies can also be used. For research protocols, one or more pieces of tissue can be snap frozen for molecular studies.

Gross examination. The gross description should include the demographic data of the patient, the number of tissue pieces, the average size, and color. In most instances careful gross examination provides important information regarding the presence of myocardium, thickened endocardium, adipose tissue, chordae tendineae [12], and blood clot or small endocardial thrombi. In our institution, we require that the number of pieces submitted be stated in the requisition form, verified on gross examination, and always correlated with the number of pieces present in the glass slides for microscopic examination.

Handling of the tissue. The tissue should be handled gently and not with forceps during gross examination in the pathology laboratory. It can be safely transferred directly from the specimen container into a mesh bag and then securely folded into the processing cassette. Do not triage the tissue. All the pieces obtained should be submitted, as they may have valuable information when examined with the microscope. Pieces that look white, suggesting that they are made up of thick endocardium, may actually be curled up, thus concealing some myocardium that will be visible on histologic sections (see section on Pitfalls and caveats). Likewise, pieces that look like blood clot may harbor a piece of myocardium in their core.

In our center, which is the third largest heart transplant center in the United States, the entire biopsy (i.e. all four or more pieces) is processed as a frozen section specimen. This procedure allows us to evaluate the sample for both acute cellular rejection and antibody-mediated rejection (AMR) in all the pieces, with a turnaround time of only a few hours. Careful preparation of the specimen prevents

Table 83.1. Acute cellular rejection (data from [10,11])

ISHLT-WF-1990	ISHLT-WF-2005
Grade 0 (no acute rejection)	Grade 0R
Grade 1A (focal, mild acute rejection) Grade 1B (diffuse, mild acute rejection) Grade 2 (focal, moderate acute rejection)	Grade 1R Mild (interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage)
Grade 3A (multifocal moderate rejection)	Grade 2R Moderate (two or more foci of infiltrate with associated myocyte damage)
Grade 3B (diffuse, borderline severe acute rejection) Grade 4 (severe acute rejection)	Grade 3R Severe (diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis

freezing artifacts such as ice crystals forming in the sarcoplasm of the myocytes.

Cardiac allograft rejection: morphologic aspects

After more than a decade of widespread use, the ISHLT-WF-1990 working formulation [10] was revisited during the Heart Session of the Banff Allograft Pathology group meeting in 2001. The meeting consensus was that although the working formulation had withstood the test of time, some areas for refinement in the cellular rejection grading approach existed, and the lack of standard diagnostic criteria for AMR needed to be addressed [13]. The formal revision was published in 2005 and it is the current working formulation for acute cellular rejection [11]. The ISHLT-WF-2005 provided for the first time the criteria for diagnosis of AMR [11,14] (Table 83.1). A further revision of the working formulation for the diagnosis of AMR was published in 2011 (ISHLT-WF-AMR-2011) [15]. Like in any other solid organ, cardiac allograft rejection can be divided into humoral and cellular rejection. These can in turn be subclassified into hyperacute, acute, and chronic rejection on the basis of mechanism and duration of the process.

Hyperacute rejection consists of a violent complement-mediated immune attack to the graft that is triggered by preformed antibodies and occurs very quickly, usually within minutes to hours, after the implantation of the graft. Mechanistic specifics of hyperacute rejection can be found in Chapter 6. Predisposing factors that may have a role are preformed antibodies to epitopes of the ABO and human leukocyte antigen (HLA) systems, multiple pregnancies, multiple surgeries with the use of blood products, and even previous cardiac transplantation. The activation of the complement cascade produces severe endothelial cell damage as well as platelet activation, followed by the clotting cascade and thrombosis. The morphologic findings include swelling of the endothelial cells (not easily documented by light microscopy), microthrombi, extravasation of red blood cells and even microscopic hemorrhages, interstitial edema, and subsequently polymorphonuclear inflammatory infiltrates, followed by tissue necrosis. On gross examination, the changes may be inconspicuous or may show hemorrhages or the combination of pallor and hemorrhages. Immunohistochemical studies may show deposits of IgM, IgG, and complement in the vessel walls as well as interstitial fibrin. Unfortunately, the pathologic examination of this type of rejection is usually carried out during the autopsy [16].

Acute cellular rejection: effector cells. Morphologically, acute cellular rejection is a mononuclear inflammatory response, predominantly lymphocytic, directed against the cardiac allograft. Its mechanistic features are detailed in Chapter 5. In severe cases, there is also participation of the granulocytes in the rejection process. The subtleties and variations of this response are used in the grading of rejection. The rejection grades proposed in the working formulation are based mainly on the amount of inflammatory infiltrate and the presence of myocyte damage; the pattern of inflammatory infiltration plays a minor part. Studies that have characterized the phenotype of lymphocytes in cardiac biopsy tissue have shown no good correlation between the extent and composition (CD4:CD8 ratio) of T lymphocytes infiltrating the graft and the histologic grading of rejection [17,18]. However, other studies report a good correlation between the mean number of $CD8^+$ T cells and the severity of rejection grade [19]. The discrepancy in these studies may be related to the fact that the immune response to the allograft is a continuous process in flux that is usually dissected in small "time-lapsed" views for pathologic study. Some support to this notion is provided by the observation that if subsets of T lymphocytes are further classified on the basis of the presence of naive cells (CD45RA) and memory or activated cells (CD45RO), naive cells of the CD4 phenotype are more abundant in biopsy tissue during mild rejection. A shift toward activated CD8 phenotype is seen in moderate rejection [20]. An increase in the number of antigen-presenting cells (i.e. macrophages and dendritic cells) is also observed as a function of the severity of rejection [21–24]. The role of regulatory cells in human cardiac allograft rejection is not well defined. However, recent quantitative methods in biopsy material will allow better characterization of this subtype of T cells in cardiac allografts [25].

B-cell infiltrates are rarely present in mild rejection. However, a substantial increase in activated B lymphocytes and natural killer cells are seen in moderate rejection, suggesting their important role as promoters and effectors of cellular rejection [24].

Grading of acute cellular rejection in endomyocardial biopsies

The rejection grades in the current ISHLT-WF-2005 have the added suffix R after the grade (Table 83.1), to indicate that these are the "revised" grades as follows [11]:

Grade 0R (no acute cellular rejection). In Grade 0 R, there is no evidence of mononuclear (lymphocytes/macrophages) inflammation or myocyte damage (Figure 83.2).

Grade 1R (mild, low-grade, acute cellular rejection). Mild or low-grade rejection may manifest in one of two ways. First, perivascular and/or interstitial mononuclear cells lymphocytes/histiocytes) are present. In general, these cells respect myocyte borders, do not encroach on adjacent myocytes, and do not distort the normal architecture (Figure 83.3). Second, one focus of mononuclear cells with associated myocyte damage may be present.

Grade 2R (moderate, intermediate-grade, acute cellular rejection). In Grade 2R, two or more foci of mononuclear cells (lymphocytes/macrophages) with associated myocyte damage are present. Eosinophils may be present. The foci may be distributed in one or more than one biopsy fragment. Intervening areas of uninvolved myocardium are present between the foci of rejection (Figure 83.4). Low-grade rejection can be present in other biopsy pieces.

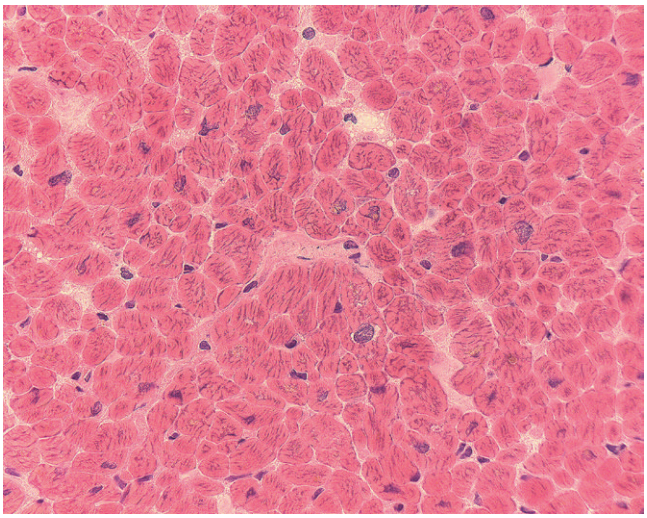


Figure 83.2. Endomyocardial biopsy of an allograft with rejection Grade 0R. The myocytes show minimal hypertrophy with enlarged hyperchromatic nuclei, but no evidence of any inflammatory infiltrates in the interstitium or around a small arteriole seen in the center of the image (H&E, original magnification ×400).

Grade 3R (severe, high-grade, acute cellular rejection). A diffuse inflammatory process, either predominantly lymphocytes and macrophages, or a polymorphous infiltrate, is present (Figure 83.5). In most cases, the majority of biopsy fragments are involved, although the intensity of the infiltrate may vary between pieces. Multiple areas of associated myocyte damage are present. In the most severe forms of cellular (and humoral) rejection, edema, interstitial hemorrhage, and vasculitis may be present.

Additional information to be included in the biopsy report (non-rejection biopsy findings) (Table 83.2)

Statement on adequacy of biopsy. The term inadequate biopsy should be used when the specimen consists of less than three pieces [10,11]. In addition, the term inadequate biopsy should also be used even when the number of pieces examined under the microscope is four, but the myocardium present in one or more of these pieces is less than 50% of the piece. The inadequate pieces usually consist of mostly or only endocardium, thrombus, granulation tissue from a previous biopsy site, or adipose tissue. However, it should be recognized that in some instances, valuable information such as rejection is present in the other adequate biopsy pieces. Although a diagnosis of rejection cannot be rendered, the findings can be

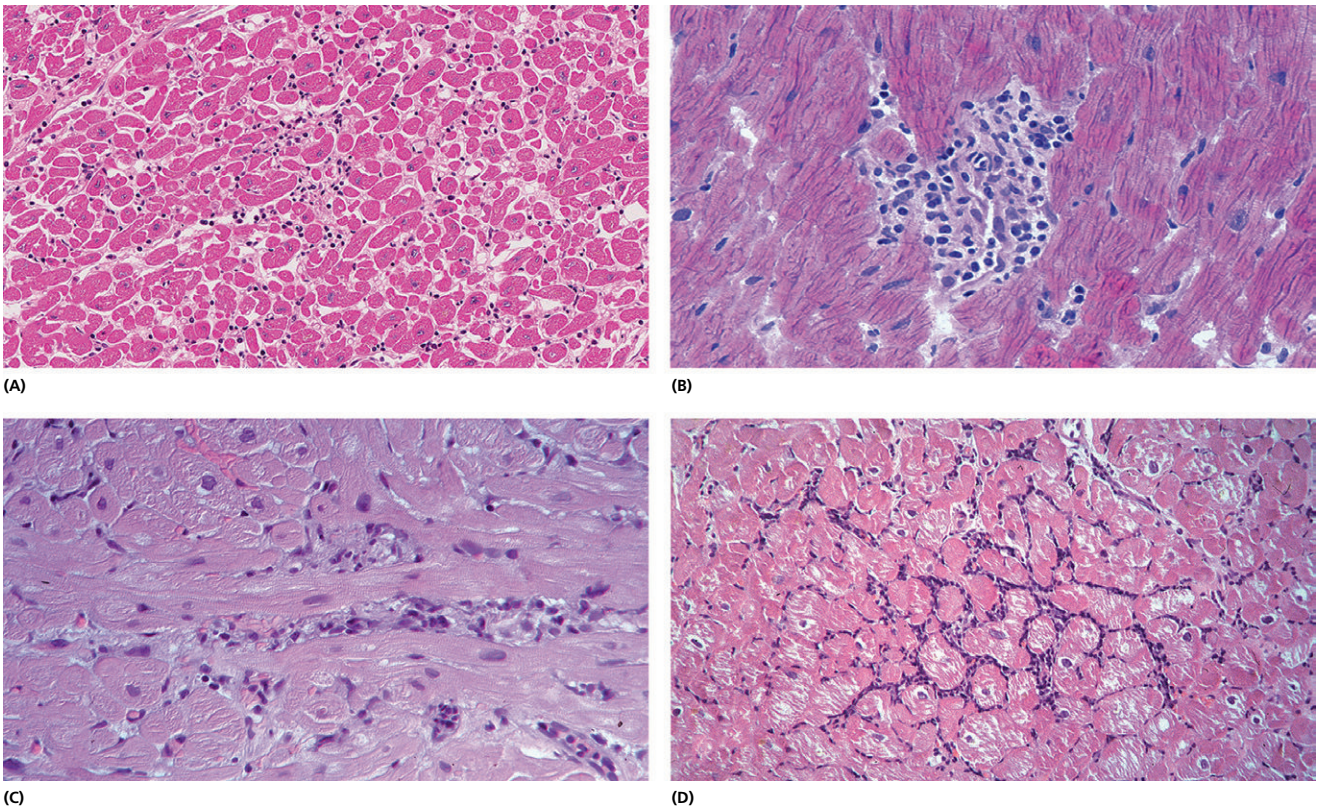


Figure 83.3. Rejection Grade 1R. (A) Light micrograph showing scant but conspicuous lymphocytes in the interstitium of the myocardium without evidence of necrosis, injury, or dropout of the myocytes (H&E, original magnification ×100). (B) A cluster of lymphocytes is present surrounding a small arteriole and also extending into the interstitial space. The infiltrate is discrete and clearly "vasculocentric" (H&E, original magnification ×200). (C) A slightly different pattern of lymphocytic infiltrate present in both perivascular and interstitial pattern (H&E, original magnification ×200). (D) This micrograph shows a more abundant infiltration, with the lymphocytes distinctly surrounding individual myocytes in their perimysial spaces. This "chickenwire" pattern was designated as the 1B pattern in the ISHLT-WF-1990 (H&E, original magnification ×150).

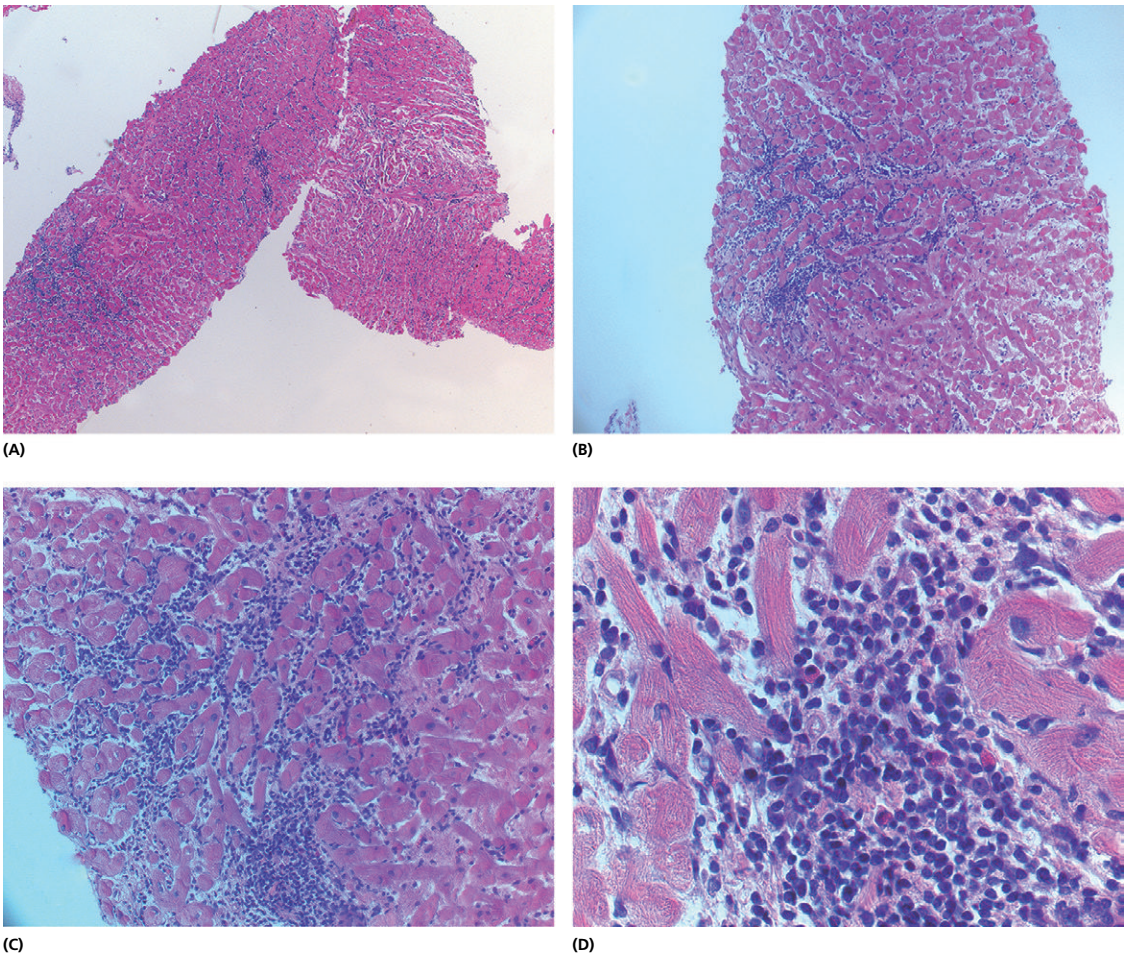


Figure 83.4. Rejection Grade 2R. (A) This biopsy shows multifocal lymphocytic infiltrates that expand the interstitial space. Two distinct foci of more abundant inflammatory infiltrates with an intervening area of myocardium without inflammation are shown (H&E, original magnification ×20). (B,C) Myocyte dropout is clearly present as well as distinct encroachment of the myocytes by the lymphocytic infiltrates (B, H&E, original magnification ×40, and C, original magnification ×200). (D) In this close up of C, the expansion of the inflammatory infiltrate clearly replaces myocytes (dropout) and in addition shows some eosinophils (H&E, original magnification ×400).

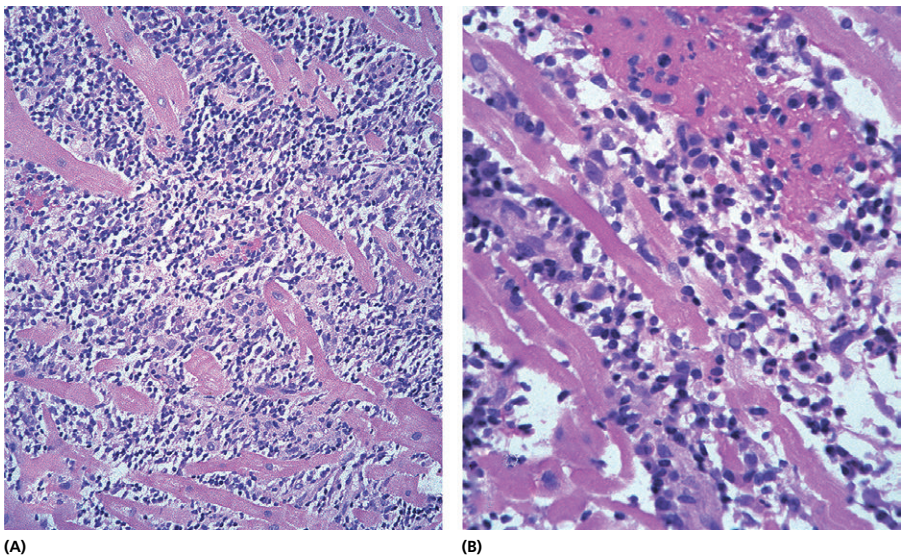


Figure 83.5. Rejection Grade 3R. (A) The myocardium is diffusely and extensively infiltrated by dense mononuclear inflammatory cells. These represent a grade 3B in the ISHLT-WF-1990 (H&E, original magnification ×100). (B) The extensive inflammation can progress to the stage in which neutrophils and even vascular necrosis can be seen. In addition, marked interstitial edema accompanies the inflammatory infiltrate (H&E, original magnification ×400).

documented in the report as part of the histologic description or as a note.

Perioperative ischemic injury. This is an early process of ischemic injury related to prolonged ischemic time and characterized by contraction band necrosis or coagulation necrosis (Figure 83.6A,B). Early perioperative necrosis may be due to events that affect the donor such as catecholamine discharge, pressor therapy given during acute care, severe donor trauma, reimplantation damage, or early postoperative damage [26]. A biopsy of the septum taken at the time of implantation is informative although the changes can be very subtle. During allograft monitoring, the working formulation makes a distinction between ischemia commonly seen up to the third week post-transplant representing perioperative injury and late ischemia that occurs after 3 months or more. Morphologically there is clear evidence of myocyte necrosis, usually coagulative type, with hypereosinophilic myocytes, pyknotic nuclei, and even some karyorrhexis. Myocyte necrosis is usually out of proportion to the inflammatory infiltrate; myocyte vacuolization may be seen. Healing may be delayed due to immunosuppression [26]. Although this type of necrosis can be clinically silent, these changes can compromise the function of the graft in various degrees if severe. These areas are usually conspicuous, especially when stained with Masson trichrome. Calcification of myocytes sometimes occurs (Figure 83.7). Myocyte vacuolization and fat necrosis may also be seen [26]. As healing ensues, the biopsy may show mixed inflammatory infiltrates, including neutrophils, lymphocytes, macrophages, and eosinophils. This phenomenon may be seen in biopsies

Table 83.2. Non-rejection findings (data from [10,11])

ISHLT-WF-1990	ISHLT-WF-2005
Ischemic injury	*Ischemic injury*
A = up to 3 weeks post-transplant	Early – up to 6 weeks post-transplant
B = late ischemia	Late – related to allograft coronary disease
Quilty effect	*Quilty effect*
A = no myocyte encroachment	
B = with myocyte encroachment	
Infection	*Infection*
Lymphoproliferative disorder	*Lymphoproliferative disorder*

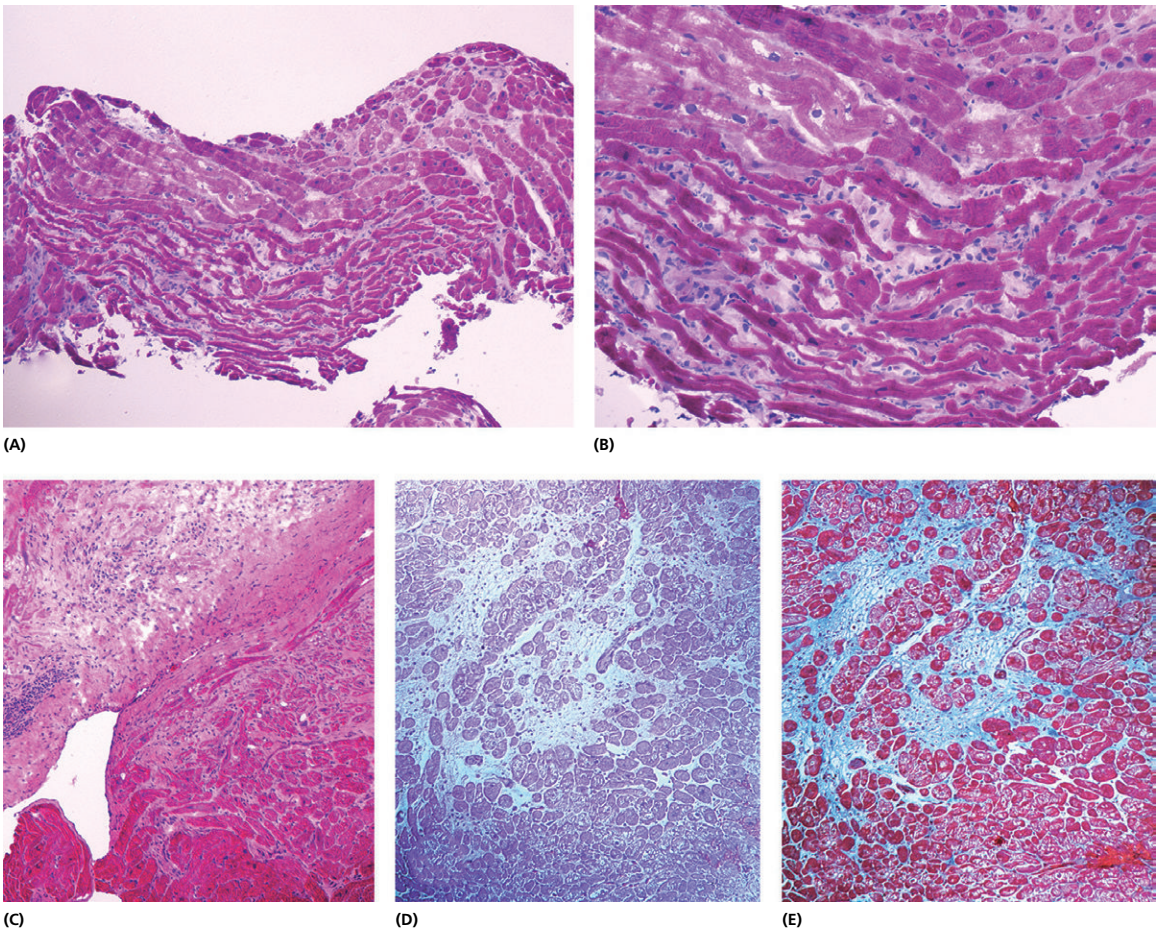
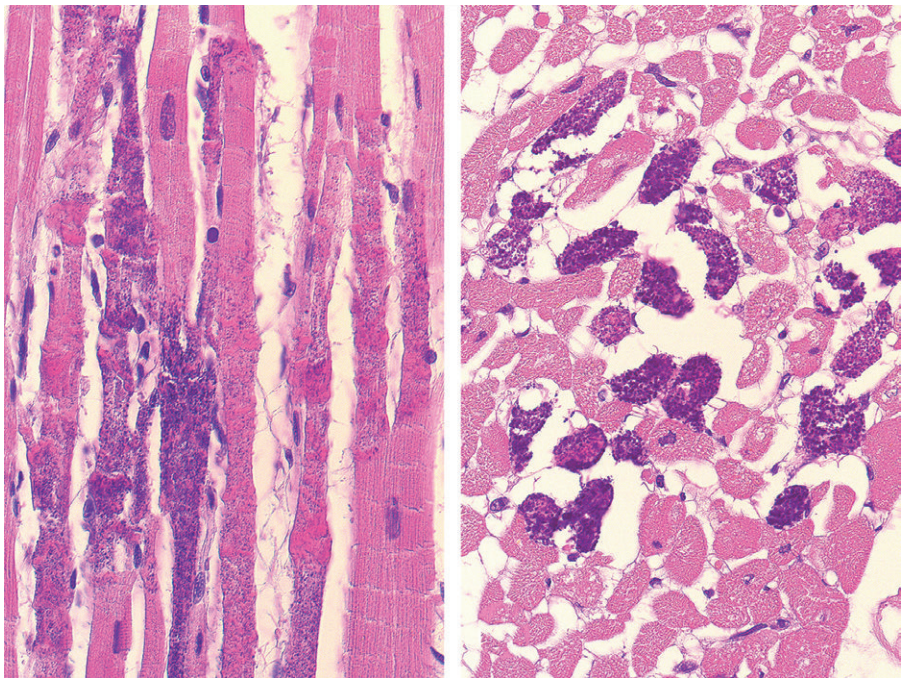


Figure 83.6. Ischemic injury. (A,B) Hypereosinophilic wavy thinned myocytes with distinctly pyknotic nuclei and/or loss of nuclei which represent acute ischemic injury (H&E, A original magnification ×40; B original magnification ×200). (C) Healing ischemic focus with loose granulation tissue and mild lymphocytic infiltrates (H&E, original magnification ×40). (D,E) Replacement and interstitial fibrosis representing healed areas of ischemic injury are shown. When present late in the post-transplant period, they should always raise the suspicion for the presence of allograft vasculopathy (D, Masson's trichrome, original magnification ×40).



(A) (B)

Figure 83.7. Calcification of myocyte mitochondria in ischemic injury. Mitochondrial calcification can be recognized as basophilic sarcoplasmic granules in these necrotic myocytes. (A) The myocytes in longitudinal section show that the basophilic mitochondria are in register with the striations of the sarcoplasm (H&E, original magnification ×400). (B) In cross-section, the pattern of distribution of the calcified mitochondria in the sarcoplasm is more haphazard (H&E, original magnification ×400). As a function of time, the myocytes become completely calcified (see Figure 83.12).

during the first 6 weeks. The injury to the myocytes is greater than the inflammatory infiltrate in this lesion [11].

A possible early injury to the allograft during the peritransplant period is the development of interstitial fibrosis [27]. The only way to ascertain that the fibrosis may be the result of peritransplant ischemic injury is to obtain a baseline biopsy piece at the time of implantation of the graft. Another caveat with regard to fibrosis is the fact that the size of the actual myocardial piece may influence the perception of the amount of fibrosis [28].

Late ischemic injury. Assessing the arterial changes of allograft vasculopathy is usually precluded by the lack of vessels large enough to permit such an evaluation in the biopsy specimen. However, secondary myocardial changes, such as colliquative myocytolysis and coagulation necrosis, can be easily identified. In the setting of cardiac allograft vasculopathy, ischemic foci are not associated with any significant mononuclear infiltrates. Thus, the diagnosis of late ischemic injury may be helpful in ruling out other potentially treatable etiologies that are part of the differential diagnoses for cardiac failure in transplant recipients, such as acute cellular rejection or AMR [11].

Endocardial lymphocytic infiltrates (Quilty effect). Endocardial lymphocytic infiltrates [29], also known as "Quilty effect" [30], are collections of T and B cells with histiocytes [31] seen in the endocardium of transplanted hearts (Figure 83.8). Plasma cells are present in about half of these lesions. Occasional eosinophils and neutrophils may be seen [32]. Capillaries with red blood cells and sometimes prominent endothelial cells may be seen within the infiltrate. They vary in size from 0.007 to 1.89 mm^2 [29]. Several hypotheses have been proposed to explain the pathogenesis of these infiltrates which include the use of cyclosporine [33] to concomitant infection with Epstein–Barr virus [34], low local levels of cyclosporine in the areas of endocardium where Quilty effect infiltrates develop [35], and idiosyncrasy to cyclosporine [33] but none of these has been proven. However, one striking observation is that the Quilty effect does not occur in the hearts of patients who are taking cyclosporine as a result of having received other organ transplants (i.e. liver, kidney) [36]. It seems to be a phenomenon that only occurs in the endocardium of cardiac allografts.

Quilty effect infiltrates may or may not be associated with rejection. The size of these infiltrates varies greatly, from a small cluster of mononuclear cells to grossly visible 2 mm lesions. Two morphologic patterns were recognized in the ISHLT-WF-1990: types A and B. Type A was confined to the endocardium. Type B extended into the subjacent myocardium [10] (Figure 83.8C,D). The ISHLT-WF-2005 removed the requirement to classify the Quilty effect lesions into A and B, because there is no clinical relevance to the distinction of these two patterns [11]. However, there is a third rarely seen pattern in which lesions identical to Quilty effect lesions are found deep in the myocardium besides the typical endocardial lymphocytic infiltrates.

Quilty effect and moderate rejection. The possible confusion of Quilty infiltrates with rejection [37] has been a problem for pathologists because of the obvious implications for therapy. One may imagine how a tangential section through the deeper myocardial end of a biopsy may show inflammatory infiltrates that look like rejection if only a few levels of section are examined. However, if deeper sections are made, or better yet, if all the biopsy tissue is

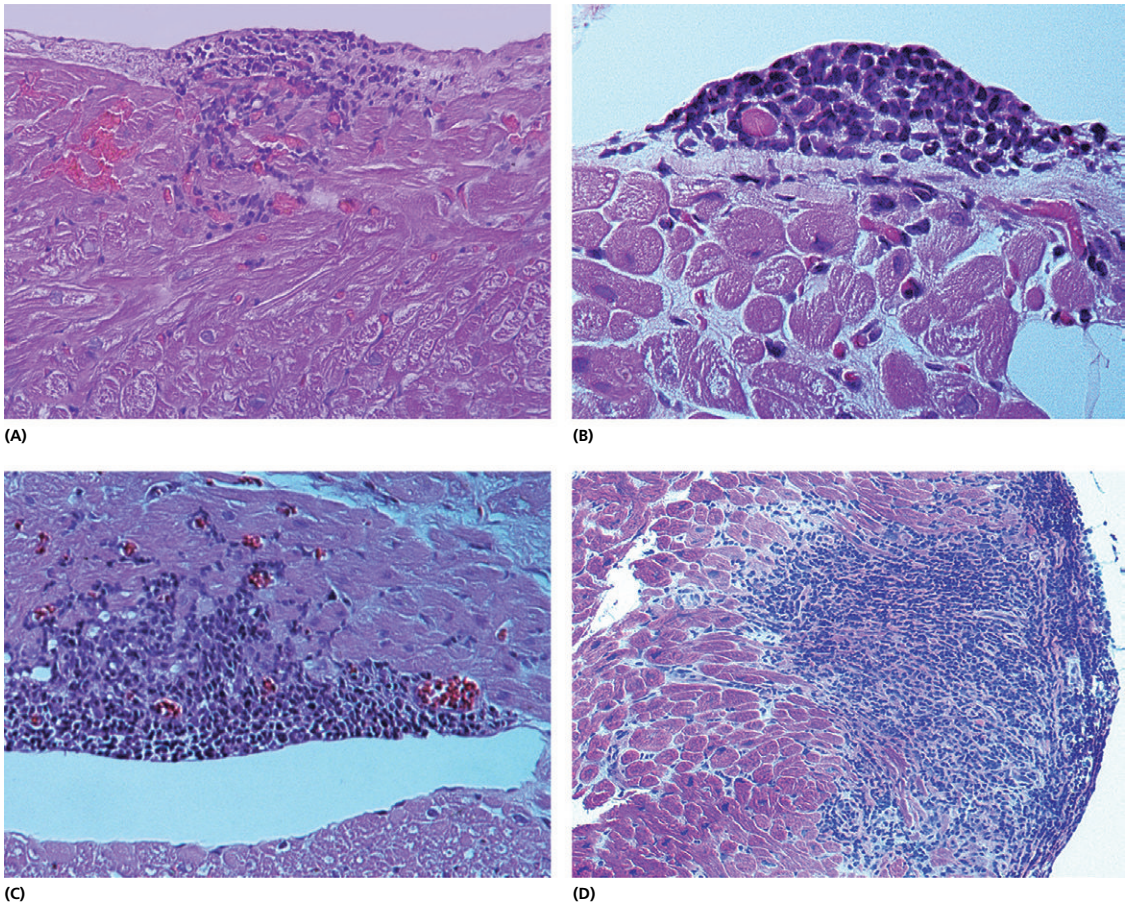


Figure 83.8. Endocardial lymphocytic infiltrates (Quilty lesions). (A) Lymphocytic infiltrate contained within the endocardium and extending into the myocardium is shown in this biopsy (H&E, original magnification ×150). (B) This Quilty lesion is distinctly contained within the endocardium and does not penetrate the subjacent myocardium. Such lesion used to be called Quilty type A in the ISHLT-WF-1990 (H&E, original magnification ×400). (C) This infiltrate is clearly within the endocardium and showing early infiltration of the myocardium. In addition, it shows conspicuous blood vessels filled with red blood cells. These are very characteristic and common in Quilty lesions. However, in frozen sections, these vessels may not be as conspicuous (H&E, original magnification ×200). (D) In this biopsy, the lymphocytic infiltrate clearly extends into the subjacent myocardium with finger-like projections. This used to be called Quilty B in the ISHLT-WF-1990 (H&E, original magnification ×100).

examined, it would soon become very obvious that the inflammatory infiltrate in the myocardium is connected to a Quilty type lesion in the endocardium.

Quilty effect and lymphoid neoplasms. Exuberant Quilty lesions can be easily distinguished from lymphoid neoplasms by their cellular composition and location. Lymphoid neoplasms are reported to occur in 6% of all transplanted patients [38]. The role of Epstein–Barr virus in the pathogenesis of post-transplant lymphoproliferative disorders (PTLD) is established [39–42] but not necessarily the sole cause of this disorder [43,44]. In most instances, these neoplasms are monoclonal B-cell type [45] and their clinical presentation involves the lymph nodes, the central nervous system, systemic organs, or the transplanted organ itself [39]. Nevertheless, T-cell lymphomas also occur [46], but primary cardiac presentation of these neoplasms is not common [39]. Development of multiple myeloma is rare [47]. Additional discussion of PTLD and malignancies post-transplant can be found in Chapters 93 and 95, respectively.

In summary, endocardial lymphocytic infiltrates (Quilty effect) are lesions that are commonly seen in cardiac transplant biopsies but until now lacked any clinical relevance. In a recent report including 217 adults and 22 children studied for 10 years, the authors show that 49% of adults and 68% of children show these infiltrates [30]. The important points in this study are that there seems to be no association between the endocardial infiltrates and: (1) grade of rejection; (2) subsequent development of vasculopathy (vascular chronic rejection); (3) development of lymphoma; and (4) viral infections (cytomegalovirus or Epstein–Barr virus). Lastly, because there is no difference whether the infiltrate is type A or type B the ISHLT-WF-2005 no longer requires this distinction [11].

Pitfalls and caveats

Although an enormous effort has been made to create a standard method for grading rejection [10,11], there are several controversial points that are often identified by both pathologists and cardiologists in using the working formulation.

Myocyte injury. A difficult issue in the interpretation of endomyocardial biopsies from human allografts has been the inability to obtain consensus about the definition of myocyte damage even among experienced pathologists trained in, and dedicated to,

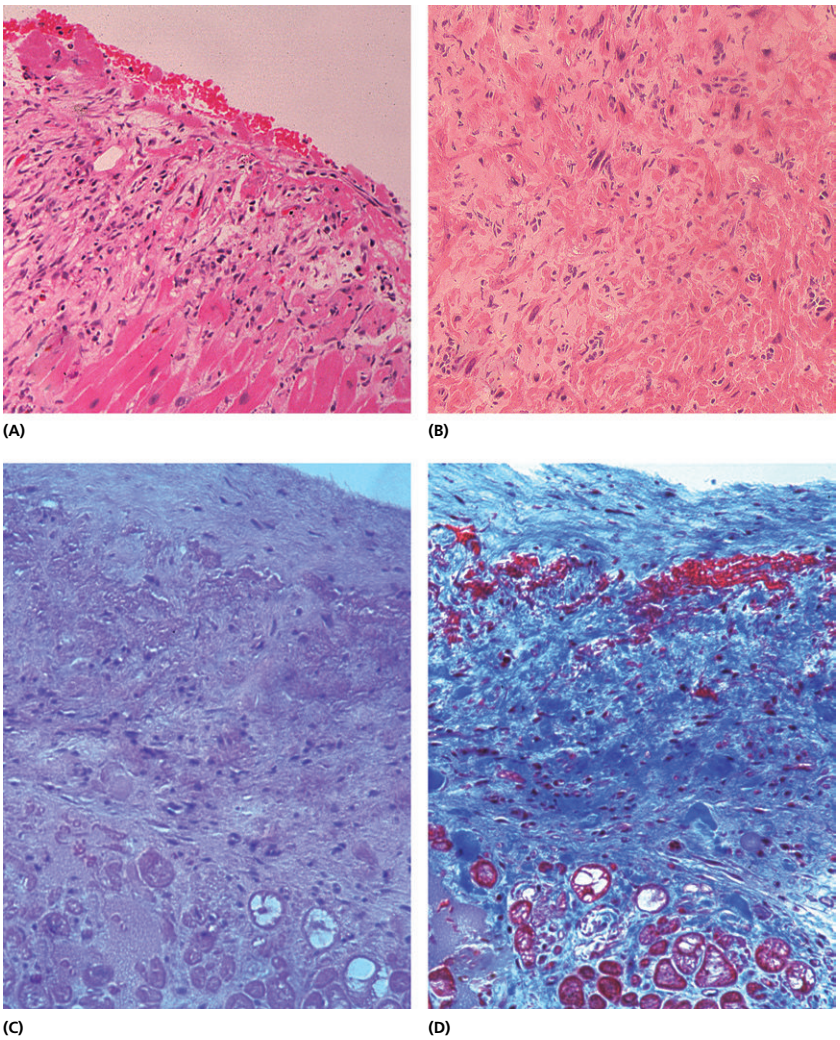


Figure 83.9. Previous biopsy sites. (A) This image illustrates an area of granulation tissue in the endocardium with abundant fibroblasts and some red blood cells on the surface. The fibrous tissue is still loose. It represents an area of healing from a recent previous biopsy site (approximately a week old) (H&E, original magnification ×100). (B) This area of granulation tissue shows further healing, with somewhat fewer fibroblasts and distinct maturing denser fibrous tissue than the lesion in A. Note the mild disarray of myocytes within the scar shown in this field. This lesion may represent a healing biopsy site of approximately 2–3 weeks' evolution (H&E, original magnification ×200). (C,D) These two images show a well-healed previous biopsy site with endocardial thickening and subjacent scar. The trichrome stain in D shows a distinct increase of dense fibrous tissue forming the scar (C, H&E, original magnification ×200; D, Masson's trichrome, original magnification ×200).

cardiovascular pathology [13]. The suggestion at the Banff 2001 conference was to focus on terminology that was more precise for pathologists, such as myocyte injury [13]. On light microscopic examination, this type of injury can encompass a spectrum of subtle to ominous changes. The subtle changes are more difficult to be noticed by the occasional observer. In a strict sense, simple changes in the myocytes such as vacuolization, hydropic change, or perinuclear halos [48] are at the somewhat milder end of the spectrum whereas coagulation necrosis, myocytolysis, and nuclear pyknosis are at the very worse end of the spectrum. Thus, the term damage is rather ambiguous. Unless there is clear coagulative necrosis or fragmentation of the sarcoplasm or typical nuclear changes such as pyknosis in the myocytes, the identification of "damaged" cells in hematoxylin and eosin stained paraffin sections is a subjective matter. Ultrastructural studies have clearly shown that subtle myocyte damage is present [49]. Myocyte degeneration is easily distinguished from necrosis. Damage to endothelial cells, basal lamina, or other components is also easily recognized [50]. Myocyte necrosis as defined by ultrastructural criteria is common in humoral rejection, whereas myocyte degeneration with the potential for recovery is more common in cellular rejection [51]. Ultrastructural studies have suggested that some of the myocyte damage seen during rejection may actually be a reversible process [49,52]; however, by light microscopy some of the myocyte changes that represent sublethal damage are indistinguishable from actual early necrosis.

Previous biopsy site. This is a common finding in transplant monitoring biopsies (Figure 83.9). In fact, it can be seen in 53–69% [53,54] of biopsies. This high frequency is because, for a given patient, the anatomy of the inflow tract to the right ventricle is constant. During the biopsy procedure using the jugular approach, the ridges of the atrial anastomotic site, the right ventricular trabeculations, and the moderator band all contribute to guide the tip of the bioptome towards the same site in the interventricular septum. Gross examination at autopsy may show a patch of thickened endocardium measuring 1–2 cm in diameter in the mid-third of the right ventricular septum in patients who survived several months to years after the transplant. On light microscopy, the findings of this repetitive sampling of a small area of the septum vary with the interval between biopsies.

Opportunistic infections. It is well-known that chronic immunosuppressive drug therapy predisposes transplant recipients to a large number of opportunistic infections including viruses, bacteria, fungi, and protozoa. This is covered in detail in Chapters 92,

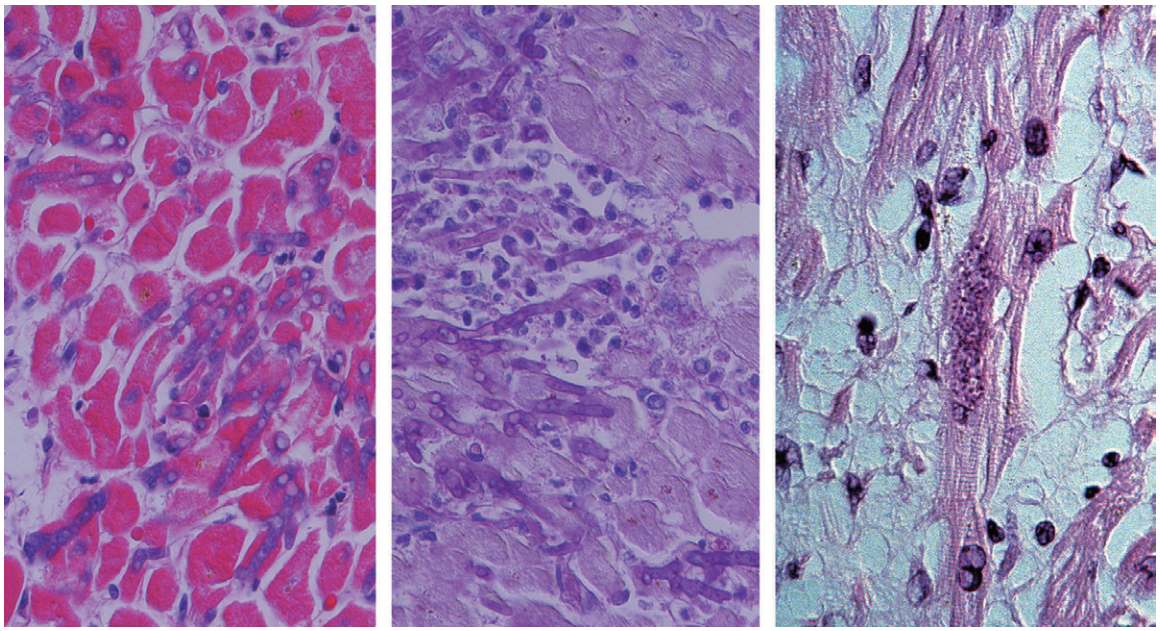


Figure 83.10. Agents in endomyocardial biopsy. (A,B) *Aspergillus* species hyphae growing in the myocardium (A, H&E, original magnification ×400; B, PAS stain, original magnification ×400). (C) In this biopsy there is a myocyte showing a sarcoplasmic cyst filled with bradyzoites of *Toxoplasma* (H&E, original magnification ×800).

93, and 94. Bacterial infection is the most common type of infection [55]. Viral infections are second in frequency, with fungal and protozoal pathogens being responsible for the rest [56,57]. The two most commonly reported opportunistic infections seen in EMB specimens are *Toxoplasma* and cytomegalovirus [58–60]. Other organisms reported to occur in heart transplant patients include the following:

Viruses: hepatitis B [61], hepatitis C [62], enteroviruses, parvovirus B19 [63], Epstein–Barr virus [63] and other herpes viruses, adenovirus [63].

Bacteria and mycobacteria [64–66].

Fungi: Aspergillus [67,68], *Candida* [68], *Nocardia* [55], *Mucor* [69] (Figure 83.10A,B).

Protozoa: Pneumocystis [70], *Toxoplasma* (Figure 83.10C) [71], *Leishmania* [72] reactivation of Chagas' disease [73].

In the pediatric heart transplant population, the pathogens reported include viruses (cytomegalovirus, herpes simplex, varicella-zoster, respiratory viruses, and Epstein–Barr virus), bacteria (mycobacteria, Gram-positive and Gram-negative), toxoplasmosis, and pneumocystis [68,74,75].

It should be noted that the identification of micro-organisms in endomyocardial biopsy is difficult and should never be the only method to rule out infection. When examining a biopsy, always look for hints of infection such as unusual inflammatory infiltrates. Unusual in this context may be the presence of granulocytes, plasma cells, and/or macrophages in a focus of inflammation without overt myocyte necrosis or dropout. Areas like these may harbor intracellular parasites (such as *Toxoplasma*) or fungal organisms, which can be elusive on hematoxylin and eosin stain. Special stains for micro-organisms should then be performed as needed. The pathologist should also look for viral inclusions in the nuclei of endothelial cells, smooth muscle cells, or miscellaneous perivascular cells. Viral inclusions visible by light microscopy within the cardiac myocytes are quite rare.

Adipose tissue: perforation and infiltration. Adipocytes are normal cellular components of the heart mostly present in the epicardium. Microscopic foci of adipose tissue are usually present in the subendocardium and within the myocardium. These foci can be seen in all chambers but are more commonly found in the right ventricular wall in obese patients and patients taking steroids. Thus, the pathologist should bear in mind that finding adipose tissue per se is not pathologic. An attempt should be made to define whether the adipose tissue seen in an endomyocardial biopsy is part of the ventricular wall because it may also come from the epicardium (Figure 83.11). Although the aim of a right ventricular biopsy procedure is to obtain samples from the right side of the interventricular septum, the bioptome may actually sample the right ventricular free wall. Therefore, when a focus of adipose tissue is found in an endomyocardial biopsy, the pathologist should make an effort to determine if this is subendocardial or subepicardial adipose tissue. This can sometimes be easily determined by looking for the presence of mesothelial cell lining, which indicates the surface is actually epicardium (Figure 83.11B). The presence of ganglion cells or nerves is not indicative of perforation. However, because of the fibrinous and eventually fibrous pericarditis that usually develops after the transplant, it may be difficult to find mesothelial lining. In the latter case, the presence of nerves and ganglion cells may be suggestive of epicardial location, as these types of peripheral nerve tissue are common in the epicardium. However, a few weeks after the transplant, the organized pericarditis usually forms a dense protective layer around the myocardium that prevents the development of tamponade if there is perforation. The presence of adipose tissue in endomyocardial biopsies has been reported [76]

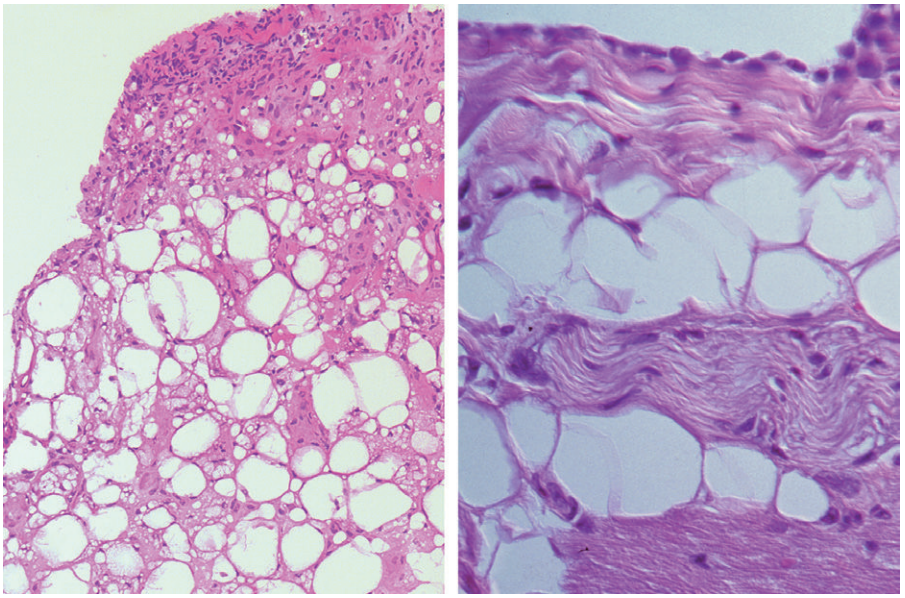


Figure 83.11. Adipose tissue in endomyocardial biopsy. (A) Endomyocardial biopsy showing abundant adipose tissue with macrophages (H&E, original magnification ×100). (B) Cuboidal mesothelial cells are present (top) as well as adipose tissue and nerve fibers in this biopsy. These findings distinctly indicate perforation of the myocardial wall and sampling of epicardial structures such as mesothelial cells and epicardial nerve fibers (H&E, original magnification ×400).

and classified [77] in non-transplant patients. The presence of adipose tissue has been reported to occur in 4.62% of transplant biopsies in one study [77]. In transplant biopsies, there is also some tendency to see fat deposits in areas of previous biopsy site or perhaps foci of healing ischemic damage. Whether the use of steroids for the treatment of rejection increases the amount of adipose tissue in the endocardium is not known.

Other artifacts: contraction bands. This is a very common artifact seen in transplant and non-transplant heart biopsies. Several factors may influence the presence of contraction bands in the biopsy (Figure 83.12A). It may be the result of trauma to the myocardium induced when the bioptome cuts the tissue. It may also be induced by poor osmolarity of the medium in which the biopsy is placed before and during fixation, as well as the cold temperature of the medium of fixative (i.e. 4°C or 22°C). Because of the high likelihood of finding contraction bands, they alone should never be the only criterion used to make a diagnosis of myocyte necrosis or ischemic injury.

Pinching or forceps artifact. This represents mechanical distortion of the tissue due to manipulation (Figure 83.12B). The bioptome itself can induce this artifact, specifically if its cutters are not very sharp (in the case of reusable bioptomes). It can also be induced during processing of the tissue in the pathology laboratory. An effort should be made to handle it with care because this artifactual deformation may render the specimen uninterpretable.

Foreign bodies. Occasional foreign bodies introduced at the time of the transplant can be seen, such as gel foam. It is also possible that the bioptome may actually sample fragments of indwelling catheters or the soft plastic cover of pacemaker leads.

Pseudohemorrhage. During the biopsy procedure,the spoon-shaped cutters of the bioptome can trap red blood cells and, at the time of biopsy, embedd these cells into the myocardium by the pressure of the bioptome on the tissue (Figure 83.12C). This produces artifactual pools that mimic hemorrhage. They are usually not accompanied by inflammatory cells or pathologic changes in the myocytes, thus making the distinction between artifact and rejection fairly easy.

"Telescoping" or intussusception of small arteries. This occurs when a small muscular artery is sampled by the bioptome. Just before the bioptome cutters actually cut the tissue, the small artery is stretched and, as soon as it is cut, recoils into its own lumen. This can give the appearance of an occluded vessel or a small artery with vasculopathy. However, the elastic lamina if present in small arteries is usually determinant in showing that there is an intussusception of the artery, thus making the distinction from vasculopathy rather obvious. It is uncommon to see this artifact occur in arterioles (Figure 83.12D).

Dystrophic calcification. There have been reports of various forms of calcification in the heart after transplantation (Figure 83.12E). In some patients, radiographic evidence of calcification has been shown in the native atria [78,79] and in biopsy tissue [78]. In our experience, it is also uncommon to see dystrophic calcification of the ventricular myocardium in biopsies (Figure 83.7). In most other reports, the myocytes and specifically the mitochondria show calcification. On light microscopy, the dystrophic calcification of the mitochondria is easily recognized as dark blue granular material within the myocytes. These granules are 1–2.5 μm in diameter. The granules may be seen in perinuclear location and also following the pattern of the sarcomeres. When abundant, they follow the contour

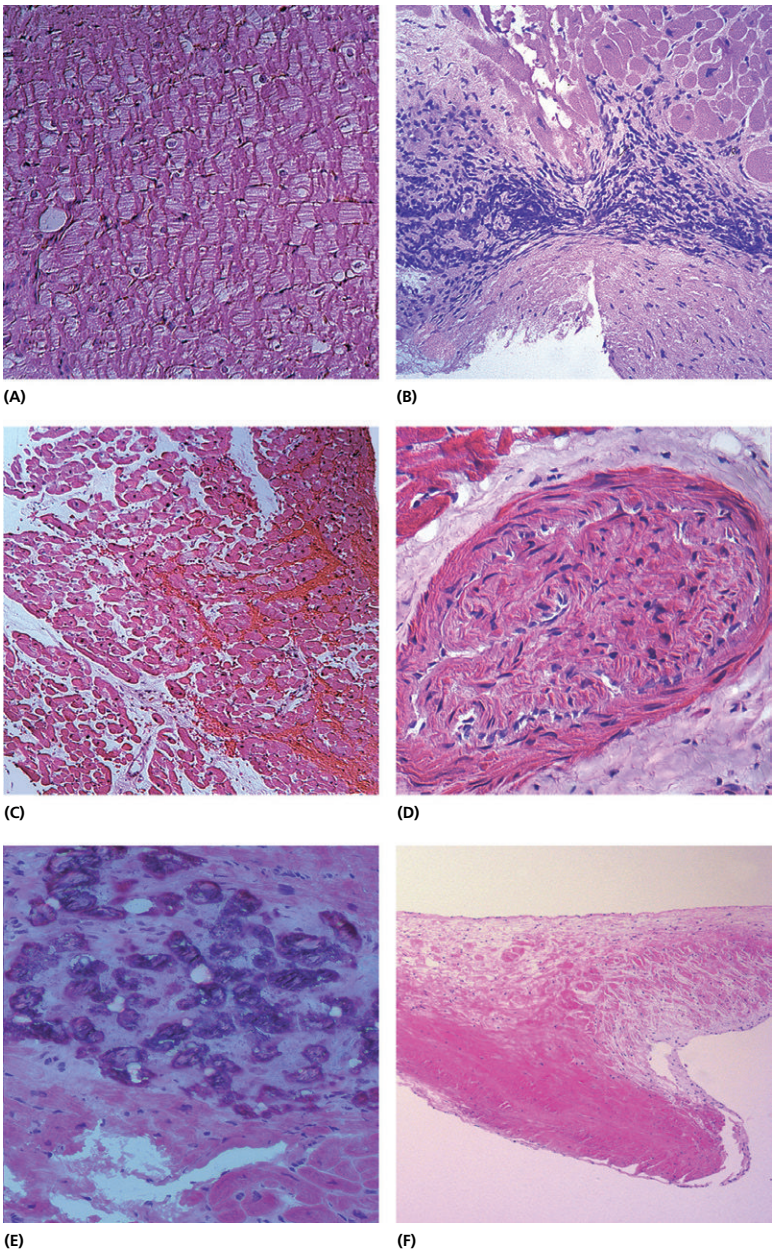


Figure 83.12. Miscellaneous artifacts in endomyocardial biopsies. (A) Contraction band artifact is relatively common in endomyocardial biopsies (H&E, original magnification ×100). (B) Pinching artifact with distortion of the tissue can occur when the bioptome does not cut the tissue, but instead compresses it. This image shows extreme distortion of a Quilty type lesion in the endocardium. Creating crushing artifact of the lymphocytes in the Quilty lesion (H&E, original magnification ×200). (C) Pseudohemorrhage. The cutters of the bioptome can trap blood and embed these into the myocardium, creating the false impression that there is a hemorrhagic infiltrate (H&E, original magnification ×100). (D) Occasionally, arterioles and small arteries can be sampled by the bioptome. In some instances, these arteries can be overstretched as the biopsy piece is cut and they recoil into their own lumen forming an intussusception or "telescoping" artifact. This should not be misinterpreted as allograft vasculopathy (H&E, original magnification ×400). (E) Dystrophic calcification of entire myocytes is also possible (H&E, original magnification ×300). (F) Chordae tendineae tissue can be cut by the bioptome, as many of the chordae tendineae from the septal leaflet of the tricuspid valve insert directly into the interventricular septum (H&E, original magnification ×20).

of the whole myocyte. In some cases, a relationship between calcification of the mitochondria and cyclosporine therapy has been suggested [80]. Some conditions that have been associated with dystrophic calcification are sepsis, temporary uremia, hypomagnesemia, steroid therapy, alcoholism [78], and ischemic injury.

Chordae tendineae and valvular tissue. Fragments of chordae tendineae (Figure 83.12F) are occasionally seen as part of the biopsy specimen [32]. They should be described and processed along with the biopsy. The clinical significance with regard to tricuspid dysfunction secondary to chordal rupture is varied [12,81].

Biopsy-negative allograft dysfunction. The concept of biopsy-negative rejection or biopsy-negative allograft dysfunction has appeared in the heart transplant literature in recent years. It refers to cases in which there is nil or no cellular rejection in a biopsy to explain dysfunction of the allograft. Thus, suggesting that in those instances the dysfunction may be secondary to AMR [82]. However, in those centers that routinely evaluate biopsies for cellular and AMR and rule out these two entities [83], the concept of true biopsy-negative allograft dysfunction usually indicates that cardiac allograft vasculopathy may be the process responsible for the dysfunction and not cellular or AMR.

Antibody-mediated rejection (humoral arm of the rejection immune response)

AMR is an immunopathologic process in which donor-specific antibodies bind to antigen within the graft, leading to activation of the complement system and, in turn, result in injury to the graft (see Chapter 6). This type of rejection was first recognized as a distinct clinicopathologic entity in kidney transplant patients as an acute

allograft rejection associated with the production of anti-donor reactive antibodies and poor prognosis [84]. AMR is poorly responsive to conventional immunosuppression, which targets the cellular arm of the immune response. It has been postulated that the allorecognition and production of antibody may occur in any transplant and can span from subclinical to fully overt clinical AMR [85]. Old terminology in heart transplant literature such as vascular rejection, microvascular rejection, and humoral rejection should be avoided, as it has only led to confusion in the literature. The preferred terminology is AMR. For many years, it was said that AMR occur early post-transplant (i.e. in the first 3 months) and the ISHLT-WF-1990 recommends AMR monitoring by immunofluorescence on all biopsies only up to 6 weeks post-transplant. This is clearly incorrect, as it is now known that AMR can and most commonly occurs months and even years after transplantation [83,86,87].

Risk factors for developing AMR include pregnancy, previous transplantation, blood transfusions, sensitization by OKT3 induction therapy, use of ventricular assist devices, use of homografts in infants, presence of positive B-cell flow cytometry cross-match, and elevated panel-reactive antibodies [11,88,89]. The long-term outcome of AMR is not yet fully established in heart transplantation but it has been suggested that an association exists with the development of cardiac allograft vasculopathy (CAV) and decreased survival [90,91].

The ISHLT-WF-1990 did not provide a detailed pathologic classification of "humoral rejection" in biopsies (Table 83.3). Consequently, the true incidence of AMR was unknown and recognition of AMR as a real entity was not widely accepted. For more than a decade, there was no uniform set of diagnostic criteria provided to guide different transplant programs in the detection of this entity until the Banff Allograft Pathology group stressed the need for defined criteria for AMR [13]. In turn, the ISHLT-WF-2005 defined an initial set of standards to guide heart transplant centers around the world on how to evaluate endomyocardial biopsies for AMR (Table 83.3). This set included histologic and immunopathologic features. It suggested that the histologic features of AMR (see below under Diagnostic criteria of AMR) should be identified in biopsies and this should prompt the use of immunopathologic examination [11,14]. However, soon after the ISHLT-WF-2005 was published, there was evidence that the histologic criteria had rather low sensitivity and specificity for AMR [92].

The antibodies used in immunofluorescence evaluation for the diagnosis of AMR were varied and included antibodies to detect IgG, IgM, C3, C1q, fibrinogen, fibrin, and HLA-DR. But the presence of these antibodies did not always correlate with hemodynamic compromise or incidence of CAV and thus resulted in decreased usefulness of this test [93]. Furthermore, a recent survey of North American centers regarding their diagnostic approach to AMR showed a chaotic situation with many more immunopathologic markers that have not been evaluated in large cohorts for sensitivity or specificity [94]. Evidence from large heart transplant centers in North America shows that while many markers such as immunoglobulins and some complement components (C3, C3c, C1q) may be detected in biopsies, the specificity and sensitivity of the immunoglobulins, and some complement components, are low as diagnostic [86] and prognostic markers of AMR [95,96] .

Diagnostic criteria of AMR. The histologic features that allow for the identification of this type of rejection on endomyocardial biopsies as defined in the ISHLT-WF-2005 and its companion article on AMR [11,14,32] include capillary injury with endothelial cell swelling and intravascular macrophage accumulation. Interstitial edema and hemorrhage can be present together with neutrophils in and around capillaries. Intravascular thrombi and myocyte necrosis without cellular infiltration can also be identified [11,14].

However, the sensitivity of histologic criteria (i.e. light microscopic features such as endothelial cell swelling, intravascular macrophages) is too low to serve as screening parameters for AMR [92]. The authors thus recommend the addition of immunostaining to screen for the presence of AMR.

Immunohistochemical staining of endomyocardial biopsies recommended in ISHLT-AMR-WF-2011 [15]*:* For immunohistochemistry (IHC) on paraffin-embedded tissue, the latest working formulation recommends C4d or CD68 (or other appropriate macrophage marker), alone or combined. A concern is the lack of experience by pathologists in the interpretation of CD68 in the context of AMR. The potential for misinterpretation is based on the fact that only intravascular macrophages should be interpreted as indicative of AMR. The interstitial macrophages should not be interpreted as indicative of AMR.

Use of C4d and C3d immunofluorescence has shown excellent correlation with AMR [83] in large transplant centers (Figure 83.13). The use of these two markers in immunohistochemistry has not been extensively evaluated in many high-volume centers [15].

The ISHLT-AMR-WF-2011 states that initial immunostaining should be avoided in the first 2 weeks because of theoretical perioperative considerations such as the presence of necrotic myocytes

Table 83.3. Antibody-mediated rejection (AMR) (data from [10,11,15])

ISHLT-WF-1990	ISHLT-WF-2005	ISHLT-WF-2011
Humoral rejection Positive immunofluorescence, vasculitis or severe edema in the absence of cellular infiltrate	**Antibody-mediated rejection** AMR 0 = Negative for acute antibody-mediated rejection AMR 1 = Positive for AMR Histologic features of AMR Positive immunofluorescence or immunoperoxidase staining for AMR (Positive CD68, C4d)	**Antibody-mediated rejection** pAMR 0 = Negative for pathologic AMR: both histologic and immunopathologic studies are negative pAMR 1 (H+) = Histopathologic AMR alone: histopathologic findings present and immunopathologic findings absent pAMR 1 (I+) = Immunopathologic AMR alone: Immunopathologic findings present and histologic findings absent pAMR 2 = Pathologic AMR: both histologic and immunopathologic findings present pAMR 3 = Severe pathologic AMR: Rare cases of severe AMR with histopathologic findings of interstitial hemorrhage, capillary fragmentation, mixed inflammation, endothelial cells pyknosis, karyorrhexis, marked edema

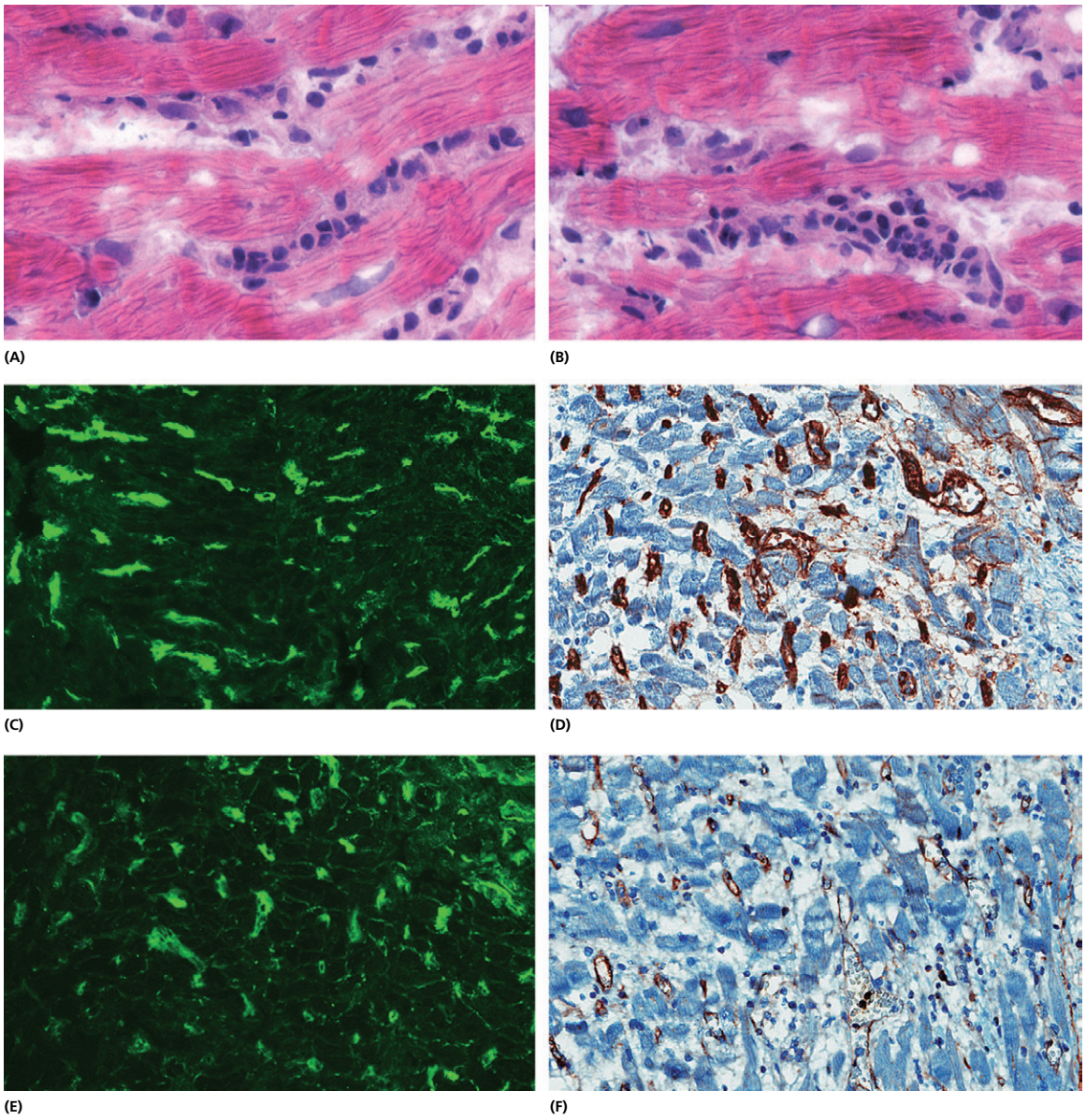


Figure 83.13. Antibody-mediated rejection. (A) This biopsy shows endothelial cell swelling and occasional intravascular macrophages (H&E, original magnification ×600). (B) In this field, a small arteriole distinctly shows intravascular macrophages (H&E, original magnification ×600). (C) Immunofluorescence for C4d shows an intense capillary pattern deposition of this complement split product in this frozen endomyocardial biopsy tissue (C4d immunofluorescence, original magnification ×300). (D) The same complement split product is present in a biopsy fixed in formalin. The pattern of positive deposition of C4d is identical (linear capillary staining) to the pattern seen on immunofluorescence (immunohistochemistry for C4d, original magnification ×300). (E,F) The same linear pattern of capillary staining is now demonstrating the presence of C3d in these two biopsies. (E) Immunofluorescence for C3d (original magnification ×300). (F) Immunohistochemistry for C3d (original magnification ×300).

binding complement early in the post-transplant period and ischemic injury [97] as potentially responsible for activating complement. However, the pattern of staining of capillaries in AMR is distinctly different from the sarcoplasmic staining of necrotic myocytes. This should not be a reason for not performing immunofluorescence during this period, particularly in highly sensitized patients.

The grading system for the latest iteration of the Working Formulation pAMR 0. Negative for pathologic AMR: both histologic and immunopathologic studies are negative.

pAMR 1 (H+). Histopathologic AMR alone: histopathologic findings present and immunopathologic findings absent.

pAMR 1 (I+). Immunopathologic AMR alone: immunopathologic findings present and histologic findings absent.

pAMR 2. Pathologic AMR: both histologic and immunopathologic findings present.

pAMR 3. Severe pathologic AMR: rare cases of severe AMR with histopathologic findings of interstitial hemorrhage, capillary fragmentation, mixed inflammation, endothelial cells pyknosis, karyorrhexis, marked edema.

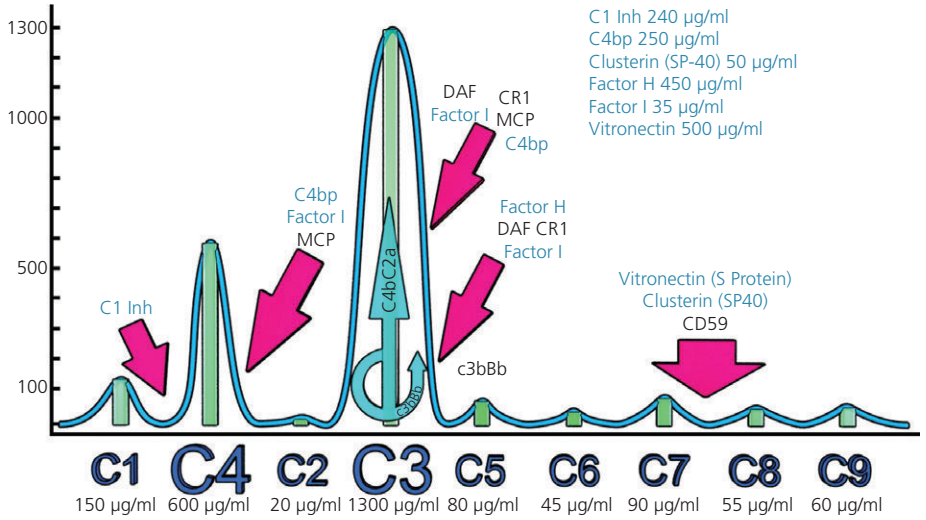


Figure 83.14. Serum concentration of complement components and regulators of complement activation. The serum concentration of the different complement factors in serum is shown in micrograms per milliliter. The activation of C3 is critical as it augments both cellular and humoral immune response. C3 is enzymatically cleaved and activated by C4b2a of the classic pathway and C3bBb through an amplification loop of the alternative pathway. Its activation is an important amplification step because C3 is present in a larger molar amount and, once activated, it can further increase the activation of the rest of the cascade. Regulators of complement activation are composed of both plasma (blue letters) and membrane (black letters) proteins that inhibit the proteolytic subunits of classic and alternative pathways, thereby preventing the progression of the complement pathway to the membrane attack complex formation. C1 Inh, C1 inhibitor; C4bp, C4 binding protein; CR1, complement receptor 1; DAF, decay accelerating factor; MCP, membrane co-factor protein.

While the ISHLT-WF-2005 recommended that these patients undergo assessment for circulating antibodies to HLA class I or II as well as non-HLA donor antigens, the ISHLT-WF-AMR-2011 does not require correlation of the histologic or immunopathologic findings with the presence of donor-specific antibodies or allograft dysfunction. An EMB with no histologic or immunopathologic evidence of AMR is graded 0 (pAMR 0). If the immunofluorescence or immunohistochemical staining supports the histologic features of AMR, the biopsy is considered positive (pAMR 2). The presence of either histologic or immunopathologic findings only is considered pAMR 1 with a qualifier of H+ or I+. Note that occurrence of severe pathologic AMR or pAMR3 is very rare in current practice. The precise clinical correlation for each of these pathologic grades will continue to be established.

Mixed acute cellular rejection and AMR. Although most AMR episodes are associated with absent or at most mild acute cellular rejection, mixed rejections have also been reported to carry a significant risk of mortality [98,99]. Mixed rejection may occur early or late [100] in the course of transplantation and is also associated with allograft dysfunction.

Practical issues in the diagnosis of AMR. Some diagnostic considerations about complement split products are germane. Immunofluorescence methods for detection of AMR in tissues have evolved in the last decade. Some complement components, specifically C3d and C4d, are found to be more readily detected than antibodies and serve as very sensitive markers of rejection in endomyocardial biopsies for several reasons [101]. Antibodies bind to antigens with different avidity and either dissociate at varying rates or are eliminated by shedding or internalization. In contrast, the process of complement activation yields split products of C4 and C3 that bind to the tissue where complement was activated. This increases the sensitivity of complement detection by prolonging their half-lives. Among the components of the complement system, C3, followed by C4, are present in the highest concentration in serum, therefore their split products are also deposited in tissues in the largest quantities (Figure 83.14) [32]. Furthermore, the amplification steps in the complement cascade results in the generation of more C3 split products [102].

While complement is activated through antibody in the classic pathway, one must remember that complement can also be activated during procedures such as extracorporeal circulation during surgery [103,104], by ischemia–reperfusion injury, and by induction therapy before transplant with antithymocyte globulin [97]. Thus, the mere presence of C4d alone or C3d alone in capillaries should not be equated with AMR.

In our experience, the use of C4d immunostaining alone is not a reliable tool. Instead, evaluation of endomyocardial biopsies for AMR should include staining for both C4d and C3d [83,86], because the presence of both markers correlates highly with allograft dysfunction and the presence of elevated donor-specific antibodies.

Discrepancy between pathology and clinical presentation. Activation of the complement cascade detected by immunostains for C4d and/or C3d is not always accompanied by dysfunction of the graft. Some authors have referred to this apparent lack of graft injury despite evidence of complement activation as "accommodation" in animal models [105] and in ABO-incompatible renal transplants [106]. One possible explanation is that complement activation is interrupted by a protective mechanism in the host. This suggests that unless the complement cascade proceeds to the formation of the membrane attack complex (MAC), there is no expected injury to the allograft. This complex is needed to form a "pore" that leads to loss of integrity of the cell membrane. In humans, it is well known

that there are regulators of complement activation (RCA) that can prevent the completion of the complement cascade at different stages of activation.

Regulators of complement activation. RCA exert their effects at different points in the complement activation cascade, whether the activation occurs through the classic, alternate, or mannose-binding lectin pathways. All these pathways converge at the point of generation of the enzymatic complexes known as the C3 convertases which, in turn, proceed to activate the remaining complement components required for the formation of the MAC. There are two main types of proteins that can regulate the activation of complement: membrane-bound and soluble types. In humans, the membrane-bound regulators are CD35 or complement receptor 1 (CR1), CD46 or membrane co-factor protein (MCP), CD55 or decay-accelerating factor (DAF), CD59 or protectin, and C8-binding protein or homologous restriction factor (C8bp/HRF) [107,108]. The soluble factors include the C1 inhibitor, C4 binding protein (C4bp), factor I, factor H, clusterin, and S-protein (vitronectin). Their points of action are shown in Figure 83.14 [32].

There is little information about the expression of these RCA molecules in human heart transplantation. A recent study shows that DAF or CD55 is expressed locally in the myocardium in heart transplant patients [109]. In this study, a group of patients with complement deposition in endomyocardial biopsies was studied. The biopsies were stained by immunofluorescence for C4d, C3d, and DAF. There were two subgroups identified on the basis of allograft dysfunction present or absent. All patients had biopsy-proven C4d and C3d deposits. Patients with good response to therapy and resolution of the AMR episode showed intense tissue expression of CD55 in the endothelium of the allograft. Patients with poor outcome had low or absent tissue expression of CD55. Thus, the local expression of DAF correlates with absence of allograft dysfunction in spite of C4d and C3d deposition in capillaries (Figure 83.15).

Complement staining artifacts. Common artifactual staining seen in immunofluorescence microscopy of transplant biopsy includes autofluorescent lipofuscin deposits and non-specific binding to collagen in the interstitium and to the internal elastic lamina of arteries. Necrotic myocytes likewise bind complement.

Platelets and coagulation factors in rejection. The combination of antibodies and complement with inflammatory cells has great potential to injure the endothelial and smooth muscle cells of the vessels of the allograft [110]. It is known that stimulation through receptors for IgG or complement split products can activate macrophages, but stimulation through combinations of these receptors generates synergistic results. Thus, the effect of antibodies and complement efficiently integrate the activation of endothelial cells, platelets, and macrophages [110].

Alloantibodies can induce exocytosis of von Willebrand factor and P-selectin from endothelial cells and attachment of platelets within minutes. Consequently, platelets also adhere to and stimulate leukocytes, which is potentiated by complement activation. After attachment, platelets degranulate and release preformed mediators stored in their granules such as platelet factor 4 and CXCL4 [111]. These effects may also have a role in the pathogenesis of CAV (see section on CAV below).

Thrombolytic and coagulation factors have also been implicated in allograft rejection and CAV. The notion that a hypercoagulable state may have a role in CAV has been supported by the finding that early elevation of tissue factor and fibrin and reduction of tissue plasminogen activator (tPA) and antithrombin in human cardiac allografts are associated with both onset and severity of CAV and graft failure. As expected, impairment of fibrinolysis has also been correlated with CAV [112].

Cardiac allograft vasculopathy

The most difficult obstacle to long-term successful outcome in cardiac transplantation is the development of graft vasculopathy [32]. The clinical manifestations of CAV are covered in detail in Chapter 79. This problem is not unique to the heart and occurs in any solid organ graft to some degree [113]. Allograft vasculopathy

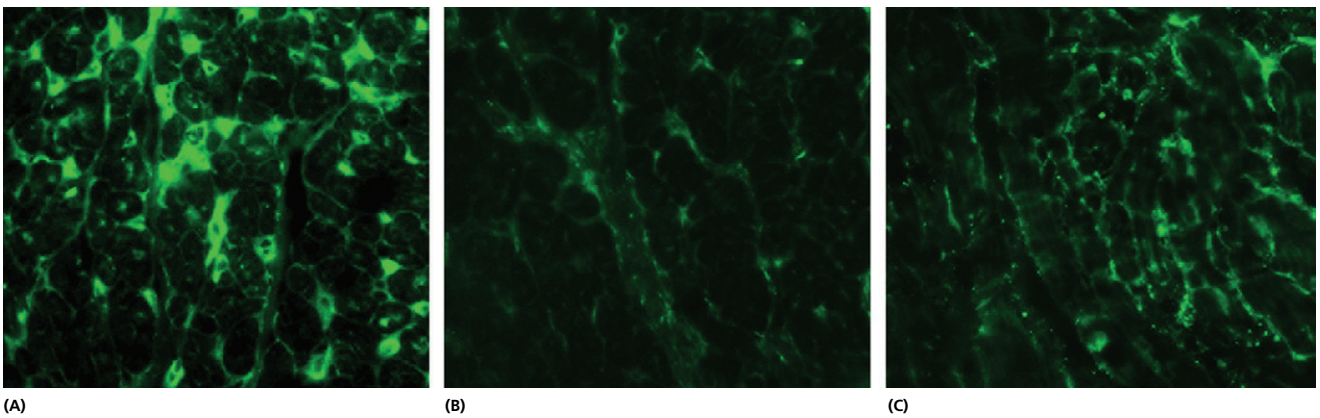


Figure 83.15. C4d positivity alone is not antibody-mediated rejection. (A) This biopsy shows distinct intense linear immunofluorescence for C4d in capillaries (immunofluorescence for C4d, original magnification ×400). (B) Staining for C3d is negative, showing no staining of capillaries (immunofluorescence for C3d, original magnification ×400). (C) Decay accelerating factor (CD55) is present in the capillaries and myocytes of this micrograph (immunofluorescence for CD55, original magnification ×400). The presence of CD55 is interpreted as the mechanism preventing progression of activation of the complement cascade from activation of C4 into activation of C3, and consequently no activation of the membrane attack complex.

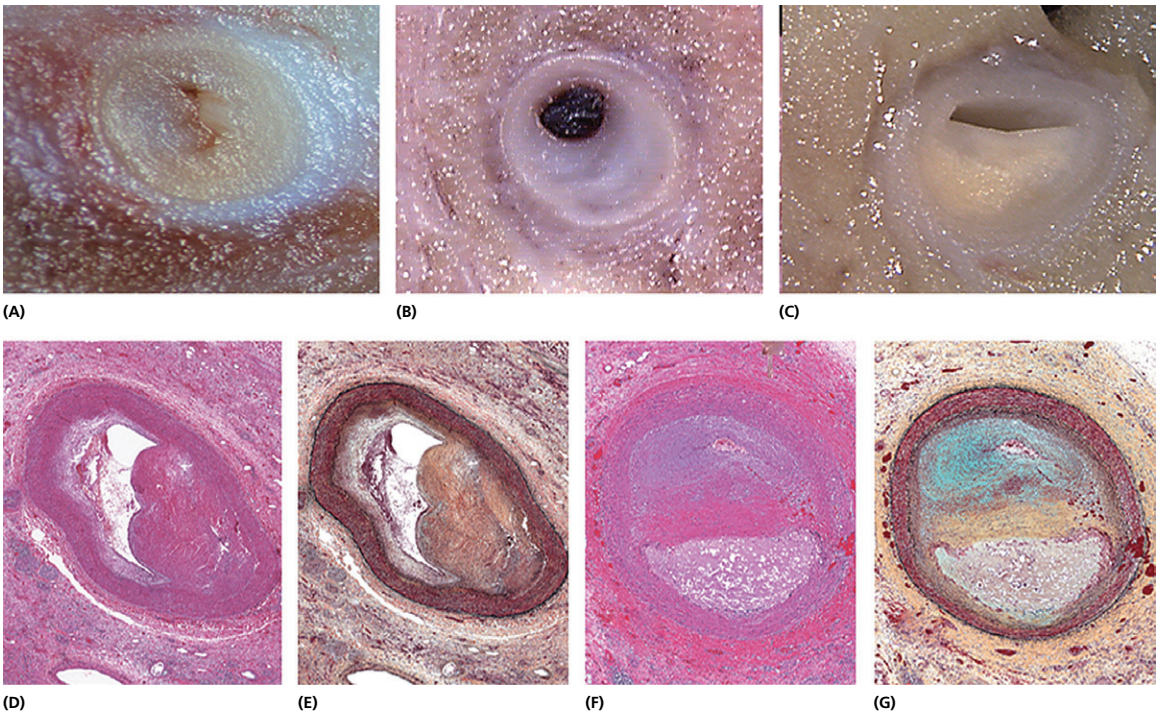


Figure 83.16. Cardiac allograft vasculopathy (CAV) I. (A) Epicardial coronary artery showing the "typical" concentric narrowing of the lumen by proliferative intima. (B) Eccentric lesions are also commonly seen. Note the pearl-white myxoid matrix of the intima. (C) If there is coronary atherosclerosis in the donor, the CAV lesions (pearl-white) are commonly superimposed upon the atheromatous plaque (yellow). (D) Microscopic examination of an eccentric plaque showing the proliferative intima in the right half of the vessel (H&E, original magnification ×10). (E) The proliferative intima in this case is formed of mature fibrous tissue (yellow). The internal elastic lamina (black) is intact, and the media of the vessel is also intact (dark red) (Movat pentachrome, original magnification ×10). (F) This light micrograph shows an almost complete occlusion of the lumen by a combination of atheromatous plaque (lower half of the image) and superimposed CAV (H&E, original magnification ×10). (G) The same artery stained with a Movat pentachrome shows the atheroma, the fibrous cap (yellow), and the proliferative neointima of the CAV process as a proteoglycan-rich extracellular matrix (green) (Movat pentachrome, original magnification ×10).

(also called chronic rejection, accelerated graft arteriosclerosis, or transplant coronary artery disease) develops, over time, in practically all transplanted hearts. In some patients it develops in a few months, while in others it develops after several years. The events leading to this vascular process are complex and include at least some of the following: native donor atherosclerosis [114], ischemic time of the graft [115], endothelial damage of diverse origins [116,117], histocompatibility issues [118], arteritis [119], chronic humoral and cellular immune attack [120–123], fibrinolysis [124,125], viral infections [126], serum lipids [127], and hormonal milieu [128]. The problem of vasculopathy is also seen in the pediatric population and follows a course similar to the one seen in adults [129,130].

Vasculopathy involves both epicardial and intramural coronary arteries [32]. To understand the pathology, one should remember that the coronary vessels have three layers: the adventitia, the media, and the intima. In turn, each one of these anatomic layers is made of many different cellular and extracellular components. There is evidence that during the development of vasculopathy a concerted series of events occurs that produce varying degrees of damage to each one of these structures (intima, media, adventitia and vasa vasorum).

On gross examination, rejection may be evident as areas of myocardial discoloration ranging from pale tan to red. Examination of the epicardial coronary arteries may reveal a combination of focal eccentric lesions and, in other areas, uniform concentric thickening of the wall (Figure 83.16). However, in some instances, while there may be little or no gross evidence of vasculopathy in these epicardial coronary arteries, there is abundant pathology in the intramyocardial vessels. Careful examination of the cut surfaces of the ventricles often reveals thickened arteries (with a diameter range of 0.2–0.5 mm) with abundant perivascular fibrosis. These vessels are quite prominent on gross examination (Figure 83.16A–C). Some allografts may show clear evidence of epicardial coronary atherosclerosis that was present in the donor prior to the transplant (Figure 83.16D–G) [32,114,131]. In these cases, the allograft vasculopathy is superimposed over the atherosclerosis. If pre-existing atherosclerotic plaques are present, the morphology of the lesion may be eccentric with more features of atheroma such as destruction of the internal elastic lamina, amorphous lipid-rich plaque, and cholesterol clefts. Long-term lesions of the epicardial coronaries may eventually look like conventional atherosclerosis and be indistinguishable from it.

The light microscopic morphology of the lesions seen as a result of vasculopathy is slightly different in epicardial from in medium or small arteries [32,132]. If no atherosclerotic plaques are present, the typical vasculopathy lesions show concentric intimal proliferation (Figure 83.17) composed of smooth muscle cells and less differentiated cells (myofibroblasts or "myointimal" cells). There is deposition of connective tissue components such as collagen and

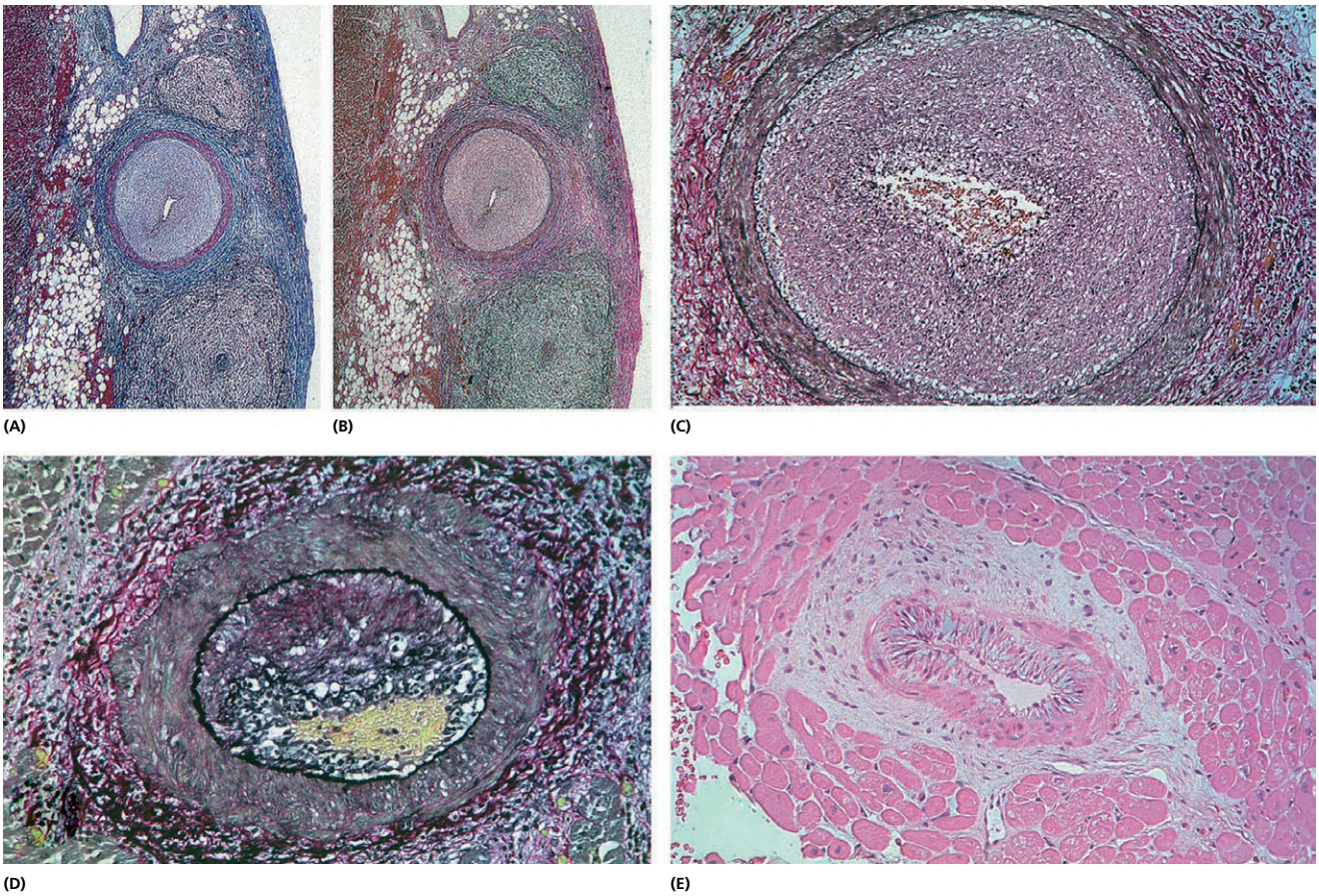


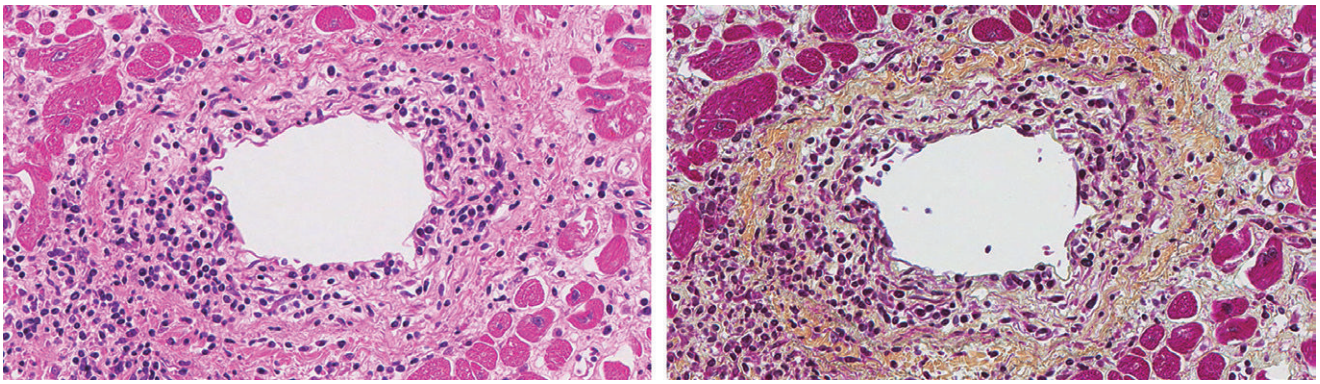
Figure 83.17. Cardiac allograft vasculopathy II. (A) Light micrograph showing an epicardial coronary artery with marked narrowing of its lumen by intimal proliferation of cells and deposition of connective tissue (red–blue). The adventitia is fibrotic and stains blue. The smooth muscle of the media and the myocardium (left) are stained red. There are two large foci of lymphocytic infiltrate flanking the coronary artery (Masson's trichrome, original magnification ×1.25). (B) An adjacent section shows the delimitation of the media and the intima with more clarity (darker red media and light pink–red intima) (Verhoeff–Van Gieson elastic stain, original magnification ×1.25). (C) Higher magnification showing the intima, media, and adventitia. The intima shows marked proliferation of cells which almost completely narrows its lumen. Note that the elastic laminae are intact (Verhoeff–Van Gieson elastic stain, original magnification ×4). (D) A smaller intracardiac coronary artery shows an eccentric lesion in the intima that partially occludes its lumen (>60%). There is a small thrombus with entrapped erythrocytes (yellow cells) in the lower part of the arterial lumen (Verhoeff–Van Gieson elastic stain, original magnification ×10). (E) Small intracardiac coronary artery shows early proliferative disease in the intima and abundant proteoglycan accumulation (light blue discoloration subjacent to the endothelial cells). Note the abundant fibrosis of the adventitia (H&E, original magnification ×20).

proteoglycans. The intramural coronary arteries usually show concentric uniform lesions consisting of intimal smooth muscle proliferation with or without lipoprotein deposition within the proliferating cells. The internal elastic lamina is usually intact or only focally disrupted, the media shows little to no lipoprotein deposition and there usually is an adventitial cuff of fibrous tissue with or without mononuclear inflammatory infiltrates. The pathology of CAV in children is practically identical [133].

In some patients, frank vasculitis has been documented (Figure 83.18) [120]. This usually affects the distal coronaries. Active vasculitis shows lymphocytes, plasma cells, and occasionally polymorphonuclear leukocytes. Fibrinoid necrosis may be seen in early stages. Thrombosed and recanalized vessels may represent healed vasculitis with thrombosis (Figure 83.19). Although small arteries or arterioles with vasculopathy may be seen in endomyocardial biopsies, there is no consistent correlation with the anatomic or functional status of epicardial coronary arteries [134]. Despite this lack of correlation, the presence of vasculopathy in the biopsy specimen, if any, should be recorded in the final report.

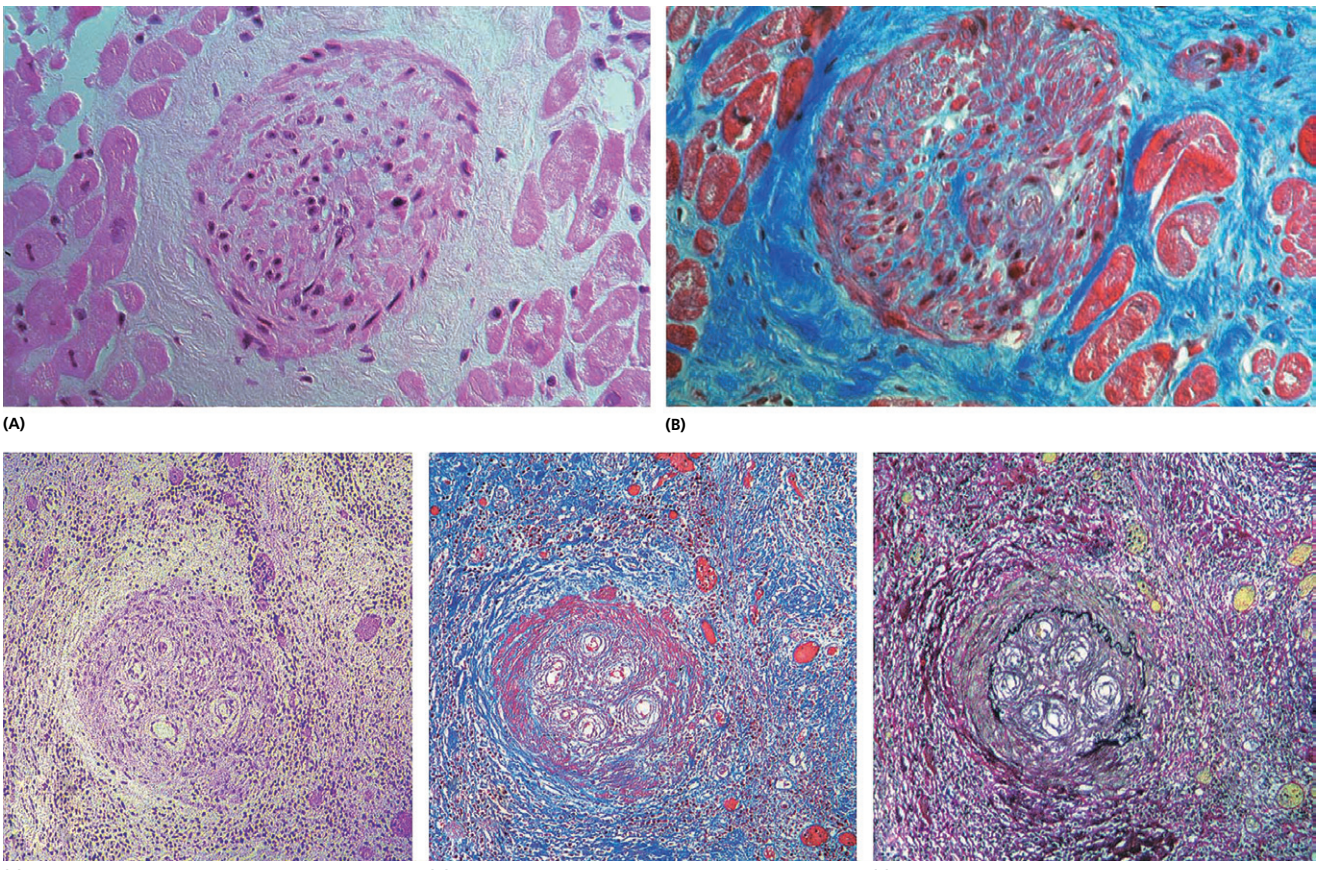
Recurrent diseases in the allograft and the patient

A number of cardiac conditions have been shown to recur in the transplanted heart, including infections [73] and amyloidosis [135]. These are discussed further in Chapter 79. Depending on the cause of iron overload leading to heart transplantation [136], the iron overload may recur in the allograft [137]. Giant cell myocarditis [138,139] can also rarely recur despite immunosuppression [140], as does sarcoidosis [141,142]. Administration of amiodarone pretransplant has been described to cause QT prolongation in the allograft [143]. Systemic disorders associated with genetic predisposition for recurrence have been reported as in the case of recurrent thrombotic complications in a patient homozygous for



(A) (B)

Figure 83.18. Allograft vasculopathy with frank vasculitis. In some instances, the cellular rejection process distinctly affects all layers of the arteries. (A) Transmural mononuclear infiltrates (occasionally with eosinophils) are noted to involve the adventitia, media, and intima of this intramural coronary artery (H&E, original magnification ×40). (B) The same artery shows that the media has been disrupted by the inflammatory infiltrate. The adventitial fibrosis is highlighted as yellow connective tissue (Movat pentachrome, original magnification ×40).



(A) (B)

(C) (D) (E)

Figure 83.19. Allograft vasculopathy in the intramural coronary arteries. (A) Occlusive proliferation of smooth muscle cells in a small intramural coronary artery. Note the adventitial fibrosis (H&E, original magnification ×40). (B) This stain proves that most of the luminal narrowing is secondary to smooth muscle proliferation (red staining cells) and little accumulation of collagen. The adventitial fibrosis is also well demonstrated (Masson's trichrome, original magnification ×40). (C) Recanalized intramural coronary artery may be secondary to healed vasculitis or thrombosis (H&E, original magnification ×20). (D) The same artery as shown in C shows marked adventitial and intraluminal accumulation of fibrous tissue (Masson's trichrome, original magnification ×20). (E) Staining for elastic fibers shows distinctly a fragmented elastic lamina suggestive of earlier vasculitis (Verhoeff–Van Gieson elastic stain, original magnification ×20).

prothrombin G20210A single nucleotide polymorphism [144] or recurrent aortic dissection after heart transplantation in a patient with Marfan's syndrome [145]. Vascular pathology complicated the postoperative course of a patient with Behçet's disease who developed a large pseudoaneurysm at the aortic anastomotic site [146]. Cardiac transplantation for unresectable primary cardiac sarcomas is controversial because of risks for local recurrence or metastatic disease. For instance, local recurrence of primary cardiac sarcomas has been described in both native right atrium [147] and in the left pulmonary artery [148]. Recurrent Epstein–Barr virus-related leiomyosarcoma has been described in young patients [149].

Examination of the allograft after failure

Gross examination. This type of examination takes place either during retransplantation or during postmortem examination. These two circumstances provide different kinds of specimens. During retransplantation, the specimen will consist of the ventricles and a small portion of atria, aorta, pulmonary artery, and in some instances the previous anastomotic sites. These should be described accordingly. Our current procedure to examine the heart is described below; it assumes that the examination is performed during the autopsy. Depending on the postmortem interval, one should decide whether some tissues may be harvested for cell culture or nucleic acid extraction. Most postmortem specimens yield useful material for DNA extraction; however, RNA is rapidly degraded after death.

The prosector should make every effort to retrieve the specimen without mutilation as there are often dense fibrous adhesions of the pericardium to the heart, the sternum, and mediastinal pleurae. This can be easily achieved with only a few more minutes of careful dissection. The heart and mediastinal structures should be inspected in situ before dissecting and sectioning the following vessels:

1 Superior vena cava at least to the level of the external jugular veins;
2 Branches of the aortic arch to the level of the common carotids and to the level of the clavicle bilaterally;
3 Aorta transected in its thoracic portion as it approaches the diaphragm;
4 Right and left pulmonary arteries to their lobar branches as they enter the lung;
5 Pulmonary veins as they arise from each of the pulmonary lobes; and
6 Inferior vena cava dissected through the diaphragm and sectioned as close to the liver as possible.

If there are any catheters or pacemaker electrodes, each of them can be secured to the respective vessel where they enter the heart (preferably by a small suture) and then cut. In this manner, the prosector will not alter the location of these devices within the heart. As an option, the prosector may choose to tie all the vessels as they are dissected and sectioned. This allows for a very clean work area and guarantees a successful harvesting of the heart in those cases in which the autopsy consent limits the autopsy to the operative incision or to the heart only.

After sectioning the major vessels, the heart can be retrieved. Any vessel tie is removed before fixing the specimen overnight in 10% buffered formalin in a container that is at least 10 volumes larger than the specimen itself (a 12-L bucket is optimal). The dissection continues the following morning. The parietal pericardium will show variable degrees of adhesion to the visceral pericardium (epicardium) (Figure 83.20). Blunt dissection is started from the anterior wall first towards the apex, then moving laterally towards the right and left ventricles and around them into the diaphragmatic surface of the heart. This will free most of the pericardial sac around the ventricles and part of the atria. Subsequently, the dissection moves into the region of the great vessels and into the native atria. Once the anastomotic sites are identified, the dissection of these structures is usually not more difficult than in a normal heart. The approach to open the heart may differ from case to case.

Depending on the expected pathology, it may be needed to open the heart through the traditional "blood flow" approach. Alternatively, a four-chamber view or even transverse sectioning may be indicated (Figure 83.20). In our laboratory, we often use the four-chamber view to section the heart because it allows us to quickly perform a very thorough examination of all the chambers, the valves, the interatrial and interventricular septa, and anastomotic suture lines. Furthermore, using this approach we can easily obtain excellent photographs of the heart for documentation and teaching purposes. In any case, examine all chambers for dilatation and hypertrophy. Note the endocardium for discoloration, thrombosis, vegetations, or fibrin deposits. Locate any cannulas catheters or pacemaker leads. Inspect the anastomotic sites (recent, healed, endothelialized) for kinks and narrowings. Describe the myocardial cut surface for any discoloration (focal, diffuse, size, location), hemorrhage or scars (perivascular, subendocardial, transmural). Record the presence of endocardial scars secondary to previous biopsy sites in the septum or mural thrombosis. The valvular apparatus integrity is checked for evidence of infection, fibrosis, or chordal rupture. The ventricles can then be thoroughly examined and if needed cut in cross-sections. In cases when the clinical history or the examination of the coronary arteries or the cut surface of the ventricles suggest ischemic disease, we prefer to proceed with transverse sections of the ventricles. This may reveal areas of necrosis, hemorrhage, or reperfusion injury. The coronary arteries and any bypass grafts are sectioned in close intervals to look for stenosis, occlusive thrombosis, and calcifications.

Occasionally, the pathologist is confronted with an unusual surgical procedure that requires modification of the dissection technique during the autopsy. For example, to examine the chest in patients with biventricular assist devices requires more extensive dissection in situ and modification of the gross examination of the heart to demonstrate the pathology and allow proper study and preservation of the specimen. In these cases, it is important to take extra time in the examination of the heart in situ. Plan the dissection method for retrieval of the heart and attached devices. Once harvested, the heart can usually be studied very thoroughly. We find it very useful to stage the study of the heart beginning with adequate fixation for a minimum of 18–24 hours after retrieval of the heart. Stages of the dissection are documented with photographs. In some instances, unusual findings will prompt to stop the dissection until photographs of good quality are available. This should not necessarily take a long time because access to a high quality digital photography system is available in most academic centers.

Conclusions

Histopathologic diagnosis is critical for proper management of cardiac allograft recipients. Indeed, given the paucity of early clinical features for allograft injury and the gravity of the consequences thereof, it is arguable that no area of transplantation is so dependent on the pathologist for care delivery. As such, protocol biopsy interpretation is an integral part of post-transplant care in all centers.

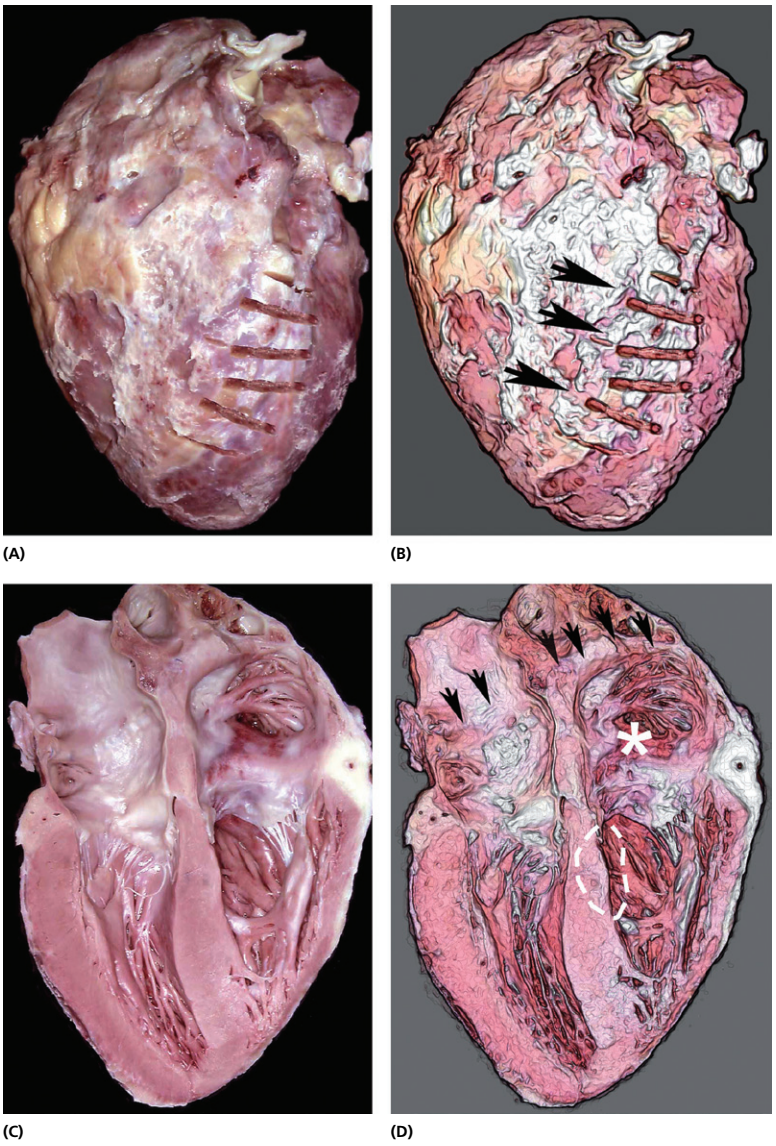


Figure 83.20. Failed allograft. (A,B) Fibrous organized pericarditis in a 16-year-old patient who died suddenly 15 months post-transplant. The coronary arteries have been sampled extensively (arrows). (C,D) Four-chamber view of the heart showing the fully endothelialized atrial anastomotic sites. Obviously, the atrial chambers are large, as they represent part of the recipient atria and the donor atria anastomosed. There is a focus of endocardial thickening (white patch) representing previous biopsy site in the trabeculae of the right ventricle (dashed line). There is ecchymosis in the endocardium of the donor right atrium (asterisk). The left ventricle shows mild hypertrophy.

The biopsy represents the intersection of mechanistic understanding and clinical effect, and its study will provide substantial insight into the biology influencing transplant outcome and should inspire the clinician to engage the immunosuppressive armamentarium with specificity.

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CHAPTER 84

Histopathological Syndromes of Lung Allograft Rejection and Recurrent Disease

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Introduction

Allograft rejection is a common and clinically serious problem in the lung. Compared to other organs, the incidence of acute cellular rejection in the lung allograft is quite high. The reported incidence in the first year post-transplantation ranges from 36% [1,2] to 55% [3]. This compares to 40% for the heart [4] and 11% for the kidney [5]. These episodes are rarely fatal but their accumulative effect on the lung results in the chronic rejection of the vessels and, more importantly, of the airways, ultimately leading to graft failure. Details on the clinical presentation and differential diagnosis of lung allograft rejection can be found in Chapters 72 and 80.

Because of the ongoing potential for rejection-related graft injury, lung allograft recipients undergo routine monitoring for clinical and pathologic signs of acute rejection. Bronchoscopic biopsy is the main diagnostic tool used to evaluate the allograft for acute rejection and its sensitivity to detect rejection if present is greater than 80% [6]. Through this procedure both histologic and cytologic specimens can be obtained and evaluated for morphologic evidence of acute rejection. As with other allograft teams, comprehensive lung transplant teams require a strong relationship with the pathology team for optimal patient management. This chapter reviews the specific histopathologic features of lung allograft rejection that are important for the clinical lung transplant physician or surgeon to comprehend to facilitate dialogue between the clinical and pathology services.

Histologic grading of lung allograft rejection

The classification system used to grade histologic rejection in these biopsies was first put forth by the Lung Rejection Study Group of the International Society for Heart and Lung Transplantation (ISHLT) in 1990 and is now in its third iteration [7]. The original Working Formulation was developed by pathologists in active lung transplantation centers to allow for the comparison of outcomes data among these institutions and to be a simple, reproducible, and easily taught system. This original schema, which used a grading system of A and C for vascular rejection and B and D for airways rejection, was adopted by most of the centers and used for the subsequent 5 years with considerable success. In 1995, an expanded group of pathologists convened to further improve the grading schema to respond to new developments in the field. The second Working Formulation, published in 1996, was based on published data and practical experience of the multiple centers and emphasized that the pathologic data needed to be interpreted within a broad clinical context to provide the optimum patient care. Most specifically, infection and other sources of inflammation in the biopsy tissue needed to be excluded clinically for more accurate and reproducible grading [7].

The most recent revision of this grading system was published in 2007 and continues to be used today (Table 84.1). The major changes in this document were the revision of the acute airway rejection grading system from a four-tier system (B1–B4) to a two-tier system with low and high grades and the grading of chronic airway rejection (C) without reference to the presence of inflammation. Finally, though recommendations were made for the pathologic evaluation of antibody-mediated rejection (AMR), there was no consensus as to its role or diagnostic criteria in lung allograft rejection [7].

General recommendations

Appropriate technical handling of the transplant biopsies is important to obtain optimal information from the tissue and recommendations for this have been standardized in the ISHLT classification system [7]. The biopsies should be fixed in buffered formalin and undergo paraffin embedding. Paraffin tissue sections are cut and placed on microscopic glass slides (2–3 sections per slide; total three slides) and stained with routine hematoxylin and eosin stains. Two additional slides are cut and one is stained with silver to evaluate for fungal organism, including *Pneumocystic jiroveci*, and the other stained for elastic fibers to highlight evidence of fibrosis, a marker of chronic rejection, especially in the airways. Additional studies for other infectious organisms including immunohistochemical studies for cytomegalovirus (CMV) and Epstein–Barr virus (EBV) can be performed, if clinically needed.

The number of tissue fragments needed in the biopsy for an adequate evaluation for the presence of rejection is not definitively stated in the most recent (2007) Revision of the Lung Rejection Study Group (LRSG) Working Formulation. The original 1990 Working Formulation and its successor in 1997 recommended that five pieces of alveolated lung or at least 100 alveoli were needed to evaluate for the presence of rejection and that standard is continued

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 84.1. 2007 Revision of the Working Formulation for the classification and grading of pulmonary allograft rejection

A. Acute rejection	Grade 0 – None Grade 1 – Minimal Grade 2 – Mild Grade 3 – Moderate Grade 4 – Severe
B. Airway inflammation–lymphocyte bronchiolitis	Grade 0 – None Grade 1R – Low grade Grade 2R – High grade Grade X – Ungradeable
C. Chronic airway rejection–bronchiolitis obliterans	Grade 0 – None Grade 1 – Present
D. Chronic vascular rejection–accelerated graft vascular sclerosis	

Based on [7] Stewart S, Fishbein MC, Snell GI, et al. Revision of the 1996 Working Formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant* 2007;26:1229–1242. Copyright 2007, with permission from Elsevier.

by most centers today [8]. For those biopsies that have insufficient tissue for evaluation, a definitive diagnosis on the presence of absence of rejection is usually not given (see later).

Histologic features of acute rejection

Acute cellular rejection in the lung is graded using two scales: A for vascular rejection and B for airway inflammation, the latter not labeled definitely as rejection because it may be a result of acute rejection of the airways, but may also have other etiologies. Rejection in the vessels is usually accompanied by rejection in the airways, especially in cases of moderate and severe vascular rejection [9] but vascular rejection is the clinical trigger that prompts therapy even in the absence of airway rejection.

Acute vascular rejection is graded on an A0–A4 scale, each increment representing an increasing amount of inflammation, first surrounding the vessels and then infiltrating out into the alveolar walls. The infiltrate is composed of lymphocytes, some with reactive faintly eosinophilic or "transformed" nuclei, macrophages, eosinophils, and neutrophils, the latter two acute inflammatory cell populations usually seen only in grade A2 and above. A diagnosis of acute rejection has no minimal number of vessels involved, requiring only a single vessel to show histologic evidence of rejection. Veins and venules are more commonly involved than arteries and arterioles. Finally, though the vascular rejection may include a spectrum of grades, the grade given to the biopsy represents the highest grade present in the tissue.

Grade A0 (no acute rejection) is the grade given to the biopsy when no acute vascular rejection is seen and adequate tissue is available for grading.

Grade A1 (minimal acute rejection) indicates the presence of lymphocytes encircling the vessels in layers 2–3 cells thick in the adventitia (Figure 84.1). Though previous Working Formulations have accepted incomplete vascular cuffing consistent with rejection, the most recent Working Formulation states that the infiltrate must completely surround the vessel [7]. Unlike higher grades of acute rejection, no eosinophils or neutrophils are seen in this grade. Though subtle, this pathology can usually be seen even at low magnification if the tissue is free of artifacts.

Grade A2 (mild acute rejection) also has a mononuclear infiltrate around vessels, containing lymphocytes, many activated and plasmacytoid variants, as well as eosinophils. The infiltrate is more prominent than in Grade A1 with cell layers of 3–5 cells thick and easily visible at low magnification (Figure 84.2). There may be subendothelial involvement of these mononuclear cells (endothelialitis). In addition, though airway inflammation is not commonly see in Grade A1, it is commonly found co-existing with Grade A2 [10]. No extension of this mononuclear infiltrate into the alveoli is seen in this grade, which distinguishes it from Grades A3 and A4.

Grade A3 (moderate acute rejection) consists of a dense mononuclear infiltrate cuffing the vessels, with frequent eosinophils and neutrophils and a prominent endothelialitis. The infiltrate, by definition, must extend into the adjacent alveolar walls, involving airspaces and septa and in some cases can cause features of early acute lung injury including reactive Type 2 pneumocyte hyperplasia and intra-alveolar fibrin. This infiltrate commonly extends into the alveolar space resulting in clusters of alveolar macrophages, lymphocytes, and occasionally focal fibrin deposition, giving

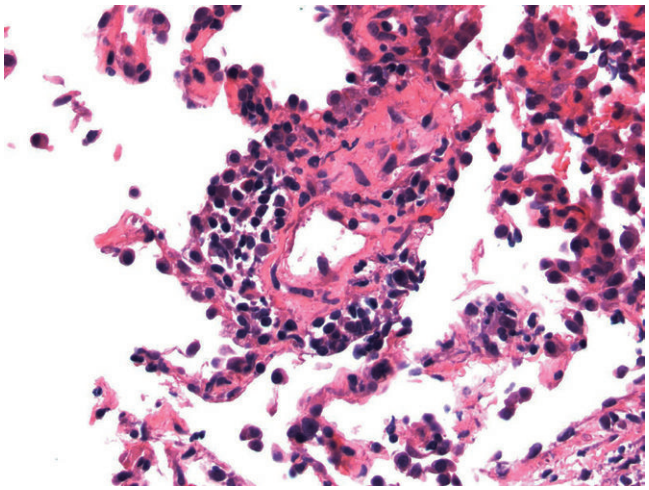


Figure 84.1. Acute cellular rejection Grade A1 (minimal). A small vessel is cuffed by plasmacytoid and transformed lymphocytes present in a layer of 2–3 cells (hematoxylin and eosin).

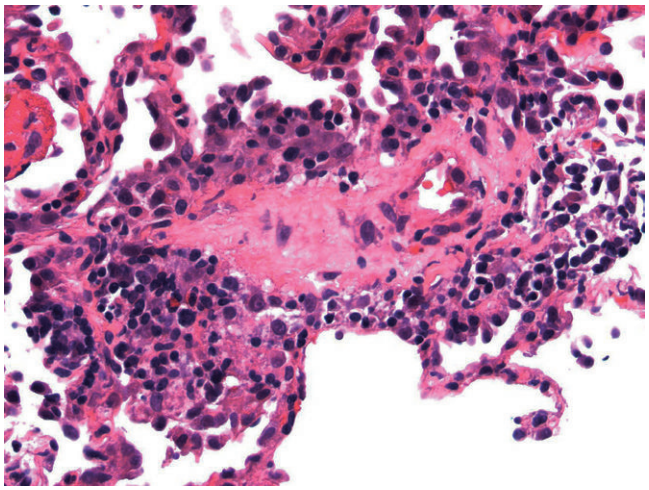


Figure 84.2. Acute cellular rejection Grade A2 (mild). Inflammatory cells greater than three layers thick surround a small vessel. This infiltrate includes lymphocytes and scattered eosinophils and neutrophils (hematoxylin and eosin).

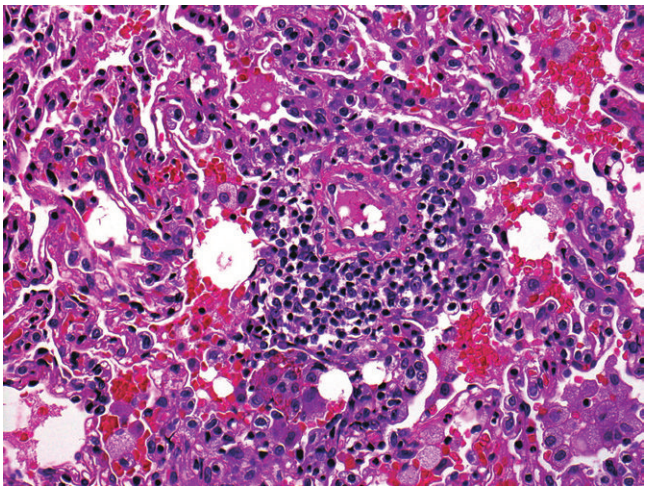


Figure 84.3. Acute cellular rejection Grade A3 (moderate). An inflammatory infiltrate with lymphocytes, eosinophils, and neutrophils extends into the alveolar septa (hematoxylin and eosin).

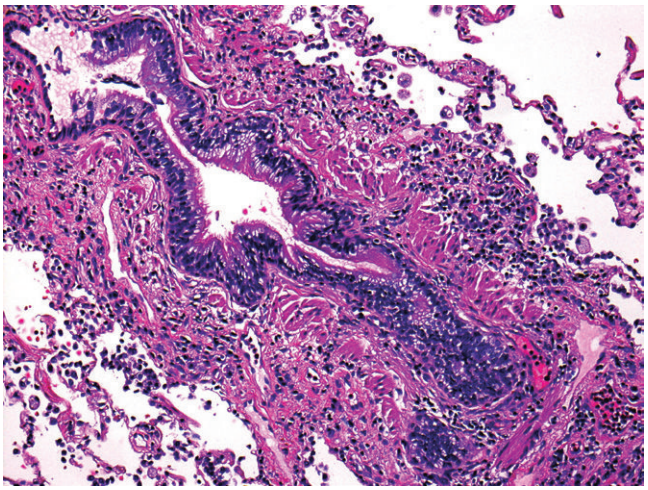


Figure 84.5. Low-grade lymphocytic bronchiolitis Grade B1R. Lymphocytes are present in a band-like infiltrate in the submucosa and as single cells infiltrating the overlying bronchiolar epithelium (hematoxylin and eosin).

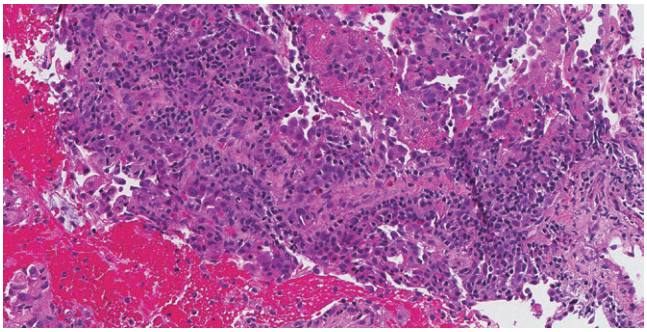


Figure 84.4. Acute cellular rejection Grade A4 (severe). Diffuse perivascular, interstitial, and air space infiltrates are present with evidence of acute lung injury and hemorrhage (hematoxylin and eosin).

the lung parenchyma a more inflamed and atelectatic appearance (Figure 84.3).

Grade A4 (severe acute rejection), a grade seldom seen in transbronchial biopsies, is defined as diffuse involvement of the lung vessels and alveoli with mononuclear cell infiltrates and severe acute lung injury. The histologic features are those of diffuse interstitial pneumonia with intra-alveolar macrophages, fibrin, and perivascular inflammation with areas of both necrosis of the parenchyma and fibrinoid necrosis of the vessels (Figure 84.4). The alveolar space may be filled with macrophages, fibrin, hemorrhage, and hyaline membranes and the abundance of neutrophils is characteristic of Grade A4 rejection. The vasculitis and perivascular prominence of the inflammation may be helpful in distinguishing Grade A4 acute rejection from diffuse alveolar damage. The latter has multiple etiologies including infection or reperfusion injury in the immediate post-transplantation period [11]. Both the reactive Type 2 pneumocyte hyperplasia and the endothelialitis are more prominent than that seen in Grade A3. With Grade A4 acute rejection, the inflammation commonly spills over into the adjacent large and small airways, resulting in infiltration of the epithelium with these inflammatory cells and ulceration [12]. Because of the abundance of acute inflammation including neutrophils, the primary differential diagnostic consideration to Grade A4 rejection is usually an infectious process. Organism stains and clinical information is often necessary to support the correct diagnosis.

For biopsies that have fewer than five pieces of tissue and are insufficient to evaluate for rejection, a Grade of AX is given.

Grading of the small airways for acute rejection is based on the extent of a lymphocytic infiltrate of the airway and epithelium injury and is applicable to small airways only [7]. In the original 1990 Working Formulation, acute airway rejection was simply listed as present or absent, at the discretion of each institution [12]. In the 1996 revision, the grading of airway inflammation expanded to include B0 (no inflammation) to B4 (severe airway inflammation) [12]. However, not all members of the LRSG were convinced that the inflammation could be used solely to grade rejection because it was also found in other settings (most notably infection) [13], thus, the consensus was that this schema would need ongoing study. In the 2007 revision, the difficulty in discriminating the causes for the inflammation (predominantly rejection or infection) was officially acknowledged with the introduction of the generic term "airway inflammation" into the grading schema. In addition, because a consensus could not be reached regarding the criteria needed to reproducibly separate the airway inflammation into four separate grades, it was decided to collapse the grading schema down to a two-tier system, B1R and B2R (R for revised grade), retaining B0 for the absence of inflammation and BX for those biopsies in which the inflammation could not be adequately assessed for a number of reasons. These included cases in which no airways were present for evaluation, other inflammatory processes capable of producing the inflammation were present (such as infection), or tangential cuts of the tissue or crush artifact obscured the small airway present [7].

In airways with Grade B1R, there are mononuclear cells forming patches or, in more prominent cases, a band within the submucosa of the small airways. The infiltrate may extend into the muscular wall of the bronchiole but does not tend to involve the overlying bronchiolar epithelium and no significant epithelial injury is present (Figure 84.5). Grade B2R has a much more exuberant infiltrate and forms a dense band of more activated

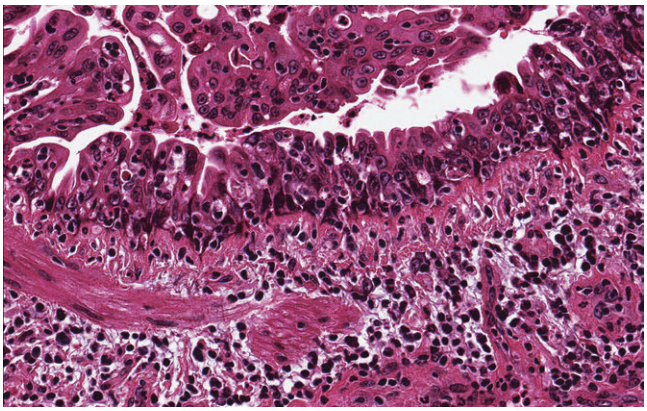


Figure 84.6. High-grade lymphocytic bronchiolitis Grade B2R. Lymphocytes are present in the submucosa with extensive infiltration into the overlying epithelium producing extensive epithelium injury (hematoxylin and eosin).

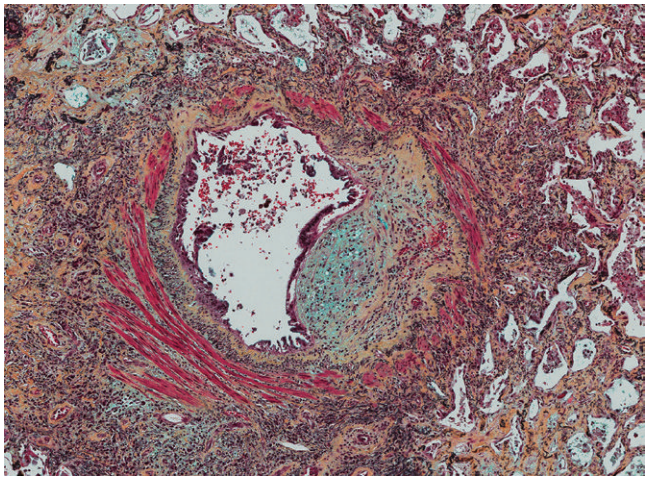


Figure 84.7. Chronic airway rejection–bronchiolitis obliterans Grade C1. A small airway has early fibrosis and asymmetrical narrowing of lumen (Movat).

lymphocytes with plasmacytoid variants and significant numbers of eosinophils and neutrophils. This inflammation infiltrates the overlying bronchiolar epithelium causing significant epithelial injury, taking the form of both apoptotic cells and areas of frank ulceration with cellular debris (Figure 84.6). The acute inflammatory cells make it difficult to distinguish high-grade rejection from infection; however, a more prominent presence of neutrophils within the epithelium and lumen of the airway may be evidence of infection rather than rejection. Viral bronchiolitis may induce a similar degree of airway-centered inflammation and epithelial injury. In these cases, careful review of the injured epithelial cells may reveal viral inclusions and immunohistochemistry studies, especially for CMV and herpes simplex virus, may demonstrate the viral inclusions.

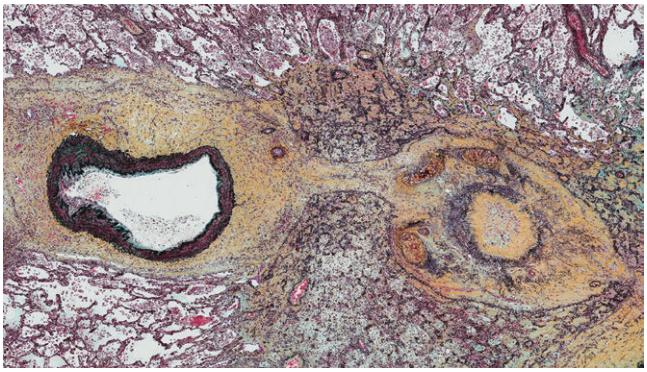
Given the importance of the transbronchial biopsy in assessing rejection and dictating treatment, it is important to examine research that has studied the validity and reproducibility of the current classification system by assessing the interobserver variability. Studies show that agreement between expert lung pathologists for acute rejection is variable from poor (kappa 0.183) [13] to moderate (kappa 0.47–0.79) [8,14,15], with most disagreement occurring for Grade A1 (minimal acute rejection) and most concurrence for Grade A2 (mild acute rejection). For airway inflammation, there was predominantly poor interobserver variability (kappa 0.035–0.25) [14,16] with significant concurrence occurring for Grade B0, no airway inflammation. These data argue for continued education to optimize and increase uniformity of the pathologic grading of acute rejection and for ongoing refinement of the criteria to further enhance diagnostic reproducibility [13].

Chronic rejection

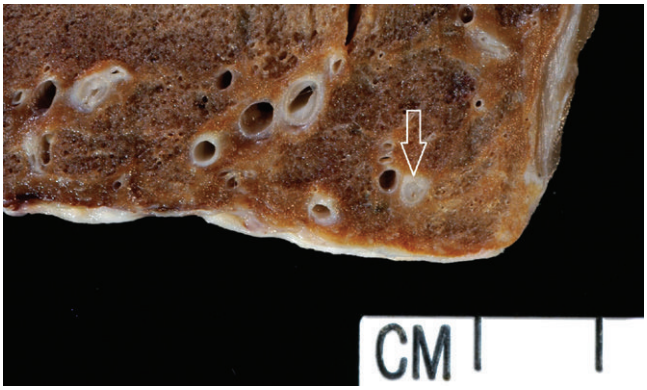
As with acute rejection, the histologic features of chronic rejection in the lung allograft are most clearly defined in the vessels and the airways, with the latter currently thought to be the most clinically significant. Chronic airway rejection, or obliterative bronchiolitis (OB), is a process by which collagenous type fibrosis occurs in the small airways as a sequela of the lymphocytic bronchiolitis, perhaps as a result of the airway inflammation of acute rejection [17–19]. This abnormal repair reaction may be mediated via transforming growth factor-beta (TGF-β) over-expression in response to acute injury and inflammation [19]. This airway fibrosis may appear anywhere from a few months to several years after transplantation. The median time to presentation is 16–20 months [20]. The sensitivity of transbronchial biopsy (TBB) for the detection of chronic airway rejection at most centers ranges 15–40% [3,21] because of its patchy nature in the allograft. This sensitivity may increase with the number of tissue fragments, the experience of the bronchoscopist and the pathologist, but it remains a less sensitive measure than pulmonary function testing and it is more expense and invasive [20]. Therefore, surveillance TBBs for OB are no longer performed in most clinical transplant centers. Instead, a standardized formulation for the clinical diagnosis of OB or bronchiolitis obliterans syndrome (BOS), developed by the ISHLT and based on pulmonary function test results, is used to dictate therapy [22].

The primary histologic marker for chronic airway rejection is the presence of dense hyalinizing fibrosis comprised of type 3 collagen in the subepithelial area of the bronchiolar mucosa [19]. This may be either concentric or eccentric and it gradually narrows (Figure 84.7) and eventually occludes the small airways (Figure 84.8). As the fibrosis increases, the bronchiolar epithelium is lost and identification of the obliterated airway may be difficult. Its proximity to a pulmonary artery or residual smooth muscle from its wall may be helpful in finding it. Also, connective tissue stains such as elastic or pentachrome stains are helpful in highlighting the elastica of the obliterated airway as well as identifying evidence of early fibrosis. Though previous Working Formulations considered the amount of chronic inflammation in the schema, the most recent grading system does not include this and consists of just two grades: Grade C0 if absent and Grade C1 if present.

Chronic inflammation, fibrosis, and eventually bronchiectasis may be present in large airways in lung allografts with both acute and chronic small airway rejection. These histologic findings may represent chronic injury from a number of insults that the allograft has suffered, including infection and aspiration as well as rejection.



(A)



(B)

Figure 84.8. (A) Chronic airway rejection–bronchiolitis obliterans with early chronic vascular rejection Grade C1D. Remnants of a cartilaginous airway (right), outlined by elastic fibers (black), is completely obstructed by collagenous fibrosis. The pulmonary artery (left) shows some evidence of intimal fibrosis consistent with an early chronic vascular rejection. (Movat). (B) Chronic airway rejection. A transplanted lung resected for chronic airway rejection has small airways some partially narrowed and others completely obliteration by fibrosis (arrow) (Gross).

Because these pathologic findings are not specific for rejection, they are not included in the 2006 Working Formulation and are not graded as chronic airway rejection.

The pathologic features of chronic vascular rejection have not received significant attention because the transbronchial biopsy does not reliably sample these lesions and the clinical significance of this pathology is not yet clear. The pathologic features of chronic vascular rejection have not been well documented. Features described include intimal fibrosis and variable transmural chronic inflammatory infiltrates (Figures 84.8 and 84.9). One report states that the intimal proliferation could be non-laminar eccentric and concentric and that the media of vessels can demonstrate mild extracellular matrix deposition with smooth muscle atrophy [23]. Grading chronic vascular rejection is similar to chronic airway rejection in that only its absence (Grade D0) or presence (Grade D1) is noted.

The concurrence of chronic airway and chronic vascular rejection is well-documented leading to speculation regarding a causal link [24]. Recent research suggests that intimal fibrosis of the small vessels that supply the airways, the pathology seen in chronic vascular rejection, may correlate with the presence of OB [25,26].

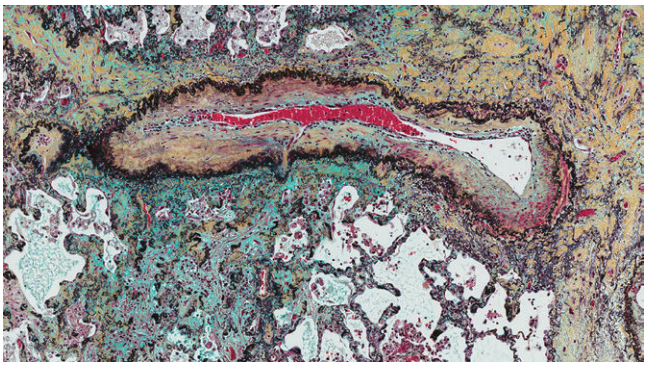


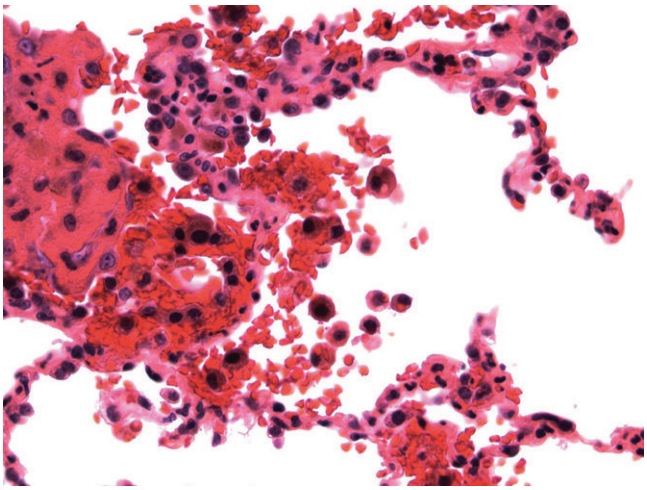
Figure 84.9. Chronic vascular rejection: vein. A large vein within an interlobular septa in a transplanted lung resection for chronic rejection shows marked intimal fibrosis (Movat).

Because the bronchial circulation, the high pressure circulation that supplies the oxygen to the lung, is not reconnected during the transplantation procedure, the lung tissue including the small airways, are more susceptible to hypoxic injury [27]. These studies suggest that the pathologic changes of chronic vascular rejection may further compromise oxygenation in the lung allograft small airways, leading to epithelium injury, fibrosis, and eventually OB. Early studies suggest that in lungs where the bronchial circulation has been reconnected, there is a decreased incidence of OB [28]. Further studies of the chronic vascular changes and their effects in lung allografts are needed to clarify these findings.

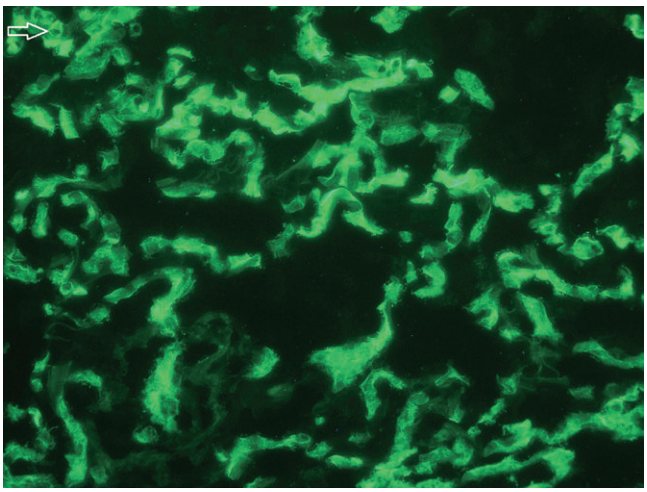
Antibody-mediated rejection

Antibody-mediated rejection (AMR) results from injury to the allograft from donor specific anti-HLA antibodies post-transplantation [29]. This form of allograft rejection is clinically important in other solid organ transplants such as kidney and heart [30]. However, its clinical significance in the lung allograft including its effect on allograft survival is not known. The detection of HLA antibodies among lung transplant recipients has been shown to decrease lung allograft survival [31,32] and humoral rejection in the form of so-called hyperacute rejection in the lung, resulting from preformed antibodies in the recipient directed against donor HLA antigens has been well-documented [33]. In the latter, the most common pattern of injury takes the form of diffuse alveolar damage (DAD) and may also reveal evidence of vascular injury such as thrombi formation in vessels and neutrophils and necrosis within alveolar septal capillaries [30,34]. In the two previous Working Formulations, AMR was not in the classification or grading for lung rejection [12] and though the current Working Formulation includes a discussion of AMR, it concludes that the pathologic features for AMR in the lung are not yet definitive and require further studies. Further, it introduces the term capillary injury (in place of capillaritis) and suggests this may be the pattern of injury for AMR but may have a wider etiologic spectrum including infection and high grades of cellular rejection [7].

Currently, the focus of study on AMR in the lung has been on defining both the clinical and the pathologic features. The working group from the National Conference to Assess Antibody-Mediated Rejection in Solid Organ Transplantation has proposed a general



(A)



(B)

Figure 84.10. (A) Antibody-mediated rejection. Lung parenchyma shows features of acute lung injury including reactive pneumocytes and intra-alveolar fibrin. Neutrophils are present with the septal capillaries (hematoxylin and eosin). (B) Antibody-medicated rejection. Immunofluorescent staining for C4d shows capillaries with linear deposition and areas of cross-sectional vessels with a characteristic "donut" like pattern of staining (arrow) (polarized light).

classification system for humoral rejection that proposes four necessary features for humoral rejection: detection of circulating HLA or donor-specific antibodies; C4d deposition in the allograft tissue; the presence of tissue pathology; and graft dysfunction [30]. Studies that have focused on defining the tissue pathology have found that, in general, it includes neutrophils within alveolar septal capillaries if not definitive necrosis or evidence of pulmonary hemorrhage (Figure 84.10), features at least suggestive of pulmonary capillaritis [35]; however, C4d staining was not performed. In studies where C4d staining was performed, the results have been somewhat discrepant. Some studies have found evidence of C4d deposition in lung allografts with this pattern of injury [36–39], while others have not seen C4d deposition in this setting of capillaritis [40]. These discrepancies may be a result of differing methodologies, nonspecific staining of elastic tissue and fibrin, or the extremely low incidence of capillary-specific C4d positivity in lung allograft biopsies. Currently underway are large-scale multi-institutional studies with standardized protocols, including those for C4d tissue staining, to help gather more conclusive data regarding the pathologic features of AMR in the lung [41].

Role of bronchoalveolar lavage fluid in the diagnosis of lung allograft rejection

The use of bronchoalveolar lavage fluid (BALF) analysis in monitoring rejection in the lung allograft remains an area of controversy. The monitoring of the active cellular dynamics between the lung parenchyma including alveoli and the interstitial compartment and the small and large airways becomes possible with bronchoscopy and bronchoalveolar lavage (BAL) through the counting and immunophenotyping of inflammatory cells within the BALF. Through lavaging the lung allograft, it is estimated that over 1 million alveoli may be sampled [42], a considerably larger area of the lung than TBB can access. This would seem to give BAL a considerable advantage over TBB given the patchy nature of rejection. However, though BAL is a primary tool used in assessment of the lung for infection [43] and is a common tool used when infection is suspected in lung transplantation patients [44], the promise of BAL as a more sensitive tool for diagnosing rejection has not been borne out for a number of reasons.

First, the cellular composition of the BALF changes during the post-transplantation period. In the immediate aftermath of surgery, the total cell count is elevated, especially the neutrophils, probably as a result of a number of inflammatory insults including reperfusion injury [44,45]. This cell count may remain elevated for years, making it difficult to establish a firm baseline with which to compare subsequent samples. Second, though acute rejection in the lung allograft is characterized by an increase in mononuclear inflammatory cells within the interstitium and the airspaces that increases with the grade of both acute vascular and airway rejection [7], granulocytes (eosinophils and neutrophils) also increase [46] making it difficult to separate infection from rejection. Studies that compared the inflammatory cell findings in TBB specimens with those in BALF, found the inflammatory cells in TBB are highly specific for rejection. However, the cellular changes in the BALF from lungs with rejection had increased proportions of lymphocytes in early rejection and neutrophils in later occurring rejection but were not specific for rejection [47,48].

To assess for features of AMR in BALF, one study evaluated soluble C4d with C4d fragment enzyme immunoassay and correlated the findings with tissue C4d deposition and anti-HLA donor-specific antibodies. The study found positive correlation of soluble C4d with tissue C4d deposition and donor-specific antibodies, but the findings were not specific and a high false-positive result was found in the setting of CMV infection [49]

Finally, given the importance of T cells in rejection [50], studies that focused on subtypes of T cells in the BALF did show some correlation with $CD4^+$ and $CD8^+$ ratios with the presence of acute cellular rejection in the lung allograft [51] and with the development of chronic airway rejection [43,52]. However, further studies to confirm these findings are needed before BALF is useful clinically in diagnosing rejection.

Conclusions

Lung transplant recipients remain at risk of alloimmune-mediated rejection of their grafts throughout life. This manifests as either

overt acute rejection or indolent chronic injury in the form of OB and its related BOS. Continuous patient surveillance supported by histopathologic diagnosis of tissue, or BALF analysis remains a critical part of lung transplantation.

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CHAPTER 85

Histopathological Syndromes of Pancreas and Islet Allograft Rejection and Recurrent Disease

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Introduction

In patients with type 1 diabetes mellitus, insulin independence can be achieved either through whole pancreas transplantation (WPnTx) or with islet transplantation (IT). Successful β-cell replacement positively impacts quality of life for the diabetic patient [1] and, if long-term normoglycemia is maintained, can lead to improvement of secondary diabetic lesions that were previously considered irreversible [2,3].

The first pancreas transplant was performed in 1966, but achievement of acceptable results with this procedure has been slow – mostly because of a series of technical and immunological difficulties [4–8]. Since the 1990s, however, results for WPnTx have continuously improved and this procedure is currently considered a standard treatment for selected patients [9]. Routine histological evaluation of pancreas allograft biopsies and standardized interpretation of the pathology findings have contributed to better outcomes in WPnTx [5,10–15]. A thorough discussion of the procedural evolution and long-term benefits and outcomes in pancreas transplantation can be found in Chapter 107.

Since the first series of successful IT reported in 2000, excellent short-term results have been achieved; however, most patients require insulin therapy within 5 years post-transplantation. Despite this serious setback, other metabolic advantages such as disappearance of hypoglycemic episodes and overall improvement of glycemic control appear to persist longer in IT recipients [16,17]. Islet transplant outcomes are discussed in detail in Chapter 108.

In comparison to other types of transplants, systematic histopathological analysis of WPnTx and IT have been limited largely by technical limitations of obtaining suitable graft tissue by percutaneous biopsy. The interpretation of pancreas biopsies is still in a developmental phase, but continuous research efforts are increasing our understanding of the mechanisms of graft loss. Although the fundamental pathways of graft loss may differ in these two types of transplants, both eventually suffer a steady and irreversible deterioration of graft function that correlates with progressive graft fibrosis and sclerosis in WPnTx, and loss of a sufficient islet mass in IT [14,16–18]. The islets of Langerhans, the essential component in both these transplant modalities, can be affected by common processes (i.e. allograft rejection, recurrence of type 1 diabetes, amylin deposition, and susceptibility to cytomegalovirus (CMV) related effects) [18–21].

Principles and practical aspects of histopathological diagnosis of allograft rejection in whole pancreas transplants

Immunological aspects of rejection

Histopathological features of acute allograft rejection in the pancreas are not different from those in other solid transplants [6,8,22–24]. Acute T-cell-mediated rejection (ACMR) and antibody-mediated rejection (AMR) are characterized by tissue infiltration by effector inflammatory cells and by antibody and complement deposition in the vascular walls, respectively; these fundamental mechanisms are discussed in Chapters 5 and 6, respectively. In both of these pathways, graft injury typically results from antigen-specific immune damage in combination with other non-specific factors [22–24].

Graft rejection depends highly on the degree of incompatibility between recipient and donor major histocompatibility complex (MHC) antigens [24]. The MHC Classes I and II are expressed differentially in exocrine and endocrine pancreatic tissues, both in normal and inflammatory conditions (Table 85.1) [13,25–27]. MHC disparities, specifically in Class II, have been associated with an increased risk of AMR and graft loss [28].

In the early post-transplantation period, histopathological manifestations related to ischemia and surgical trauma predominate [24,29–31]. The development and progression of ACMR have been characterized in various animal models. ACMR manifests with inflammatory infiltrates in the interstitium, involvement of small veins, and variable more heterogeneous involvement of ducts [32–39]. Acinar inflammation and acinar cell damage, including apoptosis, is also characteristic of acute rejection [5,12,32,35–39]. The severe, more advanced forms of ACMR manifest with intimal arteritis, necrotizing vasculitis, thrombosis, and eventual parenchymal necrosis [14,36,39].

Although islets are not a primary target in ACMR, severe rejection leading to extensive parenchymal necrosis is typically associated with secondary islet damage or necrosis and hyperglycemia [40]. Furthermore, repeated or untreated episodes of ACMR lead to chronic injury characterized by acinar damage or dropout and chronic vascular injury both associated to a fibrogenic reaction that obliterates the pancreatic architecture and leads secondarily to loss of adequate islet function. Graft sclerosis represents the main feature of chronic rejection [41,42].

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Table 85.1. Class I and II HLA expression in normal and abnormal pancreas tissue

Cell type	Normal pancreas histology sections		Tissue culture – inflammatory milieu (β-IFN, γ-IFN, IL-2)		Diabetes mellitus (DM)	
	Class I	Class II	Class I	Class II	Class I	Class II
Acinar cells	–	–	+ Aberrant expression	+ Aberrant expression	n/a	n/a
Ductal cells	++	–	++	+ Aberrant expression	n/a	n/a
Islet cells	+/– (Weak)	–	++ Over-expression	++ Aberrant expression	++ all islet cells Over-expression, (with insulitis – early DM)	+ ß cells Aberrant expression, +/– insulitis)
Capillary endothelium	++	++	n/a	n/a	n/a	n/a
Large vessel endothelium	++	Variable	n/a	n/a	n/a	n/a

n/a, data not available.
Adapted from [13] Drachenberg et al. *American Journal of Transplantation*. 2011;11:1792–1802, with permission from Wiley.

In AMR, deposition of antibodies in the vascular walls cause direct injury by activation of the complement cascade and also through antibody-dependent cell-mediated toxicity (ADCC) [28,43,44]. Therefore, vascular injury and necrosis, development of thrombosis, and secondary ischemic parenchymal necrosis typically characterize well-developed acute AMR; hyperacute rejection is the most extreme example of this process [24,43,45,46]. Although initially thought to be associated only with immediate graft loss in the form of hyperacute rejection, more protracted forms of AMR (acute and chronic active) are now recognized. Macrophages and neutrophils are recruited in abundance in AMR; however, these cell types can be present also in the more severe forms of T-cell mediated rejection as well as in any form of severe parenchymal injury [11,45,47–49]. Several documented cases of AMR have presented with hyperglycemia, suggesting that the islets may be susceptible to microvascular injury associated with antibody deposition in this form of rejection [11,47,50,51].

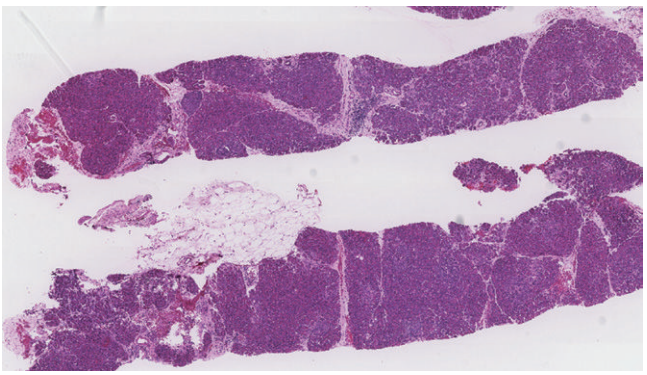


Figure 85.1. Low power view of a pancreas transplant biopsy stained with hematoxylin and eosin. The majority of the tissue sampled consists of exocrine pancreas (acinar lobules) separated by thin connective tissue septa. There is negligible septal fibrosis (i.e. graft sclerosis) in this biopsy (Stage 0).

Histological diagnosis of allograft rejection

Important efforts are being made to discover and validate non-invasive biomarkers that could potentially circumvent the performance of allograft biopsies [52–54]; however, tissue sample evaluation continues to be the gold standard for the diagnosis of acute pancreas rejection. ACMR and AMR cannot be distinguished from each other on clinical grounds alone. A complete discussion of the clinical recognition of pancreas and islet transplant rejection is found in Chapter 73. With both processes, patients present most commonly with exocrine abnormalities or a combination of exocrine and endocrine abnormalities; isolated endocrine dysfunction (hyperglycemia) is a less common indication for allograft biopsy (<10%) [10,11,47,55,56].

An ideal pancreas allograft biopsy contains at least three lobular areas and the associated interlobular connective tissue septa to allow for the evaluation of septal structures including ducts, veins, and smaller arterial branches, which are typically affected in T-cell-mediated rejection (Figure 85.1). The larger arterial branches are sampled with more difficulty. The absence of arterial branches in the biopsy core should be noted in the pathology report due to the diagnostic importance ascribed to the arterial lesions [14].

The biopsy is first examined at lower magnification for assessment of the overall lobular architecture and semiquantitative determination of the amount of septal and interacinar collagen (fibrosis), if present. Inflammation in the septal areas is identified easily at lower magnifications; however, acinar inflammation requires more detailed, systematic evaluation as the inflammatory cells tend to blend with the acinar cells, particularly in early forms of rejection [51,57].

Biopsy types

Percutaneous needle core biopsies are usually performed under ultrasound or computer tomographic guidance, with 18 or 20 gauge needles [12,15,58–63]. Adequate tissue can be obtained in 88–90% of instances [15,58–61,64–66]. Significant complications have been reported in 2–3% of cases (e.g. bleeding, mild pancreatitis), none leading to graft loss [59,60].

Performance of a laparoscopic biopsy is an alternative to the percutaneous approach when access to the graft is impossible, particularly in enteric-drained grafts (e.g. interposition of bowel loops) [67,68]. Pancreas transplantation with retroperitoneal portal-enteric drainage may allow for better access through the percutaneous route [69,70].

Cystoscopic transduodenal pancreas biopsies were often used in the past when bladder drainage was commonly utilized. Pancreatic tissue could be obtained in only 57–80% of cases and therefore interpretation relied also on the examination of duodenal mucosa from the grafted duodenal cuff [71–74]. Enteroscopic biopsy of

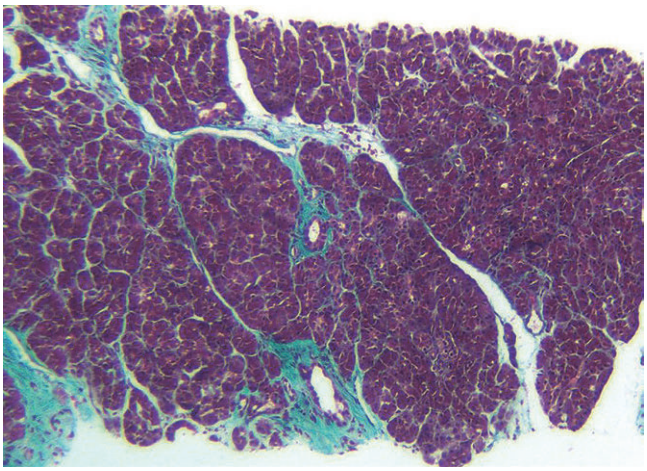
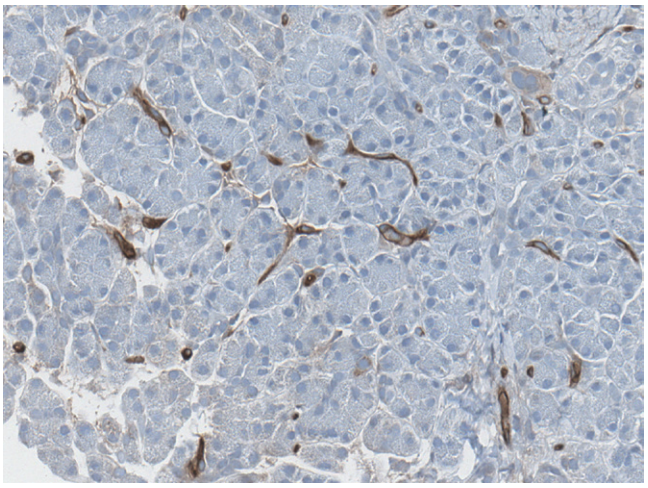


Figure 85.2. Masson's trichrome stain demonstrates thin delicate fibrous septa each of which contains branches of pancreatic ducts.



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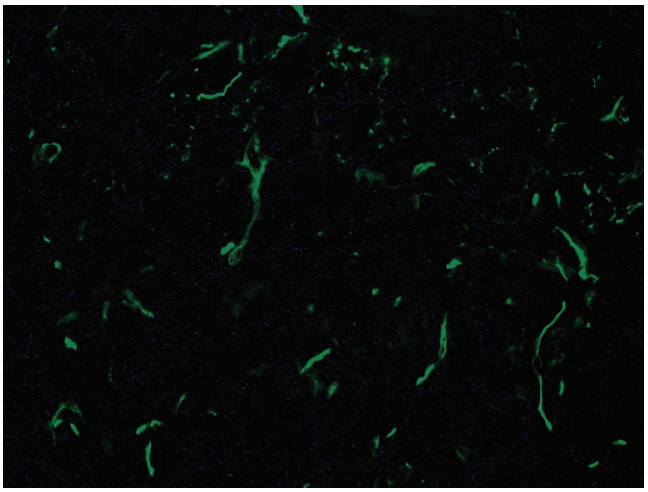


(B)

Figure 85.3. C4d stains using the immunohistochemical (A) and immunofluorescence (B) techniques demonstrate a similar staining pattern of the interacinar capillaries (IAC) in antibody-mediated rejection (AMR).

pancreas allografts, similarly, relies on the evaluation of mucosa from the duodenal cuff obtained with upper gastrointestinal endoscopy [75]. This is considered further in the section on Duodenal graft pathology.

Open (surgical) biopsies carry a higher risk of parenchymal leak and related complications, so they are performed only in selected cases when all other methods fail to provide tissue adequate for diagnosis [76].

Histological evaluation: technical guidelines

Routine light microscopy includes evaluation of at least two hematoxylin and eosin stained sections from different levels of the core. Intervening sections are stained for C4d (see diagnosis of antibody-mediated rejection below), and Masson's trichrome stain (Figure 85.2). The latter is a collagen stain used for quantitation of fibrosis and it also aids in the identification of specific structures or pathological changes (e.g. arterial walls, fibrinoid necrosis) [13,14]. With respect to the C4d stain, comparable results have been obtained with paraffin-embedded/immunohistochemical and frozen section/immunofluorescence methods (Figure 85.3) [13].

In patients biopsied due to hyperglycemia, it is essential to perform stains for *insulin and glucagon* to rule out selective loss of β cells [77–79]. Additional stains should be evaluated as needed (e.g. Epstein–Barr virus (EBV), CMV stains) [80,81].

Congo red stain is required for the identification of amylin (islet amyloid) [82] (see section on Banff pancreas allograft rejection).

If frozen tissue is available, deposition of immunoglobulins and complement in vascular walls can easily be demonstrated with immunofluorescence studies. These studies can be useful in cases suspected to represent hyperacute or fulminant acute AMR [42]. Electron microscopic preparations may be used to demonstrate selective β-cell loss in recurrence of type 1 diabetes or β-cell degeneration in calcineurin inhibitor toxicity [83].

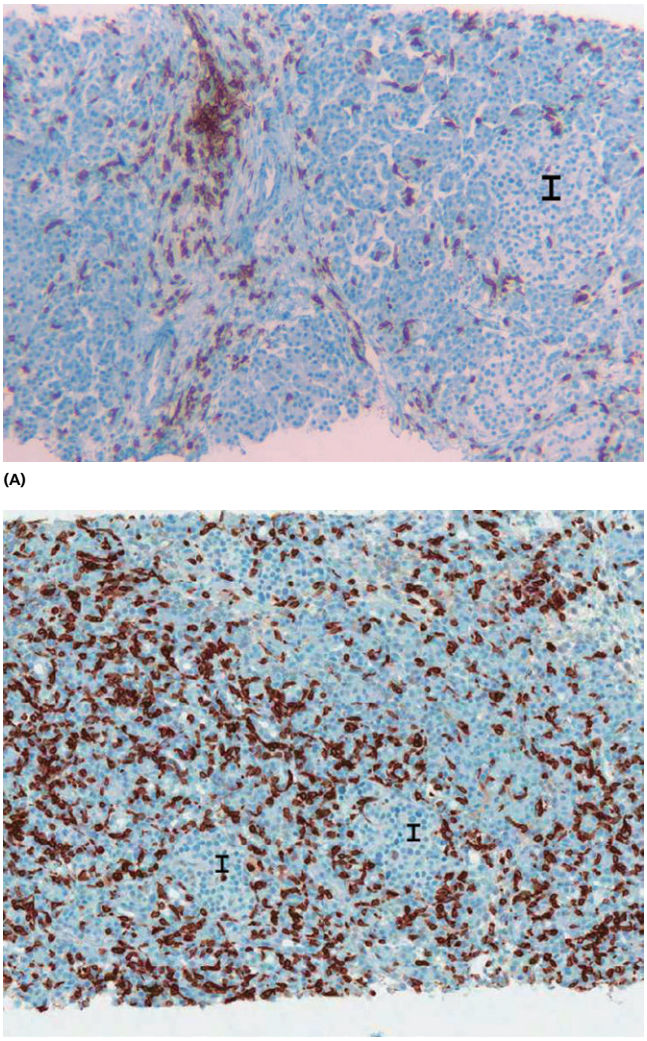
Acute T-cell-mediated allograft rejection

ACMR is characterized by septal inflammation and edema, venulitis, ductitis, and focal of diffuse "spilling" into adjacent or discontinuous acini (acinitis) [10,12,14,61]. Arterial lesions in pancreatic ACMR are morphologically identical to their counterparts in other solid organ transplants and by definition are classified with the more severe rejection grades due to their association with immediate or late graft thrombosis [14,84].

C4d staining in interacinar capillaries (≥5%) is not present in stereotypical ACMR [11].

Inflammatory cell types in ACMR

ACMR is defined by the presence of T-cell infiltrates. In milder forms of ACMR, more dense T-cell accumulation is predominantly confined to the septal areas (Figure 85.4A); in contrast, diffuse parenchymal T-cell infiltration is seen in extensive severe ACMR (Figure 85.4B). Eosinophils are commonly seen in acute ACMR and may even predominate in some cases [85]. Similar to ACMR in the kidney, macrophages are also present, but typically do not predominate unless there is a concurrent process present, such as ischemic pancreatitis or peripancreatic abscess. [86]. Plasma cells are uncommon in early acute ACMR but tend to accumulate along with the other mononuclear cells in protracted or partially treated or late acute-on-chronic rejection. Significant B-cell infiltrates are not characteristic of ACMR in the early post-transplantation period,



(A)

(B)

Figure 85.4. (A) Mild acute cell-mediated rejection (ACMR) (Banff Grade I). CD3 stain demonstrates heavier T-cell infiltrates in a septal area, with milder involvement of the acinar areas. (B) Severe ACMR (Banff Grade III) can present with various types of changes (see Banff schema). In this example, there is diffuse T-cell acinar inflammation as demonstrated by the CD3 stain. Note that in both images the islets (I) are to a large extent spared, because they are not specifically targeted by the inflammatory cells in ACMR.

but they commonly appear, often forming aggregates, in chronically inflamed grafts. B cells tend to increase with time, particularly in patients with previous episodes of rejection or ongoing ACMR. Their role is complex and poorly understood in the kidney and has not been systematically studied in pancreas allografts [87–89].

Therapeutic implications

Excellent response is achieved in most cases of ACMR with standard anti-rejection treatment (pulse steroids, antilymphocytic antibody). Conflicting results in the earlier series, where a significant proportion of severe ACMR rejection cases characterized by parenchymal and vascular necrosis were unresponsive to anti-rejection treatment, may have actually represented acute AMR [65,90]. Additional discussion on rescue therapy in general, and treatment of pancreatic acute rejection specifically are found in Chapters 67 and 73, respectively.

Differential diagnosis of ACMR

Negative or minimal pathological findings

For patients biopsied for graft dysfunction who have a "non-diagnostic" biopsy lacking obvious inflammation, the possibility of a sampling error should be considered. Unusual cases of mild ACMR may present with absence of septal infiltrates but focal acinar inflammation that may be underestimated at low-power evaluation [8,14]. Similarly, mild early AMR may present with extremely subtle acinar inflammation which may not be appreciated at first glance [13,91].

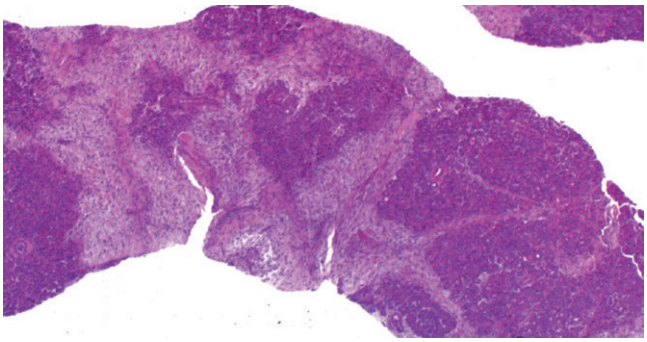
Septal and/or acinar inflammation with features different from ACMR

The main differential diagnosis includes peripancreatitis, CMV infection and post-transplant lymphoproliferative disorders (PTLD).

Infectious peripancreatitis, fluid collection, or peripancreatic abscess is a relatively common early complication of pancreas transplantation often secondary to ischemic graft pancreatitis [92,93]. Needle biopsies obtained from the grafted pancreas during post-transplantation laparotomy show variable degrees of mixed inflammation, predominantly septal. A typical finding in these biopsies is the presence of dissecting bundles of active tissue culture-like connective tissue with abundant fibroblasts. The fibrous bands run between the exocrine lobules giving the biopsy a "cirrhotic" appearance (Figure 85.5). The fibrosis becomes more pronounced as time passes if the peripancreatic infected fluid collection persists. The main differential diagnosis in biopsies from grafts with intra-abdominal or peripancreatic abscesses is with acute and with chronic rejection. In order to distinguish each of these processes it is necessary to integrate the histological and clinical information, including imaging and microbiological studies. From the morphological point of view, active fibrosis (or any active septal fibroblastic proliferation) is not typically found in acute ACMR unless there is an underlying component of chronicity. Overall preservation of the exocrine lobules is found in peripancreatitis whereas extensive atrophy of the central parts of the exocrine lobules is typically found in chronic rejection or graft sclerosis [14,41,86]. Although the degree of septal fibrosis seen in biopsies from patients who require one or more re-laparotomies may be very pronounced, these changes are usually confined to the periphery (surface) of the graft and therefore superficial fibrotic biopsies may not represent the status of the whole organ. In contrast to ACMR, the acinar parenchyma typically shows proportionally little inflammation and acinar damage in peripancreatitis [86].

Necrotizing infectious duodeno-pancreatitis with abscess formation can present at any time post-transplantation, but occurs more often in the first months. A variety of organisms can be cultured from these grafts, most commonly enterobacteria and methicillin-resistant *Staphylococcus aureus* (MRSA). Fungal infections, more often from *Candida* sp. and mixed bacterial and fungal infection, are not uncommon [94,95].

CMV allograft pancreatitis has become a rare diagnosis with the widespread use of antiviral prophylaxis. Klassen et al. [80] reported biopsy-proven CMV pancreatitis in four patients with the diagnosis made 18 weeks to 44 months after transplantation. With prolonged ganciclovir treatment, clinical and histological resolution of the infection was achieved in all patients [80]. On percutaneous needle



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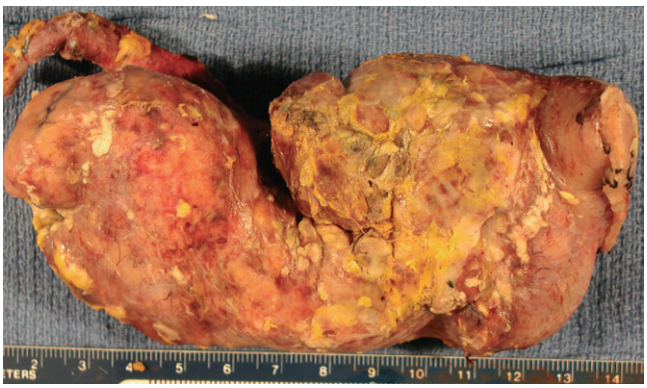


(B)

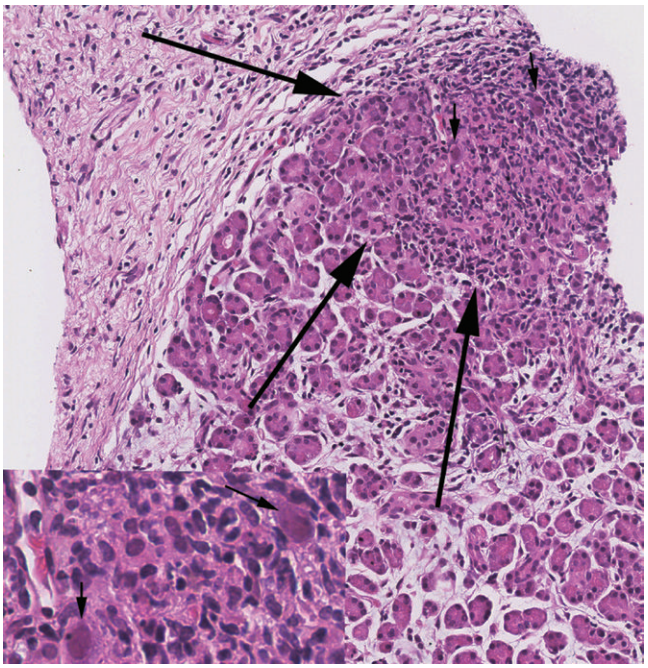
Figure 85.5. (A) Microscopic image of post-transplant peripancreatitis often shows mixed, predominantly septal inflammation and active septal fibrosis. The fibrous bands run between the exocrine lobules giving the biopsy a "cirrhotic" appearance. The inflammatory and fibrotic changes involve the peripheral areas of the graft. (B) Macroscopic appearance of pancreas transplant resected due to post-transplant peripancreatitis. The organ is partially covered by purulent exudates.

biopsies, both ACMR and mild CMV pancreatitis may present with modest, predominantly lymphocytic, acinar inflammation (Figure 85.6). Eosinophils are more common in rejection but may be present in small numbers in CMV infection as well. Neutrophils may be present in association with areas of necrosis or acinar cell damage in both rejection and CMV pancreatitis. Multiple tissue sections and CMV stains should be performed if deemed necessary to rule out viral infection [80,86].

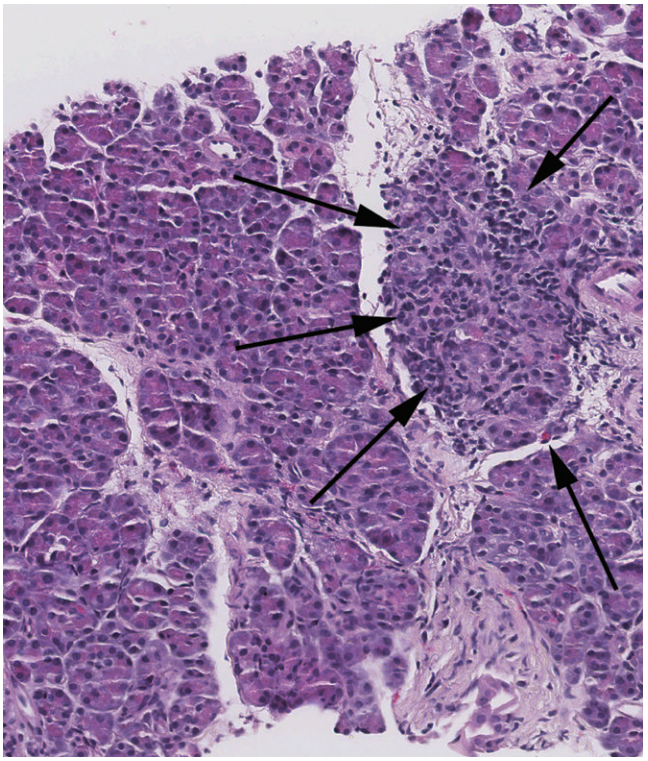
The clinical presentation of CMV graft pancreatitis is indistinguishable from acute rejection (e.g. increase in serum amylase and lipase). Severe cases of CMV infection may present with intractable gastrointestinal hemorrhage or duodenal-cuff perforation [96–100]. Necrotizing CMV infection with abscess formation and development of a CMV-related arteriovenous fistula have been reported [101–103].

Post-transplant lymphoproliferative disorder

PTLD occurs in 1–3% of pancreas transplant recipients [81,104–106]. Most PTLD is EBV-related, and of B-cell lineage. EBV-related PTLD includes a wide range of processes from benign hyperplastic to overtly malignant lymphoid proliferations. At the most benign end of the spectrum (plasmacytic hyperplasia), graft biopsies usually do not have a role in the diagnostic work-up. Graft involvement is not unusual in the other forms of PTLD (polymorphic



(A)



(B)

Figure 85.6. (A) Inflammatory focus due to CMV infection (large arrows). Enlarged cells with cytomegalovirus (CMV) cytopathic changes (small arrows) are present in the main image and in (B). Note the overall similarity with a focus of acinar inflammation due to ACMR, also marked with large arrows in (B).

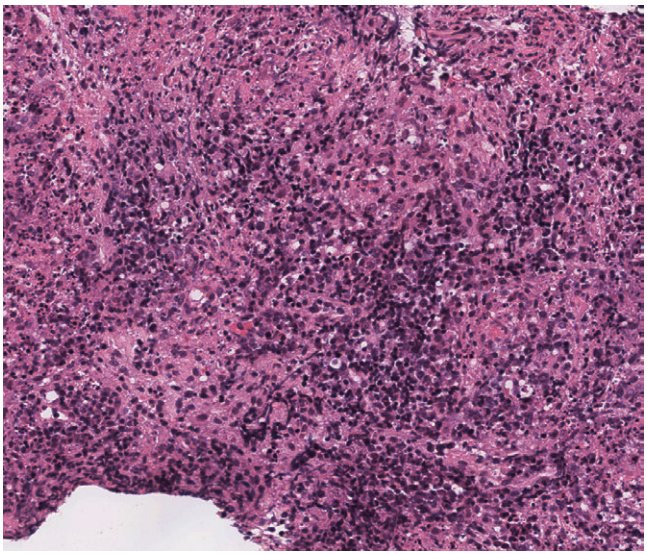


Figure 85.7. Post-transplant lymphoproliferative disorder (PTLD) is characterized by cytologically atypical immunoblastic or plasmacytoid infiltrates that infiltrate the parenchyma with a destructive expansive pattern. Necrosis, which is represented here in the lighter staining areas of the section, is commonly found in the more aggressive forms of PTLD.

B-cell hyperplasia/lymphoma, immunoblastic lymphoma) [81,107, 108]. The diagnosis of PTDL is more problematic in the earlier stages, particularly if there is a component of concurrent acute rejection. Evaluation of T-cell and B-cell markers (e.g. CD3, CD20) can aid in the evaluation of the infiltrates. Acute rejection is characterized by a predominant population of T cells (typically more than 75%) and a minor population of B cells that form aggregates no larger than a few hundred microns. In contrast, the identification of large confluent nodular B-cell aggregates are strongly suggestive of graft involvement by PTLD. The presence of marked cytological atypia, in infiltrates predominantly composed of immunoblastic and plasmacytoid cells, also favors a diagnosis of PTLD (Figure 85.7). Eosinophils are present in variable proportions both in acute rejection and in PTLD. Immunoglobulin light chain restriction is typically found in lymphoma [81,107,108]. EBV-related PTLD is confirmed with in situ hybridization for EBV-encoded RNAs (EBER) which marks a significant proportion of the atypical cells. Also, stain for LMP-1 (EBV latent membrane protein) is usually positive in a variable proportion of the cells. The random involvement seen in cases of PTLD with areas of parenchyma that may be completely free of infiltrates contrasts with the more diffuse involvement of the pancreas seen in the severe forms of cell-mediated acute rejection [5,81,107].

Acute antibody-mediated allograft rejection

There is a wide spectrum of histological changes in AMR, ranging from very mild inflammation to diffuse coagulation necrosis [43]. Intimal arteritis (endarteritis), a lesion that was considered typical of ACMR, is now recognized to also be associated with acute AMR [48,109,110].

C4d staining in >5% of interacinar capillaries is typically present in cases of acute AMR (Figure 85.3). C4d staining is characterized as focal (>5–50%) or diffuse (>50%), according to the extent of interacinar capillary staining in the lobular surface areas [11,13].

Box 85.1. Histological severity grading of acute antibody-mediated rejection (AMR) (see Banff grading schema in Box 85.2 for other diagnostic components)

Grade I, mild acute AMR
Well-preserved architecture, mild monocytic–macrophagic or mixed (monocytic–macrophagic/neutrophilic) infiltrates with rare acinar cell damage

Grade II, moderate acute AMR
Overall preservation of the architecture with interacinar monocytic–macrophagic or mixed (monocytic–macrophagic/neutrophilic) infiltrates, capillary dilatation, capillaritis, congestion, multicellular acinar cell dropout, extravasation of red blood cells

Grade III, severe acute AMR
Architectural disarray, scattered inflammatory infiltrates in a background of interstitial hemorrhage, multifocal and confluent parenchymal necrosis, arterial and venous wall necrosis and thrombosis

Table 85.2. Morphological features in acute T-cell-mediated rejection (ACMR) and antibody-mediated rejection (AMR)

	ACMR	AMR
Septal infiltrates	+++	− to +
Eosinophils	+ to +++	− to +
Neutrophils	− to ++	+/− to +++
T lymphocytes	++ to +++	+/− to +
Macrophages	++	++++
Venulitis	++	−
Ductitis	++	−
Acinar cell injury	+/− to ++	+++
Acinar inflammation	− to +++	+ to +++
Acinitis (mononuclear infiltrates within the basement membrane of individual acini)	+ to +++	− to +/−
Interacinar capillaritis	− to +/−	+ to +++
Intimal arteritis	+	+
Necrotizing vasculitis/thrombosis	− to +	+++
Confluent hemorrhagic necrosis	− to ++	+++

Adapted from [13] Drachenberg et al. *American Journal of Transplantation*. 2011;11:1792–1802, with permission from Wiley.

Inflammatory cell types in acute AMR

Mononuclear cells of monocyte–macrophage type constitute the most predominant cell type in the inflammatory infiltrates in acute AMR. Neutrophils are commonly also present and are proportional to the extent of tissue damage or necrosis. T lymphocytes are typically present, scattered throughout the parenchyma, but clearly represent a minority of the infiltrates. Rare eosinophils may be present (Box 85.1) [13,14,39,86,91].

Treatment implications

Histological grading of acute AMR (mild, moderate, or severe) is based on the extent of the interacinar infiltrates or capillaritis and tissue damage (Table 85.2) [13]. Severe acute AMR with diffuse vascular thrombosis and parenchymal necrosis is essentially untreatable [5]. Early, mild, or more indolent forms of acute AMR are potentially amenable to treatment but, as with other solid organs, results vary considerably. AMR in pancreas allografts continues to present a formidable therapeutic challenge [18].

Differential diagnosis of acute AMR

Negative or minimal pathological findings

Minimal early AMR can be very subtle and the biopsy may be erroneously considered "non-diagnostic" unless the sample is evaluated with a high degree of suspicion for identification of incipient

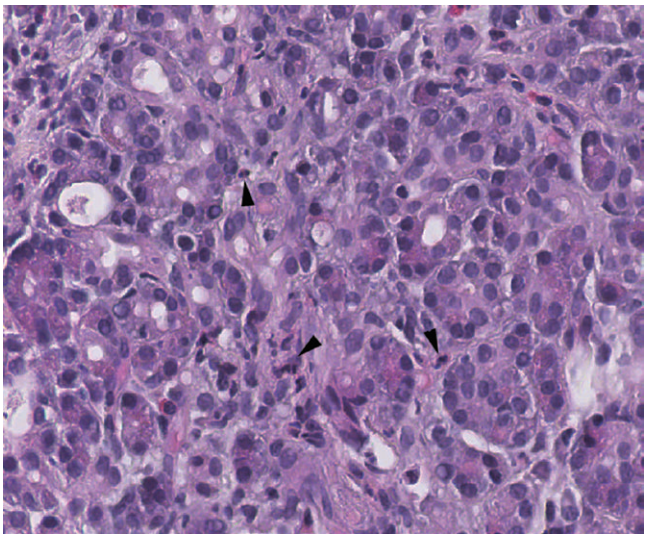


Figure 85.8. Mild ischemic pancreatitis is characterized by acinar cell injury (swelling, dropout of isolated or multiple cells) and mild neutrophilic (arrowheads) and macrophage infiltrates. This entity should be differentiated from acute AMR.

lobular infiltrates, accompanied by C4d staining and correlation with donor-specific antibody (DSA) status [13].

Recurrence of autoimmune diabetes [111], or other islet-related abnormality [83], should be considered in biopsies with minimal or absent inflammation, isolated insulitis, and particularly in patients with hyperglycemia [112].

Post-transplantation pancreatitis

AMR characterized by mixed lobular inflammation with or without microscopic or confluent hemorrhages, or cases with vascular thrombosis, require differentiation from ischemic pancreatitis. Critical evaluation of the samples is necessary for distinction of these entities [42].

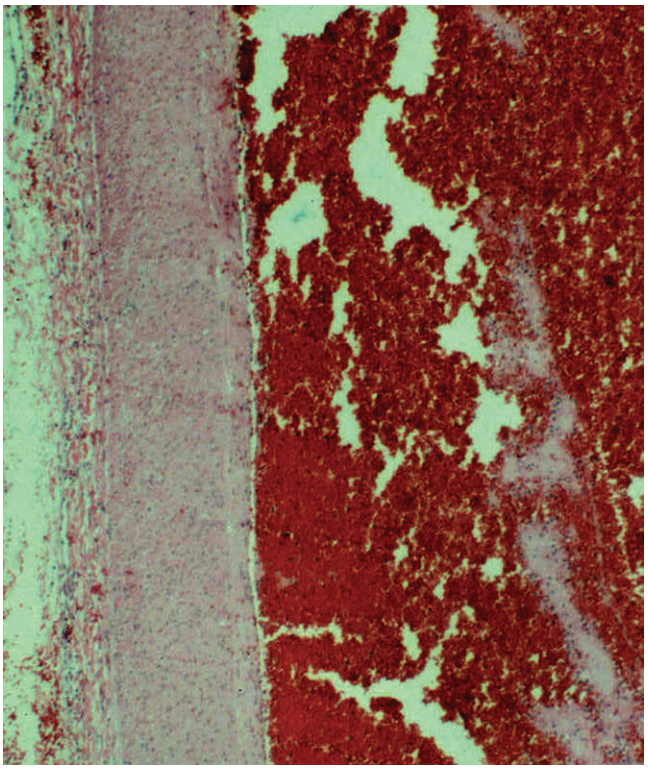
Mild ischemia–reperfusion injury is characterized by spotty acinar cell dropout, spotty apoptosis, flattening of the acinar cells, and otherwise minimal inflammation (Figure 85.8) [30,113,114]. In the absence of AMR, biopsies with ischemic pancreatitis fail to demonstrate C4d staining in interacinar capillaries. C4d stains often highlight necrotic cells and necrotic tissue in a non-specific manner but this is not diagnostic of AMR [13]. Another manifestation of ischemia is the presence of marked acinar and islet cell cytoplasmic swelling and vacuolization which should be distinguished from islet drug toxicity.

The incidence and severity of graft pancreatitis correlates with the length of cold ischemia time and is directly related to disturbances of the microcirculation in the reperfusion period [29,92,94,115].

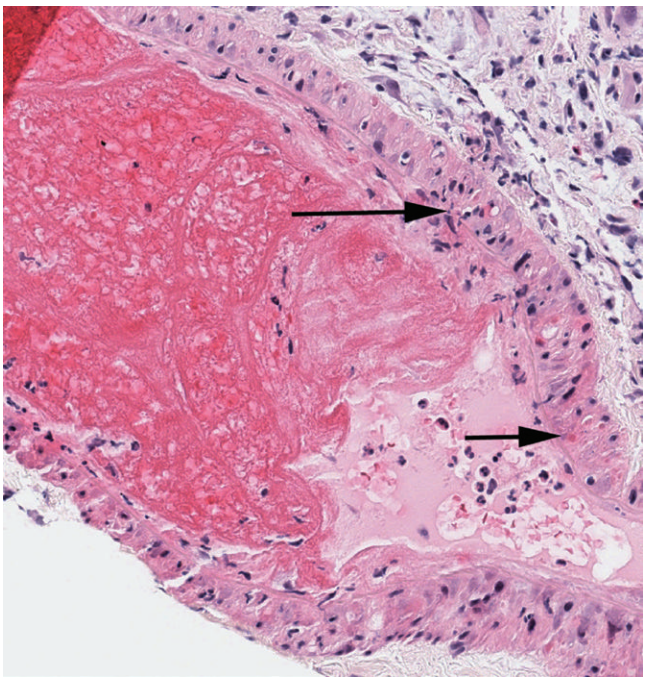
Graft thrombosis

Pancreas graft thrombosis occurs in different settings, including the following:

- *Early graft thrombosis in otherwise normal pancreas* (true technical failure) typically occurs within the first week post-transplantation [42]. In these grafts the only pathological changes consist of recent vascular thrombosis and bland ischemic parenchymal necrosis. There is no underlying vascular pathology or any other specific histological change (Figure 85.9A).



(A)



(B)

Figure 85.9. (A) Large vessel thrombosis due to "technical failure." The arterial wall is completely normal. (B) Arterial thrombosis in association with severe rejection. The arterial wall shows areas with loss of nuclei (necrosis, lower arrow) and intramural inflammatory infiltrates (upper arrow).

Perioperative inflammation and edema, as well as microvascular and endothelial damage relating to donor or procurement factors and organ preservation, all contribute to an increased incidence of early graft thrombosis [42,116].

- *Thrombosis in association to acute rejection* may occur at any time post-transplantation and is typically the result of acute or chronic vascular injury (endarteritis, transmural arteritis, transplant arteriopathy). Both AMR and ACMR can present with necrotizing arteritis (Figure 85.9B). Transmural inflammation and various degrees of intimal arteritis (endarteritis) are more common in ACMR. Systematic histological evaluation of failed grafts is necessary for accurate classification of the cause of graft loss, including generous sampling of large vessels, sections from the parenchyma to include an adequate number of medium-sized and small vessels and C4d staining [8,13,14,42,86]. High level panel reactive antibody (PRA) and circulating DSA were identified retrospectively in early cases of severe AMR with fulminant graft thrombosis [5,42].
- *Late graft thrombosis* is a recognized cause of late graft loss. This process is also associated with underlying vascular pathology that may be immune-mediated (acute or chronic active allograft rejection) or may be non-immune-related (e.g. atherosclerosis) [42,84].

Chronic allograft rejection and graft sclerosis in whole pancreas transplants

Despite excellent and prompt response to anti-rejection treatment, variable degrees of irreversible parenchymal fibrosis may develop after acute ACMR. Scarring is directly proportional to the extent of the inflammatory infiltration and to the severity of the acinar cell damage. Whereas pure septal inflammatory infiltrates (mild or Banff Grade I ACMR) may disappear without sequelae, confluent acinar inflammation and necrosis is invariably followed by secondary collagenization of the interacinar areas and eventual loss or disappearance of the exocrine component in the affected area [41].

Acute AMR refractory to treatment leads to graft failure in a short period of time (weeks or months), mostly due to widespread microvascular and large vessel obliteration and thrombosis. Thrombosis and ischemic necrosis predominate in severe acute AMR due to diffuse arterial and venous necrosis [43]. In more protracted forms of AMR, graft failure results from acute or chronic immunoglobulin and complement-induced smoldering microvascular injury and remodeling that eventually leads to graft fibrosis [13,117].

In general terms, chronic rejection is manifested histologically with progressive parenchymal sclerosis, defined by progressive fibrous expansion of the interlobular connective tissue septa and proportional degrees of atrophy of the acinar lobules. All exocrine tissue is eventually lost, with some areas becoming unrecognizable except for occasional residual islets embedded in the dense scar tissue. Disappearance of the acinar component forecasts progressive disappearance of islets as well, and eventual loss of glycemic control [41,118]. Narrowing of the arterial branches due to proliferative intimal endarteritis or transplant arteriopathy is also characteristic of the process [32,34,41,42].

In patients with long-term grafts immunosuppressed with calcineurin inhibitors (cyclosporine, tacrolimus), there may be widespread arteriolar hyalinosis, but it is not clearly established whether this contributes to the process of graft sclerosis [8]. Furthermore, in patients with generalized atherosclerotic disease, atheroemboli have been demonstrated to lodge in the pancreas allograft causing late pancreatitis and further ischemic compromise [119]. Criteria for staging of chronic rejection and graft fibrosis are described below (diagnostic category 6 of the Banff grading schema) (Box 85.2).

Duodenal graft pathology

In whole organ pancreas allografts, the exocrine drainage anastomosis is typically carried out through a segment of the graft duodenum [4]. A variety of pathological processes can affect the duodenal graft segment including technical problems (e.g. ischemia, anastomotic leak), CMV infection, and rejection.

Biopsy types

Cystoscopic duodenal biopsies were often performed in the 1990s when pancreas transplant exocrine secretions were routinely managed through bladder drainage. Significant experience was developed with the interpretation of these samples during that period [34,66,71,73,74,120,121]. Duodenal tissue can be also obtained fortuitously through the percutaneous route.

More recently, there has been renewed interest in the evaluation of duodenal samples from enteric-drained pancreas allografts with anastomosis to the proximal jejunum and obtained through upper gastrointestinal endoscopy. The technique for this type of enteroscopic graft biopsy is described in detail in the study of Margreiter et al. [75] who indicate that the duodenal cuff (area of duodenal-enteric anastomosis) was not accessible in 25% of cases; however, adequate duodenal material could be obtained in the remaining cases (76 out of 102 procedures, 75%). Microscopic pathology was identified in approximately one third of samples ranging from inflammation of undeterminate significance to severe rejection. CMV enteritis and *Giardia lamblia* infection were identified, one case of each. When the procedure was performed for suspected pancreas allograft rejection, the duodenal sample confirmed rejection in 65% of cases [75].

Pathological findings in the grafted duodenal cuff

Technical issues (duodenal leak): infections

Ischemia and poor surgical technique may lead to dehiscence of the anastomosis between the duodenal cuff and the urinary bladder or small intestine [94]. Pathological examination of grafts resected for this reason may show specific causes such as CMV-related perforation or may only show non-specific changes such as necrosis in the area around the anastomosis and acute inflammation. The adjacent peritoneal surfaces typically show acute fibrinous or purulent serositis [122].

Acute allograft rejection

From early experience with pancreas allografts drained externally (cutaneous graft duodenostomy) it was concluded that inflammation of the exposed segment of the graft duodenum correlated with acute rejection in the pancreas proper [123]. This was confirmed with earlier studies of duodenal and pancreatic samples which showed that acute rejection in the duodenum commonly occurred concurrently with pancreas rejection (72–100% concordance) [71,73]; but discrepant results with respect to rejection and even CMV infection could be observed in each of these organs [71,124].

Studies in dogs [125], pigs [126], and rodents [127] demonstrated a high degree of concordance between rejection in the duodenum and the pancreas but, interestingly, treatment response

Box 85.2. Banff pancreas allograft rejection grading schema, 2011 update diagnostic categories*

1. Normal
Absent inflammation **or** inactive septal, mononuclear inflammation not involving ducts, veins, arteries, or acini. There is no graft sclerosis. The fibrous component is limited to normal septa and its amount is proportional to the size of the enclosed structures (ducts and vessels). The acinar parenchyma shows no signs of atrophy or injury

2. Indeterminate
Septal inflammation that appears active but the overall features do not fulfill the criteria for mild cell-mediated acute rejection

3. Acute T-cell-mediated rejection‡
Grade I, mild acute T-cell-mediated rejection
Active septal inflammation (activated, blastic lymphocytes, ± eosinophils) involving septal structures: venulitis (subendothelial accumulation of inflammatory cells and endothelial damage in septal veins, ductitis (epithelial inflammation and damage of ducts)
and/or
Focal acinar inflammation. No more than two inflammatory foci† per lobule with absent or minimal acinar cell injury.
Grade II, moderate acute T-cell-mediated rejection (requires differentiation from AMR)
Multifocal (but not confluent or diffuse) acinar inflammation (≥3 foci† per lobule) with spotty (individual) acinar cell injury and dropout
and/or
Mild intimal arteritis (with minimal, <25% luminal compromise)
Grade III, severe acute T-cell-mediated rejection (requires differentiation from AMR)
Diffuse (widespread, extensive) acinar inflammation with focal or diffuse multicellular /confluent acinar cell necrosis
and/or
Moderate or severe intimal arteritis, >25% luminal compromise.
and/or
Transmural inflammation – necrotizing arteritis

4. Antibody-mediated rejection (see diagnostic components below*)
***Confirmed circulating donor-specific antibody**
***Morphological evidence of tissue injury (interacinar inflammation/capillaritis, acinar cell damage swelling/necrosis/apoptosis/dropout, vasculitis, thrombosis)**C4d positivity in interacinar capillaries (≥5% of acinar lobular surface)
- *Acute AMR:* three of three diagnostic components*
- *Consistent with acute AMR:* two of three diagnostic components*
- *Requires exclusion of AMR:* one of three diagnostic components*

See separate table for histological grading of acute AMR†

Chronic active antibody-mediated rejection
Combined features of categories 4* and 6 in the absence of features of category 3.

5. chronic allograft arteriopathy
Arterial intimal fibrosis with mononuclear cell infiltration in fibrosis.

6. Chronic allograft rejection/graft fibrosis
Stage I (mild graft fibrosis)
Expansion of fibrous septa; the fibrosis occupies less than 30% of the core surface but the acinar lobules have eroded irregular contours. The central lobular areas are normal
Stage II (moderate graft fibrosis)
The fibrosis occupies 30–60% of the core surface. The exocrine atrophy affects the majority of the lobules in their periphery (irregular contours) and in their central areas (thin fibrous strands criss-cross between individual acini)
Stage III (severe graft fibrosis)
The fibrotic areas predominate and occupy more than 60% of the core surface with only isolated areas of residual acinar tissue and/or islets present

7. Islet pathology
Recurrence of autoimmune diabetes mellitus
- Insulitis
- Selective β-cell loss
- Islet amyloid (amylin) deposition
- Islet cell drug toxicity

8. Other histological diagnosis
Pathological changes not considered to be due to acute and/or chronic rejection (e.g. CMV pancreatitis, PTLD)

AMR, antibody-mediated rejection; CMV cytomegalovirus; PTLD, post-transplantation lymphoproliferative disorder.
* Categories 2–8 may be diagnosed concurrently and should be listed in the diagnosis in the order of their clinicopathological significance.
† Histological grading of acute AMR (see Box 85.1).
‡ Histological features of stereotypical ACMR and AMR (see Table 85.2).
See Box 85.3 for morphological definition of specific lesions and Table 85.3 for other histological diagnosis.

occurred promptly in the duodenum but not in the pancreas in rodents [127].

Criteria for grading allograft rejection in the duodenal cuff mucosa have not been defined. In general terms, mild acute duodenal rejection is manifested by an increase in the inflammatory cells in the lamina propria, including neutrophils, with mild villous blunting and apoptosis of epithelial cells (Figure 85.10). In severe rejection there is confluent epithelial necrosis with total loss of the epithelial lining. Necrosis can extend to all layers, including the muscularis propria. Arterial involvement (e.g. endarteritis, necrotizing arteritis), which is diagnostic of rejection in the pancreas, may be seen also in the duodenum [71]. A case of duodenal perforation caused by acute rejection has been reported [128].

Chronic rejection and graft fibrosis

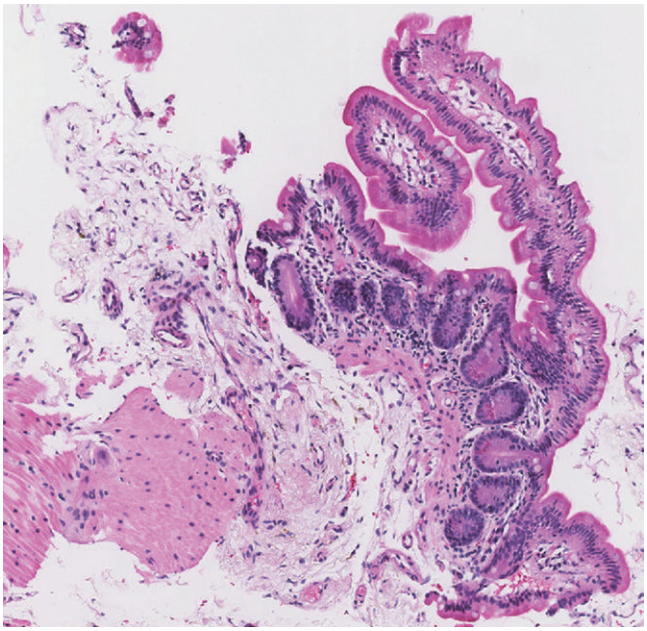
Examination of failed pancreas allografts with advanced chronic rejection and fibrosis consistently demonstrate similar sclerotic changes in the duodenal cuff (Figure 85.11). The latter typically shows marked flattening, atrophy, and attenuation of the duodenal mucosa, which may be reduced to the thickness of a few layers of reactive epithelial cells. The deeper muscular and connective tissue layers are irregularly replaced by fibrotic tissue and often contain scattered inflammatory infiltrates similar to those found in the pancreatic parenchyma itself.

The specific features of acute and chronic allograft rejection in the duodenal cuff are poorly defined. Systematic studies of the features of ACMR and AMR in the grafted duodenum are necessary to definitely determine if this type of tissue can be used as a reliable surrogate of the pancreatic parenchyma itself. Current studies in this area are underway as part of the Banff 2013 pancreas task groups [129].

Banff pancreas allograft rejection: Banff working grading schema (2011 update, Box 85.2)

Diagnostic categories: specific considerations

The Banff schema includes eight diagnostic categories that cover the range of histopathological changes that can occur in pancreas allografts. Similar to other transplanted organs, two main forms of allograft rejection are recognized: T-cell mediated and antibody-mediated [13,14,22,23,45]. For each rejection type, acute and chronic histological manifestations are identified and criteria



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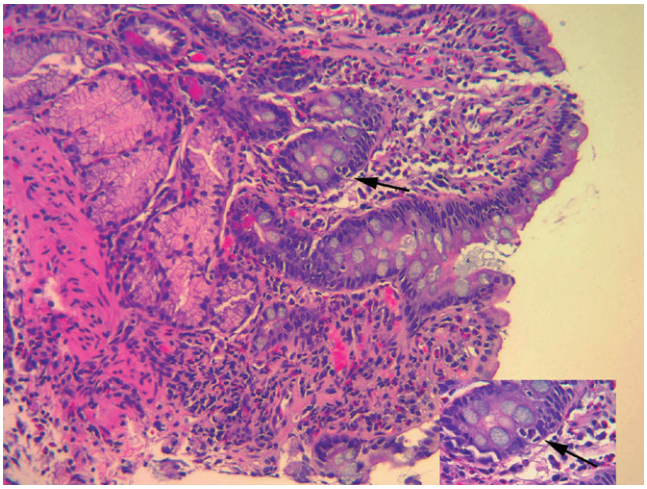


(B)

Figure 85.10. (A) Fragment of duodenal mucosa from the grafted duodenal cuff in a patient with normal graft function. The duodenal mucosa has preservation of the intestinal villi and a normal amount of infiltrates in the lamina propria. There is no evidence of acute rejection. (B) Mild acute rejection in duodenal cuff mucosa shows blunted intestinal villi, increase in the inflammatory cells in the lamina propria including numerous eosinophils, and occasional apoptosis of enterocytes (arrows, insert).

defining severity grades are presented. The specific histological features utilized in the pancreas Banff schema are presented in Box 85.3.

Normal

Inflammatory infiltrates are absent, or very sparse, inactive, mononuclear (small lymphocytes, rare plasma cells). If there is slight inflammation, this is focal and confined to the septa with lack of involvement of any of the septal structures (vessels, ducts, nerves) (Figure 85.2).

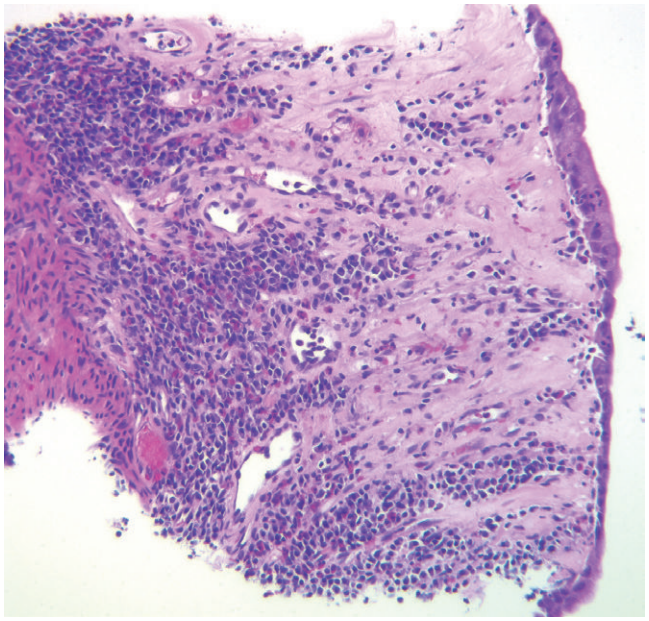


Figure 85.11. Advanced chronic rejection of the duodenal cuff segment. There is complete flattening of the intestinal villi, marked fibrosis and sclerosis of the lamina propria, and chronic inflammatory infiltrates with large numbers of plasma cells.

An adequate biopsy with these histological characteristics practically rules out a diagnosis of ACMR. Accordingly, these types of findings are more often encountered in protocol biopsies of well-functioning grafts [65,90,130]. "Normal"-appearing biopsies may also be encountered under other clinical circumstances. Specifically, in patients biopsied for hyperglycemia, the differential diagnosis includes:

1 Late phase of recurrent autoimmune disease (i.e. after resolution of isletitis) [111];
2 Drug toxicity, characterized by vacuolization or damage of islet cells [83]; and
3 Early or mild AMR [51].

Indeterminate for rejection

This category is defined by the presence of focal septal inflammation that displays features of activation (blastic changes ± eosinophils), but the overall features do not fulfill the criteria for mild rejection (e.g. partial cuffing of a septal vein or duct but lacking any evidence of endothelial or epithelial involvement) (Figure 85.12).

These histological features can be seen in protocol biopsies of well-functioning grafts as well as in patients biopsied for graft dysfunction. Similar to the "borderline" category in the kidney, these changes may represent early as well as treated acute rejection, or alternatively may be entirely non-specific [90,130].

The treatment of patients with biopsies showing "indeterminate" features may vary depending on the indication for biopsy, and ultimately depends on clinical judgment. In accordance with the heterogeneous nature of the "indeterminate" histological changes, response to treatment varies significantly in comparison to biopsies with definite acute cell-mediated rejection which are usually responsive to treatment [90].

Box 85.3 Histological definition of lesions used for the diagnosis of rejection

Acinar cell injury-necrosis. Cytoplasmic swelling and vacuolization and/or nuclear pyknosis, apoptotic bodies, lytic necrosis leaving empty spaces equaling the size of individual cells (cell dropout)
Acinar inflammation. Inflammatory infiltrates with characteristics similar to the septal infiltrates amidst the exocrine acini
Acinar inflammatory lesion/focus. Collection of ≥10 lymphocytes/eosinophils within an acinar area
"Active" transplant arteriopathy. Narrowing of the arterial lumen by a subendothelial proliferation of fibroblasts, myofibroblasts, and smooth muscle cells with infiltration of the subintimal fibrous proliferation by mononuclear cells (T cells and macrophages).
Capillaritis. Neutrophil and mononuclear cell margination in dilated interacinar and islet capillaries
C4d semiquantitative grading. Diffuse positive, ≥50% of interacinar capillaries; focal positive, 5–50% of interacinar capillaries; minimal positive/negative, <5% of interacinar capillaries
Ductitis. Infiltration of ductal epithelium by mononuclear and/or eosinophillic inflammatory infiltrates and ductal epithelial cell damage. May lead to epithelial denudation
Focal acinar inflammation. ≤2 inflammatory foci per lobule with no evidence of acinar cell injury
Minimal intimal arteritis. Rare, occasional, clearly defined subendothelial (intimal) inflammatory infiltration by mononuclear cells
Moderate–severe intimal arteritis: Easily identifiable (more than six to eight) lymphocytes within the intima of an involved muscular artery with some evidence of intimal injury (i.e. endothelial cell hypertrophy, fibrin leakage, coating neutrophils and/or macrophages, activation of intimal myofibroblasts)
Multicellular/confluent acinar cell injury-necrosis: Acinar cell damage/apoptosis involving multiple acinar cells (clusters)
Multifocal acinar inflammation: ≥3 foci of inflammation per lobule with single/isolated acinar cell injury/necrosis. Intervening uninflamed acinar areas
Necrotizing arteritis: Focal or circumferential fibrinoid necrosis of the arterial wall with or without transmural inflammation
Neural and perineural inflammation: Septal inflammatory infiltrates in and around nerve branches (rare finding in needle biopsies)
Septal inflammatory infiltrates: Predominantly mononuclear, including "blastic" (activated) lymphocytes and variable numbers of eosinophils. Eosinophils may be the predominant cell type
Severe/extensive acinar inflammation: Confluent diffuse (widespread) acinar inflammation with focal or diffuse multicellular/confluent acinar cell injury-necrosis. None or very rare uninflamed acinar areas
Single cell/spotty acinar cell injury-necrosis: Only isolated cells are affected, with the vast majority of cells appearing preserved
Transplant arteriopathy: Fibrointimal arterial thickening with narrowing of the lumen. Grading is carried out in the most affected artery as mild, up to 25% of luminal area; moderate, 26–50% of luminal area and severe, >50% of luminal area
Venulitis: Circumferential cuffing of septal veins with subendothelial accumulation of inflammatory cells and endothelial damage/lifting

Adapted from [14] Drachenberg et al. *American Journal of Transplant*. 2008;8:1237 with permission from Wiley.

Acute T-cell-mediated rejection

ACMR is graded as mild, moderate, or severe (Grades I, II and III, respectively), based on the identification of lesions that have been shown to prognosticate progressively worse outcomes [5,24,32,33,36–40,131]. Diagnosis of acute ACMR presupposes the concurrent evaluation of C4d stain to rule out a component of AMR [13,14].

Mild, ACMR Grade I

This grade is defined by the presence of septal inflammatory infiltrates that have not only features of activation ("blastic" lymphocytes, variable numbers of eosinophils), but also involve septal structures (veins, ducts) ± focal acinar inflammation. These findings may vary from septal area to area; however, any degree of venulitis (subendothelial accumulation of inflammatory cells and endothelial damage in septal veins) or ductitis (epithelial inflammation and damage of pancreatic ducts) is sufficient for the diagnosis of mild Grade I T-cell-mediated rejection (Figure 85.13). Inflammation of peripheral nerve branches coursing through the parenchyma is also a feature of rejection.

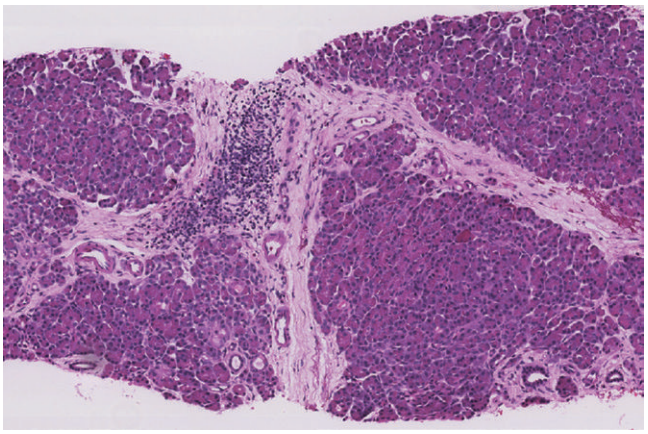


Figure 85.12. Banff grade indeterminate for rejection. The image demonstrates a single focus of mononuclear septal inflammation which does not involve acini, arteries, or ducts.

Focal acinar inflammation in biopsies with the features described above (mild, Grade I), is not uncommon, and is typically seen in the interface between the septal connective tissue and the acinar lobules (e.g. periphery of the exocrine areas).

The mild cell-mediated rejection, Grade I, also includes cases in which due to sampling, septal involvement is not present and only focal acinar inflammation is appreciated.

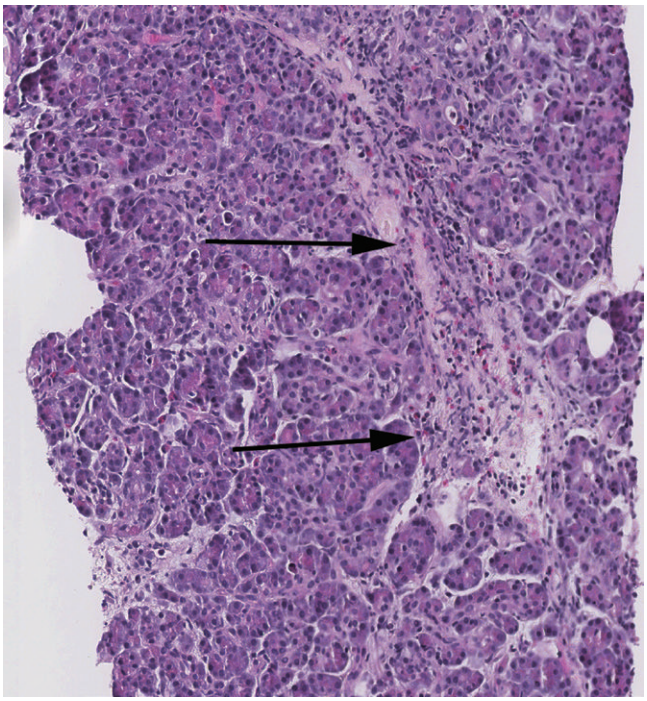
In any case, in mild, Grade I rejection, the acinar inflammation should be clearly focal (i.e. no more than two inflammatory foci per lobule and should be lacking evidence of acinar cell injury – apoptosis, necrosis). The composition of the acinar inflammation is typically similar to that of the septal infiltrates.

Biopsies with the features defining this rejection grade are occasionally found in patients with well-functioning grafts [130] but are more commonly seen in biopsies performed for graft dysfunction (typically, increase in amylase/lipase in serum, or decrease in urinary amylase in bladder-drained grafts) [12,36,65].

Moderate, ACMR Grade II

Typically found in association with the septal infiltrates described above, this grade is defined by two additional histological features which may be identified either in isolation or concurrently.

Multifocal acinar inflammation. The most common presentation of this grade consists of multiple foci (≥3 foci per lobule) of acinar inflammation and associated spotty (individual) acinar cell injury and dropout. The acinar inflammatory involvement in this grade is identified with ease; however, the involvement should not be confluent or diffuse. From a practical point of view, completely uninflamed acinar or exocrine areas are easily identified between the inflamed foci. Absence of confluent inflammation will differentiate this grade from the next higher category (see later). Significant



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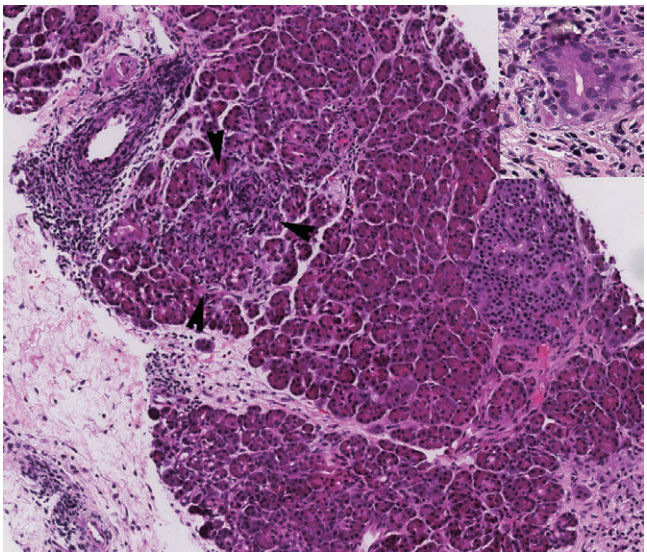
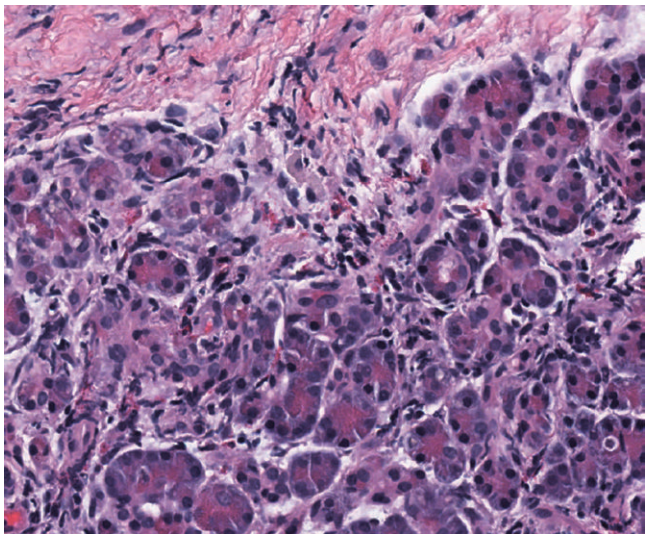


(B)

Figure 85.13. (A,B) Mild ACMR (Banff Grade I). Septal inflammation and edema (long arrows), with venulitis and acinar inflammation (arrowheads) characterize ACMR. The insert demonstrates ductitis, also a common feature of ACMR.



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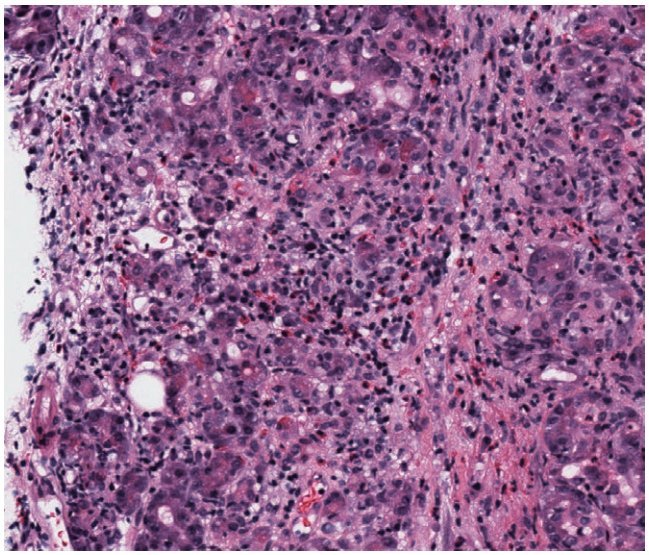


(B)

Figure 85.14. (A) Acinar inflammation (acinitis) and acinar cell injury. (A) demonstrates the edge of an exocrine lobule with acinar inflammation. Although distortion of the acinar arrangement by the inflammatory infiltrates can be appreciated, there is only minimal acinar cell injury. (B) demonstrates extensive diffuse acinar inflammation associated with multifocal areas of acinar injury and loss (cytoplasmic swelling and clearing, spotty cellular dropout appearing as empty spaces).

acinar inflammation is always associated with evidence of acinar cell injury, but in this grade the latter should be spotty (isolated) (Figure 85.14). Specifically, acinar cell injury may appear as any of the following: cellular dropout (empty spaces equaling the size of individual cells), cytoplasmic swelling and vacuolization, nuclear pyknosis, apoptotic bodies, or single cell lytic necrosis.

Minimal intimal arteritis. Alternatively, depending on sampling variations, the category of moderate, ACMR Grade II, can be defined by the sole presence of mild focal intimal arteritis consisting of rare, occasional but clearly identifiable subendothelial (intimal) mononuclear cells (Figure 85.15). The latter changes may or may not be accompanied by the complete constellation of inflammatory changes described earlier in the septa and lobules.

From a clinical point of view, biopsies with features of moderate ACMR Grade II are typically found in patients with graft dysfunction (usually increase in amylase or lipase in serum or decrease in urinary amylase in bladder-drained grafts) [12,36,65].

Severe, ACMR Grade III

This grade can be defined by three histological features which may be identified ether in isolation or concurrently. These morphologi-

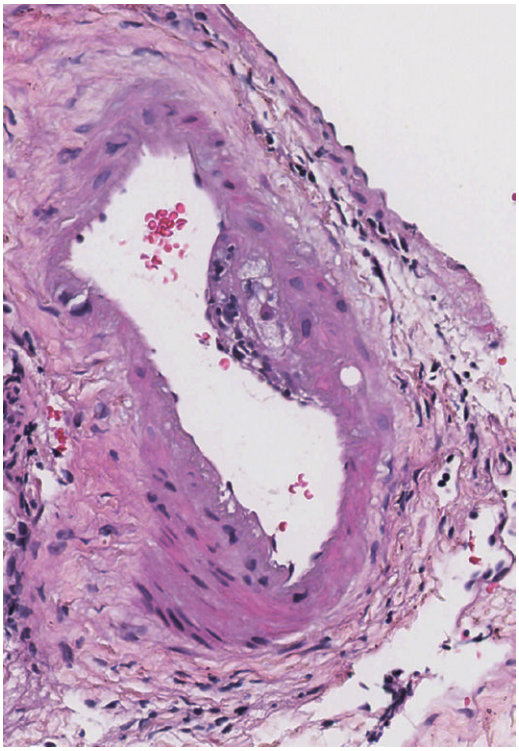


Figure 85.15. A small focus of intimal arteritis (endarteritis) can define moderate ACMR (Banff Grade II, see Banff grading schema). A focal accumulation of foamy macrophages beneath the focus of endarteritis signal the likely development of transplant arteriopathy, particularly if there is persistent vascular inflammation.

cal features also require differentiation from cases of moderate and severe AMR. The latter typically presents with more pronounced interstitial hemorrhage and focal or widespread vascular thrombosis.

Severe acinar inflammation and acinar cell damage. This entity is characterized by confluent or diffuse (widespread, extensive) acinar inflammation with associated focal or diffuse multicellular or confluent acinar cell necrosis (Figure 85.14B). The inflammation may be predominantly lymphoid or may contain abundant eosinophils or variable amounts of neutrophils. By definition, there should be none or only rare focal areas of completely uninflamed acinar or exocrine parenchyma, and C4d staining should be negative.

Moderate or severe intimal arteritis. This feature, defined as easily identifiable (e.g. more than six to eight) lymphocytes within the intima of an involved muscular artery, is by itself sufficient to justify a diagnosis of severe, ACMR Grade III. Moderate or severe intimal arteritis is usually associated with some evidence of intimal injury (i.e. endothelial cell hypertrophy, fibrin leakage, coating neutrophils and/or macrophages, activation of intimal myofibroblasts).

Necrotizing arteritis. Transmural arterial inflammation leading to complete or partial circumferential necrosis also defines severe ACMR (Figure 85.9B). Transmural fibrinoid arterial necrosis is, however, more often associated with AMR. C4d staining and search for DSA is therefore necessary to rule out AMR if necrotizing arteritis is identified.

Each of the three lesions used to define severe ACMR portend poor outcome to the graft, because they are associated with, or lead to, irreversible parenchymal damage. The short- and long-term impact to the organ will depend on the extent of acinar damage and the size and number of arteries affected by intimal arteritis or necrosis. Similar to other solid organ transplants, intimal arteritis is associated with an increased risk of immediate or delayed thrombosis (Figure 85.9B). This lesion is also a precursor of transplant arteriopathy [42,132].

Biopsies with histological findings corresponding to this category are characteristically associated with graft dysfunction and failure, often including hyperglycemia [37,65,90].

Acute antibody-mediated rejection

In recent years, awareness of the possibility of AMR in the pancreas has led to better recognition of this process [56,133–135]. Based on our current knowledge of AMR, the diagnostic criteria defined by the Banff schema rely on the combination of three elements as described in Box 85.2: confirmed circulating DSA, morphological evidence of tissue injury, and C4d positivity in interacinar capillaries (IAC). It is worth noting that a significant proportion of cases of AMR may present with an incomplete constellation of findings. This may relate to our inability to demonstrate DSA (i.e. non-HLA antibodies) or to the lack of sufficient microvascular complement deposition in the graft (i.e. C4d-negative AMR).

Therefore, the presence of two of the three diagnostic elements leads to a diagnosis of "suspicious" for acute AMR, and prompt initiation of treatment for acute AMR is the rule, particularly if the patient has graft dysfunction.

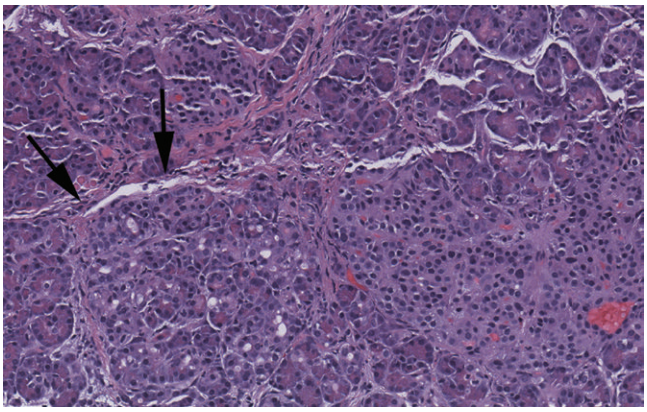
The morphological findings in acute AMR consist of various degrees of inflammation and tissue injury as detailed below.

Acinar or interacinar inflammation is inconspicuous in the milder forms of acute AMR and is accompanied by subtle spotty acinar cell dropout or apoptosis (Figure 85.16).

The identification of acinar cell injury in an otherwise bland-appearing biopsy should alert the pathologist to the possibility of subtle interacinar inflammation or capillaritis, and warrants correlation with the C4d staining and DSA studies. More pronounced inflammation leads to the recognition of *interacinar capillaritis* consisting of marginating and intraluminal inflammatory infiltrates in dilated and congested IAC (Figure 85.17). Interacinar capillaritis is morphologically and, presumably, pathogenetically similar to peritubular capillaritis in renal allografts, but in comparison to the renal peritubular capillaries, the pancreatic IACs have a less predictable distribution and are relatively sparse. In fully developed acute AMR there is extensive microvascular injury leading to focal or confluent interstitial hemorrhage, edema, and multicellular cell necrosis of interstitial and acinar cells making the identification of interacinar capillaritis more difficult. Identification of interacinar infiltrates with associated interacinar capillaritis has been found to be strongly associated with C4d positivity and detection of DSA [11,56].

Very severe or advanced forms of acute AMR have morphological features approaching those found in hyperacute rejection. These findings consist of widespread vascular necrosis and thrombosis in small or larger vessels and small or confluent foci of parenchymal necrosis (Figure 85.18).

Acute AMR is graded histologically (mild, moderate, or severe) based on the extent of the interacinar infiltrates or capillaritis and tissue damage (Box 85.1).



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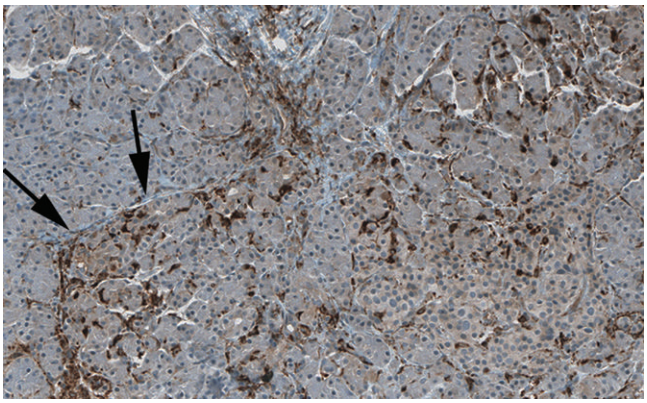


(B)

Figure 85.16. Mild acute AMR. (A) On hematoxylin and eosin stain there is only mild acinar inflammation; however, acinar cell injury is prominent focally (arrows). (B) CD68 stain demonstrates significant increase in macrophages in multiple areas of the biopsy with more pronounced accumulation in the area with acinar cell injury (arrows).

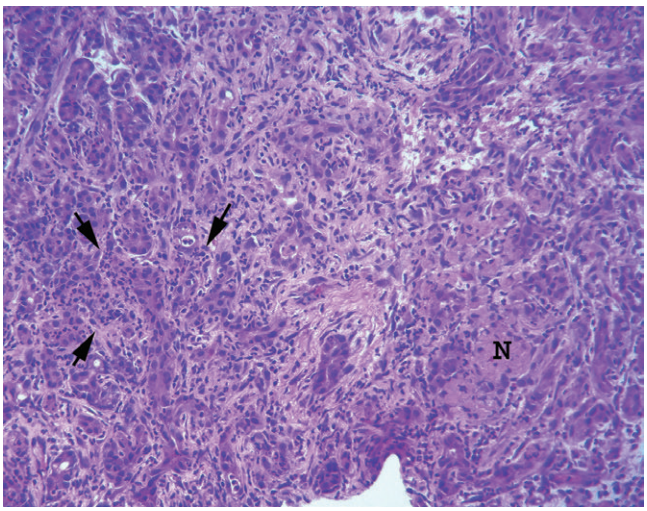
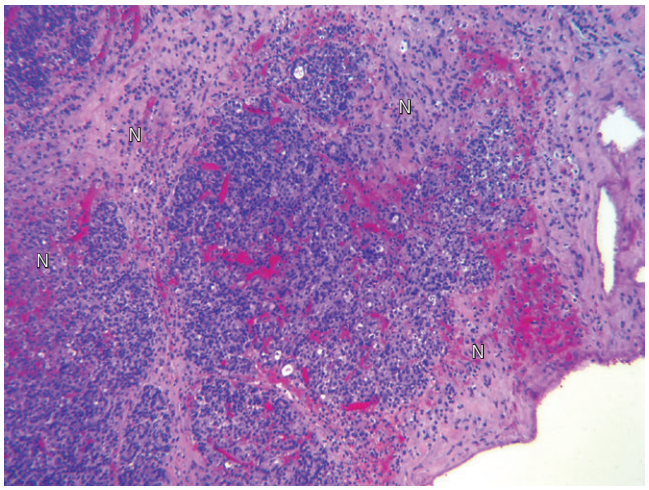


Figure 85.17. A case of moderate acute AMR shows abundant interacinar inflammatory infiltrates including neutrophils (arrows). There is a focus of necrosis (N).



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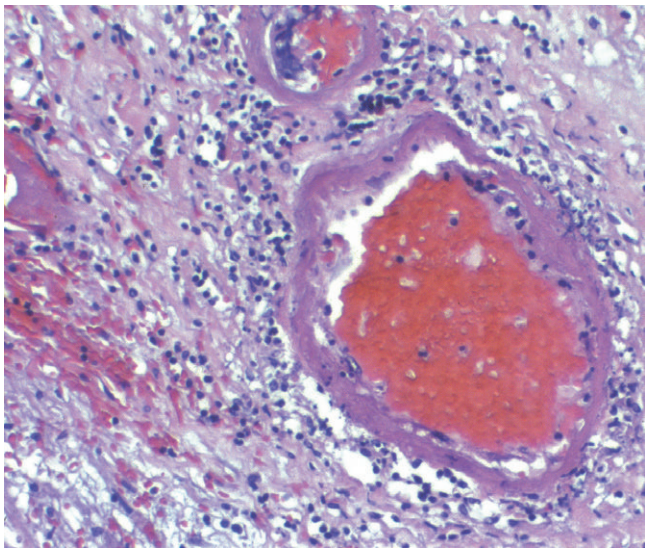


(B)

Figure 85.18. (A) Moderate to severe acute AMR is defined by more pronounced tissue damage secondary to microvascular injury. In addition to patchy areas of severe congestion and extravasation of red blood cells, there are geographic areas of parenchymal necrosis (N). (B) Severe fulminant acute AMR has features overlapping with hyperacute rejection. The image demonstrates fibrinoid necrosis of all vascular walls.

Chronic active AMR

The histological diagnosis of chronic active AMR is based on the following triad:

1 Features of acute AMR as described in the previous section (also see Box 85.2 AMR diagnostic components);
2 Absence of features of ACMR; and
3 Underlying graft fibrosis (Banff diagnostic categories 5 and/or 6).

The utilization of this diagnostic category presupposes that the main cause of graft fibrosis is ongoing AMR, and therefore requires that other causes of graft fibrosis or sclerosis are ruled out, such as previous episodes of ACMR. In clinical practice this conclusion would be most accurate when serial biopsies are available for evaluation.

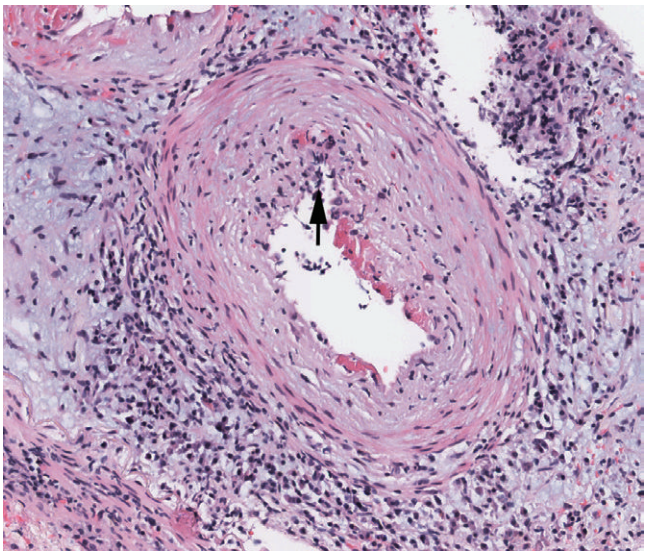
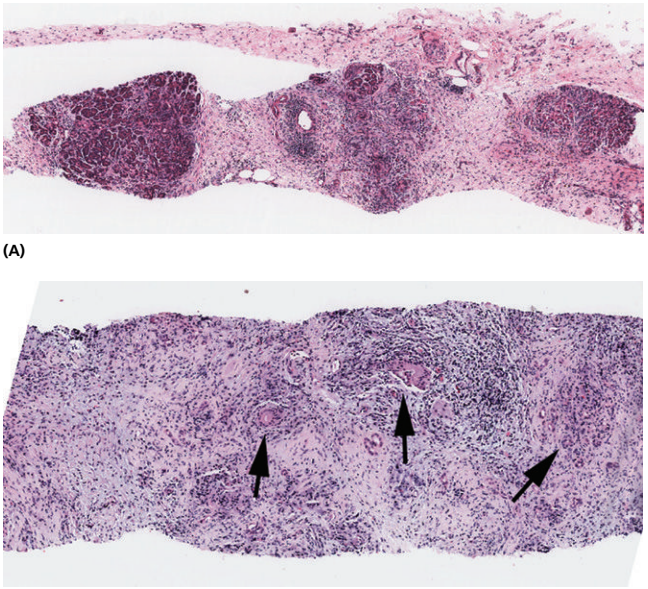


Figure 85.19. Active transplant arteriopathy characterized by a narrowed arterial lumen due to subendothelial proliferation of spindle cells (fibrosis and sclerosis). In addition, the area of fibrosis is infiltrated by mononuclear inflammatory cells (and occasional eosinophils). There is a focus of endarteritis (arrow) and small thrombi adhering to the right and lower left areas of endothelium.



(A)

(B)

Figure 85.20. Chronic allograft rejection and graft fibrosis. There is pronounced architectural distortion due to marked increase in connective tissue and corresponding loss of exocrine (acinar) parenchyma. (A) Three atrophic lobules are recognized, separated by markedly expanded fibrous septa. (B) The parenchyma has been completely replaced by connective tissue. Rare ductal and acinar structures can be recognized (arrows). Note that in both images prominent inflammatory infiltrates remain in the connective tissue indicating ongoing rejection (acute on chronic T-cell-mediated rejection).

Chronic allograft arteriopathy

This diagnosis is defined by the presence of active transplant arteriopathy, which consists of narrowing of the arterial lumen by a subendothelial proliferation of fibroblasts, myofibroblasts, and smooth muscle cells with superimposed evidence of ongoing inflammatory activity (typically T cells and macrophages) (Figure 85.19). Chronic allograft arteriopathy was initially considered to be an expression of T-cell-mediated allograft rejection [14,22,23,45], but recent studies have shown that acute and chronic arterial lesions can also be associated with DSA and AMR [48,109,110]. Accordingly, this lesion is listed as a separate morphological category (independent from ACMR and AMR).

Although rarely seen in needle biopsies due to sampling issues, this lesion is consistently present in pancreatectomies from failed grafts due to chronic rejection [14,41,42].

Recognition of chronic allograft arteriopathy in biopsy samples is clinically important because it indicates ongoing (chronic) alloimmune injury and for its association with late graft thrombosis [42].

Chronic allograft rejection and graft fibrosis

Graft fibrosis is staged in pancreas biopsies according to the proportion of fibrosis replacing the lobular component as mild stage I, moderate stage II, and severe stage III ($\leq$30%, $\geq$30% but $<$60%, and $>$60%, respectively) (Box 85.2; Figure 85.20) [14,41].

Islet pathology

As a result of the increasing acknowledgment of the importance of islet integrity for long-term graft outcome, a separate category has been created in the 2011 Banff schema update for lesions specifically involving these structures [13]. The main purpose of this category is the recognition of recurrent autoimmune diabetes mellitus (characterized by insulitis (isletitis) and/or selective β-cell loss), deposition of islet amyloid (amylin) and islet cell drug toxicity (see discussion later).

The impact of AMR in pancreatic islets remains unclear. Whereas hyperglycemia was documented in early reports of AMR [51], this was a rare indication for biopsy in subsequent larger studies [11,47,55,56]. C4d staining in islet capillary endothelium was found in one fifth of samples from patients with DSA but this did not correlate with hyperglycemia [11]. Hyperglycemia typically develops in severe necrotizing AMR (as well as severe ACMR), correlating with the extent of parenchymal necrosis.

Other histological diagnosis

Acute rejection episodes have become less common under current immunosuppressive protocols and a variety of other pathological processes can be encountered in pancreas biopsies from patients with graft dysfunction. These entities can be identified in isolation or concurrently with other diagnostic categories in the schema (Table 85.3).

Histopathological syndromes of islet dysfunction

Non-specific islet pathology in whole pancreas allografts

The vast majority of pancreas allograft biopsies contain one or more islets of Langerhans (95%) [136], even if these structures are distributed unevenly throughout the normal pancreas parenchyma.

Table 85.3. Other histologic diagnosis: Banff diagnostic category 8.

Diagnosis presentation	Main histological findings	Clinical
Post-transplant ischemic pancreatitis	Inflammation: neutrophils, foamy macrophages Location: septal if mild or diffuse if severe Other features: fat necrosis, edema and interstitial hemorrhage. Patchy coagulation necrosis of clusters of acinar cells may be present. No fibrosis, the septa may be expanded due to edema/fat necrosis	Increase in amylase and lipase in serum Decrease in urinary amylase* Hyperglycemia if there is extensive necrosis
Peripancreatitis/ peripancreatic fluid collection	Inflammation: mixed (lymphocytes, plasma cells, eosinophils, neutrophils) Location: septa and periphery of lobules Other features: dissecting bundles of active fibroblastic proliferation with obliteration of septal structures, relative preservation of the center of lobules ("cirrhotic appearance")	Local or systemic infectious symptoms, abdominal pain, peritonitis. Peripancreatic fluid accumulation.Increase in amylase and lipase in serum
Cytomegalovirus pancreatitis	Inflammation: mostly mononuclear Location: septal and acinar, patchy Other features: cytomegalovirus cytopathic changes in acinar, endothelial, or stromal cells	Increase in serum amylase and lipase Decrease in urinary amylase* Systemic symptoms if generalized disease Other: duodenal cuff perforation
Post-transplant lymphoproliferative disorder	Inflammation: ranging from polymorphic with lymphoblasts, plasma cells, eosinophils in low grade disease, to monomorphic, predominantly lymphoid in high grade disease (lymphoma). Other features: lymphoid proliferation is nodular, expansive. Necrosis may be present	Asymptomatic, or increase in serum amylase and lipase. Lymphadenopathy Tumor mass May co-exist with acute rejection
Bacterial or fungal infection	Inflammation: variable; acute, chronic, purulent, necrotizing (abscess), granulomatous Location: random Other features: same as bacterial and fungal infections in other organs	Systemic and/or localized infectious symptoms. Peritonitis, duodenal cuff perforation. Increase in serum amylase and lipase

* In bladder-drained grafts.

There is no experimental or clinical evidence that in WPnTx the endocrine islets are specifically targeted in alloimmune rejection reactions. It appears that islet pathology in acute and chronic allograft rejection is in essence non-specific, secondary to the overall degree of acute parenchymal injury and ensuing fibrosis or sclerosis. In these entities, the pathological findings consist of islet inflammation and occasional necrosis that involve the islets at a degree proportional to the severity of acute rejection. The inflammation is therefore random and the cells infiltrating the islets are similar to the surrounding inflammatory infiltrates, more or less representing a "spillover" phenomenon [5,33,37,39,136,137].

In long-term pancreas allografts, islet distribution and morphology to a large extent reflects ongoing injury and repair. Extensive collagenization in chronic rejection leads to fragmentation of exocrine lobules, as well as of islets that acquire irregular shapes and may consist of only a few cell clusters with irregular distribution, but still containing a mixture of both insulin and glucagon-producing cells. Conversely, in some grafts, aggregates of large but otherwise normal-appearing islets may reflect compensatory hyperplasia [136,137]. Islet cell atypia, hyperchromasia, is seen in less than 5% of cases independently of the cause of the biopsy and is of unknown etiology or significance [136].

Nesidioblastosis, defined by the identification of insulin-producing cells in adult pancreatic ductal epithelium, appears to represent aberrant differentiation and is most likely regenerative in nature. Nesidioblastosis was demonstrated in an animal model of pancreas transplantation [138] and was found in approximately 4% of samples, with or without evidence of graft rejection in needle biopsies. The process had no discernible clinical significance [136,137]. Marked nesidioblastosis in a pancreas allograft was associated with hypoglycemia in one patient [139].

Histological studies in islet transplantation

Obtaining diagnostic graft samples has proven to be difficult in the case of IT because the infused donor islets (typically weighing 5–10 g in aggregate) are distributed throughout the recipient's whole liver, which has an average weight of 1.5 kg. Toso et al. [140] studied morphological changes in islets and adjacent liver tissue in percutaneous liver core biopsies and autopsy samples from 16 and 2 patients, respectively. Islets were present in 31% of liver core biopsies, with the likelihood of their identification being in direct relationship to the size of the biopsy. As expected, multiple foci of graft islets were visualized in liver tissue collected at the time of autopsy. The adjacent liver parenchyma often showed localized steatosis [140]. Overall, the transplanted islets had similar cellular composition to native pancreatic islets and only minimal inflammation (insulitis) was seen in a few cases independently of the clinical presentation at the time of the biopsy [140]. Similar lack of immunological damage (either allo- or autoimmune) and normal islets of insulin-producing cells were found in liver tissue obtained at the time of autopsy from one patient with two IT, each of which failed 6 months after transplantation [141]. C4d staining has been consistently negative in the samples evaluated histologically [21], although a significant proportion of IT recipients develop DSA [140,141].

The scarcity of available morphological studies highlights the limited scope of our understanding of the causes of IT failure. It is speculated that islet loss may result from metabolic or microenvironmental stresses that may be related to localized islet ischemia, toxic injuries, failure of islets to regenerate, etc. A concurrent low-grade immunologic rejection reaction is likely, but its true impact is unclear [140–142]. Non-invasive techniques to monitor IT are currently being developed [143,144].

Post-transplant diabetes mellitus

Post-transplant diabetes mellitus (PTDM) can develop after an otherwise functional and technically successful pancreas transplantation, presumably resulting from excessive insulin requirements that are beyond what is produced by even a perfectly functioning WPnTx [145]. Although PTDM is more commonly found in patients with solitary pancreas and has also been associated with higher rates of rejection, systematic clinicopathological studies are not available and the understanding of the mechanisms leading to PTDM remains poor [145,146].

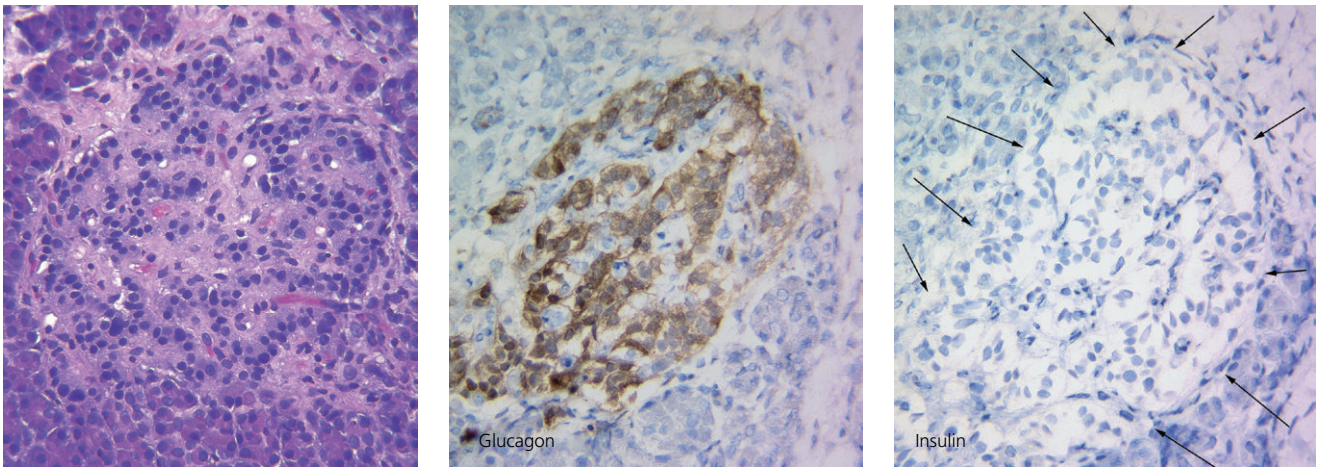


Figure 85.21. Recurrence of type 1 diabetes mellitus. (A) Hematoxylin and eosin image demonstrating an islet of Langerhans from a failed whole pancreas transplant. On this stain the islet lacks any specific pathological features and although minimally represented, the adjacent exocrine parenchyma is within normal limits (i.e. lacks features of chronic rejection and/or graft fibrosis that could explain the graft failure). (B) Glucagon stain demonstrates numerous glucagon-roducing cells (α cells). (C) Insulin stain demonstrates complete absence of β cells.

In a study of a large cohort of pancreas transplant recipients, the incidence of PTDM was 14%, 17%, and 25% at 3, 5, and 10 years post-transplantation, respectively [146]. Factors associated with increased risk for developing PTDM were older donor age, a solitary pancreas transplant ($P = 0.01$), higher recipient body mass index (BMI), donor-positive recipient-negative CMV status, post-transplant weight gain, higher 6-month fasting glucose, HBA_{1C} and triglyceride to high-density lipoprotein ratio, and higher pretransplant BMI and high pretransplant insulin needs [145,146].

Recurrence of autoimmune type 1 diabetes mellitus

This process is defined by the development of autoimmunity with destruction of β cells, loss of insulin secretion, and return to insulin dependence after a period of insulin independence had been achieved with a successful WPntx or an IT. Recurrence of type 1 diabetes occurs despite immunosuppression and regardless of HLA matching [77,111].

Fundamental clinical and pathological features required for the diagnosis of recurrent type 1 diabetes include: selective loss of insulin secretion in the absence of clinical or pathological features of rejection, histological evidence of insulitis and/or selective loss of β cells; and persistence or reappearance of autoantibodies (i.e. glutamic acid decarboxylase (GAD)) prior to the recurrence [77]. Vendrame et al. [147] showed clear evidence of recurrent autoimmune diabetes mellitus in three patients by demonstrating reappearance of autoreactive T cells, associated with documented insulitis and destruction of the β-cell population [147].

It is estimated that at least 5% of WPnTx recipients develop recurrent type 1 diabetes; however, accurate diagnosis of type 1 diabetes recurrence requires exhaustive prospective follow-up, which is not done routinely, certainly leading to under-recognition of this process [77,137].

The cardinal histological features of recurrent type 1 diabetes are insulitis (or isletitis) and selective β-cell loss (Figure 85.21). These two processes occur sequentially in individual islets but are not temporally uniform throughout the pancreatic parenchyma. Insulitis is characterized by selective peri-islet and intra-islet lymphocytic infiltrates that conspicuously do not involve other areas of the parenchyma (Figure 85.22). The distribution of the inflammatory involvement is opposite to that characterizing ACMR. Progression of recurrence of type 1 diabetes leads to selective destruction of the β cells, with eventual clearing of the inflammation in the affected islet when complete disappearance of β cells has occurred. If only evaluated with routine stains, macroscopic and microscopic examination of failed grafts lost to recurrence of type 1 diabetes are remarkably normal architecturally, both with respect to the exocrine and the endocrine components. Immunohistochemical stains

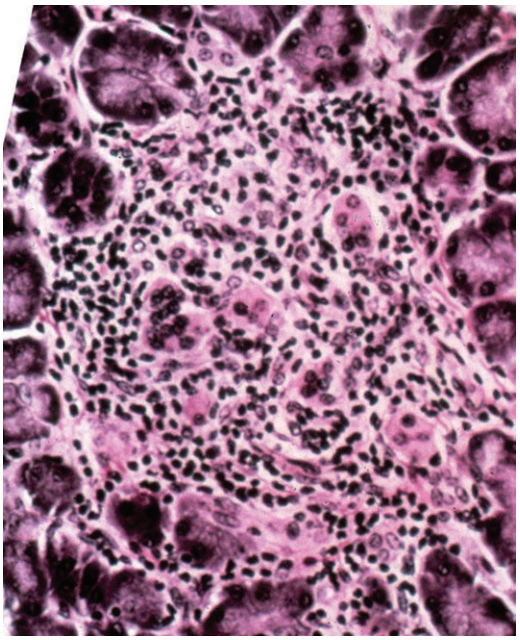


Figure 85.22. Early stages of recurrent type 1 diabetes mellitus is characterized by selective islet and peri-islet lymphocytic inflammation (insulitis).

for insulin and glucagon, however, demonstrate the characteristic selective loss of β cells [77,111,147,148]. Because the insulitis lesions are transient, actual documentation of the inflammatory stage is rare. Such a case was reported by Ishida-Oku et al. [149], who described a patient with documented seroconversion to positive anti-GAD antibody concurrent with abrupt development of hyperglycemia 48 months after transplantation. On tissue samples, loss of β cells was noted with associated insulitis and peri-insulitis. The inflammatory cells consisted predominantly of T lymphocytes and macrophages [149].

Islet amyloid

Islet deposition of amylin (also known as islet amyloid polypeptide (IAPP)) appearing as amorphous Congo red positive material, is increasingly recognized as a pathological process involving transplanted islets. Amyloid deposition in the native islets of a large proportion of patients with type 2 diabetes has been known to occur for many decades, but its role in the development of the disease is unknown [82].

In normal circumstances, IAPP, a protein normally co-secreted with insulin by β cells, is present in the halo region of the β-cell secretory granules together with C peptide and other substances [82]. Under pathological circumstances, and particularly in hyperglycemic states (e.g. type 2 diabetes mellitus), excessive or abnormal IAPP forms insoluble aggregates with the conformational characteristics of amyloid. Accumulation of IAPP amyloid results in islet cell injury and decreased numbers of insulin-producing cells. The mechanisms leading to IAPP aggregation are unknown, but it is possible that abnormalities in the molar ratio between IAPP and other secretory products contribute to the amyloidogenic condition. Deposition of amylin in otherwise normal islets of pancreas allografts is usually associated with loss of glycemic control (hyperglycemia) [19,82,150,151].

Widespread deposition of IAPP was found in the islets of a patient who died of myocardial infarction 5 years after the first of three intraportal islet infusions [150]. Exhaustive studies of liver samples from the autopsies of patients who had previously received IT showed amyloid deposition in the islets of three of four patients, further indicating the possibility that amyloid deposition has a role in the long-term fate of transplanted islets [19]. In rare instances, amyloid deposition has also been seen in the islets of WPnTx (Figure 85.23).

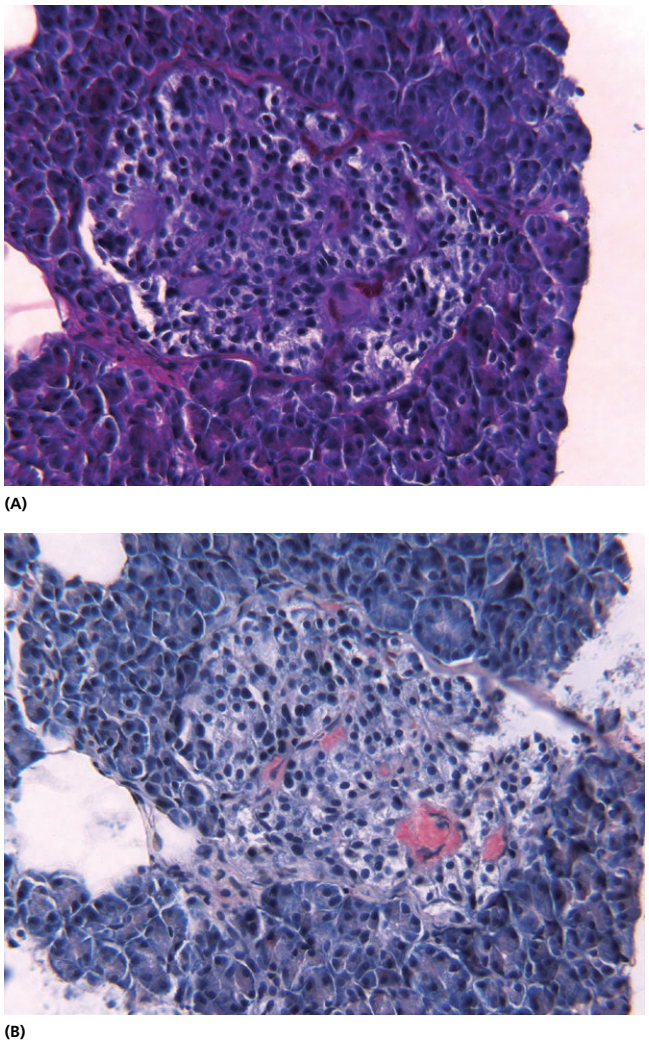


Figure 85.23. Deposition of islet amyloid in a whole pancreas transplant demonstrated by hematoxylin and eosin stain (A) and confirmed by positive staining of the extracellular accumulation with the Congo red stain (B).

Islet cell drug toxicity

In animal studies, cyclosporine administration has been associated with reduction in insulin secretion, diminished β-cell density, decreased insulin synthesis, and defective insulin secretion. Similar morphological findings have been seen also with tacrolimus [152].

The morphological findings in pancreas biopsies from patients with clinical evidence of calcineurin inhibitor drug toxicity consist of cytoplasmic swelling and vacuolization of islet cells. The islets appear optically clear and stand out from the more eosinophilic acinar parenchyma. In more severe cases, islet cell dropout with formation of empty spaces (lacunae) can be seen if there is confluent islet cell dropout. Rarely, intra-islet apoptotic cell fragments can be identified [83].

Immunoperoxidase stains for insulin and glucagon show diminished staining for insulin in β cells in comparison with controls. This is the light microscopic counterpart of the marked loss of insulin-dense core granules seen in β cells by electron microscopy. The histological changes as well as the clinical findings are reversible with reduction or discontinuation of the drug. Hyperglycemia and the histological evidence of drug toxicity are worsened with the concurrent use of pulse steroids to treat acute rejection [83].

Viral infections and pancreatic islets

The role of CMV infection in the development of PTDM and recurrence of type 1 diabetes is unclear but a statistical association between PTDM donor-positive recipient-negative CMV status has been described [146]. Additional discussions of CMV and other viral infections can be found in Chapter 94. There is clear evidence from a rodent model that β cells are susceptible to CMV infection and can sustain ongoing low levels of viral gene expression [153]. After the infection, β-cell immunogenicity is markedly enhanced and there is increased cellular expression of immune cell-activating ligands [20,153,154].

Thus, it is possible that CMV infection can induce changes that may impact β-cell survival as well as development of rejection or recurrent diabetes in WPnTx or IT. From the clinical point of view, it has been observed that recurrent CMV infections were associated

with recurrence of humoral and cellular autoimmunity to islet autoantigens in one pancreas transplant recipient [155].

Recent studies have demonstrated that rotavirus, through molecular mimicry between viral epitopes and pancreatic islet autoantigens, may induce islet cell destruction by autoreactive T cells. Thus, this virus is being implicated as a potential cause of type 1 diabetes [156]. The potential relationship between viral infections and shortened survival of transplanted islets in WPnTx and IT should be better investigated.

Conclusions

Pancreas and islet allografts are subject to numerous alloimmune, autoimmune, inflammatory, metabolic, and technical maladies, and accurate systematic histopathological diagnosis is important in aiding the clinician in determining the proper course of action. Numerous approaches to obtaining tissue have been developed although, in general, tissue acquisition is more difficult in pancreas (and particularly in islet) transplantation than other forms of allotransplantation. Nevertheless, the clinician should seek definitive diagnosis and partner with the pathologist in the care of patients following these forms of β-cell replacement.

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CHAPTER 86

Histopathological Syndromes of Intestinal Allograft Rejection and Recurrent Disease

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Introduction

Maturation of the field of intestinal (ITx) and multivisceral transplantation (MVTx) has been a welcome advance in the treatment of short gut syndrome (SGS) and intractable gastrointestinal compromise secondary to catastrophic abdominal compartment injury, medical disease, and certain tumors [1–4]. The core failure for ITx and MVTx recipients is a loss of the absorptive function of the intestines, ultimately resulting in malnourishment of the patient. Some of these conditions are listed in Table 86.1 and covered in more detail in Chapters 33 and 117. Although there are special considerations beforehand, both pediatric and adult patients can receive alimentary tract grafts [5], and the disorders compromising gut function are often noticeably different according to the age group of the patient (Table 86.1). The majority of these patients are maintained by parenteral feeding prior to transplantation. Medical management of SGS has had an enhancement comparable to the option of allograft transplantation [6], although chronic parenteral feeding can encumber the patient with life-threatening complications, high costs, and altered quality-of-life issues. In contrast, the successful implementation of an ITx or MVTx allows the recipient to regain a functioning gastrointestinal tract devoid of, or with minimal, dietary restrictions, enhanced growth and development, and the prospect of an improved and independent lifestyle [7].

The surgical procedure of an intestinal allograft varies according to the underlying conditions compromising gut function and presented by the transplant candidate. The intestinal allograft can be provided as an isolated graft (ITx) or as part of an MVTx; the latter is an abdominal organ bloc that can include stomach, small and large intestine, liver, pancreas, and spleen [8,9]. (Note: the omission of liver transplant is known as a modified multivisceral transplant.) Technical details of the procedures can be found in Chapters 62 and 117. The implementation of an isolated intestine or multivisceral graft depends upon the underlying pathological conditions in the recipient. In recent years, the use of MVTx has increased, with graft and patient survival being comparable to isolated ITx [9].

The experience of experimental large and small animal models utilizing orthotopic and heterotopic gut transplantation has contributed to the advancement of procedures and therapies in human grafts [10,11]. As such, multiple issues with ITx and MVTx have notably improved including the pretransplant evaluation and selection of appropriate donors and recipients, surgical mechanics and techniques, exponential growth of our knowledge regarding gut physiology and mucosal immunology, and overall post-transplant maintenance, thus contributing to an observed improvement in graft and recipient survival. A critical member of the monitoring team is the transplant pathologist, a recognized subspecialist who optimally should incorporate anatomic pathology, clinical laboratory, and basic scientific aspects to the solid organ transplant or bone marrow transplant program that he/she is supporting. Thus, the transplant pathologist in gastrointestinal transplantation is a vital component of the clinical team's vigilance of post-transplant complications via coordination of biopsy evaluation, general and specialized clinical laboratory analysis, and comprehension of pathophysiological mechanisms associated with allograft intestine or the other transplanted abdominal viscera [12].

In spite of these advancements, the intestinal graft introduces a massive alloantigenic hematolymphoid and parenchymal cellular mass to the recipient that typically initiates a vigorous host-effector immune response, requiring continuous modulation and interruption by powerful and sustained immunosuppressive reagents. While induction and maintenance immunosuppressive regimens evolve and novel targeted pharmaceutical compounds and antibody-based immunosuppressive reagents are being developed [13], the host-derived immune response [14], and sometimes the graft immune response to the host (i.e. graft-versus-host disease (GVHD) [15], remain significant complications of ITx and MVTx recipients. The level and type of immunosuppression is not the only variable impairing the deleterious effects of the host alloimmune response, because a quiescent immune system (under "appropriate" immunosuppression coverage) can also be the result of many variables such as the type of host innate genetic polymorphisms present that may influence the level of host responsiveness [16], the ratio of effector to regulatory cell populations [17], and the degree and type of genetic disparity between the host and donor. Regardless, intestinal and multivisceral graft recipients more often than not demonstrate immune-based acute rejection episodes that are clinically demonstrable or that may be "subclinical" in severity [18,19]. This is supported by data showing that unlike the present state of other solid organ transplants, in ITx and MVTx acute rejection remains the principal complication in the post-transplant period.

As with other patients undergoing vigorous immunosuppression, the introduction of unrelenting modulation of the immune response places the ITx or MVTx transplant recipient at elevated

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Table 86.1. Anatomic and functional causes of intestinal failure in patients receiving intestinal or multivisceral transplants

Pediatric	Adult
Gastroschisis	Ischemia (venous or arterial infarction)
Midgut volvulus	Crohn's disease
Necrotizing enterocolitis	Trauma
Pseudo-obstruction	Volvulus
Intestinal atresia	Motility disorders
Aganglionosis/Hirschsprung	Desmoids
Retransplant	Retransplant
Microvillous inclusion disease	Radiation enteritis
Malabsorption	Other tumors
	Gardner's syndrome

Box 86.1. Complications and pathological processes in intestinal and multivisceral transplant patients

1 Donor organ pathology and preservation injury
2 Acute rejection
  a. Antibody-mediated rejection (AMR)
    i. Hyperacute and accelerated acute rejection
    ii. Acute antibody-mediated rejection (AAMR)
  b. Acute T-cell-mediated (cellular) rejection (ACR)*
    i. No evidence of acute rejection, Grade 0
    ii. Indeterminate for acute rejection, Grade IND
    iii. Acute T-cell-mediated acute rejection (ACR), mild, Grade 1
    iv. Acute T-cell-mediated acute rejection (ACR), moderate, Grade 2
    v. Acute T-cell-mediated acute rejection (ACR), severe, Grade 3
  c. ACR in colon and stomach
  d. "Mixed" acute rejection – concomitant ACR and AAMR
3 Chronic rejection
4 Infections
  a. Viruses (e.g. rotavirus, adenovirus, calicivirus, cytomegalovirus (CMV), herpes simplex virus (HSV), and Epstein–Barr virus (EBV))
  b. Miscellaneous (e.g. bacteria, fungi, *Cryptosporidium*, mycobacteria)
5 Recurrent disease
  a. Inflammatory bowel disease, tumors
6 Other entities
  a. Persistent ulcers of undetermined etiology
  b. Active enteritis of undetermined etiology
  c. Eosinophilic syndromes of undetermined etiology
7 Regenerative changes
8 Graft-versus-host disease (GVHD) in native tissue
9 Post-transplant lymphoproliferative disease (PTLD)

* Grading of ACR performed based on [60] Ruiz et al. *Transplantation Proceedings*. 2004;36:335–337.

risk for the development of infections and malignancies [20]. Moreover, the drugs themselves often impart direct toxicities on several organ systems (e.g. renal, neurological) (see Chapters 94 and 95) [21,22]. Aside from complications associable with immune system perturbation, ITx and MVTx recipients are also susceptible to other complications that may be related to pre- and post-transplant alimentation protocols, former surgeries to the abdominal region, or co-morbidities associated with the underlying disease(s) of the host. A list of the complications in the post-transplant period is present in Box 86.1.

The continuous post-transplant scrutinizing of early and late intestinal graft function and patient status by the ITx and MVTx surgical and medical teams has been transformed and enhanced over the past quarter century. An evaluation of MVTx requires that the transplant pathologist understand histopathological changes of native organs and surgical allografts not typically encountered (e.g. stomach, colon) and an awareness of the likelihood of pathological changes depending upon which area is sampled (e.g. duodenal versus ileal). For example, monitoring of an MVTx patient could involve simultaneous procurement of biopsies from native tissues such as esophagus, rectum, skin, as well as biopsies from transplanted stomach, small intestine, large intestine, and liver. A comparison of native versus allograft tissues is a worthwhile tool in many circumstances such as trying to discern whether changes are unique to alloimmune responses (e.g. rejection), generalized pathologies (e.g. infection, post-transplant lymphoproliferative disease (PTLD), or graft-derived changes (GVHD, preservation injury).

Histopathological evaluation

Pathological evaluation of allografts by routine histology involves all stages of ITx and MVTx pretransplant phases and continues through the evaluation of long-term surviving grafts. Histological assessment by biopsies remains the gold standard for evaluating pathological processes in allografts; however, ancillary testing of tissue and concomitant systemic biomarker evaluation is the norm in evaluating these complex processes. ITx and MVTx biopsies must be correlated with concurrent recipient medical laboratory values. The transplant pathologist requires the clinical history of the recipient (e.g. date of transplant, native disease or condition that necessitated the transplantation, current clinical symptoms), previous biopsy results, and the endoscopic results. A description of where the biopsies were procured (e.g. lesional, perilesional, or non-involved areas) is critical. A preponderance of ITx and MVTx allograft tissue samples are endoscopically visualized and superficial mucosal biopsies are obtained. Typically, tissue obtained from an exteriorized ostomy (e.g. ileostomy or colostomy) stoma is discouraged because there may be ongoing inflammatory responses related to surgical wound healing, etc. reflected by histological inflammatory processes, fibrosis, and distorted architecture [23].

As the level of changes provided to the pathologist is typically limited to the mucosa and submucosal layers of the gastrointestinal site, it is critical that the endoscopist have some experience with gastrointestinal transplants in order to determine areas of interest and which regions to biopsy. The endoscopist and pathologist should have ongoing communication regarding the endoscopic changes seen in the mucosa, including normal versus abnormal; if abnormal, the lesion(s) should be characterized as to the graft location and distribution, its limitation to the graft or involvement of native tissue, and its characteristics (e.g. erythematous mucosa, ulcers). Full-thickness evaluations of the gastrointestinal allograft are not the norm and tend to occur when there is surgical revision of problematic areas or when grafts are explanted.

Most of the complications affecting gastrointestinal transplants demonstrate clinical urgency and could rapidly lead to allograft dysfunction and potential graft loss. Hence, the histology laboratory and pathology service providing transplant pathology support must operate to provide particularly rapid turnaround time 7 days a week, with the capacity to incorporate specialized immunohistochemical (IHC), immunofluorescence (IF), and in situ hybridization (ISH) techniques. Moreover, the modern laboratory should also serve as a launching point for initiation of (or performance of) graft tissue-derived molecular assays such as quantitative infectious agent evaluations and specific gene arrays.

One or two gastrointestinal transplant tissue fragments per area should be placed immediately in an appropriate fixative (typically,

buffered formalin) for at least 1 hour. Multiple sections are typically cut at 0.5 cm, and hematoxylin and eosin (H&E) stains are used for the initial evaluation. ITx or MVTx biopsies typically require a variety of special stains; the following should be available: IHC (e.g. for infectious agents such as cytomegalovirus (CMV), adenovirus); ISH for viruses such as Epstein–Barr virus (EBV); viral load measurements (e.g. of EBV), and other molecular assays such as measurement of antigen receptor rearrangement studies for T and B cells (when evaluating the possibility of PTLD). Finally, the transplant pathologist should also have access to, and ideally be involved in, the interpretation of other critical laboratory tests for ITx and MVTx patients such as citrulline levels, immune function assays (e.g. Cylex, Elispot, cytokines), human leukocyte antigen (HLA) typing of donor and recipient, and alloantibody measurements. Because of the evolving nature of MVTx and ITx management, specialized gene testing (e.g. *MBL-2* or *NOD* polymorphisms) and other assays of certain potential biomarkers are currently being evaluated and appear to hold promise as useful adjuncts to the histopathological and clinical presentation of the patient.

Pathological processes

A variety of complications associated with the process of transplantation can befall the ITx or MVTx recipient. As listed in Box 86.1, many of these complications can occur concomitantly, thus requiring their specific identification as therapies may differ or have a negative impact on resolving the principal issue (e.g. therapy for PTLD or infection versus acute rejection differ dramatically).

Donor organ pathology and preservation injury

Successful modern-day procurement of intestinal or multivisceral organs for use as transplants relies on a sophisticated, highly coordinated system whereby potential donors are identified based on an algorithm that incorporates various factors including size (e.g. size of the recipient abdominal cavity; pediatric age), donor clinical history suitability, and an elimination of most transmissible risk factors [24]. The testing laboratory must rapidly provide a comprehensive battery of tests for measuring potential infectious pathogens in the proposed donor, and the clinical team must try to identify the status of the donor organs for issues such as ischemic injury, possible tumors, and ongoing medical conditions that could compromise gut function. Typically, gross inspection of the donor organs by the surgeon retrieving the organs suffices to assess any possible lesions; rarely, frozen section evaluation by a pathologist is needed for ITx or MVTx. Therefore, unlike other allografts such as kidney and liver in which pretransplant biopsies are obtained when there is a questionable clinical history or the organ appears grossly suspicious, it is not usual for the pathologist to be asked to evaluate a donor bowel or stomach prior to being transplanted. Pathological evaluation of residual donor tissue has typically not shown any significant pathological changes aside from minimal ischemic injury, as would be expected from graft preservation.

The majority of transplanted organs undergo varying periods of preservation at cold temperature (depending upon the organ transplant) in solutions followed by warm reperfusion of the grafts upon vascular anastomosis with the recipient blood supply. Gastrointestinal grafts tend not to have much "cold ischemic" time. Regardless, some degree of preservation-associated or ischemia–reperfusion (IR) injury to the allograft often results from this revascularization [25]. IR injury can occur in the gut [26] and cause a cascade of physiological changes that may influence function. In the large majority of the cases, these IR changes are mild and have no great consequence to the organ's short-term function, although there may be significant effects on the allograft organ's eventual susceptibility to immune-mediated injury and physiological function. Evidence is mounting that non-inflamed cadaveric organs have remarkable changes in many genes at the transcriptional level, including genes associated with inflammation [27,28]. The implication of these transcriptional changes in donor bowel grafts remains unknown and there can only be speculation as to whether these changes in the procured organs could impact long-term graft function; no pathognomonic changes are yet known in long-standing allografts that had early IR injury. The use of "marginal" donors [29], while not currently a major issue in ITx or MVTx, would be supposed to predispose the recipient to IR injury, primary nonfunction or delayed graft function, and complications such as rejection.

Morphology. The majority of ITx and MVTx transplants have "protocol" biopsies, particularly in the initial weeks post-transplant. As such, it is not unusual for mucosal biopsies obtained in the early post-transplant period to demonstrate preservation injury changes [30]. Small and large intestine grafts experiencing mild preservation injury can demonstrate diffuse edema and swelling of the villi without a significant increase in the inflammatory cell infiltrate, some vascular congestion, and a separation of the surface epithelial lining from the underlying lamina propria (Figure 86.1). As IR injury progresses and becomes more severe, there are additional changes such as epithelial cell necrosis which can extend from the surface of the mucosa to the deep submucosa. These latter changes have been best described in the controlled environment of experimental models of IR injury; for example, several murine models show gradable and progressive changes from the mucosa which eventually progress to transmural necrosis of the intestine [31,32]. These latter changes are not typically apparent microscopically in a clinical situation unless there is severe IR injury along with prolonged vascular compromise, in which case the allograft tissues

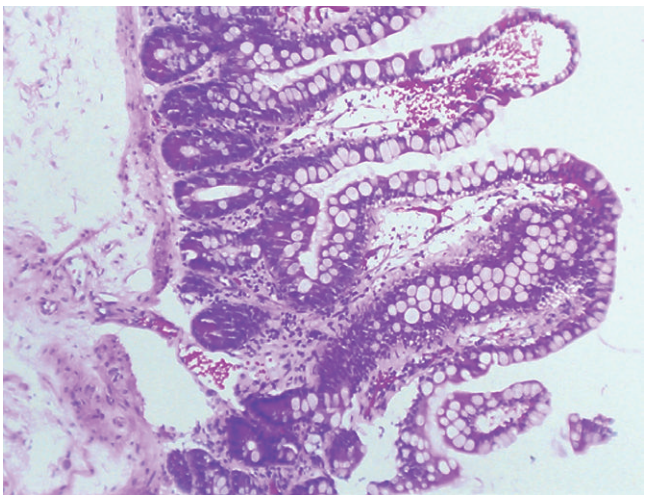

Figure 86.1. Preservation injury. Small intestinal biopsy several days after transplant shows swelling of the villi without a significant increase in the inflammatory cell infiltrate, some vascular congestion, and a separation of the surface epithelial lining from the underlying lamina propria (H&E, original magnification 200×).

show ischemic changes similar to bowel ischemia in native organs. Currently, the morphology of stomach allografts undergoing IR injury is not well described.

Acute rejection

The initiation and propagation of recipient alloimmune effector cell populations (cell-mediated or T-cell rejection), host B-cell-derived alloantibodies (humoral or antibody-mediated rejection), in conjunction with non-specific innate immune mechanisms can constitute an onslaught of anti-graft inflammatory responses that is defined as acute rejection [17]. The fundamental biology of these conditions is described in detail in Chapters 5, 6 and 7. This potent assemblage of immune-based cell populations and soluble molecules can act in concert or individually to manifest different forms of injury that have classically been delineated morphologically and sometimes behaviorally as distinct forms of acute rejection. As a result of the enormous potential for acute rejection to reduce graft function and lead to organ loss, the detection and effective interruption of acute rejection in ITx and MVTx recipients, as with other solid organ transplants, remains a continuous challenge and goal for transplant clinicians. However, in spite of the application of powerful immunosuppressive drugs, allograft acute rejection remains a common complication in gastrointestinal transplantation. The broad mechanisms involved in T-cell-mediated and antibody-based forms of acute rejection are similar to other organ systems and beyond the scope of this chapter. Basically, recipient-derived B and T cells (which represent that adaptive immune response), in a complex interplay with innate immune populations including natural killer cells, dendritic cells, and macrophages, and an assortment of immunomodulatory molecules [33], centrally participate in the genesis and culmination of immunological pathways that recognize the allogeneic gastrointestinal tissue. There are multiple potential stimulatory targets in gastrointestinal tissue for immune effector cells and molecules, including parenchymal or epithelial cells (e.g. enterocytes, Paneth cells), vascular endothelial cells, muscle, endocrine, and neural cells. Acute rejection-mediated injury or death of these various target cell populations in bowel leads to clinical consequences such as fever, malabsorption, dysmotility, and ischemia [7,24,34]. Acute rejection of the bowel can progress rapidly because of the marked antigenicity and huge cellular mass of the bowel and in some circumstances can lead to exfoliation of the mucosa and submucosa [35,36], transmural ischemia, and predisposition to translocation of luminal bacteria [37,38], the latter scenario evolving to sepsis. It is therefore of vital importance to diagnose acute rejection in small bowel and MVTx promptly and precisely in order to avoid the menacing clinical consequences of rejection continuing unabated in the host.

Clinical and endoscopic correlation is critical when considering the potential diagnosis of acute rejection. Bowel acute rejection can be associated with an assemblage of symptoms that include increased fecal output (early on from the patient's stoma), fever, and swelling [9]. These symptoms are not specific and other processes such as infection can also induce similar effects. In this regard, the endoscopically derived gastrointestinal biopsy is often central to distinguishing these processes. Morphologically defined acute rejection in the absence of clinical symptoms is known as **subclinical rejection** (SCR) which has been described in bowel allografts [18], as in other transplants including liver and kidney [19,39,40]. Typically, SCR is detected when biopsies are taken as part of protocol surveillance and the patients are clinically stable. SCR is potentially important because patients have a higher rate of eventual graft loss. Some protocols treat SCR with additional immunosuppression. Adjunctive non-invasive laboratory and biomarker assays, though currently lacking optimal specificity and sensitivity, are growing in use in order to further support a morphological diagnosis of acute rejection on the biopsy [41]. These tests include cytofluorographic analysis of peripheral immune cell populations [42], cytokine profiling, and the quantitation of distinct gene set changes [33]. Serial measurements of peripheral blood levels of the amino acid citrulline, though not specific for rejection, appear to provide a snapshot of viable intestinal mass, and this analyte is routinely measured at our institution in monitoring post-ITx and MVTx patients [43–45].

Morphology. The histopathology associated with acute rejection in biopsies after ITx or MVTx represents a wide range of changes, and differs according to many variables with recipient, graft, and time post-transplant. The categorization of acute rejection in small bowel and MVTx, as with other solid organ allografts, utilizes terms that originate from basic immunology (e.g. hyperacute rejection, accelerated acute rejection, acute vascular rejection); with time these terms have become more standardized. Morphological changes associated with these subtypes of acute rejection also appear to be able to co-exist (e.g. "mixed rejection"). We utilize a general classification, based on the general underlying etiology of the rejection: antibody-mediated or T-cell-mediated (Box 86.1).

Antibody-mediated rejection

Hyperacute and accelerated acute rejection

Hyperacute and accelerated acute rejection are expressions that define the scenario infrequently encountered in which an allograft organ is exposed to extremely high levels of alloantibodies that cross-react with antigens on the organ (i.e. donor-specific antibodies) and is subsequently severely rejected within minutes to hours (**hyperacute rejection**) or a few days (**accelerated acute rejection**) following implantation [46,47]. These potentially devastating forms of acute rejection occur in the presensitized patient (i.e. pre-existing antibodies) and are secondary to a severe antibody-mediated response in which the vasculature endothelium is the principal target; vascular injury, thrombosis, and ischemic lesions characterize it histologically. Experimental evidence of these forms of rejection in bowel allografts exists but documented clinical cases of hyperacute rejection in ITx or MVTx are rarely described [48]. This latter point is a testament to the predictive success of cross-matching of recipient sera by the histocompatibility laboratory with donor cells before gastrointestinal transplantation. Pretransplant cross-matching was historically not the norm, although this is changing since bowel hyperacute rejection episodes associated with the presence of pretransplant donor-specific antibodies have been described [49–51]. Grossly, the donor graft upon anastomosis immediately turns dusky in color and becomes hyperemic, resembling changes seen in other solid organ grafts experiencing hyperacute rejection.

Morphologically, there is extensive mucosal congestion and necrosis with neutrophils and margination around vessels (Figure 86.2). A substantial amount of vascular congestion extends from the mucosa through the entire thickness of the graft (transmural). Native tissue is unremarkable. Curiously, these patients have the capacity to overcome this severe form of antibody-mediated acute rejection with aggressive intervention with plasmapheresis, treatment with anti-CD20 antibody (among other immunosuppressives) and close monitoring [48,50]. One patient we described with

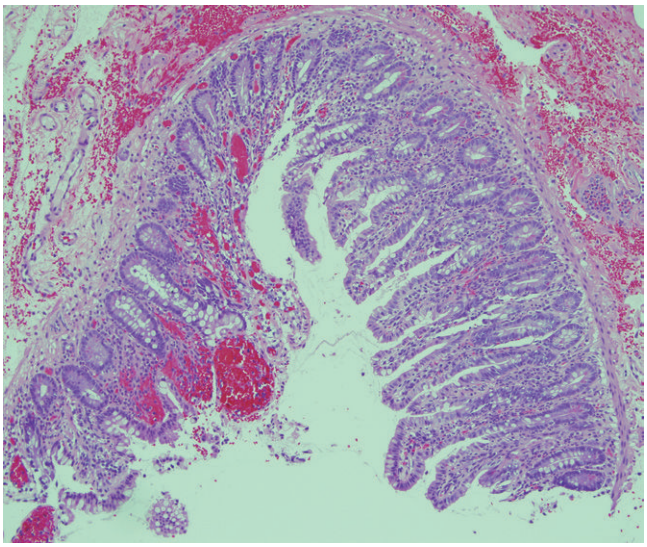

Figure 86.2. Hyperacute rejection. Small intestinal biopsy 1 day after transplant shows severe vascular congestion, neutrophils, early vasculitis, and necrosis (H&E, original magnification 200×).

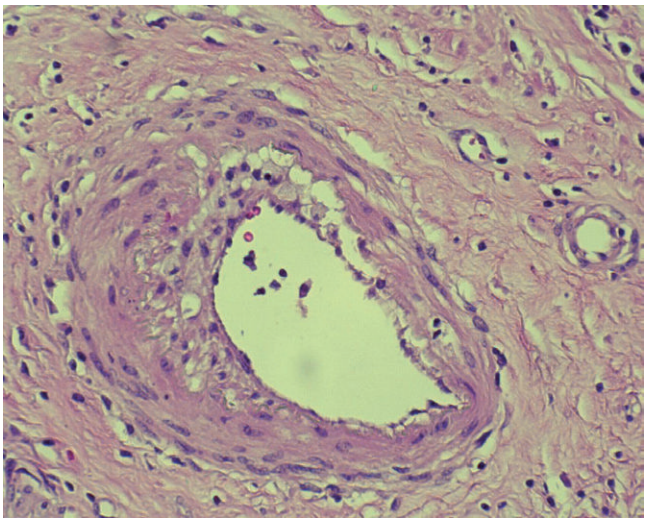

Figure 86.3. Acute antibody-mediated rejection (AAMR). Small intestinal biopsy showing large artery with vasculitis (H&E, original magnification 400×).

hyperacute rejection exhibited a full salvage of bowel graft function (normal graft morphology and asymptomatic), achieved concurrent with a reduction of titers of anti-donor antibodies and the development of normal endoscopic appearance [48]. Crossmatching is routinely performed at our center for all ITx and MVTx recipients.

On occasion, we have also found an accelerated antibody-mediated rejection (AMR) that occurred in the first several days following small bowel of MVTx in which the recipients were sensitized with pre-existing alloantibodies and which morphologically demonstrated features of AMR described below, but not to the degree seen for hyperacute rejection.

Acute antibody-mediated (humoral) rejection

Until recently, acute antibody-mediated rejection (AAMR) in human small bowel and MVTx was an entity whose frequency and severity were not well understood. The pathophysiology of this entity originates with antibodies directed to alloantigens that then initiate a variety of sequelae [49,52]. Accordingly, identification of AAMR cannot be accomplished as a form of rejection without additional techniques and evidence of antibody pre- and/or post-transplant. Vascular inflammation can be one of the histological components of this process; however, this is not specific to antibodies because the cell-mediated arm of the immune response (T-cell-mediated vasculitis) can also be an underlying cause of vasculitis in bowel allograft vessels. **Severe forms** of AAMR in small bowel transplants have been described and are often associated with alloantibody post-transplant sensitization of the recipient to donor antigens and subsequent rises in titers of pretransplant antibodies that were present at very low amounts before the transplant. The graft often demonstrates widespread inflammatory changes, with a critical lesion being vasculitis of large to small arterial branches (Figure 86.3). The vasculitis tends to show an infiltration of acute and chronic inflammatory cells in the intimal layer of the artery, with intimal edema and endothelial cell reactivity. The complement components C4d and C3d are often present in lamina propria and larger vessels; monitoring and grading changes in the immunohistochemical expression of these molecules in sequential lesions is an important requirement to the pathologist. Left unchecked, the vasculitis evolves with transmural inflammation, fibrin deposition, and necrosis of the artery, causing severe ischemic injury to the graft. On occasion, selective arteries in the graft are affected more severely than others by the vasculitis. For example, mesenteric arteries can undergo AAMR and this can lead to sclerosing mesenteritis [53]. This uncommon phenomenon can be associated with increased alloantibodies. Stomach and colon allografts can also demonstrate the severe vasculitis patterns, as seen in the small bowel. When considering a differential diagnosis for this form of severe vasculitis only involving the transplant, other causes such as infectious agents (such as fungal or viral agents) should also be contemplated. Other causes of vasculitis such as neoplasia, drugs, and autoimmune processes are not common causes and would be distributed in native organs as well as the allograft.

Larger vessel vasculitis is typically identified in explants or rejected organs or autopsies. Thus, the diagnosis of AAMR with severe vasculitis lesions in mucosal biopsies can be challenging in small bowel transplants, often only with morphological evidence of extensive necrosis.

However, **less severe forms** of AAMR also occur in small bowel, stomach, and colon transplants and do so at a higher frequency than previously believed. We have described alterations identifying early, mild, or evolving AAMR that can occur isolated or in conjunction with T-cell-mediated acute rejection [52]. These early and/or mild forms of AAMR are represented as mucosal morphological changes and appear associated in many cases with pre-existing or post-transplant alloantibody formation; these changes tend to occur particularly in the early period after transplantation. The microvasculature of the allograft mucosa shows mild to diffuse, significant vascular congestion with erythrocyte extravasation (Figure 86.4). Villous region and lamina propria microvasculature is principally affected and there may be no evidence of any significant vasculitis. Chronic inflammatory cells may or may not be increased and usually there is preservation of epithelial cells. Ancillary tests can be very useful for evaluation of humoral-based forms

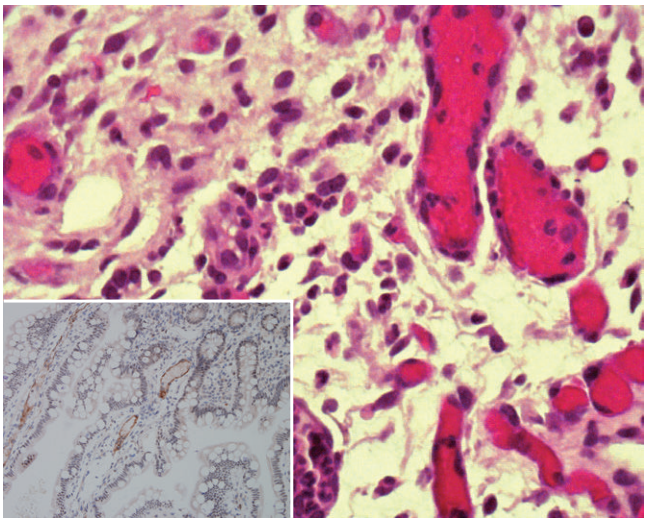

Figure 86.4. AAMR. Small intestinal biopsy with antibody-mediated rejection showing severe vascular congestion of mucosal vessels with neutrophil margination, edematous interstitium, and mixed chronic inflammatory cell infiltrate (H&E, original magnification 400×). (Insert) Lamina propria vasculature with positive C4d staining (C4d immunohistochemistry, original magnification 200×).

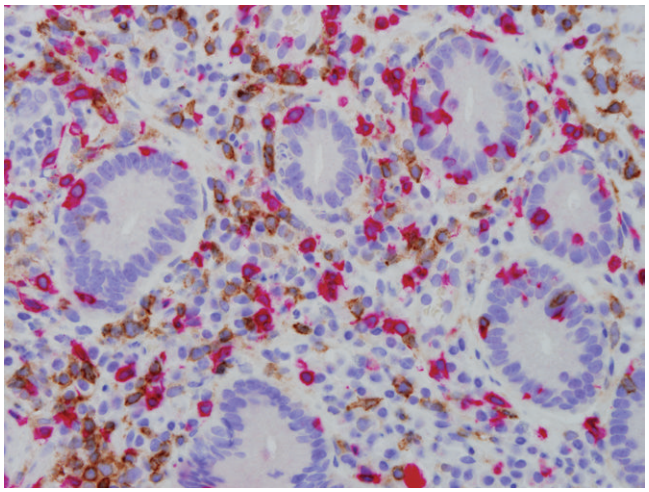

Figure 86.5. Acute T-cell-mediated rejection (ACR) colon allograft undergoing ACR, mild (grade 1) with significant mixed population of $CD4^+$ and $CD8^+$ T cells within interstitium and crypts (two-color immunohistochemistry, original magnification 400×, $CD4^+$ T cells = brown, $CD8^+$ T cells = red).

of acute rejection. For example, in suspected AAMR cases, an immunofluorescence panel is performed to determine the presence of immunoglobulins (IgG, IgA, IgM) and complement components (C3, C4, C1q); fibrinogen, C3d and C4d are performed in our laboratory by IHC optimized for bowel. In AAMR, immunoglobulins can be deposited along vessels and within interstitium along with complement components. As in other transplanted organs and gastrointestinal transplants undergoing severe AMR (mentioned earlier), we have also found C4d, C3d in small arteries and small capillaries in patients with milder or evolving forms of AAMR. These more subtle forms of AAMR do not demonstrate specific histopathological findings, and some of these alterations can be found in ischemia, non-specific enteritis, viral infections, and mechanical vascular problems. Consequently, it is essential to integrate the clinical history and laboratory values (e.g. alloantibody anti-donor titers), lesion distribution (allograft versus native tissue), other morphological findings (e.g. the presence of an acute inflammatory cell infiltrate or superficial epithelial changes with enteritis) and culture results. Moreover, there should be a relationship of symptom and histopathological resolution with augmented immunosuppression concomitant with lessening of alloantibody concentrations.

Acute T-cell-mediated (cellular) rejection

Acute T-cell-mediated rejection (ACR) is the most commonly recognized form of acute rejection in gastrointestinal transplants and can be present concurrently with AAMR, PTLD, infections, or other complications. The primary pathophysiological process operative for this form of rejection is the recipient's T-cell-mediated response to donor alloantigens. Histologically, this is represented by a heterogeneous lymphocyte-rich chronic inflammatory cell infiltrate that is primarily dispersed within the interstitial regions of organs with infiltration and damage of specific tissue parenchyma components [54]. These gastrointestinal elements are principally cells within crypts, glandular structures, and lining of the surface epithelium; however, muscle, endothelial and nerve cells may also be affected. There are several functional and structural sequelae of this injury to the parenchyma, such as metaplasia, apoptosis, and altered regulation of cell pathways.

Vascular compromise from the alloimmune effector cell-based injury to the vessels can be particularly devastating (as with AAMR) because the vascular compromise subsequently leads to extensive necrosis. ACR in gastrointestinal allografts is a complex, multifactorial process in which both $CD4^+$ and $CD8^+$ T cells are involved (Figure 86.5), analogous to other forms of allograft rejection. Crypt epithelial cell apoptosis is one of the chief features associated with ACR in intestinal allografts [55], appearing due to $CD8^+$ cytotoxic T cells' induction of target cell apoptosis via the granzyme B–perforin-dependent granule-exocytosis pathway and Fas and Fas ligand-mediated cytotoxicity [33]. Interestingly, non-$CD8^+$ T cells also appear to contribute to crypt epithelial cell apoptosis and acute allograft rejection in experimental animal models [56,57].

Several classification systems for grading acute rejection in small bowel and colon transplants have been described [54,58], as well as a classification system for stomach [59]. A unified grading scheme for ACR in small bowel allografts was developed in 2003 at the Eighth International Small Bowel Transplant Symposium by an international group of pathologists and clinicians experienced in small bowel transplant morphology (Box 86.1) [60]. This scheme is now widely used and has been employed at our institution for more than 4000 biopsies [61]. To date, there appears to be good correlation between the morphological grading system and the clinical symptoms displayed by the recipient as well as with inter-observer studies between different institutions (unpublished data).

No evidence of acute rejection, Grade 0

The changes associated with this grade are essentially minimal or none (i.e. histomorphology is indistinguishable from normal bowel) in regards to acute rejection; however, other concurrent conditions (non-rejection) may be present.

Indeterminate for acute rejection, Grade IND

The morphological changes seen in biopsies with this grade can be seen at any stage including the early or resolving stages of ACR when there is a minor amount of epithelial cell injury or destruction but nevertheless there is an increased inflammatory infiltrate within the parenchyma. The inflammation is composed of lymphocytes, eosinophils, immunoblasts, some plasma cells, and occasional neutrophils, varying in proportion, but with diffuse or localized intensity visibly increased above normal. Often also present are villous blunting, edema, and vascular congestion, but these features are not necessary for the diagnosis. Cryptitis with lymphocytes or eosinophils and epithelial apoptotic bodies are present. However, the number of apoptotic bodies does not reach the level designated for Grade 1 (mild) ACR.

T-cell-mediated acute rejection, mild, Grade 1

Mild ACR utilizes six apoptotic bodies or more per 10 crypts as the minimal cutoff, as designated in the International Grading Scheme [60]; other features typically include edema, congestion, and altered architecture such as villous blunting. The crypt cell injury, inflammation, and all the other changes listed above for the indeterminate category (Grade IND) are also present in ACR, mild (Grade 1), but at higher levels, including the level of apoptosis. The mild to moderate-intensity mixed chronic inflammatory cell infiltrate tends to be diffusely distributed (Figure 86.6), often with deeper extension to the submucosa, and can involve muscle. All of these morphological features, particularly the character and intensity of the infiltrate, can fluctuate according to the time after transplantation. Regenerative features such as mucin loss, epithelial cell nuclear enlargement, and hyperchromasia may also be present, depending on the duration of the rejection. Vascular congestion and endothelialitis can be present concomitantly with this and higher forms of ACR.

Acute T-cell-mediated acute rejection, moderate, Grade 2

In ACR, moderate (Grade 2), there are the features of mild ACR but with intensified crypt cell injury containing multiple, sometimes confluent apoptotic bodies in single crypts. There may be whole gland necrosis and crypt abscesses. The inflammatory infiltrate in the lamina propria and submucosa is stereotypically more intense than with mild ACR with the nature of the infiltrate being typically mixed but predominantly a mononuclear inflammatory cell population, including blastic or activated lymphocytes and mucosal architectural alteration that tends to be significant. The moderate to severe-intensity infiltrate is less affected by the time after transplantation, and villous blunting, edema, and vascular congestion are inclined to be more widespread with this higher degree than with Grade 1 rejection.

Acute T-cell mediated acute rejection, severe, Grade 3

ACR, severe (Grade 3) is a clinically and morphologically striking form of ACR. This is often also a "mixed" acute rejection (ACR plus AAMR) such that alloantibodies can be present. Crypt cell injury and apoptosis, gland destruction, and related mucosal ulceration are ubiquitous features. The level of crypt epithelial apoptosis is variable; in fact, there may be a normal level of apoptosis among the surviving crypts. There is a marked diffuse inflammatory infiltrate with blastic or activated lymphocytes, eosinophils, and neutrophils. The endoscopist often finds the tissue friable and thus fragments of tissue with significant architectural alterations are obtained. Protracted severe rejection typically results in complete loss of the bowel histological architecture, and there may be predominantly granulation tissue and/or fibrinopurulent (pseudomembranous) exudate, with mucosal sloughing (Figure 86.7). Mucosal ulceration in the absence of active crypt cell injury should not be classified as Grade 3 ACR but rather "consistent with ACR, severe" because ischemic and infectious processes can also lead to mucosal necrosis. The culmination of extended and extensive severe rejection – effectively with only necrotic tissue – has also

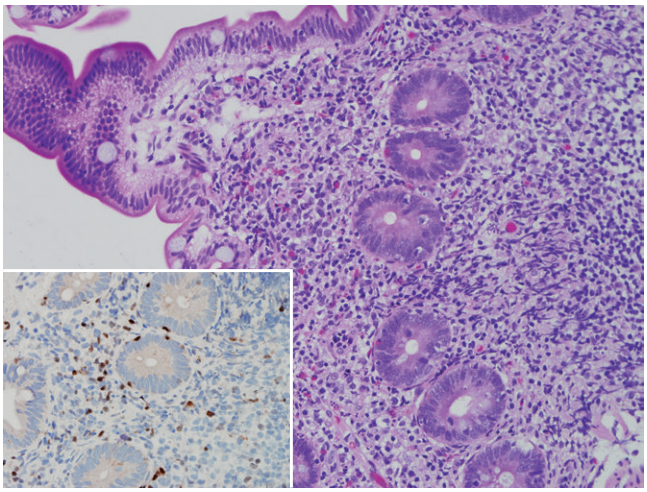

Figure 86.6. ACR, mild. Mixed inflammatory infiltrate and several apoptotic bodies are seen in crypts (more than six apoptotic bodies in 10 crypts) (H&E, original magnification 200×). (Insert) Tbet+ T cells (Th1 type cells) increased with ACR (Tbet immunohistochemistry, original magnification 400×).

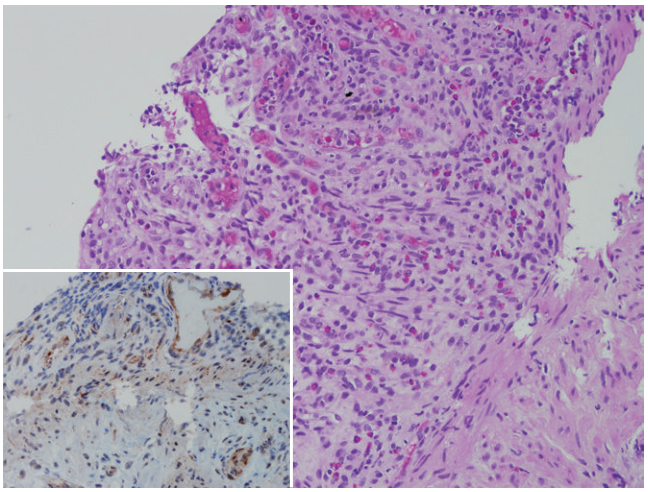

Figure 86.7. ACR/AAMR ("mixed rejection"), severe. Small intestinal allograft architecture mucosal architecture is extensively distorted and replaced by granulation tissue. The evaluation of crypt epithelial cell apoptosis is difficult to assess because of complete loss of crypts. Dense inflammatory infiltrate is seen which consists of mixed but predominantly mononuclear cell population with blastic and activated lymphocytes, eosinophils, and neutrophils (H&E, original magnification 200×). (Insert) Positive C4d within the vasculature. The patient also had donor-specific alloantibodies (C4d immunohistochemistry, original magnification 400×).

been called **exfoliative rejection** [35,36,62]. Therefore, it is very useful to obtain tissue obtained from areas that grossly appear less involved. Severe ACR can lead to intestinal graft loss.

In general, particular regions within the small intestine appear to be more susceptible to ACR; for example, the ileum displays ACR more commonly and often more severely than duodenum or jejunum [63]. There may be severe infectious complications as a consequence of the heavy immunosuppression to treat ACR and alloimmune-mediated injury and impairment of mucosal barrier function (intestinal epithelial cells, intercellular tight junctions, and basement membranes) can result in bacterial translocation into the peritoneal space [38,64].

ACR in colon and stomach

In MVTx patients, segments of the grafted alimentary tract aside from the small intestine can also be involved by acute and chronic rejection. ACR in colon allografts shows similar changes as seen with small intestine, and our experience with biopsies from this organ allograft is increasing as colon segments are included more often now with MVTx [65]. The pattern and composition of the inflammatory cell infiltrate ongoing in colon ACR displays the same pattern as small bowel, as well as the epithelial cell injury in crypts. There can also be architectural distortion, goblet cell loss, and attenuation of the thickness of the surface epithelial cells. We employ the same criteria and grading system for colon as we use in small intestine [60,61,66]. There is often contemporaneous native colon in MVTx patients; thus, obtaining tissue from native and allograft simultaneously can be useful to the pathologist in distinguishing alloreactive versus other inflammatory processes.

Stomach allografts can display ACR in all regions of this organ, exclusively or in combination with other inflammatory processes such as various forms of chronic gastritis and infectious processes. There is epithelial injury in the form of apoptosis and reactive changes, similar to small bowel and colon allografts. The degree of inflammation during acute rejection in the stomach is reduced compared to intestine such that corresponding grades of acute rejection in stomach do not demonstrate the same level of inflammation and epithelial injury as in small bowel or colon. However, we have seen many situations where there is isolated gastric rejection or the grades of gastric rejection exceed other regions of the small bowel or colon. A grading scheme that generates a numerical score to provide different levels of ACR can be a useful approach to evaluating stomach allograft pathology [59].

Chronic rejection

Chronic allograft enteropathy (CAE) or chronic rejection of small bowel allografts is growing in incidence and recognition as graft survival steadily improves in ITx and MVTx [2,34]. Actually, it is becoming one of the principal causes of late graft loss in gastrointestinal transplantation. The pathophysiology of chronic rejection of the bowel, as with other organ systems, appears influenced by an interaction of several non-immune [67] and immune factors [68–71]. The clinical symptoms for CAE tend to be non-specific (e.g. protein-losing enteropathy) and endoscopic information can include loss of villous structure with flattening; these findings can be very beneficial in correlating the pathological findings of the mucosal biopsies. Bowels that are removed with severe chronic rejection grossly show a matted organ bloc due to abundant serosal adhesions, with transmural thickening, an irregular flattened mucosal surface, and intermittent ulcerations.

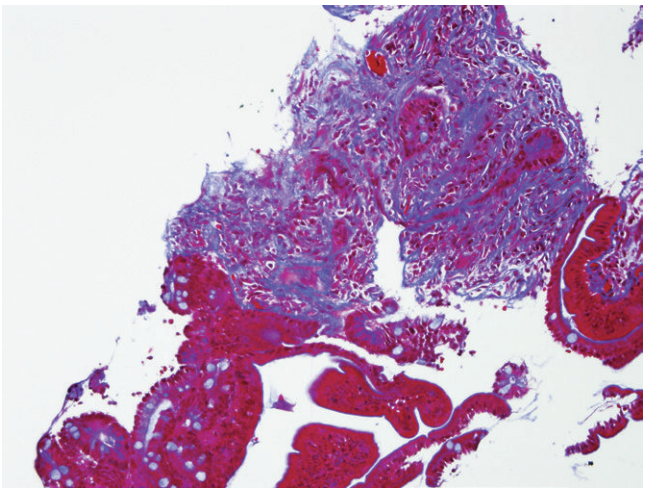

Figure 86.8. Chronic rejection. Trichrome stain shows marked fibrosis in submucosa and lamina propria (trichrome stain, original magnification 200×).

The pathognomonic lesion of bowel chronic rejection is arterial concentric intimal thickening with fibrous changes, medial hypertrophy of smooth muscle cells interspersed with foam cells, and adventitial fibrosis. As such, mucosal biopsies of small bowel are typically limited from being able to demonstrate the large vessel changes of chronic rejection [72,73] because these arteries are not usually present in endoscopic biopsies. Occasional thrombi in arterial branches at various stages can sometimes be seen along with occasional chronic inflammatory cells within the intimal space, the latter identical to "active" chronic allograft arteriopathy described in kidney allografts. There is mucosal, submucosal, and muscular layer fibrosis with crypt loss, crypt separation, villous blunting, mucosal atrophy, and small arterial branches with evidence of transplant arteriopathy. There can be ganglion cell destruction and hyperplasia and there may be fibrinous serositis. Chronic inflammatory cell infiltrates are typically present and there may be ulceration and superimposed acute rejection.

Mucosal biopsies from gastrointestinal allografts with CAE can be very useful in identifying the chronic injury (e.g. fibrosis, crypt loss and distortion, altered architecture) and consequently, with the clinical and endoscopic history provide a practical suspicion for chronic rejection (Figure 86.8). Comparison with adjacent native tissue is very useful because the native tissue is typically unremarkable. A semiquantitative scoring system for the mucosal biopsy evaluation of chronic rejection along with immunohistochemical characterization of lymphoid and macrophage cell populations is also useful in the identification and prognostication of CAE [73].

Infections

Among the serious adverse effects associated with ITx and MVTx immunosuppression is the appearance of opportunistic infections and complications [74–76]. These infections can manifest themselves systemically and/or locally in the graft and adjacent native tissue (e.g. infectious gastritis, enteritis, or colitis). The infectious pathogens can compromise graft function and place the host at risk of death without identification and therapy. Since gastrointestinal graft infections with clinical symptoms can sometimes mimic acute rejection (e.g. diarrhea, fever), these diagnoses must be

differentiated by culture and/or biopsy appearance because treatment often includes reduction in immunosuppression.

This brief overview describes a number of viruses that can involve the bowel allograft and are recognizable by characteristic morphological changes and confirmed by immunohistochemical or molecular techniques or culture. Among these viruses are rotavirus, adenovirus, calicivirus (human calicivirus (HuCV)), CMV, herpes simplex virus (HSV), and EBV. The presence of these viruses in gastrointestinal tissue is accompanied by an interstitial inflammation (e.g. gastritis, enteritis) that is composed of a mixed acute and chronic inflammatory cell infiltrate with focal or diffuse epithelial damage, altered cell proliferation, and cytological changes [77]. Necrosis may be focally present in more severe cases. Concomitant acute rejection may be present; thus, it remains important that the transplant pathologist consider the patient's clinical history and culture and molecular test results when evaluating the histology.

Adenovirus infection can be a perplexing and dangerous infection for gastrointestinal transplant patients, with spotty reports of cases in ITx and MVTx [78,79]. There is crypt cell apoptosis and a mixed chronic inflammatory cell infiltrate with disarray of the surface epithelial cells associated with the presence of enlarged, often hyperchromatic cells; there can be eosinophilic nuclear inclusions as well as "smudge cells" with enlarged basophilic nuclei and proliferation of surface enterocytes [80]. Immunostains, electron microscopy, and viral polymerase chain reaction (PCR) assays (of tissue) for this virus are very useful to help identify the presence of this pathogen. Some of the histopathological changes associated with adenovirus infection can also be evident in acute rejection; therefore it is important to use all tools necessary to distinguish the processes. Rapid diagnosis of adenovirus enteritis is essential because without proper treatment the clinical condition of patients tends to quickly deteriorate.

The general pediatric population commonly experiences *Rotavirus* and it can likewise complicate ITx and MVTx patients. The histopathological alterations associated with this virus are obscure and biopsies are not commonly procured to identify this pathogen. There may be superficial hyperplastic changes in the epithelium, a mixed mucosal surface inflammatory infiltrate with occasional neutrophils and cell debris. Deeper crypts tend not to be affected by the epithelial injury. Co-existence of acute rejection and rotaviral infections is possible [81,82].

Calicivirus (HuCV) is composed of two pathogenic strains: Norwalk-like virus and Sapporo virus. HuCV is a common cause of mild gastroenteritis and/or prolonged high-volume diarrhea in the general population and is usually detected by reverse transcriptase-PCR (RT-PCR) in fecal specimens. Histopathological alterations that have been reported include blunting and flattening of villi, mixed lymphoplasmacytic infiltrate with a small number of neutrophils in lamina propria, disarray and reactive changes of the superficial epithelium with loss of cellular polarity, increased apoptosis in the superficial epithelium and in the crypts, as well as in macrophages in the superficial portion of the lamina propria [83].

Cytomegalovirus (CMV) infection is common in gastrointestinal transplant recipients [84–87] and can be systemic or localized in its distribution. CMV enteritis can present as diarrhea, epigastric pain, and abdominal discomfort. Mucosal erosions and ulcers are frequently found endoscopically in stomach and in small intestine. There can be characteristic large CMV-infected cells that exhibit eosinophilic intranuclear inclusions surrounded by a clear halo and thickened nuclear membrane. Intranuclear inclusions are seen in endothelial, stromal, smooth muscle, and, less often, epithelial cells. These cells are present within a chronic inflammatory infiltrate, composed of lymphocytes and histiocytes, with neutrophils at times observed in the lamina propria. Isolated intranuclear inclusions are sometimes hidden in dense chronic inflammatory infiltrates and hard to identify. Immunohistochemical staining for CMV, and PCR-based assessment of tissues for CMV are helpful to confirm the diagnosis of CMV enteritis, which sometimes is obscured by severe inflammatory cell infiltrates.

Herpes simplex virus (HSV) infection in transplant patients is most frequently found in the oral cavity, esophagus, perianal area, and rectum, whereas HSV enteritis is relatively uncommon [88]. In endoscopic examination, HSV enteritis shows aphthous and necrotic ulcers, mucosal erythema and friability, and inflammatory pseudopolypoid lesions. Microscopically, there are lymphoplasmacytic inflammatory changes with scattered eosinophils. Virally infected cells can demonstrate eosinophilic intranuclear inclusions and multinucleation. Culturing of the biopsy sample and immunohistochemistry is useful to confirm the diagnosis of HSV enteritis.

Epstein–Barr virus (EBV) acute infection is low in the ITx and MVTx population but chronic infection is commonly associable with the development of PTLD (see below).

Bacterial overgrowth in bowel allografts (compared with the density of the normal flora) can be seen and the pathologist should communicate this information. Among potentially important bacterial infections in bowel allografts are **atypical mycobacteria** that can cause significant graft dysfunction [89]. Also, several fungal and parasitic pathogens, including *Candida* and *cryptosporidium* [90], are in the gastrointestinal tract and can involve the allograft.

Recurrent disease and other entities

There is a small proportion of ITx or MVTx patients whose original systemic or intestinal disease has the potential to recur in the bowel allograft. In general, disease recurrence with gastrointestinal transplants is not as frequent a complication as with other solid organ allografts (e.g. liver, kidney). Patients with inflammatory bowel disease (IBD) (e.g. Crohn's) [91,92] may show re-involvement of the bowel and this may be evident in the mucosal biopsies. There may also be IBD involvement of gastrointestinal allografts with certain systemic diseases such as primary sclerosing cholangitis [93]. MVTx patients whose original disease is among certain intra-abdominal neoplastic disorders (e.g. desmoid tumors) [94,95] may show tumor recurrence after transplantation. The recurrences of those neoplasms can be intra-abdominal but also occur outside the gastrointestinal tract or extraperitoneal space.

Gastrointestinal transplants can have inflammatory lesions and processes that have an ambiguous or unexplained origin. Later in the course of treatment, some ITx and MVTx patients may develop **persistent ulcers** that can involve graft, native gastrointestinal tissue, or both [96,97]. These ulcers can originate from several causes, including EBV-positive PTLD (the most common cause), smoldering acute rejection, infections, and some cases that remain of undetermined etiology.

Aside from alloimmune reactions, there are also other miscellaneous inflammatory conditions that can affect the small intestinal allograft and native small intestine [98,99]. **Active enteritis of undetermined etiology** is characterized by acute inflammation (e.g. polymorphic neutrophil infiltration) in lamina propria and/or surface epithelium with focal ulceration and crypt abscess, occurring on a background of chronic inflammation. Several potential

pathogenic mechanisms of this entity have been proposed, including stasis, altered bacterial flora, ischemia, prolapse, and mucolysis. **Eosinophil-rich enteritis and colitis** is an inflammatory condition sometimes associated with allergic sensitization. Some *NOD2* gene polymorphisms are associated with altered bacterial clearance and increased inflammatory infiltrate [16,91,100,101].

Regenerative changes can occur due to healing after acute rejection, infectious enteritis, or after ischemic injury.

Graft-versus-host disease (GVHD) can occur in ITx or MVTx patients with involvement of skin, native alimentary tract, and other systems [15,102–104]. The histopathological features of GVHD in native gastrointestinal tissue can resemble ACR, with increased crypt epithelial cell apoptosis and inflammatory infiltrate. Thus, it is critical to know the clinical history and origin of the tissue sample (whether from allograft or native) in order to diagnose GVHD. PTLD has a high incidence in ITx and MVTx patients Because of their prolonged immunosuppression [20,87,105,106]. This common complication increases in frequency as the time post-transplant increases and often *does* demonstrate involvement of the allograft. EBV infection is often associated with PTLD, although EBV-negative PTLD can occur [107,108]. The histopathology of PTLD in the bowel includes a progression from plasmacytic hyperplasia (an early lesion), polymorphic PTLD, monomorphic PTLD, and frank lymphoma. Lymphoplasmacytic infiltrates (i.e. plasmacytic hyperplasia), the suspected precursor lesions of PTLD, are commonly seen in bowel allograft biopsies as the time from transplant extends and epithelial structures may be effaced by the infiltrate (Figure 86.9). EBV staining by Epstein–Barr Early Region (EBER) gene products and immunostaining for the presence and relative composition of B and T cells within the infiltrate is useful in evaluating possible PTLD [109]. Antigen receptor gene rearrangement studies for T- and B-cell-antigen receptors from the paraffin block are helpful for an assessment of potential monoclonality [107,108]. Monomorphic PTLD can be of T- or B-cell origin [110,111], and are recognized as neoplastic. PTLD lymphomas are classified according to their architectural and cytological features in fashion identical to lymphomas occurring in native tissue [109].

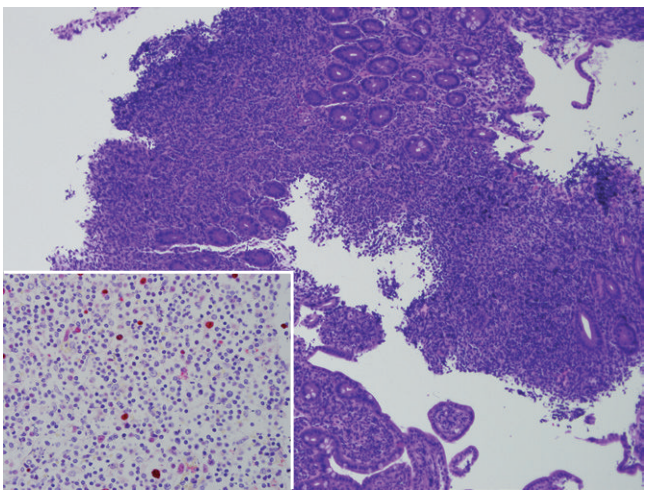

Figure 86.9. Post-transplant lymphoproliferative disease (PTLD). Expansile mild lymphoplasmacytic infiltrate with focal atypia, also indeterminate for acute rejection (H&E, original magnification 40×). (Insert) Epstein–Barr virus (Epstein–Barr Early Region) in situ hybridization. EBV-positive cells within the infiltrate (original magnification 400×).

Conclusions

The success of ITx and MVTx over the past two decades has been multifactorial, with the development of pathological assessment of the transplanted viscera an area of particularly notable improvement. There has been precise delineation of multiple pathological entities affecting these particular organ allografts, development of grading schemes for several conditions, and acceptance that multiple conditions often co-exist in the graft. Moreover, additional biomarker tools are increasingly available for intragraft and systemic tissue evaluation that help refine the morphological impression from the biopsy. The prospect is good that future clinical and basic science findings will further enhance the central diagnostic capacity of the allograft biopsy.

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CHAPTER 87

Histopathological Syndromes of Vascularized Composite Allograft Rejection and Recurrent Disease

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Introduction

Vascularized composite allotransplantation (VCA) refers to the non-autologous transfer of tissues from diverse embryological origins as a functional unit, with a common example being a hand transplant. VCA is performed to reconstruct tissue defects that are otherwise non-reconstructable. It differs from other vascularized allografts in the diversity of tissues comprising the graft, and in the common inclusion of donor skin. To date, over 90 patients have received VCAs as reported to the International Registry on Hand and Composite Tissue Transplantation: 51 limbs, 7 faces, 9 abdominal walls, 6 knees, 3 femoral diaphyses, 2 digits, 1 lower limb, and 1 larynx [1].

Essentially, all VCA recipients have experienced episodes of rejection. At the time this chapter was written, acute rejection episodes had been observed in up to 85% of VCA recipients within the first year [2]. As noted in Chapter 110, the high incidence is likely multifactorial; one factor being the presence of highly antigenic skin, which could condition a robust alloimmune response. As an external organ exposed to the environment, the skin also allows for visualization of changes not necessarily secondary to alloimmunity, but considered alloimmune in nature and treated as rejection. This adds a unique nuance to the role of histopathological diagnosis not evident in other types of transplanted organs.

Although VCA rejection has been presumed to be mediated by mechanisms similar to those in other organs, it has not been determined whether VCA tissues have a hierarchical predilection for the development of an allospecific infiltrate, or if some elements are spared rejection. Studies in preclinical models have shown different infiltrates between tissues and different survival times when multiple tissues are transplanted rather than one vascularized tissue [3]. Because of the infrequent application of VCA, no single group has accumulated sufficient experience to determine histologic patterns of rejection. As such, this single chapter focuses on the histopathological aspects of graft rejection within the context of the early stage of VCA development. We also provide a historic perspective of the study of VCA histopathology primed by the Banff Working Group [4] and considerations on non-alloimmune pathology.

Background

The first mention of a VCA dates back to the year AD 348, when twin brothers from Arabia, Saints Cosmas and Damian, posthumously transplanted a lower extremity (Figure 87.1). Throughout the years, advances in surgery, immunology, and transplantation have made possible the emerging field of VCA. Soon after Joseph Murray performed the first successful kidney transplantation in identical twins, the first hand transplant was performed in Ecuador in 1964. The graft was rejected after 2 weeks as a result of deficiency of immunosuppressive therapy [5]. Large animal models of VCA emerged shortly thereafter. These models relied on manipulations of the immune system that were not translatable to humans [6]. With the advent of cyclosporine in the early 1980s, interest in VCA was renewed. Large animal models in primates and miniature swine showed improved allograft survival with the use of calcineurin inhibitors. These models also showed the skin as a principal site of rejection [7]. Some consider that the modern era of VCA began after the introduction of cyclosporine with a unilateral hand transplant performed in Lyon, France, in 1998 [8].

Large animal models of VCA have yielded important insights into the tempo and character of immunological rejection in this setting which have been important for the classification of human VCA rejection. Early studies in swine models assessed rejection based on cellular infiltrates involving vessels: dermal connective tissue, skin appendages, and epithelium. Studies in outbred swine correlated visual and histologic findings after forelimb osteomyocutaneous free flaps. The visual scale ranged 0–4 based on skin color, bleeding from biopsy site, and blister formation. Histologic grades were assigned based on the degree of vasculitis, folliculitis, dermal inflammation, and epidermal degeneration [9]. Interestingly, their findings included the presence a visual Grade I rejection with no evidence of rejection histologically, and specimens demonstrating +/– 1 histologic grade on pathologic examination compared to the visual scale. Studies in major histocompatibility complex (MHC) matched miniature swine described acute rejection after heterotopic transplant of hind legs. The animals received a 12-day course of cyclosporine immediately postoperatively. Acute rejection was described as an erythematous indurated appearance of the skin with correlating histologic epidermal cellular infiltrate. Complete sloughing of the epidermal layer followed these incidents. Despite rejection and loss of the epidermis, the dermis, muscle, and bone showed no sign of histologic rejection. Re-epithelialization of the dermis occurred subsequent to these episodes [3].

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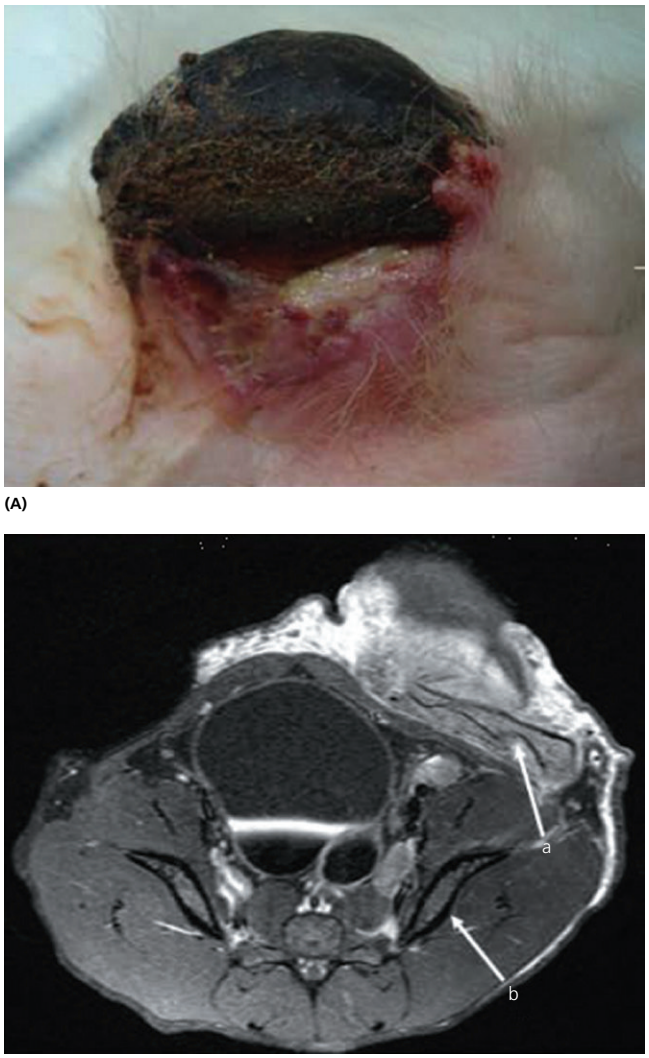
Figure 87.1. Saint Cosmas and Damian performing a posthumous limb transplant. Source: A verger's dream: Saints Cosmas and Damian performing a miraculous cure by transplantation of a leg. Oil painting attributed to the Master of Los Balbases, ca. 1495. http://www.wdl.org/en/item/3251/ (last accessed 23 January 2014).



(A)

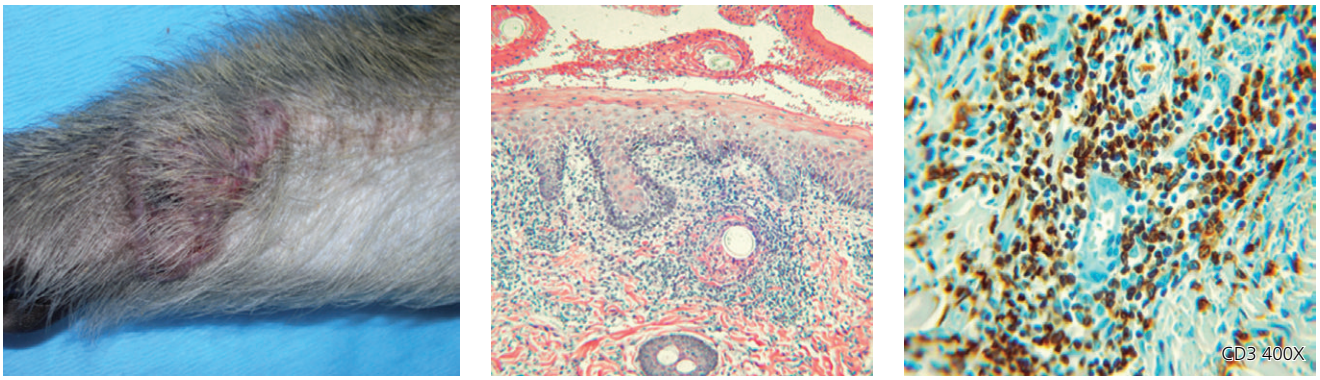
(B)

Figure 87.2. Differential rejection of a vascularized composite facial allograft in a non-human primate: (A) eschar formation; (B) imaging demonstrating viable muscle and bone. (Reproduced from [10] Barth R, Nam AJ, Stanwix MG, et al. Prolonged survival of composite facial allografts in non-human primates associated with posttransplant lymphoproliferative disorder. Transplantation. 2009;88:1242–1250, from Wolters Kluwer Health.)

Studies in non-human primates include a model of facial VCA containing components of bone, muscle, and skin [10], and a model using a sensate osteomyocutaneous radial forearm flap [11]. Compete rejection of the dermis in the facial VCA model was demonstrated by eschar formation but revealed viable underlying muscle and bone (Figure 87.2) [10]. Immunosuppressed animals in the sensate osteomyocutaneous radial forearm flap experiencing acute rejection developed skin changes such as a patchy erythematous rash without blanching, at times preceded by vesicle formation. Biopsies showed a lymphocytic perivascular and perifollicular dermal infiltrate, as well as a lymphocytic infiltrate at the dermal–epidermal junction. Skeletal muscle had multifocal areas of necrosis and perivascular lymphoid infiltrate. Sections of nerve, arteries, and veins demonstrated moderate lymphoid infiltrates. The histologic pattern of rejection was similar to that described in episodes of rejection in human VCA recipients (Figure 87.3) [11].

As is the case for all transplanted organs, histopathology has a key role for VCA in diagnosing rejection, understanding the pathophysiology of rejection, and facilitating management. Standardization is necessary for reporting clinical results and establishing objective end points for clinical trials. In 2005 a collaborative relationship was established between investigators with experience in clinical VCA worldwide to initiate the groundwork for a universally accepted histological classification. In 2007 the first working classification of VCA rejection was published after the first international consensus meeting at the ninth Banff Conference on Allograft Pathology [4]. The participation of the VCA group at the Banff Conference of Allograft Pathology was the first time the field was recognized as a partner in transplantation. The VCA conference reviewed the four published grading systems on human VCA rejection and noted their similarities: the progressive severity of perivascular lymphocytic infiltrates, which parallels the severity of rejection; moderate rejection marked by inflammation extending to the dermal stroma, epidermis, and adnexa; and severe rejection comprising epidermal apoptosis and necrosis. One system scored rejection based on the extent of vessels infiltrated, with $<10\%$ signifying mild rejection and $>50\%$ signifying severe rejection [12].

The Banff conference sought to answer several questions regarding the diagnosis of acute rejection in VCA recipients: (1) specimen adequacy and biopsy preparation; (2) features required for the diagnosis of acute rejection; (3) grading severity of acute rejection;



(A) (B) (C)

Figure 87.3. Skin rejection in a vascularized composite allograft (VCA) in a non-human primate on immunosuppression. (A) Rash circumscribed to the allograft. (B) Parakeratotic hyperkeratosis with a serocellular crust is evident. A moderate dermal, perifollicular, and perivascular mononuclear cell infiltrate is present (hemtoxylin and eosin, original magnification 100×). (C) Lymphocytic infiltrates are positive for CD3 immunostaining (original magnification 400×). (Reproduced from [11] Cendales L, Xu H, Bacher J, et al. Composite tissue allotransplantation: development of a preclinical model in nonhuman primates. Transplantation. 2005;80:1447–1454, from Wolters Kluwer Health.)

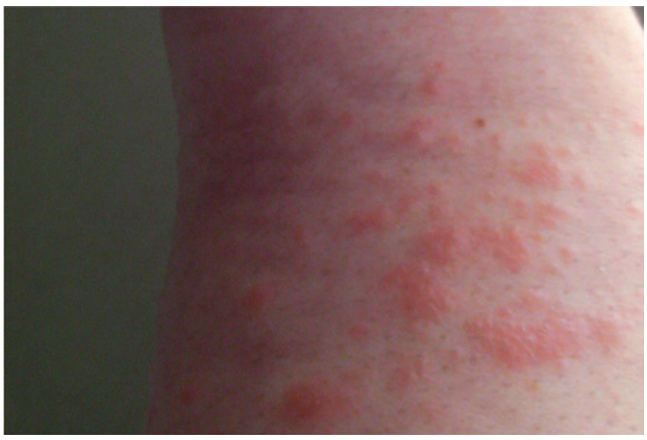


Figure 87.4. Hand transplant. Red macules localized to the allograft. Courtesy of the Emory Transplant Center, Atlanta, Georgia, USA.

(4) features required for the diagnosis of chronic rejection; and (5) diagnosis of humoral rejection. Currently, the treatment of rejection in VCA parallels the treatment of rejection in other solid organs.

Specimen adequacy

Allografts that contain skin are unique in that rejection can be appreciated by visual inspection and appreciation of lesions that are palpable to the clinician. The tempo by which the visual features of rejection appear in the graft is undefined. Efforts have been made to incorporate this unique feature of VCA into the scoring system and to evaluate its role in distinguishing VCA from solid organ allografts. The area of clinical involvement should be documented at the time of rejection as <10%, 10–50%, >50%, or no visible changes (Figure 87.4). Gross features of rejection include ulceration, rash, edema, erythema, vesiculation, desquamation, and necrosis. Biopsies are directed toward the area of the graft with the most severe skin changes that is still viable. In contrast to other organs such as kidney, and considering that rejection may be visible, at this time, one 4-mm punch biopsy is recommended for diagnosis. Although the site of the biopsy is visible, sampling bias is still to be determined. Some centers have transplanted a vascularized sentinel skin graft (consisting of donor forearm) with face transplants and hand transplants as a site to obtain biopsies in an effort to avoid disfiguring the allograft [13]. This is not a universal approach and validation studies have not been reported.

The biopsy should contain the epidermis and its adnexal structures, dermis, subcutaneous tissue, and vasculature. Slides should be prepared with hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) stains. Immunohistochemical stains may be performed depending on H&E findings or for research purposes: CD3, CD4, CD8, CD19, CD20, CD68, HLA-DR, and C4d. Trichrome staining is also performed on an as needed basis but is not currently required for diagnosis.

Acute cell-mediated rejection: VCA Banff scoring system

The histopathological diagnosis of acute rejection is recognized by predominantly lymphocytic inflammatory infiltrates progressively involving small dermal vessels, skin adnexa, and the epidermis. The infiltrate includes several cell types, including lymphocytes and neutrophils, and both the cellular density as well as location is noted. Similar to the Banff classification for acute renal allograft rejection, the degree of perivascular infiltrate parallels the extent of rejection.

The Banff 2007 working classification for VCA pathology grades rejection 0–IV in order of increasing severity. Grade 0 comprises no infiltrate or rare inflammatory infiltrate. Grade I, or mild rejection, is described as mild perivascular infiltrate without involvement of the overlying epidermis. Grade II, moderate rejection, includes moderate to severe perivascular involvement with or without mild epidermal or adnexal involvement. Grade III, or severe rejection, is described as a dense inflammatory infiltrate with epidermal involvement including epithelial apoptosis, dyskeratosis, or keratinolysis. Grade IV, necrotizing acute rejection, consists of necrosis of the epidermis or adnexa (Figure 87.5) [4].

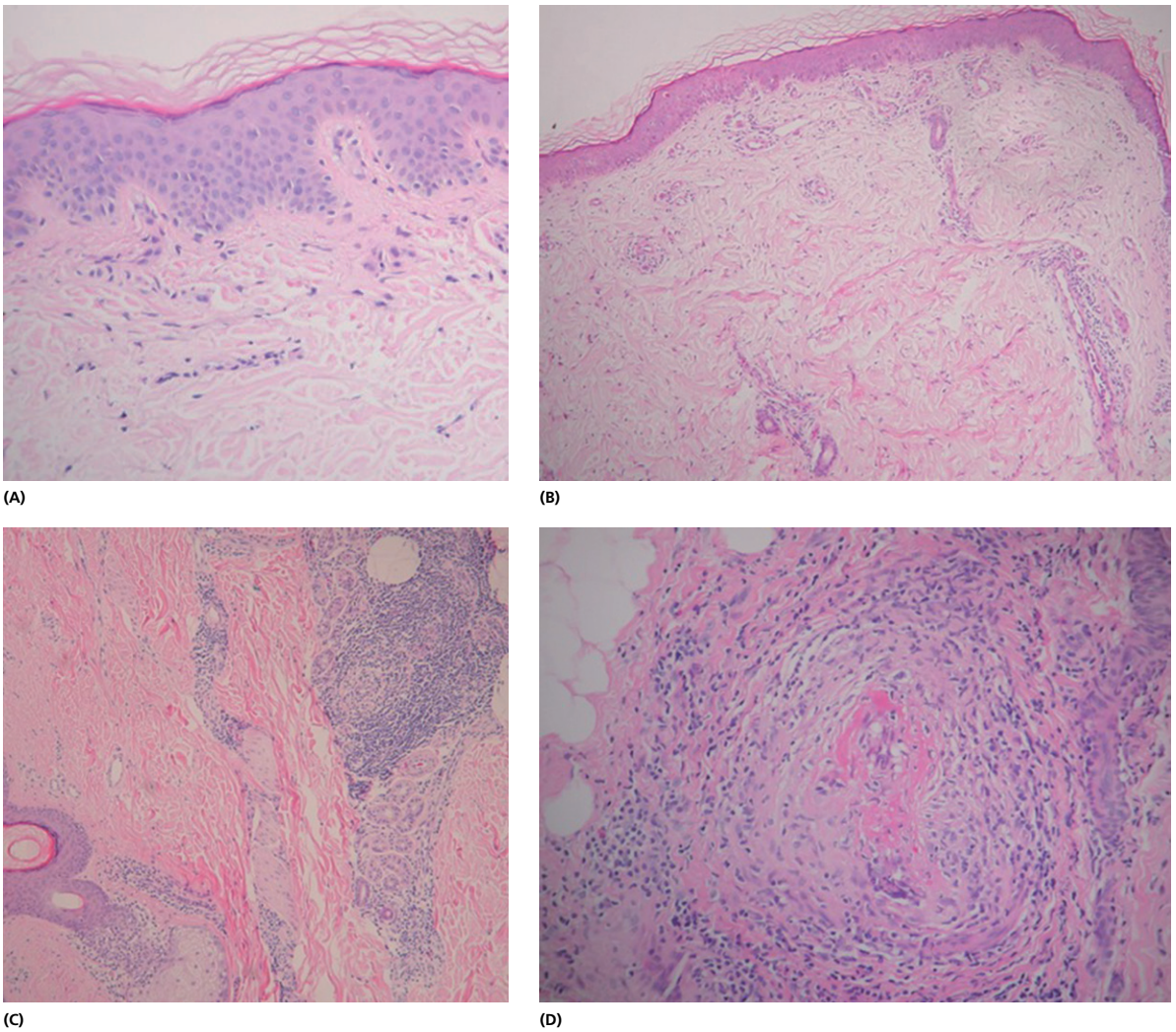


Figure 87.5. Banff classification system for skin containing vascularized composite allografts. (A) Banff Grade 0. No or rare inflammatory infiltrates. (B) Banff Grade I. Mild perivascular infiltration. No involvement of the overlying epidermis. (C) Banff Grade II. Moderate to severe perivascular inflammation with or without mild epidermal and/or adnexal involvement. No epidermal dyskeratosis or apoptosis. (D) Banff Grade III. Severe rejection with fibrinoid degeneration. (Reproduced with permission from [30] Liapis H, Wang HL (eds.) Pathology of Solid Organ Transplantation. Springer, 2011, pp.393–399.)

Vasculitis may be present in other pathologic processes besides rejection, particularly if ulceration or necrosis is involved. Rejection-mediated vasculitis is distinguished by involvement of multiple vessels within the biopsy, especially involvement of multiple sizes of vessels at different depths within the dermis; multifocal involvement of a single vessel; involvement of vessels distant from ulceration; and vasculitis without a history of trauma. Similar to the diagnosis of acute rejection in solid organs, the histopathology of rejection and other pathologic processes may overlap, and these should be considered in the differential diagnosis [4].

Non-alloimmune pathology

Skin-containing VCAs are in constant contact with the environment and the skin can undergo changes unrelated to alloimmune processes. When biopsied, skin changes may exhibit lymphocytic infiltrate not necessarily specific for rejection; thus, a broad differential diagnosis must be considered: infections, drug toxicity, lymphoproliferative diseases, insect bites, graft-versus-host disease (GVHD), and allergic or irritant contact dermatitis [4].

Rejection can be manifested clinically by erythematous macules, which progress to confluent areas of violaceous lichenoid papules if left untreated [14]. Grade I rejection presents clinically as faintly erythematous macules scattered over the allograft or as a generalized rash in the allograft. The clinical appearance at this stage may be similar to viral infections. The histology of Grade I rejection similarly overlaps with viral infection; a lymphocytic perivascular infiltrate concentrated in the upper dermis. Grade II rejection has been clinically observed as erythematous macules which may be scaly. Histologically, the perivascular infiltrate is denser and extends to the mid-dermis; epidermal exocytosis and spongiosis with vesicular changes may be present. The differential diagnosis is broader at this stage and includes viral and drug-related reactions, contact dermatitis, dermatophyte infections, and insect bites.

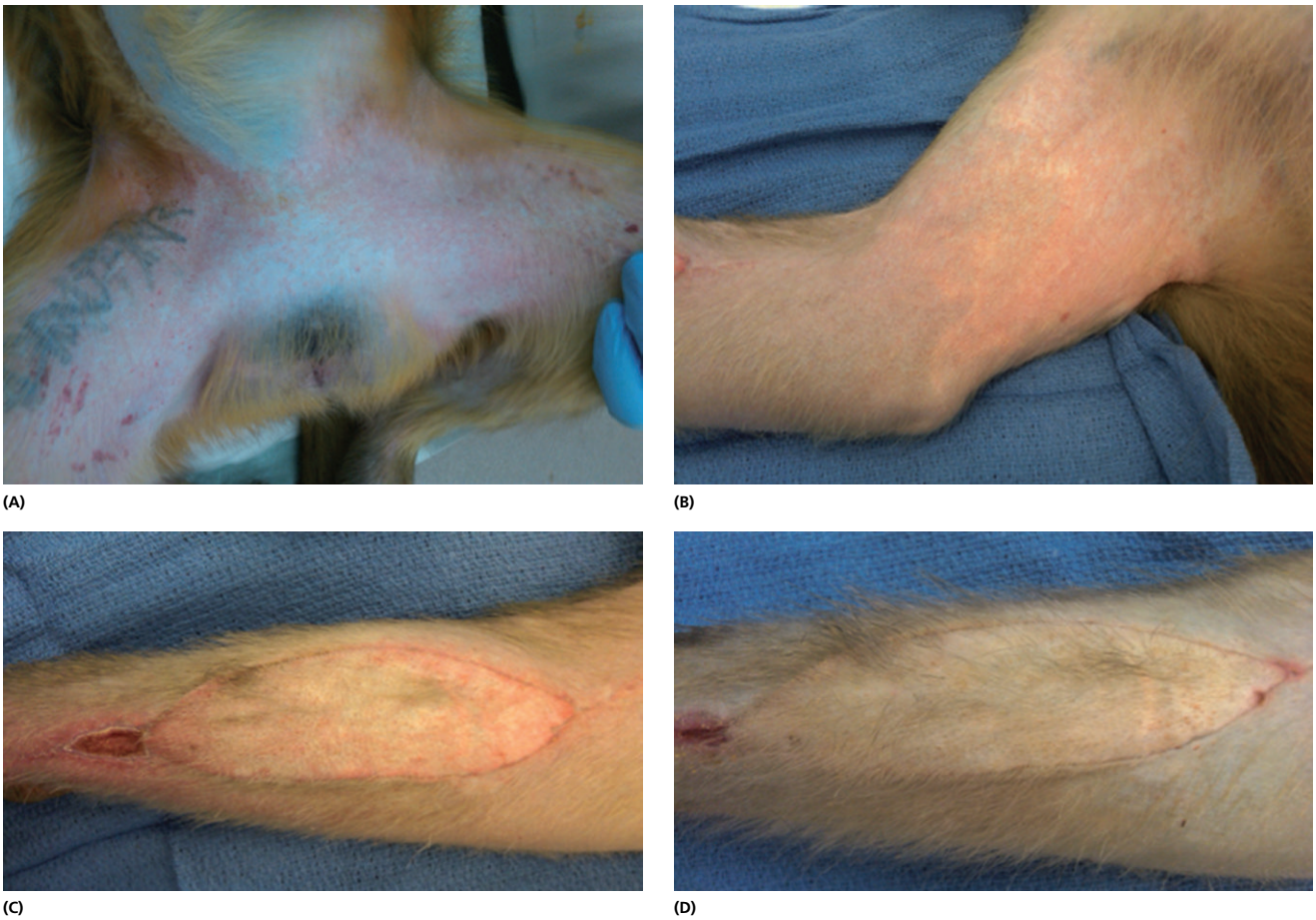


Figure 87.6. Non-specific dermatitis in a non-human primate after a vascularized composite allograft: (A) native skin of groin; (B) native skin of axilla; (C) coinciding rash on allograft skin; and (D) resolution of allograft rash without treatment. (Source: Emory Transplant Center.)

Differentiating between rejection and other causes of inflammation requires a detailed history, physical and pathologic examination. Viral infection and drug reactions are not generally limited to the allograft. Histologically, and different from an alloimmune reaction, samples may contain extravasated erythrocytes in the dermis. Contact dermatitis may have a similar clinical and histologic appearance as rejection and requires history and clinical examination of the native skin to differentiate the two. Dermatophyte infections are characterized clinically by eczematiform skin changes and pathologically by a perivascular dermal infiltrate. Additional cultures and staining such as PAS may be helpful in the differential diagnosis. Insect bites have a high density of eosinophils in the dermis generally not seen with early grades of VCA rejection [15].

More severe rejection can include lichenoid-like plaques and papules. Pathologically, the dermal infiltrate is denser still, extending to the epidermis and surrounding blood vessels and adnexa. The differential diagnosis includes cutaneous lymphomas, pseudolymphomas, lichenoid dermatoses such as GVHD and erythema multiforme. Cutaneous B-cell lymphomas are distinguished by a predominance of B cells in the dermis, epidermal sparing, and presence of Epstein–Barr virus (EBV). Cutaneous pseudolymphomas are characterized by a mixed lymphocytic infiltrate of T and B cells as well as eosinophils. Drug rash with eosinophilia and systemic symptoms syndrome is also part of the differential diagnosis. Lichenoid dermatoses such as erythema multiforme, lichen planus, lichenoid lupus erythematosus, and GVHD are distinguished pathologically by melanin incontinence, a feature not seen with rejection.

The most severe grade of rejection encompasses a dense dermal–epidermal lymphocytic infiltrate similar to Grade III but with the addition of epidermal necrosis and necrosis of the epidermal adnexal structures. Clinically, the appearance may mimic toxic epidermal necrolysis [14,15]. An important visual clue in differential diagnosis is the limitation of the lesions to the allograft. Current research in animal models of VCA include differentiating between rejection and non-rejection-associated inflammation by distinct cytokine signatures [16].

Observations by our group in a non-human primate model of VCA include lacy flat pink rashes noted both on the allograft and in the native intertriginous areas (Figure 87.6). The rash was not treated as rejection and it cleared from both the native and allograft skin. Other lesions included areas of erythema on the allograft that progressed to a thickened scab. A total body skin examination revealed several similar scabs on the native skin which upon investigation were determined to be local reactions to medication injection sites. What was initially thought to be a harbinger of rejection was a misplaced injection into the allograft skin (Figure 87.7).

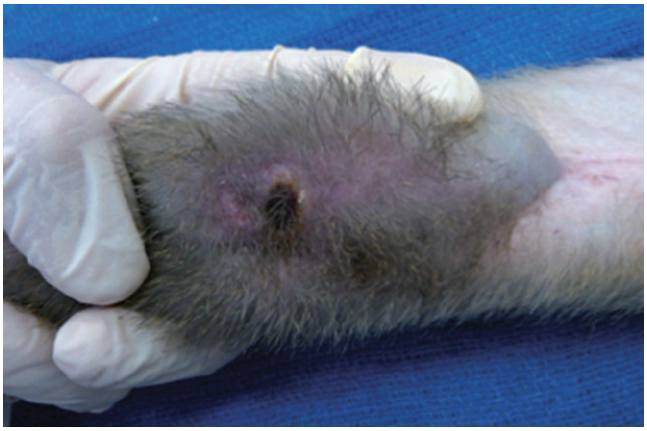


Figure 87.7. Injection site reaction on allograft skin in a non-human primate. (Source: Emory Transplant Center.)

Chronic rejection

As anticipated, chronic rejection has been reported after limb transplantation and the numbers will certainly increase as longer follow-up data are gathered. Chronic rejection in solid organ transplantation is characterized by a gradual loss of graft function, accompanied by transplant vasculopathy, or intimal hyperplasia, often with evidence of C4d deposition and circulating donor-specific antibody [17,18]. Chronic injury to an allograft may be caused by non-immune mechanisms, and its common phenotype belies multiple etiologies. Chronic allograft pathology in VCA is described as vascular narrowing, skin and muscle atrophy, loss of adnexal structures, deep tissue fibrosis, myointimal proliferation, and nail changes [4]. Data are limited regarding the prognosis of composite allografts because of the limited number of transplants that have been performed, as well as the relatively short follow-up when compared with visceral organ transplants, which have been performed for over half a century. None the less, it is anticipated that fibrotic changes and atrophy will decrease range of motion and strength, and long-standing neuritis may translate into sensory changes.

Graft vasculopathy is primarily caused by intimal hyperplasia of vessels and has been observed in a number of human and animal composite allograft models [19–22] . The findings have been likened to myointimal proliferative changes seen in other allograft organs, including cardiac allograft vasculopathy [21]. Based on data from a center with experience in six hand recipients, it appears that vessels, both the arteries and veins, are targets of rejection [19]. Graft vasculopathy should be distinguished from donor arteriosclerosis. If arteriosclerosis is detected on early biopsies, then pre-existing donor arteriosclerosis can be suspected as a contributing culprit of the vascular changes.

Changes in other tissue types have been described in addition to changes that have been seen in vessels. The muscles and tendons can have mild chronic inflammation [23]. Muscular hypotrophy has been described as primarily affecting the intrinsic muscles [24]. This finding could be secondary to slow nerve regeneration. Fatty degeneration may accompany the muscular hypotrophy [24]. Some investigators have reported bone marrow and joints to be spared from chronic changes [25]. However, synovial tissue has been shown to experience rejection. Animal models have demonstrated that the donor bone marrow is replaced with recipient marrow based on short-tandem repeated genotypic analysis [20]. Bone union is observed between the donor and recipient tissue [20]; however, the bone trabecular density decreases in allografts [24]. This particularly occurs in recipient tibia and hand-grafted graft radius. Graft radius changes can also affect the cortices [24].

A series of 15 hand transplants in China with 1–9 year follow-up described the clinical phenotype of chronic rejection as skin erythema, uneven nails, allograft stiffness, muscle atrophy and loss of function, cold intolerance, and pain. These symptoms occurred in most patients over time, according to the authors. Histologically, chronic rejection in this series of patients was described as stromal edema of the epidermis and superficial dermis as well as the vascular endothelium. Perivascular lymphocytic infiltrate and vascular narrowing was also described [26]. Several patients in their series received graft irradiation prior to transplant to reduce donor-derived lymphocytes in the bone marrow. Certain pathologic features of radiation injury overlap with chronic rejection, such as stromal fibrosis, epithelial atrophy, and arteritis, which makes the diagnosis of chronic rejection difficult to differentiate definitively from radiation damage in this patient cohort [26].

Imaging and other ancillary technique findings

Radiologic methods have been proposed to assess chronic changes in VCA. Ultrasound biomicroscopy has been used to assess graft vasculopathy in which vessel wall thickness increases [19]. Magnetic resonance imaging [24], biomechanical analysis, angiography [20], and high-resolution ultrasonography [24,31] may also be useful. Although helpful, it is likely that once the changes detected by imaging are observed, they may be irreversible.

Antibody-mediated rejection

Antibody-mediated rejection (AMR) has been established in multiple organs in both acute and chronic forms. Specifically, donor-specific human leukocyte antigen (HLA) antibodies are risk factors for organ transplant recipients if present at the time of transplantation or produced de novo following transplantation. Thus, monitoring of antibodies directed against antigens is crucial to maximize allograft survival [27,28]. Staining for C4d has been as a marker for antibody-mediated rejection using both immunofluorescence and immunohistochemistry. However, examination of C4d staining in VCA has not yielded a characteristic staining pattern with the current limited data available [11,29]. The development of donor-specific antibodies in combination with vasculopathy has been reported in VCA recipients after hand allograft loss [19]. Although, to date, there is not enough information to draw conclusions on AMR in VCA recipients, it is prudent to assume that several clinical and histological data points should be gathered to help define AMR in VCA. These include C4d deposition on VCA biopsies along with presence or absence of serum donor-specific (anti-HLA) antibody. Additionally, the presence of pathologic markers of chronic injury such as vasculitis, thrombus formation, neutrophilic margination, and necrosis should be noted. A history of patient sensitization is performed before transplantation, including PRA (panel reactive antibody), pregnancy, transfusion, and previous allograft. Additionally a T-cell and B-cell cross-match is performed pretransplant.

Conclusions

The fundamental biology of VCA is sufficiently similar to that of other solid organs that additional phenotypes will surface as clinical experience grows. VCA candidates of a skin-containing transplant should undergo a detailed dermatological examination, including a history of pre-existing dermatosis and a detailed family history, as some dermatoses and malignancies will become evident or develop after transplantation. As a field in development, many questions remain unanswered in VCA. The VCA Banff classification is an international effort that lays the groundwork to advance the understanding and the study of VCA pathology. Similar to other Banff classifications, the VCA scoring system is a living document that changes as more data become available. Further refining of the diagnostic criteria through the analysis of systematic data and clinical trials is needed.

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CHAPTER 88

Cardiovascular Disease and Preventive Healthcare after Organ Transplantation

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Introduction

Cardiovascular disease (CVD), including myocardial infarction (MI), heart failure, stroke, and peripheral vascular disease, accounts for a significant portion of global morbidity and mortality in the general population [1–3]. As patients survive longer with improvements in immunosuppressive therapy, surgical technique, and a better understanding of infection management, CVD has become a leading cause of morbidity and mortality in solid organ transplant (SOT) recipients [4–6]. CVD is much more prevalent after SOT than in age-matched controls, as shown in Figure 88.1 for kidney transplant patients [7,8]. Although all of the factors contributing to this increase in CVD prevalence are not clear, the side-effects of immunosuppressive therapy are believed to play a substantial role in increasing cardiac risk, in part through the augmentation of traditional risk factors [5,6,9]. This chapter will review CVD in SOT patients. The identification and management of risk factors in the general population, as well as factors specific to transplant recipients will also be discussed. This discussion will complement the organ-specific discussions of waitlist management in Chapters 37–42.

Solid organ transplant recipients and independent risk factors

CVD is the leading cause of mortality among kidney and heart transplant recipients, and one of the main causes of death among liver and pancreatic transplant patients after the first year [4,5,10–13]. Lung transplant recipients have a higher graft failure and mortality rate compared to other SOT patients [13,14]; this shorter life span is associated with lower CVD mortality [5,14,15].

Many independent risk factors for CVD are present in patients prior to transplantation, or are induced or exacerbated by immunosuppression, including hypertension, dyslipidemia, diabetes mellitus, obesity, and the metabolic syndrome [3,13]. Compared to the general population, these risk factors are seen with a higher prevalence in SOT candidates and recipients alike, and have a higher associated CVD mortality [12,16]. In an effort to decrease post-transplant CVD, thorough pretransplant evaluation with imaging (nuclear or echocardiogram), stress testing (exercise and chemical), and cardiac catheterization have been employed [12,16].

Hypertension

Hypertension in a transplant recipient is often defined as a blood pressure of >140/90 mmHg or an increase in blood pressure requiring use of antihypertensive medications [3,17]. A known correlation between hypertension and cardiovascular events exists in the general population [18]; it is stated to be the most common, identifiable, and reversible risk factor for CVD [18]. In transplant patients, hypertension is not only associated with an increase in CVD morbidity and mortality, but has also been correlated with decreased graft function and survival [3,19].

Although this risk factor is often present prior to transplantation, immunosuppressive medications, particularly calcineurin inhibitors (CNIs) and corticosteroids, increase the prevalence of hypertension [3,4,20]. Neither sirolimus nor mycophenolate mofetil (MMF) appears to elevate blood pressure [3]. Use of antihypertensive medications is appropriate to maintain a blood pressure of <140/90 mmHg. Calcium channel blockers are commonly prescribed [20]; their benefits extend beyond optimizing blood pressure as they may decrease CNI-associated renal vasoconstriction [3]. Angiotensin-converting enzyme (ACE) inhibitors and beta-blockers play a significant role in blood pressure management in the general population and have an added morbidity and mortality benefit in post-MI patients. Their benefit in the SOT population is still under debate. In experimental models, ACE inhibitors and angiotensin receptor blockers (ARBs) attenuate the development of transplant arteriosclerosis and chronic rejection [3,21]; in patients they have the added benefit of reducing proteinuria. However, ACE inhibitors and ARBs decrease glomerular filtration rate (GFR), a concern for patients already taking immunosuppressive therapy as this can impair renal function [3,22]. For patients with known coronary atherosclerosis, some have suggested the use of a beta-blocker as the first line in the management of post-transplant hypertension [23]. However, the associated hypertriglyceridemia

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

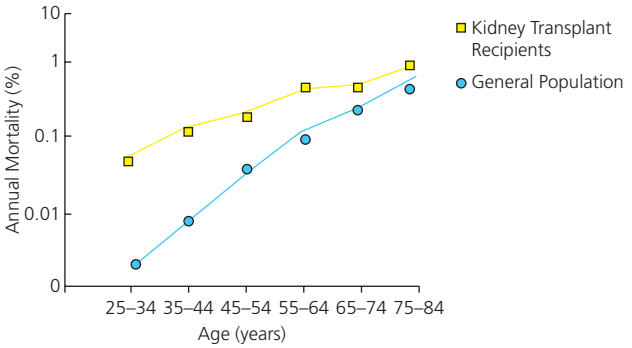

Figure 88.1. Comparison of kidney transplant recipients versus The general population of the same age and percent annual mortality by age. (Reproduced Foley et al. [7], with permission from Elsevier. Copyright © 1998, Elsevier.)

and lowering of high-density lipoprotein (HDL) with beta–blocker therapy may not make it an optimal choice [3,21,24]. The management of hypertension in specific organ transplant populations is discussed in the sections that follow.

Dyslipidemia/hyperlipidemia

Dyslipidemia has been strongly implicated in the occurrence of CVD in the general population, as well as in SOT recipients [3]. Post-transplant dyslipidemia can be seen in approximately 25–50% of patients [25]. Immunosuppressive therapy has been implicated as an etiology for the occurrence and exacerbation of dyslipidemia/hyperlipidemia [3,25]. The use of cyclosporine A (CyA) in transplant and non-transplant patients alike has been proven to increase lipid and lipoprotein levels (see below) [3,26–28]. Concomitant use of corticosteroids with CyA augments this hyperlipidemic effect [3,29]. Patients taking tacrolimus have an improved lipid profile when compared to CyA, and MMF does not adversely affect plasma lipids [3,30,31]. Sirolimus use often leads to the greatest increases in cholesterol and triglyceride levels [3,32,33].

Treatment of dyslipidemia in the post-transplant population usually requires medication [3]; specific recommendations, when available, will be discussed in the sections that follow. Given the effect of certain immunosuppressive agents on lipid levels, changing immunosuppressive therapy to medications with reduced hyperlipidemic effects may also prove beneficial [3]. In cardiac transplant patients specifically, early introduction of lipid lowering agents not only improves lipid levels, but results in decreased cardiac allograft vasculopathy, decreased rejection, decreased maximal intimal thickening, and increased overall graft survival [34–36].

Diabetes mellitus

A significant increase in CVD-related morbidity and mortality has been associated with post-transplant diabetes mellitus (DM) [37,38]. Post-transplant DM has been reported in 3–19% of transplant patients, with an incidence as high as 25% in renal transplant recipients [39–41]. Immunosuppression plays a large role in the development of post-transplant DM. The CNIs, CyA and tacrolimus induce DM by diminishing insulin synthesis and secretion [39,42]. Corticosteroids are also known to cause or exacerbate DM in both transplant and non-transplant patients via induction of insulin resistance [39,42]. However, this effect is dose related and diminishes with decreasing corticosteroid dosing [3,43,44]. When corticosteroids are used in combination with CNIs, the diabetogenic effects are typically additive. Sirolimus use has also been associated with diabetes [45]; MMF has not been found to elevate blood glucose.

The treatment of post-transplant DM is discussed further in the sections that follow, but is similar to the treatment of diabetes in the general population. Dietary modification according to the American Diabetes Association (ADA), with restrictions on the consumption of carbohydrates, is recommended, as well as the use of oral hypoglycemics and/or insulin. Reduction in diabetogenic immunosuppressive medications can be considered in some SOT patients; however, this must be carefully balanced against the risk of rejection.

Obesity

Post-transplant improvements in nutrition, along with immunosuppression with steroids and CNIs, are associated with increased body weight [46–48]. Recipients of renal transplants have been noted to be overweight or obese prior to transplant more often than their heart, liver, and lung counterparts [49]. Up to 50% of renal transplant recipients have been reported to be obese, gaining an average of 10 kg in their first year post transplant [50]. Studies of liver transplant recipients have found a significant increase in body mass index (BMI) within the first year; 20–40% actually become obese (BMI $>30\,kg/m^2$) several years after transplant [46,51–53]. Of interest, BMI alone does not appear to be a risk factor for cardiovascular events in liver transplant patients [46,51–53].

A well-accepted association between obesity and several risk factors for CVD exists in the general population. Hypertension, DM, and hyperlipidemia are exacerbated by weight gain and improve with weight loss. Interestingly, obesity is also associated with increased inflammation, the newest identified risk factor for CVD (see Chronic inflammation section below) [54]. We can extrapolate that a similar relationship exists between obesity and cardiovascular risk in transplant recipients. Measures to optimize nutrition with attenuated caloric intake and increased physical activity are needed to decrease the influence of obesity on CVD [50,55].

Chronic inflammation

Atherosclerosis is a known chronic inflammatory process. C-reactive protein (CRP) levels predict cardiovascular events, CVD mortality, and cerebrovascular events in the general population [54,56]. These findings, along with increased mortality, extend to the SOT population, particularly to renal and heart transplant recipients [5,57]. Evidence of increased inflammation is present early post transplant, but appears to resolve in most patients over time [9]; liver transplant recipients have the lowest levels of inflammatory markers [9]. Smoking and obesity can both increase inflammation in transplanted and non-transplanted patients alike; however, other etiologies for increased inflammation exist for transplanted individuals [58]. Although it is debatable whether evidence of cytomegalovirus (CMV) infection is associated with CVD in the general population, elevated CMV antibody titers are highly associated with CVD in heart transplant patients [57]. Rejection also appears to play a key role in increased systemic inflammation, and can increase the risk of CVD in the heart transplant population [5,57]. Reducing the occurrence of rejection, and prophylaxis against and treatment of CMV may reduce chronic systemic inflammation and potentially reduce the risk of CVD (see Heart transplant section below).

Mechanisms of the adverse cardiovascular effects of immunosuppressive medications

Modern immunosuppressive therapy has made transplantation a practical treatment for organ failure. However, immunosuppressive medications come at a cost, resulting in an increased risk of infection, malignancy, and CVD. This section will review our understanding of the mechanism of this increase in cardiovascular risk; the clinical manifestations of this risk pertaining to different transplanted organs will be discussed in subsequent sections. Detailed discussions of the targeted mechanism of immunosuppressive drugs are found in Chapter 17.

Calcineurin inhibitors

The CNIs, CyA and tacrolimus, are chemically different but both exert their immunosuppressive effects by inhibiting the phosphatase activity of calcineurin, preventing the passage of nuclear factor of activated T cells (NF-AT) into the nucleus and thereby preventing the transcription of interleukin (IL)-2; this results in the suppression of T-cell activation, prevents the formation of cytotoxic T cells, and inhibits T-helper cell-dependent proliferation of B cells, effectively blocking the immune response [59]. However, the effects of these medications are not limited to the immune system; they are known to adversely affect cardiac risk by raising blood pressure, elevating plasma lipids, and impairing glucose metabolism. It is presumed that these adverse effects are the result of calcineurin inhibition beyond calcineurin's effects on T cells [60,61] as calcineurin is expressed in many tissues throughout the body [61].

Although incompletely understood, CNIs are known to have multiple effects that raise blood pressure [62,63]; CyA appears to have more potent vascular effects than tacrolimus. The primary mechanism of CNI-induced hypertension is through widespread arterial vasoconstriction that results in increased systemic vascular resistance [64]. The effect of vasoconstriction on the kidney is to promote sodium reabsorption and volume expansion. CyA potently activates the renin–angiotensin system (RAS) both by directly affecting juxtaglomerular cells, as well as secondarily activating the RAS through vasoconstriction of the renal vascular bed [65]; CyA also augments the vasconstrictive effects of angiotensin II [64]. CyA affects the balance between vasoconstrictor and vasodilatory metabolites of arachadonic acid, which results in net renal vasoconstriction [60]; it increases local release of the vasoconstrictor thromboxane and decreases the production of the vasodilator prostacylin from vascular endothelial cells [66]. Wadei et al. studied patients after liver transplantation [64] and reported that CyA and tacrolimus both suppressed the vasodilator prostacyclin for up to 2 years post transplant. They also reported marked elevations of urinary endothelin over this period, which was associated with decreases in renal blood flow and GFR, supporting an important role of both CNIs in causing renal vasoconstriction. Further, endothelin receptor antagonists improve renal blood flow and blood pressure in animal models of CyA toxicity [64]. Both CyA and tacrolimus have also been reported to inhibit the production of nitrous oxide [60].

Increased sympathetic nerve activity has been postulated as an important contributing mechanism to CNI-related hypertension, but this is not clear. Studies in renal transplant recipients [67] have demonstrated elevated sympathetic nerve activity, but this effect did not appear to be related to CyA use. However, more recent studies of sympathetic nerve activity in normal volunteers show that CyA, but not tacrolimus, does result in an acute rise in blood pressure and sympathetic tone [62]. Mechanisms of CNI-induced hypertension are listed in Table 88.1.

Table 88.1. Reported mechanisms of calcineurin inhibitor-induced hypertension

- Activation of renin–angiotensin system
- Augmentation of angiotensin II-induced vasoconstriction
- Renal vasoconstriction, sodium reabsorption, and volume expansion
- Increased thromboxane production
- Decreased prostacyclin production
- Increased endothelin production
- Increased sympathetic nerve activity

CNIs are also known to be important contributing factors to the marked lipid abnormalities that occur in kidney transplant patients, including elevations in total and low-density lipoprotein (LDL) cholesterol, lowering of HDL cholesterol, and hypertriglyceridemia, although the pattern of hyperlipidemia may vary markedly between patients. Radioactive tracer studies in rats demonstrate a reduction in the fractional clearance of LDL cholesterol, as well as an increase in the rate of cholesterol production [68]. The latter has been suggested to be related to reduced clearance of very low-density lipoprotein (VLDL) by the liver, resulting in increased conversion to LDL [69,70]. Studies by Rayyes et al. [71] demonstrate that CyA reduces LDL uptake and degradation into hepatic cells by 25%, which appears to be due to decreases in synthesis of the LDL receptor; CyA decreased LDL mRNA by about 40%. CyA has also been found to impair the internalization of LDL particles into human skin fibroblasts [72]. These observation imply that diminished cellular uptake of LDL may lead to elevated plasma levels. In addition, studies by Vaziri et al. in rats have found that CyA reduces the activity of cholesterol 7 alpha-hydroxylase, the rate-limiting step in the conversion of cholesterol to bile acids; reduction of the production of bile acids may contribute to the elevation of total cholesterol levels [73]. Other enzymes involved in bile acid synthesis, such as the 27-hydroxylase, may also be inhibited by CyA, leading to elevated intracellular cholesterol and effecting a reduction in the synthesis of the LDL receptor through negative feedback [69,74]. Studies in CyA-treated patients have demonstrated elevated cholesteryl esther transfer protein (CETP) levels, a protein that circulates bound to HDL and facilitates the exchange of cholesteryl ester from HDL to apolipoprotein B (apoB)-containing lipoproteins (VLDL and LDL) with reciprocal transfer of triglycerides [74]. This enrichment of VLDL and LDL with cholesteryl esters could contribute to their atherogenicity and to the atherosclerotic risk of CNIs in general. Tory et al. confirmed this elevation of CETP by CyA by in-vitro studies of human plasma, but tacrolimus did not cause this change [75]. However, both CyA and tacrolimus did markedly suppress lipoprotein lipase (LPL) activity and could account for the elevation in triglyceride levels seen in some transplant patients treated with CNIs. Follow-up studies confirmed the correlation of reduced LPL and elevated triglyceride levels in renal transplant recipients treated with tacrolimus. Total, HDL, and LDL cholesterol levels were unchanged, as were plasma CETP levels. Indeed changes in cholesterol are known to be less prominent in patients treated with tacrolimus than with CyA (see Renal transplant section below). Animal studies have consistently shown a reduction in LPL activity by CNIs [73] (see above).

Le Goff et al. [76] reported that CyA inhibits the ATP-binding cassette transporter ABCA1 in mice; ABCA1 plays a key role in removing cholesterol from macrophages in the arterial wall by mediating cholesterol and phospholipid transport to lipid-poor

HDL [77]. This effect is a possible mechanism that contributes to the low HDL cholesterol that may be seen in some renal transplant patients, and to increased atherosclerotic risk. Kockx et al. reported that CyA inhibits secretion of apoE from human macrophages via a calcineurin-dependent mechanism, but which was not dependent on ABCA1 [77]. ApoE directs movement of lipids from the periphery to the liver; binding of apoE to the LDL receptor facilitates uptake of lipoprotein particles by the liver and protects against atherosclerosis. These studies notwithstanding, the mechanisms by which CNIs affect lipid metabolism and cardiac risk are still only partly understood.

CNIs have long been known to result in impaired glucose metabolism, and to contribute to the development of new-onset diabetes after transplant (NODAT) [78,79]. CNIs impair insulin secretion [80]; tacrolimus has been found to impair insulin secretion to a greater extent than CyA, and is associated with a higher incidence of NODAT [81]. Studies by Heit et al. have demonstrated that calcineurin/NF-AT signaling is important for pancreatic beta-cell proliferation and functioning [82]; CNIs may block this signaling pathway in the beta-cell [83]. Tacrolimus has also been shown to have a direct effect to reversibly inhibit insulin gene transcription, which leads to a decline in insulin mRNA levels, resulting in decreased insulin synthesis and release [84]. A randomized study comparing tacrolimus and CyA in renal transplant recipients demonstrated reduced insulin secretion in patients who developed NODAT compared to those who remained normoglycemic; the reduction was greater in patients taking tacrolimus [81]. Further, biopsies of pancreas allografts reveal islet cell damage to be more frequent and more severe in patients taking tacrolimus compared with those taking CyA [85].

Corticosteroids

Steroid medications have long been a mainstay of immunosuppressive therapy for transplantation. However, the significant side-effects associated with this class of medications are well recognized, in particular their effects on elevating blood pressure and blood sugar, and in causing abnormalities of lipid metabolism which contribute to increased cardiovascular risk.

The primary mechanism by which steroids cause hypertension has been reported to be through promiscuous activation of the mineralocorticoid receptor [86], resulting in renal sodium and water retention with resulting volume expansion. While this may occur with the chronic use of steroids such as prednisone and methylprednisolone, which have mixed mineralocorticoid and glucocorticoid activity, the rapidity of onset of steroid-induced hypertension cannot be accounted for by an increase in renal sodium reabsorption [86,87], and spironolactone does not prevent the hypertensive response to glucocorticoid administration [87]. Peripheral vasoconstriction, causing an increase in peripheral vascular resistance, has long been recognized as a mechanism of glucocorticoid-induced blood pressure elevation [86–88]. Studies have shown that vascular endothelial and smooth muscle cells possess glucocorticoid receptors, which mediate cellular sodium and calcium uptake [89]. Glucocorticoids also up-regulate angiotensin II receptors [90]. Mangos et al. demonstrated that cholinergic vasodilation of forearm blood flow was inhibited by glucocorticoid administration, implying an impairment of the endothelial nitrous oxide system [91]. Studies by Goodwin et al. in mice, in which the glucocorticoid receptor was knocked out of vascular smooth muscle cells, revealed a marked diminution of the acute hypertensive response to glucocorticoids [86]. Taken together, these findings reveal multiple mechanisms by which glucocorticoids cause vasoconstriction that may result in hypertension.

Corticosteroids have also been associated with plasma lipid abnormalities in patients with and without organ transplants. Elevation in total cholesterol, LDL cholesterol, and triglyceride levels may be seen [92], along with decreases in HDL cholesterol [93]. Steroid-related weight gain, hyperinsulinemia, and insulin resistance are important mechanism that result in alterations in lipid metabolism [94]. Studies in animals have revealed elevations in LDL cholesterol and triglycerides resulting from increased hepatic synthesis of VLDL [95]. Insulin resistance also results in increased uptake of free fatty acids by the liver, contributing to VLDL production [92]. Inhibition of LPL activity increases plasma triglycerides. Other studies have found decreased LDL receptor and increased hydroxyl-3-methylglutaryl co-enzyme A (HMG-CoA) reductase activity [93], which results in increases in plasma LDL.

Glucocorticoids are known to play a role in worsening the blood sugar in diabetic patients and in the development of NODAT. They exert their effects through two mechanisms. First, they have been long known to produce insulin resistance that results in a decrease in peripheral glucose utilization, as well as in increase in production of glucose by the liver [96]. Glucose uptake and glycogen synthesis by muscle tissue are thereby both reduced. Insulin resistance also causes an increase in insulin secretion with fasting hyperinsulinemia. Second, glucocorticoids impair beta-cell function [96].

Sirolimus

Sirolimus causes hyperlipidemia in about 40–60% of liver and kidney transplant patients [97,98]. Hypertriglyceridemia and/or hypercholesterolemia with elevated LDL levels commonly occur. Kinetic studies by Hoogeveen et al. in renal transplant recipients demonstrated that sirolimus significantly decreases catabolism of apoB 100-containing lipoproteins [99]. The decrease in the fractional clearance of LDL apoB 100 resulted in hypercholesterolemia with elevated LDL levels. Hypertriglyceridemia was not related to changes in baseline LPL activity, but was related to a significantly reduced fractional clearance of VLDL apoB 100, resulting in decreased VLDL clearance. In vitro, sirolimus has also been demonstrated to down-regulate LDL receptor gene transcription, leading to a decrease in LDL cellular uptake [97,100]. This finding may in part explain the observed decrease in lipoprotein catabolism [98]. Further studies of sirolimus-treated renal transplant recipients by Morrisett et al. demonstrated an expanded free fatty acid pool [101]. Decreases in [$^{13}C_4$]palmitate incorporation into triglyceride suggested that sirolimus may interfere with insulin's stimulation of adipocyte triglyceride storage, resulting in an increase in hepatic fatty acid uptake and hepatic triglyceride synthesis, and contributing to hypertriglyceridemia. Recent studies in rats have found coordinated down-regulation of multiple genes involved in lipid clearance, fatty acid transport, and adipose tissue triglyceride synthesis and deposition [102], which may underlie the clinically observed elevations in plasma lipids. The pathogenesis of sirolimus-induced hyperlipidemia is only partially understood.

Sirolimus was initially thought not to adversely impact glucose metabolism [103]; however, more recent studies have clearly demonstrated the association of sirolimus with NODAT in patients after renal transplantation [45]. Johnston et al. studied over 20 000 patients from the United States Renal Data System (USRDS) and found an approximately 60% increase in the risk of NODAT when sirolimus was combined with a CNI (CyA or tacrolimus) and an almost 40% increase in risk when combined with an antimetabolite

(MMF or azathioprine) [45]. Other studies have also demonstrated the diabetogenic effect of sirolimus [104,105]. The mechanisms by which sirolimus impairs glucose metabolism are not clearly understood [45]. Proposed mechanisms include impaired insulin receptor substrate signaling [106], insulin resistance resulting from ectopic triglyceride deposition [45,107], and a toxic effect of sirolimus on pancreatic beta-cells [45,108]. A recent study in rats found that sirolimus directly induced transcriptional activation of gluconeogenic genes, resulting in severe glucose intolerance [102].

Cardiovascular disease in cardiac transplant patients

The average life span of a heart transplant recipient is approximately 10 years [5]. CVD, particularly ischemic heart disease, has a much higher prevalence among heart transplant recipients when compared to age-matched individuals in the general population, with an associated increase in morbidity and mortality [109]. Cardiovascular death is one of the top three causes of mortality among heart transplant recipients; some studies report it to be the leading cause of death during the first post-transplant year [5,110]. Given the small patient population, data regarding the benefits of risk factor modification are limited, and much of the data with regards to prevention and treatment of traditional CVD risk factors has been extrapolated from studies involving the general population.

Dyslipidemia

Dyslipidemia is one of the few risk factors that has consistently been proven to be associated with increased risk for cardiac allograft vasculopathy (CAV) [109–111]. The prevalence of this CVD risk factor is as high as 57.6% at the first year post transplant and increases to 93.3% 10 years post transplant [5]. Sanchez Lazaro et al. found dyslipidemia to be present in 68.3% of patients with CAV [110]. Pretransplant dyslipidemia has no bearing on the occurrence of CAV; however, post-transplant increase in total cholesterol, LDL, and triglycerides, as well as a low level of HDL, all have a significant association with CAV [111]. The increased incidence and prevalence of dyslipidemia post transplant is due to the use of steroids, CNIs, and/or sirolimus as a part of the immunosuppressive regimen (see above).

Treatment for dyslipidemia requires a multifactorial approach. Dietary modification in the form of reduced intake of saturated fats and cholesterol has been recommended by the American Heart Association (AHA) for the general population [25]. Omega-3 fatty acids, such as fish oils, have also proven to be beneficial in SOT recipients; this recommendation can be extrapolated to heart transplant recipients [25]. HMG-CoA reductase inhibitors are the most potent agents for treating dyslipidemia [25]. In cases of severe lipid abnormalities, dual therapy with a fenofibrate is recommended [25]. Care must be taken to assess for possible rhabdomyolysis or myopathy when using HMG-CoA reductase inhibitors in patients taking CNIs and/or fenofibrates [25]. Initiation of statin therapy, particularly pravastatin and simvastatin, early post transplant has been shown to improve survival and prevent CAV [35,36,109].

Hypertension

Hypertension is one of the most common traditional CVD risk factors among heart transplant recipients. According to the most recent International Heart Lung Transplant Registry, approximately 73.3% of patients have hypertension 1 year post transplant, which increases to 97.4% at 10 years [5]. Although there is a clear correlation between hypertension and CVD among the general population, the data regarding the association of hypertension with the occurrence of CAV are mixed. Some studies find a clear connection between hypertension and CAV, while other studies have not shown a significant association [109–113]. The increased incidence of hypertension seen after heart transplant is similar to that noted in other SOT recipients; this is due in part to the side-effects of immunosuppressive therapy. However, denervation of cardiac volume loop receptors also appears to play a role in the higher prevalence noted among heart transplant recipients [109].

Treatment of hypertension in heart transplant recipients may require more than one antihypertensive agent [109]. ACE inhibitors and calcium channel blockers have been proven to have significant benefit in this population [109]. Not only do these agents help to lower blood pressure by counteracting the renin–angiotensin–aldosterone system, and reducing renal afferent arteriolar vasoconstriction, respectively, but in small studies both have been shown to reduce coronary intimal thickening in heart transplant recipients, thereby reducing the severity of CAV [3,109,112,113]. In effect, both of these agents work to improve graft and patient survival after heart transplantation.

Diabetes mellitus

Approximately 15–27% of heart transplant recipients develop diabetes after the first postoperative year [5,109]. This value increases to almost 40% 10 years after heart transplantation [5]. The use of steroids and CNIs is strongly associated with the development of this traditional CVD risk factor, along with elevated pretransplant blood glucose, pretransplant metabolic syndrome, a family history of diabetes, and the need for insulin on the second postoperative day [109,114]. The impact of diabetes as a CVD risk factor is still debated in these patients. Some studies have reported diabetes to be a significant risk factor for CAV, while other studies have shown it to be an independent risk factor leading to increased infection and rejection, with hemodynamic compromise and decreased survival [111,115–117]. Still other studies have found diabetes to have little significance with respect to patient outcomes [118]. A report by Czerny et al. showed an 18% decreased 10-year survival in heart transplant patients with diabetes when compared to those without diabetes [116]. Interestingly, the same study also demonstrated no difference in the incidence of CAV between the two groups [116]. In contrast, Valantine et al. found elevated glucose levels to be associated with a significant increase in intimal thickness, decreased freedom from CAV (56% vs. 81%; $P < 0.01$), and decreased actuarial survival (60% vs. 92%; $P < 0.005$) when compared to those with normal glucose levels [117].

Treatment of diabetes in heart transplant recipients is similar to that in the general population. A diet low in carbohydrates, as well as the use of insulin and/or oral hypoglycemics as needed, can aid in maintaining euglycemia [109]. Reduction in the use of corticosteroids and CNIs can help decrease hyperglycemia. However, the risk of rejection with decreased immunosuppressive doses early on must be balanced against the benefit of lower glucose levels [3]. Although it appears there is a correlation between diabetes and decreased survival, increased infection, and increased CAV, it is unclear if treatment of diabetes will actually lead to improved morbidity and/or mortality [109].

Metabolic syndrome

Metabolic syndrome (MS) is defined by the AHA as central obesity, elevated triglycerides, low HDL, elevated blood pressure,

Table 88.2. Adult treatment panel III criteria for metabolic syndrome from the National Cholesterol Education Program

Abdominal obesity	Waist circumference >102 cm (40 in) for men and >88 cm (35 in) for women
Impaired glucose tolerance	Fasting plasma glucose level ≥100 mg/dL (5.6 mmol/L) or drug treatment for hyperglycemia
Hypertriglyceridemia	Triglyceride level ≥150 mg/dL (1.7 mmol/L) or drug treatment for high triglyceride levels
Low HDL levels	HDL level <40 mg/dL (1 mmol/L) for men and <50 mg/dL (1.3 mmol/L) for women or drug treatment for low HDL level
High blood pressure	Blood pressure ≥130/85 mmHg or drug treatment for hypertension

Note: The patient must have three or more of the listed criteria. The International Diabetes Federation uses the same criteria but expands the waist circumference criteria according to ethnicity (South Asians, Chinese, and South/Central Americans, 90 cm for men and 80 cm for women; Japanese, 85 cm for men and 90 cm for women; and all others, 94 cm for men and 80 cm for women). According to the International Diabetes Federation criteria, if the body mass index is >30 kg/m^2, central obesity can be assumed, and the waist circumference does not need to be measured.
Data from Watt et al. [365].

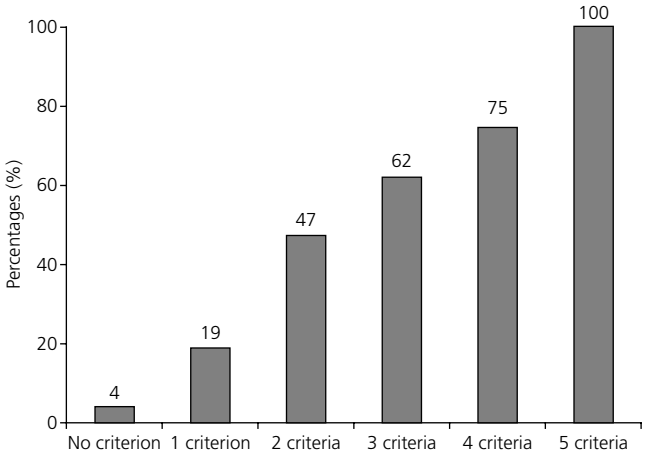

Figure 88.2. Incidence of cardiac allograft vasculopathy according to the number of components of the metabolic syndrome. (Reproduced from Sanchez-Gomez et al. [121], with permission of Wolters Kluwer Health.)

and elevated fasting glucose (Table 88.2) [119]. MS is thought to cause vascular dysfunction via a proinflammatory, prothrombotic state and is associated with increased CVD-related morbidity and mortality in the general population [120,121]. Studies have shown an association between MS and the occurrence of CAV [117,121,122]. Prevalence of MS is 18–39% in the general population; however, due in large part to immunosuppressive therapy, the prevalence of MS is approximately 43% in the heart transplant population [121,123]. Sanchez-Gomez et al. have shown that with increasing numbers of MS components, there is an increased incidence of CAV (Figure 88.2) [121]. When the coronaries were evaluated using intravascular ultrasound (IVUS), approximately 67% of heart transplant patients with MS were found to have CAV at 1 year post transplant [121,123].

Cardiac allograft vasculopathy

CVD in heart transplant patients is typically due to CAV or to rejection, and is one of the leading causes of death in this population. CAV is the leading cause of mortality after the first postoperative year, and accounts for 7% of deaths or retransplantation 5 years post transplant [5,57,109]. Typical symptoms of angina are unlikely to exist as these patients have denervated hearts (reinnervation rarely occurs). Both CAV and rejection can present as fatigue and shortness of breath due to heart failure, with ventricular arrhythmia and/or sudden cardiac death being the other possible manifestations. Given the difficulty of symptomatic recognition of either, scheduled evaluation is needed with right heart catheterization to assess hemodynamics, left heart catheterization to evaluate coronary anatomy, and endomyocardial biopsy to evaluate for rejection.

Table 88.3. Patient characteristics with regards to development of chronic allograft vasculopathy

- Pretransplant:
  - Body mass index
- Post transplant:
  - Total cholesterol
  - Low density lipoprotein
  - High density lipoprotein
  - Triglycerides
  - Diabetes

Reproduced from Chamorro et al. [111], with permission from Elsevier. Copyright 2006, Elsevier.

CAV is an accelerated form of coronary artery disease (CAD). It afflicts approximately 50% of heart transplant recipients over a lifetime and diffusely affects the arteries, capillaries, and veins of the coronary tree [111,124,125]. One-year after transplant, approximately 5.5–7.8% of these patients are diagnosed with CAV [121,126]. Independent risk factors range from those typical for CAD (hyperlipidemia, DM, hypertension, etc.) to more atypical factors such as CMV serology and episodes of rejection (Table 88.3 and see below) [121]. Several retrospective analyses of heart transplant patients have been performed looking at the association of various CAD- and non–CAD-associated risk factors in the development of CAV [110,111]. Donor age of >50 years has been proven in several studies to be an independent risk factor for CAV [121,127]. CMV seropositivity, a non-traditional risk factor for vasculopathy, has also been associated with an increased occurrence of CAV [128,129]. A decreased incidence of CAV can be seen with the use of CMV prophylaxis in heart transplant recipients who are CMV positive or receive a heart from a CMV-positive donor [128]. Dyslipidemia, diabetes, and smoking are the traditional CAD risk factors that have the highest correlation with occurrence of CAV in heart transplant recipients [110,111,121]. Statins have shown not only to be beneficial in treating dyslipidemia, but also in decreasing immunoglobulin G (IgG) autoantibody levels and reducing the incidence of CAV [111]. Detailed consideration of CAV following heart transplantation can be found in Chapter 79.

Allograft rejection

Rejection rates are highest during the first year after transplant, and rejection is one of the leading causes of post-transplant mortality. Frequent evaluation with endomyocardial biopsies and blood sample testing for donor specific antibodies (DSA) aid in screening for both cellular- and antibody-mediated rejection. Allograft rejection can present as heart failure with decreased systolic function and changes on the electrocardiogram (ECG), with increased conduction time or a supraventricular arrhythmia (atrial fibrillation, atrial flutter, atrioventricular (AV) nodal re-entry tachycardia, etc.),

ventricular arrhythmia, or sudden cardiac death [130,131]. The gravity of this diagnosis requires immediate attention with appropriate immunosuppressive treatment, which can range from high-dose corticosteroids to treatment with plasmapheresis and intravenous immunoglobulin (IVIG) or thymoglobulin. The approach taken is based on the hemodynamic compromise noted and the protocols at each individual institution. A single episode of hemodynamically significant cellular- and/or antibody-mediated rejection decreases overall actuarial survival by 8–15%, freedom from CAV by 9–15%, and freedom from non-fatal major cardiovascular adverse events by 2–18% at 5 years post transplant [132].

Conduction abnormalities and atrial arrhythmias

Conduction abnormalities are common after heart transplantation; however, the cause of post-transplant rhythm disturbances cannot always be determined. Approximately 70% of initial ECGs after heart transplantation are noted to be abnormal; right bundle branch block is the most commonly found abnormality [133,134]. Leonelli et al. noted donor heart ischemic time and severity of early post-transplant in-hospital rejection to be associated with evolution of the initial ECG [133]. Patients whose ECGs demonstrated progressive conduction system damage over time, or were initially abnormal and remained abnormal, were noted to have an increased incidence of deterioration of left ventricular function and a higher mortality rate [135]. Patients with progressive conduction abnormality had higher rates of sudden cardiac death due to complete heart block or ventricular arrhythmias [135]. Over time, heart transplant recipients with normal ECGs that remained normal had the best outcome [135].

A study of over 1000 heart transplant recipients by Cui et al. demonstrated that approximately 11% of transplanted patients demonstrate some form of AV block on initial ECG [131]. At times this AV block was associated with atrial tachyarrhythmias [131]. Approximately 88% of the time, rejection was the etiology leading to these dysrhythmias. When the AV block was not associated with atrial tachyarrhythmias, the rate of associated rejection was only 36%, and was less severe [131]. Second-degree block, both Mobitz I and II, and complete heart block were much less common (2%); when they did occur, this was often during coronary interventions or endomyocardial biopsy [131]. Sinus node dysfunction has also been reported. A case series by DiBiase et al. found the incidence of sinus node dysfunction to be about 7%; rejection and sinus node artery vasculopathy were the most common causes of this dysrhythmia [136]. Permanent pacemakers should be implanted when sinus node dysfunction, symptomatic heart block, or concerning asymptomatic heart block (Mobitz type II or complete heart block) are present. If rejection is the underlying etiology, aggressive immunosuppressive therapy is required.

Cerebrovascular events

Neurologic events have been documented in 18% of heart transplant recipients, according to a review by the Mayo Clinic [137]. Eighty percent of ischemic strokes occur in the first 60 days post transplant, and account for 11% of diagnosed neurologic events; however, it is only perioperative stroke that carries a 1-year mortality with a hazard ratio of 4.17 ($P = 0.04$) [137,138]. After the perioperative period when stroke risk is highest, heart transplant recipients have a 4.1% 5-year stroke risk [138]. Approximately 40% of patients who suffer from an ischemic stroke were transplanted for a diagnosis of idiopathic dilated cardiomyopathy, compared to 12% who were transplanted for other indications [137]. These rates are noted to be approximately ten times those seen in heart transplant recipients prior to 1993 [137,139]. In the 1980s, ischemic events had a high association with hypoperfusion or atrial fibrillation [139]. In the current era, increased occurrence of ischemic events has been coupled with the selection of higher risk patients for transplant, such as those with worse cardiomyopathies. Emboli originate from the native heart, or may be released during manipulation and/or cross-clamping of an atherosclerotic aorta [137]. Prior history of stroke, hemodynamic instability, extracorporeal circulation for >2 h, cardiac arrest, and carotid stenosis of >50% have been noted as risk factors for perioperative neurologic events [138]. When looking at risk factors for the occurrence of strokes outside of the perioperative window, history of stroke, both ischemic and hemorrhagic, had the highest associated risk [138]. In a review by the Mayo Clinic, all heart transplant recipients who suffered neurologic events made complete or near-complete recoveries with an "excellent quality of life and outcomes" [137].

Cardiovascular disease in lung transplant patients

The mortality rate after lung transplant is highest during the first year, with a recipient survival of 84%, falling to 49% 5 years post transplant. The graft failure, infection, and mortality rates of lung transplant patients are higher than those of other solid organ recipients [13,14,140], but are continuing to improve. Several factors account for this relatively high mortality (Table 88.4). CVD-related

Table 88.4. Causes of death among adult lung transplant recipients

Cause of death	0 to 30 days (n = 1622)	31 days to 1 years (n = 2781)	>1 years to 3 years (n = 2481)	>3 years to 5 years (n = 1445)	5 years to 10 years (n = 1592)	>10 years (n = 310)
Bronchiolitis	8 (0.5%)	129 (4.6%)	648 (26.1%)	412 (28.5%)	394 (24.7%)	62 (20.0%)
Acute rejection	70 (4.3%)	50 (1.8%)	40 (1.6%)	10 (0.7%)	11 (0.7%)	0
Malignancy						
Lymphoma	1 (0.1%)	74 (2.7%)	56 (2.3%)	26 (1.8%)	41 (2.6%)	15 (4.8%)
Other	2 (0.1%)	71 (2.6%)	145 (5.8%)	114 (7.9%)	151 (9.5%)	22 (7.1%)
Infection						
Cytomegalovirus	0	86 (3.1%)	25 (1.0%)	5 (0.3%)	3 (0.2%)	0
Non-cytomegalovirus	330 (20.3%)	1011 (36.4%)	578 (23.3%)	278 (19.2%)	292 (18.3%)	59 (19.0%)
Graft failure	458 (28.2%)	504 (18.1%)	462 (18.6%)	274 (19.0%)	286 (18.0%)	61 (19.7%)
Cardiovascular	180 (11.1%)	109 (3.9%)	77 (3.1%)	63 (4.4%)	76 (4.8%)	17 (5.5%)
Technical complications	134 (8.3%)	63 (2.3%)	16 (0.6%)	4 (0.3%)	11 (0.7%)	4 (1.3%)
Other	439 (27.1%)	684 (24.6%)	434 (17.5%)	259 (17.9%)	327 (20.5%)	70 (22.6%)

mortality is highest within the first 30 days post transplant and then decreases markedly (Table 88.4) [141]. However, CVD is not the leading cause of mortality among lung transplant recipients. Of interest, the use of modern immunosuppressive therapy has led to an increase in the occurrence of CVD risk factors (hypertension, DM, and hyperlipidemia) earlier and more frequently in lung transplant recipients compared to other SOT patients [13,142].

Cardiovascular mortality versus other causes

Unlike other SOT patients, lung transplant recipients have a much lower occurrence of CVD events (approximately 30%) and CVD mortality (approximately 10%) [5,14,15,142,143]. Christie et al. noted the increased 5-year mortality in lung transplant recipients to be due to a history of diabetes, human leukocyte antigen (HLA) match level, donor cause of death, and several post-transplant outcomes (Table 88.4) [140,141]. Plantier et al. noted these post-transplant outcomes to be the most significant over the lifetime of a lung transplant recipient, with sepsis the leading cause of death (34.6%), followed by bronchiolitis obliterans syndrome (18.5%), primary graft dysfunction (11.7%), and hemorrhagic complications (8.6%) [143]. Vadnerkar et al. found similar results even among recipients >60 years of age [144]. Interestingly, in a study of 73 patients requiring bilateral sequential lung transplants, Aratari et al. found bronchiolitis obliterans, not sepsis, to be the leading cause of long-term death, with three of these patients requiring retransplantation [145]. In an effort to decrease bronchiolitis obliterans occurrence, improvements in immunosuppression have been adopted with good results [141,144,145]. Cardiovascular events noted to occur most often after lung transplant include vascular events (8.9%), heart failure (7.4%), and atrial fibrillation (4.7%) [141,143]. Vascular events typically had a longer median time to event dates; 1227 days for acute coronary syndrome and 1490 days for severe symptomatic peripheral arterial disease (stage 2+), both with a lifetime occurrence rate of 2% [143]. Strokes, also a vascular event, occurred earliest post transplant at a median time to event of 191 days, with the highest rate of occurrence among the vascular events (5%) [143]. Pretransplant variables of diabetes, carotid atherosclerosis, and history of atherothrombosis events had the highest correlation with increased risk of post-transplant vascular events, with statistically significant hazard ratios (HRs) of 4.06, 3.05, and 7.7 respectively [143]. Heart failure had an incidence rate of 2.08 per 100-patient years in a study by Plantier et al. of lung transplant patients over a lifetime [143]. This is much higher than that seen in non-transplant patients aged 50–59 years [143].

Risk factors after lung transplant

Improvement in immunosuppression has caused an increased prevalence of new-onset hypertension, diabetes, and hyperlipidemia within the first year after lung transplant [13,141,142,146]. Each is an independent risk factor for CVD in non-transplant and transplant populations across the board. Approximately 90% of patients post lung transplant have developed one traditional CVD risk factor and approximately 40% have developed two or more traditional CVD risk factors by 3 years post transplant [146]. However, to date, no clear association between hyperlipidemia or hypertension and increased CVD events or mortality in lung transplant recipients has been documented [13,141–143]. A history of pretransplant diabetes does have a significant bearing on post-transplant CVD-related mortality [143]. Low cardiac index, atrial fibrillation, and elevated systolic pulmonary artery pressures prior to transplant are also related to increased CVD mortality [143].

Diabetes

Approximately 6–32% of lung transplant recipients develop new-onset diabetes in the first year after lung transplant [13,141,146]. The prevalence continues to increase with time; Christie et al. documented a prevalence of 26% at 1 year and 36% 5 years post lung transplant [141,146]. Of the typical CVD risk factors, diabetes has the strongest correlation with CVD events and mortality in lung transplant recipients. Post-transplant diabetes has an associated 5-year relative risk of mortality of 1.24 ($P < 0.05$) in patients who have survived for >1 year [141]. Silverborn et al. reported the risk factors most associated with the development of diabetes post lung transplant to be a higher mean CyA dose (7.2 ± 3.9 vs. 5.2 ± 2.0 mg/kg/day; $P = 0.006$) regardless of serum level, a diagnosis of cystic fibrosis ($P = 0.01$), and an elevated pretransplant blood glucose level ($P = 0.02$) [146].

A history of diabetes prior to transplant also confers an increased risk of both early postoperative (HR 2.3; CI 1.2–4.4) and later 5-year (RR 1.15; $P < 0.05$) mortality [141,143]. Survival is significantly decreased compared to that in non-diabetic lung transplant patients, with a decrease in 5-year survival from approximately 50% to 10% [143]. Pretransplant diabetes has also been associated with increased risk of atherothrombotic events (HR 4.06; $P < 0.05$) and CVD mortality [143]. Interestingly, the negative impact of diabetes on overall survival in lung transplant recipients may be due not only to its affect as a CVD risk factor, but also the role it plays in provoking and exacerbating infection.

There are currently no data demonstrating improvement in morbidity and/or mortality associated with optimal management of blood glucose in lung transplant recipients. Nevertheless, similar to other SOT recipients, the management of diabetes with diet and exercise, as well as the use of oral hypoglycemic agents and insulin as necessary, is recommended to decrease diabetes-associated morbidity, as well as to decrease the effects of diabetes on CVD and all-cause mortality.

Hypertension

Of the three traditional CVD risk factors discussed, hypertension has the highest prevalence among lung transplant recipients (Figure 88.3) [146]. Between 50% and 60% of these patients develop new-onset hypertension within the first year after transplant, with Silverborn et al. noting 69% having developed hypertension with a mean time to onset of 16 ± 13 months [13,141,146]. By 7 years post transplant, 75–85% of surviving patients have developed hypertension [13,141,147,148]. The mechanism of hypertension in this patient population is similar to that in other SOT recipients: vasoconstriction, chronic vascular structural changes, and sodium and water retention secondary to CNI and steroid use [13,148]. Interestingly, although the prevalence of hypertension is similar to that seen in other SOT recipients, in comparison the time to onset appears to be delayed, despite similar co-morbid conditions [148]. An abnormal circadian blood pressure profile noted by a lack of nocturnal drop in blood pressure, or nocturnal hypertension, has been documented in SOT recipients [13,149]. Vandergheynst et al. studied this phenomenon specifically in lung transplant recipients and noted that this phenomenon remains consistent over prolonged periods of time [149]. When compared to control subjects, a lack of reduction in both systolic and diastolic blood nocturnal pressure among lung transplant recipients was noted ($P < 0.01$ and $P < 0.001$, respectively) [149].

Silverborn et al. reported risk factors for developing post-transplant hypertension to be pretransplant diastolic blood pressure

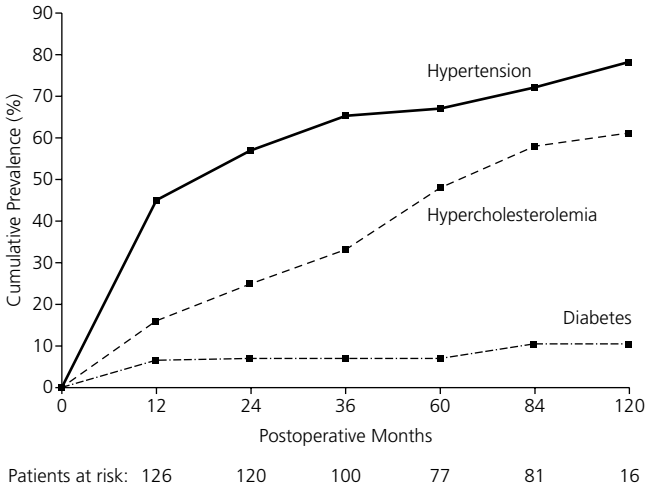

Figure 88.3. Cumulative prevalence of cardiovascular disease risk factors (hypertension, hypercholesterolemia, and diabetes) in post lung transplant patients. (Reproduced from Silverborn et al. [146], with permission from Elsevier. Copyright ©2005, Elsevier.)

and pretransplant blood glucose level on univariate testing ($P < 0.05$); only diastolic blood pressure proved to be significant on multivariate analysis ($P = 0.005$) [146]. Interestingly, no correlation could be identified between dose or serum level of CyA or prednisone and the incidence of hypertension [146]. This study found that patients with hypertension were noted to have significantly lower mean (41 ± 15 mL/min/1.73^2 vs. 47 ± 13 mL/min/1.73^2; $P < 0.05$) and minimum GFRs (33 ± 14 mL/min/1.73^2 vs. 38 ± 15 mL/min/1.73^2; $P < 0.05$) when compared to patient with normal blood pressure [146]. Surprisingly, Silverborn et al. also found that episodes of acute rejection were lower in patients with than without hypertension (0.6 ± 0.6 vs. 1.0 ± 0.8 per year of follow-up; $P = 0.002$) [146].

There are no current studies that demonstrate a correlation between hypertension in lung transplant recipients and the incidence of CVD [143]. However, based on our understanding of the progression of CVD in other SOT patients as well as the general population, standard guidelines for the treatment of hypertension should be followed in lung transplant recipients. Of note, this patient population very often requires more than one antihypertensive agent [13,148].

Hyperlipidemia

Hyperlipidemia is a well-known CVD risk factor in the general population. Goal-directed therapy based on co-existing CVD risk factors has been proven to reduce the incidence of CVD-related morbidity and mortality [150]. Due to the hyperlipidemic effects of immunosuppressive therapy, particularly sirolimus and prednisone, approximately 20% of lung transplant recipients will develop de-novo hyperlipidemia within the first year after lung transplant, almost 50% at 5 years, and 62% at 10 years, with a mean time to onset of 27 ± 21 months after transplant (Figure 88.3) [3,13,146]. Although an association of hyperlipidemia with the use of CyA has been noted, one study failed to correlate a difference in the serum concentration of CyA or prednisone with the occurrence of hyperlipidemia [146,151]. Silverborn et al. reported increased age, systolic blood pressure, BMI, and pretransplant serum triglyceride levels and serum cholesterol levels to be predictive of post-transplant hyperlipidemia on univariate analysis; however, in multivariate analysis only serum cholesterol was predictive [146]. They also observed decreased episodes of rejection among patients with higher lipid levels [146]. Interestingly, cystic fibrosis as the etiology of lung transplant has also been associated with post-transplant hyperlipidemia [146,151].

Although there is an increased prevalence and incidence of hyperlipidemia after lung transplant, no study to date has shown any increase in the occurrence of CVD-related events or mortality associated with hyperlipidemia in this population [141,146,151,152]. There is also no correlation between pretransplant hyperlipidemia and post-transplant CVD [143]. Similarly, treatment of post-transplant hyperlipidemia has not been shown to decrease the incidence of CVD-related events or mortality in this population. A relation between hyperlipidemia and the accelerated progression of chronic kidney disease (CKD) has been reported in lung transplant recipients [153]. Use of statins in this population has been shown to decrease the risk of CKD compared with patients who do not utilize statin therapy [153]. Despite the lack of a CVD-related survival benefit, an overall mortality benefit has been seen with the use of statin drugs after lung transplantation [153].

Management of hyperlipidemia and hypertriglyceridemia is based on similar strategies to those employed in the general population. Medications such as statins, niacin, omega-3 fatty acids, and fenofibrates can be prescribed to improve lipid levels. Care must be taken in the selection and combination of these medications due to drug interactions, which may occur due to the hepatic metabolism of some lipid-lowering and immunosuppressive agents.

Preoperative evaluation of cardiovascular disease

It is standard among lung transplant centers to evaluate recipients >60 years of age for CVD [141]. Some conflicting data exist regarding the correlation between pretransplant coronary disease and post-transplant outcomes. Vadnerkar et al. noted that in patients >60 years of age, pretransplant coronary disease is a risk factor for post-transplant mortality with a HR of 2.43 over a 5-year study period [144]. Sherman et al., however, noted no difference in incidence of CVD (including cerebrovascular events and MI) or survival rates among lung transplant recipients who were noted to have pretransplant coronary disease versus the control group over the same time period [154]. However, an increased risk of thromboembolic events has been associated with lung transplantation in recipients >60 years of age [144].

Post-lung transplant atrial fibrillation

Atrial fibrillation confers an increased risk of mortality in the general population, with an HR of 1.5–1.9, regardless of age [155]. This HR increases to 3.1 among lung transplant recipients with a pretransplant history of atrial fibrillation, with most events occurring in the early postoperative period [143]. In a study looking at 137 lung transplant recipients by Isiadinso et al., mortality rates as high as 43.5% were associated with post-transplant atrial fibrillation within 200 days of transplant [156]. De-novo atrial fibrillation was noted to occur in approximately 20% of lung transplant recipients, with a history of atrial fibrillation carrying an HR of 12.2 with regards to postsurgical atrial fibrillation [157]. In the study by Plantier et al., massive hemoptysis, likely due to anticoagulation for the arrhythmia, was one of the leading causes of mortality seen in lung transplant patients with a history of atrial fibrillation [143].

Although atrial fibrillation is the supraventricular tachycardia that most commonly occurs early after transplant, an increased

incidence of atrial tachycardia is noted >12 months after receiving a new lung [158]. Older age, primary pulmonary hypertension, and either obesity or being very thin increase the likelihood of developing atrial fibrillation [157]. In a study by Mason et al., peak occurrence of atrial fibrillation was seen 2 days after transplant with occurrence at a mean of 11 ± 9 days, and atrial tachycardia occurred at a mean of 1486 ± 2462 days [157]. Since atrial fibrillation originates as aberrant electrical activity around the pulmonary veins and can be induced by increased pulmonary pressures, the lower pulmonary pressures associated with double lung transplant provides protection from post-lung transplant atrial fibrillation [159].

Different strategies can be employed to treat atrial fibrillation after lung transplantation, including the use of pharmacologic agents and cardioversion. Cardioversion can be used to quickly return a patient to sinus rhythm [157]. Amiodarone is often used to control the heart rate and may result in chemical cardioversion. Interestingly, the use of amiodarone has been shown to be associated with an increased risk of mortality [156]. Isiadinso et al. observed that the increase in mortality among patients with atrial fibrillation was only seen in those who were treated with amiodarone [156]; patients who did not receive amiodarone had a mortality rate similar to that for those without atrial fibrillation [156]. This increase in mortality has been attributed to the pulmonary toxicity that is commonly associated with amiodarone use in lung transplant recipients [156]. Consideration should be given to performing prophylactic ablation to reduce the burden of atrial arrhythmias and improve post-lung transplant survival [159].

Other complications

Pericardial constriction

Pericardial constriction is extremely rare in the general population, with an occurrence of 0.1% [160]. Interestingly, it is even less common among lung transplant recipients. It is noted by a thickening of the pericardial sac, leading to limited cardiac filling, decreased end-diastolic volume, decreased stroke volume, and decreased cardiac output, or constrictive physiology. Equalization of diastolic pressures in the left and right ventricles is consistent with this diagnosis. Also, ventricular interdependence and deep "x" and "y" descents of the venous wave forms are appreciated. One may consider manipulation of the pericardium to place double lung transplant recipients at decreased risk for pericardial constriction [160]. Sever et al. performed a retrospective study of renal transplant recipients and noted the incidence of pericardial constriction to be 2.4% among this patient population, which is much higher than that documented among lung transplant recipients [161]. In an effort to prevent rejection, higher levels of immunosuppression are often maintained in the lung transplant population. It is very likely that this level of immunosuppression aids in preventing the inflammation needed to cause pericardial constriction, thereby making this an extremely rare complication in the post-lung transplant population [160].

Bronchial fistula to the pulmonary vein causing air embolism

The formation of post-surgical adhesions is a common occurrence after any surgery. After lung transplant, infectious and anastomotic complications can be the source of fistula formation between the bronchi and adjacent vasculature [162]. This fistula formation can occur as an early or late complication of transplant and can lead to air emboli being the etiology of ischemia. Although it is thought to be an extremely rare occurrence, bronchial–pulmonary vein fistulas likely occur more often than is noted [162]. This possibility should always be considered when evaluating a lung transplant recipient who presents with MI or peripheral ischemia. Prompt evaluation and diagnosis of this problem is necessary to provide a meaningful outcome for the patient [162]. Bronchoscopy, transesophageal echocardiogram, and/or cardiac computed tomography (CT) can be used to establish this diagnosis [162].

Compromise of the right coronary artery

Patients who undergo single lung transplant have been known to undergo a mediastinal shift;. this may occur immediately or late after transplant. A reported case of acute mediastinal shift was reported to have an associated "accordion" effect of the right coronary artery. Although there was good flow within the coronary artery, the kinking at the proximal portion of the right coronary artery led to positional changes in the inferior leads of the ECG [163]. Given these findings, percutaneous intervention was employed to successfully return adequate blood flow through the right coronary artery [163].

Cardiovascular disease in kidney transplant patients

Patients usually come to kidney transplant after a long period of CKD or ESRD requiring renal replacement therapy with dialysis. Hypertension and diabetes are the leading causes of ESRD and are also common in patients with other causes of renal failure. Hyperlipidemia and obesity are also frequently found in this population. These and other associated factors result in accelerated atherosclerotic CVD as well as myocardial dysfunction. The result is a 30–120% increase in the mortality of patients with CKD [164,165] and a staggering ten-fold increase in the risk of cardiovascular death in dialysis patients [166]. Although overall life expectancy is almost doubled by renal transplantation, CVD remains the leading cause of mortality in kidney transplant patients (Figure 88.4) [166]. It is fortunate that the rate of cardiovascular mortality after renal transplantation appears to be decreasing [167]; however, the risk is still three to five times that of the general population [7] and has played a major role in limiting the long-term survival of kidney transplant recipients [168]. This section will review known factors that contribute to cardiovascular risk after kidney transplantation, and review guidelines for managing these risks.

Congestive heart failure

Transplantation has been called a "state of accelerated heart failure" [168,169]. Congestive heart failure (CHF) and MI are the most common cardiovascular complications occurring in kidney transplant patients; among patients covered by Medicare, the rate of hospitalization due to cardiovascular causes in the first year post transplant was almost 15 admissions per 100 patient years at risk [166]. CHF was the most common primary cause, accounting for almost 25% of cardiovascular hospitalizations in the first 2 years, followed by approximately 17% related to coronary atherosclerosis or acute MI. A recent study by Lentine et al. of over 67 000 patients waitlisted for kidney transplant from 1995–2004, showed a more than doubling in the risk of CHF in the early post-transplant period (<90 days) compared with patients remaining on the waitlist; fortunately that risk quickly drops [170]. Rigatto et al. [169] studied 638 patients transplanted between 1969 and 1999 who were free from CVD 1 year post transplant; over 7 years of follow-up, de-novo

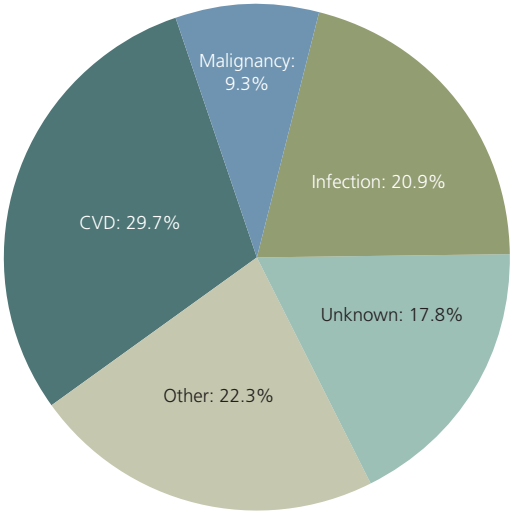

Figure 88.4. Causes of death with a functioning kidney transplant in first-time kidney-only transplant recipients aged 18 years and older, from 2005 to 2009. (Reproduced from United States Renal Data System (USRDS) [256].
The data reported have been supplied by the United States Renal Data System (USRDS). The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy or interpretation of the US government.
CVD, cardiovascular disease.

CHF occurred as frequently as de-novo ischemic heart disease (IHD) and carried a similarly high risk for mortality; this pattern is distinctly different from that of a Framingham cohort that had a similar incidence of IHD but a much lower rate of CHF. In this study hypertension and anemia were two factors associated with the risk of developing CHF. In a subsequent study, Rigatto et al. found a strong correlation between the development of new ECG evidence of left ventricular hypertrophy (LVH) 5 years post transplant and the risk of CHF or death [171]; hypertension was the major predictor of the occurrence of LVH. Although the risk of CHF persists post transplant, the Lentine et al. study found that renal transplantation reduced the risk of CHF long term, compared with the risk in patients who remain on the waitlist; patients with living related donor transplants and those who were not obese experienced the maximum risk reduction [170]. Transplant can be expected to improve factors that contribute to the "uremic milieu," such as intradialytic weight gain, volume overload, hypertension, hyperphosphatemia, and anemia, which are associated with cardiovascular mortality [172], LVH, and "uremic cardiomyopathy" [173,174]. Further, most studies reveal an improvement in left ventricular systolic function [175] and in LVH in patients after renal transplantation [176–178]. A randomized study comparing lisinopril and nifedipine found a similar degree of regression of LVH in patients with well-controlled hypertension in the first year post transplant [178]. Of note, a small study by Patel et al. employing cardiac magnetic resonance, which may be more sensitive than echocardiography, did not document regression of LVH in 25 patients 2.5 years post transplant, compared with controls who remained on dialysis; these findings remain to be confirmed [179]. It is recommended that careful attention be paid to controlling blood pressure and volume status in renal transplant recipients; normalization of these factors is expected to help improve left ventricular function, and may reduce the occurrence of episodes of CHF [176].

Coronary heart disease

IHD is a common source of morbidity and mortality in renal transplant patients. Although less common than CHF, acute MI (AMI) is a complication that may occur in the early postoperative period or late post transplant [168]. Kasiske et al. [180] studied over 53 000 Medicare patients listed for transplant from 1995 to 2002. Cumulative incidence of AMI 3 years post transplant in patients receiving a deceased donor transplant was 6.1% and 4.2% in those receiving a living donor transplant; overall, transplant patients experienced a 17% reduction in AMI compared with patients remaining on the list. Of interest, the incidence of AMI was highest in the early post-transplant period, even in the face of the screening and coronary revascularization that usually occurs during the transplant evaluation. The next section will discuss the risk factors for coronary heart disease (CHD) that are of importance in renal transplant recipients.

Non-traditional risk factors

Hypertension, hyperlipidemia, and diabetes, which may be pre-existing, are common among renal transplant recipients and are important contributors to the risk of CVD. These conventional risk factors will be discussed in detail later. However, it has long been recognized that these risk factors do not by themselves adequately predict cardiac risk in renal transplant patients [38,181]. This point was made in 1996 by Kasiske et al., who studied 1000 kidney transplant recipients from Hennepin County Medical Center [38,181]. They found that pre-existing cerebrovascular, peripheral vascular, and CHD were associated with up to a nine times increase in cardiovascular events, while smoking, hypertension, and hyperlipidemia were not predictive of this composite endpoint (CHD, MI, and cardiac death or coronary intervention). They later reported that the use of the Framingham equation underestimated the risk of IHD after renal transplantation, particularly underestimating the risk in patients with diabetes [181].

Subsequent studies have found that non-conventional risk factors were strongly associated with cardiac events in kidney transplant recipients (Table 88.5) [169,182–184]. Recently, the Patient Outcomes in Renal Transplantation (PORT) study comprehensively evaluated non-traditional risk factors. PORT analyzed data from almost 24 000 patients from 14 transplant centers worldwide [185]. The conventional Framingham Heart Study variables cholesterol, hypertension, and the use of antihypertensive medications were individually correlated with CHD beyond the first year post transplant, but they did not improve the ability of their model to predict CHD events (Table 88.5). This study confirmed the association of pretransplant co-morbid cardiac conditions with post-transplant CHD events. Non-traditional risk factors that were also verified included obesity [183], duration of dialysis [184], diabetes, and hypertension as the cause of ESRD [182], as well as risk factors associated with poor allograft function such as deceased versus living donor transplant [169], episodes of acute rejection [169], and delayed graft function [182]. CKD stage is well recognized as a risk factor for CVD in the CKD population (see below) [186]. The up to 70% increase in cardiovascular events previously reported in patients with NODAT [187] was confirmed in the PORT study, although patients with diabetes pretransplant were at even higher risk, presumably due to longer diabetic exposure.

Table 88.5. Non-traditional risk factors for coronary heart disease post transplant

- Recipient age
- Male gender
- White race
- Body mass index >35 kg/m^2
- Panel reactive antibody (PRA) >10%
- Time on dialysis prior to transplant
- Deceased donor transplants
- Delayed graft function
- Acute rejection
- History of cancer pretransplant
- Post-transplant lymphoproliferative disorder
- New-onset diabetes post transplant
- Decreasing renal function
- Number of pretransplant cardiovascular comorbid conditions*

* Includes acute myocardial infarction, congestive heart failure, coronary revascularization, cerebrovascular accident, and revascularization procedures for peripheral arterial disease.
Data from Israni et al. [185].

It is likely that the predominance of non-traditional causes of cardiac disease is a reflection of years of damage to the cardiovascular system associated with progressive CKD and dialysis. It is well known that duration of dialysis has a major association with poor transplant outcomes. Of interest, cardiovascular mortality rates have been found to fall over time in patients transplanted before the age of 21 years while infection-related mortality rates remain stable [188]. This may imply a continuing positive influence of the transplant milieu, as opposed to the uremic milieu, on the cardiovascular system in this younger population with a shorter exposure to uremia-related cardiac risk factors. This finding is in direct contrast to that of the PORT study, as well as other studies of adults [189], which report high rates of CVD and mortality in long-term survivors of renal transplantation.

The PORT investigators point out that many of these non-conventional risks are potentially modifiable, including BMI, duration of dialysis, acute rejection, post-transplant lymphoproliferative disorder (PTLD), new-onset diabetes post transplant, and post-transplant renal dysfunction. It is also important to recognize that retrospective studies in no way imply that traditional risk factors should not be identified and treated [185].

Other non-traditional risk factors that have been identified as associated with CVD in the kidney transplant population include anemia [184], elevated CRP reflecting systemic inflammation [190], and homocysteine elevations [191]. Unfortunately, the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) trial [192] found that folic acid did not reduce cardiovascular events in over 4000 kidney transplant patients randomized to folic acid or placebo, despite significant reductions in homocysteine levels in the treatment group. Studies reporting the cardiovascular effects of raising the hemoglobin concentration have shown mixed results. Heinze et al. [193] retrospectively studied almost 1800 renal transplant patients and found a decreased survival in those patients treated with erythopoietins and in those with higher hemoglobin values. On the other hand [194], a recent small study of 128 kidney transplant recipients randomized to treatment with β-erythropoeitin to normal hemoglobin values of 13–15 g/dL versus partial correction to 10.5–11.5 g/dL, showed the normalization group had significant slowing of CKD progression with improved quality of life; cardiovascular events were low in both groups. Although small, this study did not find the high rates of cardiovascular events previously reported to be associated with erythropoietin therapy [193] or in CKD patients, as previously reported in the Correction of Hemoglobin Outcomes in Renal Insufficiency (CHOIR), Cardiovascular Reduction Early Anemia Treatment Epoetin beta (CREATE), and Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) trials [195–198]. There may be a fundamental difference in the response to erythropoietin between patients with chronic allograft dysfunction and those with CKD of their native kidneys.

In the general population, CKD with reduced GFR is associated with a high prevalence of cardiac risk factors, as well as with an increased risk of cardiac events and of all-cause mortality [186,199,200]. Similar findings have also been demonstrated in the kidney transplant population; transplant patients with elevated creatinine levels and decreased estimated GFR have been shown to be at increased risk for cardiac death and for all-cause mortality [201,202]. Thus, cardiac risk may potentially be reduced through efforts to preserve renal function, such as avoiding episodes of rejection, reducing exposure to nephrotoxic medications, and controlling hypertension.

Hyperlipidemia

Hyperlipidemia is common among renal transplant recipients. Hypercholesterolemia occurs with a prevalence of approximately 40%, and hypertriglyceridemia in about 60% of patients [203]; HDL levels are usually normal, but low HDL levels may also be observed. Not only are these abnormalities of serum lipids strongly associated with an increase in cardiovascular risk, but elevations of both cholesterol and of triglycerides have each been associated with a decrease in renal allograft survival [204,205]. In the setting of hyperlipidemia, patients should be evaluated for contributing factors that may be amenable to medical therapy, including the nephrotic syndrome, hypothyroidism, excessive alcohol use, and liver disease [206]. Obesity, and diabetes are also frequently present, but immunosuppressive medications are the major contributors to lipid abnormalities. The adverse effects of CNIs, mTor inhibitors, and corticosteroids on plasma lipids are discussed in detail above. Of note, CyA has a greater adverse effect on the lipid profile than tacrolimus, although sirolimus is most likely to result in an extreme elevation of total cholesterol and triglycerides [207]. It has been theorized that the antiproliferative effects of sirolimus may mitigate some of the damage it could potentially cause to the vascular endothelium [203,208]; indeed, patients on sirolimus-containing regimens do not seem to have a noticeably higher incidence of cardiovascular events on short-term follow-up compared to CyA-treated patients [207]. Other drugs that are well known to contribute to hyperlipidemia include diuretics, beta-blockers, oral contraceptives, and highly active antiretroviral agents [206].

Drug therapy and lifestyle modification

The Assessment of LEscol in Renal Transplantation (ALERT) trial [209] published in 2003 is the only large randomized controlled trial of statin therapy in renal transplant patients, and continues to influence the use of lipid-lowering treatment in this population. In this trial, 2102 renal transplant recipients with total cholesterol of 4.0–9.0 mmol/L (150–350 mg/dL) were randomly assigned to fluvastatin or placebo and followed for up to 6 years. A significant decrease in both total and LDL cholesterol (32% reduction) was achieved. Despite a 17% reduction in the primary endpoint of major adverse cardiac events, defined as cardiac death, non-fatal MI, or coronary revascularization, the study did not reach statistical significance (odds ratio 0.83; 95% CI 0.64–1.06; $P = 0.139$). This was due in part to a lower than expected event rate; in retrospect, a study of 6800 patients would have been required to be

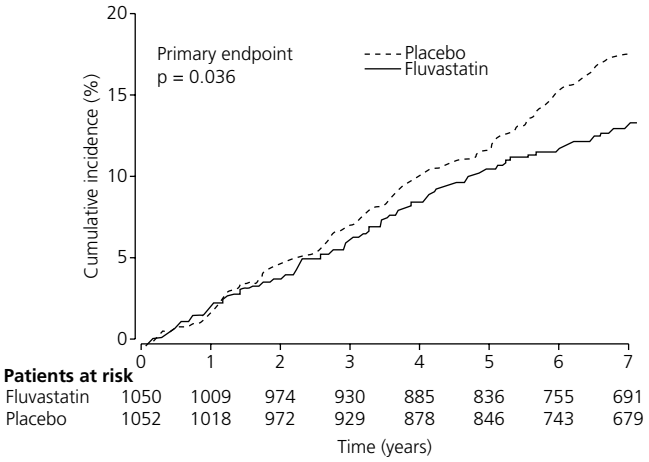

Figure 88.5. Cumulative incidence of major adverse cardiac events by original treatment group based on intention to treat (Kaplan–Meier estimates). (Reproduced with from Holdaas et al. [210], with permission from John Wiley & Sons.)

adequately powered to meet the primary endpoint. However, fluvastatin did significantly reduce the predefined secondary endpoints of cardiac death by 38% and of non-fatal MI by 32%. Further, a 2-year extension of ALERT also showed a significant 21% reduction in the primary endpoint of major adverse cardiac events (P = 0.036) (Figure 88.5) [210]. Set in the context of the many studies that have shown clear reductions in cardiovascular and cerebrovascular death and morbidity in patients without CKD [211], as well as the Study of Heart and Renal Protection (SHARP) trail [212], which demonstrated the efficacy of the combination of simvastatin and ezetimibe in reducing major atherosclerotic events in patients with advanced CKD, the ALERT trial offers strong evidence for aggressive treatment of hyperlipidemia in kidney transplant patients [203].

Although most statin drugs are generally safe and effective in lowering cholesterol [213], it is important to recognize that many statins, including simvastatin, atorvastatin, and lovastatin, are extensively metabolized through the same cytochrome P450 isoenzyme as CyA (CP3A4), resulting in elevation of plasma statin levels and a greater risk of rhabdomyolysis [214]. Simvastatin's package insert states that it is contraindicated in patients taking CyA, and although rosuvatatin clearance is not dependent on the 3A4 pathway, the manufacturer recommends that the dose be limited to 5 mg once daily in patients taking CyA to avoid increased drug exposure. Pravastatin and fluvastatin are metabolized differently and appear to be safe at their usual dosages. Significant interactions between tacrolimus and statin drugs may also occur, as well as interactions with fibrates and nicotinic acid derivatives that may increase the risk of muscle breakdown [214,215]. Most statins are metabolized in the liver, with the exception of pravastatin and lovastatin that have significant renal excretion. Although most statin drugs are not renally excreted, it is generally recommended that this class of medication be started at low doses in patients with CKD [216]. A high index of suspicion for rhabdomyolysis should be maintained for all kidney transplant patients taking statins, and CPK levels should be measured at baseline and in the presence of any muscle-related symptoms.

The 2003 Kidney Disease Outcomes Quality Initiative (KDOQI) dyslipidemia guidelines, updated in the 2009 Kidney Disease: Improving Global Outcomes (KDIGO) transplant guideline report, represent the most current assessment of the literature pertaining to the evaluation and treatment of lipid abnormalities in renal transplant recipients [206,213]. These guidelines differ in their recommendations from those for the general adult population as described in the National Cholesterol Education Program [217], although the association of lipid abnormalities and CVD is often extrapolated from that population. The general approach to detecting and managing lipid abnormalities in kidney transplant patients, as in CKD patients in general, considers these patients to be in the highest cardiovascular risk category. The KDIGO guidelines recommend that lipids should be measured before transplant, 2–3 months post transplant, and at least yearly thereafter. Drug therapy with a statin should be initiated for patients with a LDL cholesterol of 100–129 mg/dL who do not respond to 3 months of therapeutic lifestyle modification; lipids should be rechecked 2–3 months after a change in lipid-lowering therapy.

Despite the high prevalence of CVD among renal transplant patients, statin drugs appear to be underused. Pilmore et al. reported that only 62% of the PORT cohort with previous MI was treated with a statin [218], and Gaston et al. found only 59% of patients in the Long-Term Deterioration of Kidney Allograft Function Study with a history of CVD had received a statin 6 months post transplant [219]. Aspirin was also only prescribed in 75% and 59% of these two patient groups, respectively. These findings suggest that there is an opportunity to improve the management of transplant patients with and at risk for CVD through the appropriate use of lipid-lowering as well as other cardiovascular medications.

Change in immunosuppression

Withdrawal or avoidance of steroids may result in improvements of plasma lipids. A recent study by Vincenti et al. [220] found a reduction in weight gain, lipid-lowering medication use, and triglycerides in patients in whom steroids were avoided, or withdrawn early post transplant. A Cochrane review of 30 randomized controlled trials of steroid-sparing strategies found that steroid avoidance was associated with lower cholesterol levels in patients taking both CyA and tacrolimus, although a reduced need for lipid-lowering medication was only observed in the tacrolimus patients [221].

Hypertension

Hypertension is nearly ubiquitous in kidney transplant patients; 50–90% have blood pressure above 140/90 mmHg or are taking antihypertensive medications [10,64]. Ambulatory blood pressure studies of untreated patients have confirmed that 95% have BP readings over 130/80 mmHg [222]. As discussed previously, hypertension is an important risk factor for CVD in renal transplant recipients [223]. Reports, such as the Collaborative Transplant Study of over 24 000 deceased donor transplant patients, have also shown the degree of blood pressure elevation to be a major determinant of the rate of graft failure (Figure 88.6) [19].

Subsequent studies of this population showed that in patients with systolic blood pressure above 140 mmHg 1 year post transplant, who subsequently had their blood pressure lowered to below 140 mmHg, graft survival was improved compared with patients who remained hypertensive; for patients under the age of 50 years, cardiovascular deaths were also reduced [224].

In the immediate post-transplant period, CNIs and steroids are well known to cause or exacerbate hypertension. However, many

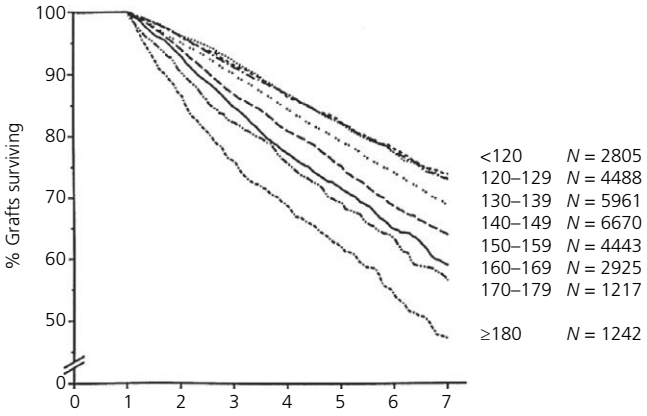

Figure 88.6. Association of blood pressure at 1 year after transplant with subsequent graft survival in recipients of cadaver renal transplants, $P < 0.001$. (Reproduced from Opelz et al. [19]: with permission of Macmillan Publishers Ltd. Copyright 1998.)

Table 88.6. Hypertension post kidney transplantation

- Donor factors:
  - Age
  - Hypertension
  - Family history
  - Renal size
- Recipient factors:
  - Pre-existing hypertension
  - Obesity
  - Native renal disease
  - Sleep apnea
- Immunosuppressive therapy:
  - Calcineurin inhibitors
  - Corticosteroids
- Transplant ureteral obstruction
- Transplant renal artery stenosis
- Transplant dysfunction:
  - Acute rejection
  - Antibody-mediated rejection
  - Thrombotic microangiopathy
  - Chronic transplant dysfunction with chronic kidney disease
  - Recurrent renal disease

Adapted from Mangray et al. [225]. Copyright © 2011 Elsevier, with permission from Elsevier.

other factors contribute to the high prevalence of hypertension currently seen in renal transplant patients (Table 88.6) [225]. Important donor factors that can result in hypertension include the background of the deceased donor; in the pre-CyA era, studies by Guidi et al. suggested that patients receiving kidneys derived from donors with a family history of hypertension were more likely to develop hypertension themselves [226]. Recipients of kidneys from older donors are also more likely to develop hypertension [227,228]. Recipients who receive a transplant that is relatively small in relation to their body size have been found to require more antihypertensive medication [229]. Recipient factors are important as well: up to 90% of renal transplant candidates are hypertensive pretransplant [164]. These patients have evidence of vascular calcification and arterial stiffness, factors that contribute to blood pressure elevation and persist post transplant [230,231]. Obesity is also prevalent in this population. Transplant dysfunction with CKD can result from episodes of acute rejection, or can occur in the setting of chronic allograft dysfunction or recurrent renal disease. Occasionally, specific anatomic abnormalities, such as transplant renal artery stenosis, or obstruction related to ureteral stenosis or extrinsic compression, may be identified through appropriate diagnostic studies (see below). Specific factors that are important contributors to hypertension in kidney transplant patients will be discussed further below.

Calcineurin inhibitors

Hypertension was appreciably less of a problem in the era prior to the widespread use of CNIs in renal transplant patients [168,232], pointing to the central role current immunosuppressive medications play as a cause of elevated BP. The widespread use of CNIs in the setting of bone marrow, liver, and cardiac transplantation has also been associated with a marked increase in the incidence of hypertension [225], as well as in patients with autoimmune diseases. It has been noted that tacrolimus-treated patients have a lower incidence of hypertension than those treated with CyA [233]. Normal volunteers treated with usual doses of CyA, but not tacrolimus, exhibit raised blood pressure and reduced GFR and renal blood flow [234]. Cross-over studies have demonstrated that patients switched from CyA to tacrolimus have significant improvement in hypertension, but this reverses when CyA is reinstituted [235].

Although incompletely understood, CNIs are known to have multiple effects that raise blood pressure [62,63]. The mechanisms by which CNIs cause blood pressure elevation are discussed above.

Corticosteroids

Steroids have long been known to contribute to hypertension. The mechanisms by which steroids elevate blood pressure are discussed above; this occurs principally through vasoconstriction that results in an increase in peripheral vascular resistance. Although a randomized trial did not demonstrate a significant difference in blood pressure among CyA-treated patients who received prednisone, or underwent steroid withdrawal or steroid-free renal transplantation [220], a systematic review of 30 randomized controlled studies concluded that steroid-sparing and steroid withdrawal strategies reduced the need for antihypertensive medication [221]. Steroid use has dropped significantly over the past two decades; over 90% of kidney transplant patients received them in 1995 but this had decreased to approximately 60% in 2007 [236].

Transplant renal vascular disease

Hypertension resulting from decreased renal perfusion may come from obstruction of the main renal artery, or from disease of the renal microvasculature. Transplant renal artery stenosis (TRAS) is estimated to occur in up to 12% of hypertensive renal transplant patients [225], usually presenting as hypertension with varying degrees of renal failure. Trauma to the vessels, occurring during organ procurement or during the transplant procedure, and malpositioning of the allograft may be causes of TRAS that presents early post transplant. Suggested risk factors contributing to TRAS that occur late post transplant include CMV infection, rejection, and long cold ischemic time [237]. Doppler ultrasound is a good screening test for TRAS, but may overestimate the incidence of hemodynamically significant arterial obstruction. In one study [238], only about 25% of patients with elevated transplant artery peak systolic velocity had angiographically confirmed stenosis. Magnetic resonance angiography (MRA) using modern techniques may be also used to identify anatomic abnormalities of the transplant renal artery [239,240]. Arteriography remains the gold

standard for diagnosis, and angioplasty is usually technically successful; even so, blood pressure may not improve in all patients [241,242]. The presence of TRAS appears to negatively affect transplant outcome [237].

Thrombotic microangiopathy may complicate CNI therapy, malignant hypertension, or transplant glomerulopathy, or result from disorders of the coagulation system such as antiphospholipid antibody syndrome [243], or recurrence of diseases such as hemolytic uremic syndrome or scleroderma [244]. The clinical picture includes one of renal ischemia with resultant hypertension and renal failure, and may include thrombocytopenia and the characteristic hematologic abnormalities of hemolysis. The treatment depends on the underlying pathophysiology, but avoidance of CNIs is recommended. Renal prognosis is often poor [245]. Dragun et al. described a syndrome of malignant hypertension in kidney transplant patients with refractory vascular rejection, but without anti-HLA antibody, associated with the presence of antibodies to angiotensin II type 1 receptors [246]. These patients responded to treatment with plasmapheresis and the ARB losartan, with prolongation of graft survival. Infusion of these antibodies into rats resulted in hypertension with lesions of endarteritis. Subsequent studies confirmed a strong association of high-affinity antibodies to the angiotensin II type 1 receptor in patients with clinically diagnosed antibody-mediated rejection, also in the absence of anti-HLA antibodies [247], although malignant hypertension was not prevalent in this study. These antibodies can be identified in 3–4% of transplant candidates; whether this class of receptor-activating autoantibodies will be further borne out as clinically significant is currently unknown [248].

Treatment post transplant

There are no large randomized studies to guide treatment of hypertension in renal transplant recipients. The KDIGO practice guidelines recommend a target blood pressure of 130/80 mmHg or less based on practice guidelines in other populations at high risk for CVD, such as patients with hypertension and diabetes or CKD [213]. This appears to be a reasonable treatment goal; it is consistent with the recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) [249]. Controlling blood pressure can be expected to reduce cardiovascular events and to prevent accelerated loss of allograft function [225].

Lifestyle modifications such as diet, exercise, and reduction of the consumption of sodium and alcohol are recommended first steps in the treatment of hypertension, and may result in an improvement in blood pressure control [250]; however, the institution of antihypertensive medication is usually required. Although there are small trials comparing antihypertensive medications [251], there are no large studies that clearly indicate the superiority of any particular class of drug. The choice of antihypertensive agent is often dictated by the patient's co-morbidities and the experience of the transplant center. No agent is contraindicated in renal transplant patients [213], except for endothelin receptor blockers; CyA decreases their hepatic metabolism and can result in elevated blood levels [64]. Dihydropyridine calcium channel blockers are often prescribed due to their efficacy, safety, and the theoretical advantage that they counteract the vasoconstriction caused by CNIs (see above) [64]. Caution should be exercised when prescribing the non-dihydropyridine calcium blockers verapamil and diltiazem as they potently inhibit the cytochrome P450 3A4 isoenzyme, and result in elevation of tacrolimus and CyA levels. Calcium channel blockers may also potentiate CNI-induced gingival hyperplasia [64]. Beta-blockers are often prescribed in renal transplant recipients, and are particularly beneficial in patients with underlying CVD. Diuretic medications are useful in the post-transplant period where volume overload may be present; however, resulting volume depletion and prerenal azotemia may result in elevation of the serum creatinine level. ACE inhibitors and ARB blockers are also effective antihypertensive agents and have been demonstrated to reduce post-transplant proteinuria [252]; their use has been advocated as cardioprotective agents [219]. There are currently no clear data to recommend blockers of the renin–angiotensin system over other classes of antihypertensive medication; adverse effects of ACE inhibitors and ARBs in the transplant population include anemia and hyperkalemia. Recent reviews of the management of hypertension in renal transplant recipients are available [63,64,225].

Diabetes

Diabetes is the leading cause of ESRD in the US; it is listed as the cause of ESRD in 46% of patients initiating dialysis [253] and also occurs in patients with other causes of ESRD. Almost 24% of transplant patients have diabetes as their primary renal diagnosis [254]. Diabetes has long been recognized as a risk factor for CVD, and many transplant programs subject prospective diabetic kidney transplant recipients to extra scrutiny for evidence of pre-existing CVD prior to listing. For instance, Cosio et al. reported on 933 first transplant recipients at the Mayo Clinic from 1998 to 2006 [187]. Patients who were diabetic at the time of transplant had a higher rate of cardiovascular events (25%), cardiovascular mortality (12%), and all-cause mortality (19.3%) over the 46 months of follow-up, compared with patients without diabetes (7.4%, 1.1%, and 6.1%, respectively; $P < 0.0001$); not surprisingly, diabetic patients also had a much higher incidence of prior CVD. Recently, Kuo et al. reported on 37 448 primary transplant recipients from 2004 to 2007 from the Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/UNOS) database who survived for 1 year post transplant [255]. They found that patients with pretransplant diabetes had an adjusted 89% increase in cardiovascular mortality ($P < 0.001$).

NODAT has been increasingly recognized as a serious problem. The most recent USRDS data report shows the cumulative incidence of post-transplant diabetes to be approximately 30% at 1 year and >40% at 3 years post transplant in adults, and >10% 3 years post transplant in pediatric patients younger than 18 years of age [256]. However, the incidence may have been over-reported, as the criteria used for NODAT were not known. Valderhaug et al. [257] studied 1410 consecutive first-time renal transplant recipients without diabetes, and performed oral glucose tolerance testing 10 weeks post transplant on those who had not already developed NODAT. Using the current ADA criteria, they found 17% of patients developed NODAT and 22% had impaired glucose tolerance. This high incidence of NODAT occurs in an era in which almost 90% of patients use tacrolimus as their initial CNI [236]; tacrolimus use has been strongly associated with NODAT (see above) [78]. The current criteria for the diagnosis of diabetes as defined by the ADA and the World Health Organization are listed in Table 88.7.

New-onset diabetes after kidney transplantation has long been recognized as a risk factor for mortality. Kasiske et al. [78] studied

Table 88.7. Current criteria for the diagnosis of diabetes as defined by the American Diabetes Association and the World Health Organization (WHO)

1 Fasting plasma glucose of ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h*
or
2 Symptoms of hyperglycemia and a casual plasma glucose of ≥200 mg/dL (11.1 mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of hyperglycemia include polyuria, polydipsia and unexplained weight loss
or
3 2-h plasma glucose of ≥200 mg/dL (11.01 mmol/L) during an oral glucose tolerance test. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water*

*In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day.

11 659 patients reported to the USRDS who had received first kidney transplants from 1996 to 2000 and were not diabetic. NODAT developed in 24% of these patients by 36 months, and was associated with a 47% increase in death from CVD ($P = 0.0140$) and an 87% increase in death from all causes ($P < 0.0001$). Cole et al. [258] reported on 27 707 first kidney-only transplants from the USRDS database transplanted from 1995 to 2002. They found that patients with NODAT experienced a relative risk of death with a functioning graft of 1.41 (1.25–1.59), which was presumed to be from CVD. In Valderhaug et al.'s study [257], post-transplant diabetes patients experienced an 80% relative increase in the risk of cardiovascular death ($P < 0.05$) and a 54% relative increase in all-cause mortality ($P < 0.05$). Each 1-mmol/L (18-mg/dL) increase in plasma fasting glucose was associated with a 19% increase in cardiovascular mortality risk ($P < 0.05$). Of interest, in Kuo et al.'s study of over 35 000 patients, those with NODAT had a 15% increase in risk of cardiovascular death, which was not statistically significant [255]. This negative finding may be related to the short 1.5-year follow-up period, or to incompleteness in the reporting of NODAT (only a 6.6% reported incidence at 1 year post transplant). The morbidity and mortality risk associated with NODAT requires further study.

Risk factors for NODAT

Many important risk factors for NODAT in renal transplant recipients are not modifiable and include African–American and Hispanic ethnicity, male gender of either the recipient or the donor, increasing number of HLA mismatches, and a family history of diabetes in a first-degree relative; infection with hepatitis C virus (HCV) or CMV also confers increased risk [78,103,142,259]. Increasing age is an important risk factor; patients who are over age 45 years have over twice the risk of NODAT compared with younger patients [78]. Autosomal dominant polycystic kidney disease has been reported to be associated with an increased risk for NODAT [260]; however, more recent studies have not confirmed that finding [261,262]. Potentially modifiable risk factors include obesity, which is increasingly common; up to 60% of renal transplant recipients have been reported to be overweight or obese [263]. In a report by Shah et al., patients who were overweight (BMI 25–30 kg/m^2) have a 1.5-fold increased risk, and those who were obese (BMI >30 kg/m^2) a 1.8-fold increased risk, of NODAT [261]. Other potentially modifiable risk factors include episodes of acute rejection (which may be related to the resulting steroid treatment) and the use of CNIs, mTOR inhibitors, and corticosteroids [103]. In particular, NODAT occurs more commonly with tacrolimus than with CyA [78,79]. The mechanisms of impaired glucose tolerance relating to immunosuppressive medications are discussed above.

Management post renal transplant

Modification of the immunosuppressive regimen

The mechanisms by which corticosteroids, CNIs, and sirolimus impair glucose tolerance have been reviewed above. Reducing the doses of, or in some cases eliminating, these medications must balance the potential improvement in blood sugar control with the risk of rejection. Studies of steroid withdrawal or avoidance, summarized in the Cochrane review by Pascual et al. [221], indicate a decrease in the rate of NODAT, particularly in patients taking CyA; patients taking tacrolimus did not seem to benefit from steroid withdrawal. Confirming these observations, two recent randomized studies of steroid avoidance, or early withdrawal, did not find a reduction in NODAT in patients taking tacrolimus and low-dose prednisone [220,264]; however, the steroid-free groups did require less antidiabetic medication. In patients at high risk for NODAT, a strategy of rapid steroid tapering, or steroid withdrawal, should be considered if a steroid-free regimen is not the standard of care. Similarly, selected patients may benefit from a change from tacrolimus to CyA, although the potential exists for worsening of hypertension and hyperlipidemia, and the resulting overall change in cardiovascular risk is unknown [263]. High tacrolimus levels above 15 ng/mL in the early post-transplant period seem to convey an increased risk for persistent impairment of glucose control and should be avoided [265].

Lifestyle changes and medical therapy

Changes in lifestyle aimed at improving diet and increasing exercise are important ingredients for controlling diabetes in transplant and non-transplant patients alike. If adhered to, active lifestyle modification has been shown to improve glucose control in patients with and at risk for NODAT [266]. However, many patients will go on to require treatment with hypoglycemic medications. There are currently no randomized studies on which to base drug selection in the management of diabetic patients after renal transplant; practice guidelines such as KDIGO offer only general advice [213]. A stepped approach, starting with monotherapy with an oral agent, and escalating through combination therapy with the eventual institution of insulin, is in keeping with consensus guidelines for the management of diabetes in the overall population [263,267]. A recent review by Yates et al. suggests institution of hypoglycemic therapy if the hemoglobin $A1_c$ ($HbA1_c$) remains above 7.0% despite lifestyle measures [263]. The KDIGO guidelines suggest considering a target $HbA1_c$ of 7.0–7.5% to avoid severe hypoglycemic reactions, which have been shown to be more common in kidney transplant patients treated with an intensive glucose control regimens [268]. All classes of hypoglycemic agents have been prescribed to this patient population. Considerations in transplant patients include the fact that many sulfonylureas raise CyA levels, which should be carefully monitored; this effect does not occur with glipizide, which may be preferred [263,269]. Gastrointestinal upset can occur with metformin use, and particularly may be problematic in patients taking mycophenolic acid; swelling and a possible increase in cardiovascular events may have been reported with thiazolidinediones

[263]. If insulin therapy is required, many endocrinologists recommend the use of morning NPH insulin, as its duration of action matches the pharmacokinetic profile of oral glucocorticoids. Recent reviews of the management of diabetes in renal transplant recipients are available, and international consensus guidelines were published in 2003 [103,142,263].

New immunosuppressive agents

Belatacept is a member of a new class of immunosuppressive agents that work by blocking costimulation signals (signal 2) between T cells and antigen presenting cells, thereby preventing T-cell activation [270]. Belatacept is a fusion protein that is intravenously administered monthly in place of a CNI. In the Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT), in which belatacept was randomly compared to CyA in 1209 patients who also received MMF and prednisone, the absence of a CNI resulted in lower systolic and diastolic blood pressure, non-HDL cholesterol, and triglycerides [271]. Further, the patients treated with a belatacept-based regimen had an estimated GFR that was 21 mL/min/1.73 m^2 higher than in the CyA group after 3 years of study [272]. Although graft and patient survival have not yet proven to be superior in patients who receive belatacept, the markedly reduced cardiovascular risk profile and improvement in renal function associated with this class of medication offers promise for decreased CVD and increased patient and allograft longevity in the future. It is not known whether existing renal transplant patients with, or at high risk for, CVD would benefit from conversion to a belatacept-based regimen.

Tofacitinib is an inhibitor of Janus kinase (JAK), a tyrosine kinase that participates in signaling from multiple cytokine cell surface receptors essential for T-cell, B-cell, and natural killer cell function [273,274]. A preliminary study compared tofacitinib with CyA in renal transplant recipients. Tofacitinib-treated patients showed improved renal function and a lower incidence of NODAT; however, there was a higher incidence of serious infections, hematologic abnormalities, and PTLD in the tofacitinib group [274]. In the future this class of medications may provide an alternative to current agents, but with a lower profile of cardiac risk.

Cardiovascular disease in pancreas transplant patients

Pancreas transplantation is a successful method for treating diabetes; simultaneous kidney and pancreas transplantation (SPK) accounts for over 70% of pancreas transplant procedures [275]. Although type 1 diabetics have traditionally been considered the only candidates for pancreas transplantation, type 2 diabetics have increasingly benefited from this operation [276].

Compared to patients who undergo deceased donor kidney transplant alone, patients with a successful SPK transplant have a significantly improved survival. Of note, SPK and living donor transplant recipients with type 1 diabetes have similar excellent patient and graft outcomes [277]. Because living donor and SPK recipients have different characteristics, it is hard to compare these two groups precisely; living donor patients are older, have higher rates of co-morbid cardiac disease, receive kidneys from older recipients who are less likely to be African–American, may receive a pre-emptive transplant, and have better HLA matching compared with SPK patients [277]. Recent multivariate analyses have tried to take these differences into account, and have come to different conclusions. Young et al. analyzed the UNOS database and found living donor transplant recipients have superior outcomes [278]. However, Weiss et al. examined Scientific Registry of Transplant Recipients (SRTR) data and reported the outcome advantage of living donor recipients to be concentrated in the first year post transplant; SPK patients with pancreatic allografts functioning >1 year had the best long-term patient and graft survival rates [277]. Patients receiving a pancreas after a living kidney transplant also have good long-term results [279]. The improved outcomes of pancreas recipients are likely due to the long-term salutary effects of euglycemia.

Restoration of euglycemia after pancreas transplantation has been shown to slow and eventually halt the progression of diabetic retinopathy [280] and neuropathy [281,282], as well as to prevent the recurrence of diabetic nephropathy in the simultaneously transplanted kidney [282]. Changes of diabetic nephropathy have been found to pathologically reverse in patients who undergo pancreas transplant alone [282].

Pancreas transplant patients have often been reported to experience an improvement in cardiac risk factors that includes a reduction in total cholesterol and serum triglycerides, as well as lowering of both systolic and diastolic blood pressure [283,284]; but not all studies have consistently found these improvements. Pancreas transplantation has also been seen to have an effect on improving macrovascular disease. Larsen et al. studied patients before and approximately 2 years after pancreas transplant; they reported improvement on ultrasound in carotid intima-media thickness (IMT), which has been correlated with the risk of cardiac events [285]. In a cross-sectional study, Fiorina et al. also found the improvement in IMT, and further reported an improvement in endothelial function; endothelial-dependent dilation of the brachial artery was found to normalize in SPK patients but not in patients with kidney transplants alone [286]. Jukema et al. performed quantitative coronary arteriography on 26 SPK patients with functioning pancreatic grafts and on six patients who had lost their grafts [287]. After a mean of 3.9 years, progression of coronary atherosclerosis was markedly slowed in patients with functioning allografts; lesion regressed in 38% of these patients compared with 0% of patients in whom the pancreatic graft was lost. Left ventricular systolic and diastolic function has been shown to improve to a greater extent in SPK patients as well [288,289]. Rates of acute MI [288,290] and congestive heart failure [288] have also been reported to be lower in patients with combined pancreas and kidney transplants, compared with patients receiving a kidney transplant alone.

Taken together these data provide strong evidence that pancreas transplantation improves both microvascular and macrovascular disease, and provides a benefit in cardiovascular morbidity and mortality. Nevertheless, CVD is still a leading cause of morbidity and death in this patient population [288,291].

Cardiovascular disease in liver transplant patients

Improvements in orthotopic liver transplantation (OLT) have resulted in patients living longer than ever before. Current 1- and 5-year survival rates are 85% and 67%, with a 10-year survival of over 50% [292]. Steadily improving patient survival rates are related to improvements in surgical technique, and in the medical management of these patients both pre- and post-operatively. Although patients with significant underlying CVD are not considered candidates for liver transplantation, now that patients are surviving

longer CVD is becoming an important cause of morbidity and mortality in this patient population [293]. Further, risk factors for CVDs, such as hypertension, hyperlipidemia, diabetes, and the metabolic syndrome are becoming increasingly prevalent post OLT. Cardiac complications are also a common cause of death in the immediate postoperative period [294]. Liver transplant patients have been reported to have higher cardiovascular death rates compared to the general population [295]. Of note, liver transplant recipients are becoming older every year, with the most recently available data showing that the average age has increased from 46 years in 2000 to almost 50 in 2009 [292]; patients over the age of 65 years are the most rapidly growing group, increasing from 7% of OLT patients in 2000 to over 11% in 2009. Along with this increase in age comes an increase in cardiac risk factors. Although less prevalent than in kidney or cardiac transplant, patients coming to liver transplant often have underlying CVD, which may be unrecognized. This section will review the factors that contribute to cardiovascular risk after liver transplantation, and discuss management guidelines.

Postoperative cardiovascular complications

Patients undergoing liver transplant are at high risk of cardiovascular complications in the days and weeks following transplant surgery; cardiac complications are a leading cause of perioperative death [294].

Although a relatively small number of patients have underlying CHD (see below), cirrhotic cardiomyopathy is a common occurrence [296]. The identification of abnormal cardiac function in cirrhotic patients is attributed to Kowalski and Abelmann [297]; later classic studies by Regan et al. [298] and by Gould et al. [299] uncovered an abnormal increase in left ventricular end-diastolic pressure, and a notable decrease in cardiac contractility in cirrhotic patients, under the stress of angiotensin administration or exercise. It is now accepted that cirrhotic cardiomyopathy occurs in alcoholic and non-alcoholic patients alike [296]. Clinically, patients exhibit abnormalities in systolic and diastolic function when challenged by stress; however, the peripheral vasodilation and reduced afterload that occurs in patients with liver disease may prevent clinically apparent CHF.

These underlying cardiac abnormalities leave patients at risk for adverse cardiac events during and after liver transplant surgery. During the operation, bleeding, third space fluid shifts, and ongoing production of ascites may result in marked drops in preload, while overly aggressive volume replacement with fluids or blood products may result in postoperative volume overload [296]. Underlying impairment of myocardial diastolic and systolic function increases the risk of reduced cardiac output and hypotension, and of pulmonary edema. Pulmonary edema is common; it has been reported to occur in 22–56% of OLT recipients in the first postoperative month [294]. The rapid increase in blood pressure and systemic vascular resistance that occurs post transplant places a stress on the heart and, in the setting of fluid administration, may account for the common occurrence of pulmonary edema; most clinical episodes resolve with appropriate medical therapy [296,300]. Even in the absence of evidence of, or risk factors for, underlying heart disease, Eimer et al. reported 7% of 86 patients with normal heart catheterizations and normal echocardiograms developed severe heart failure after liver transplantation [301]. It is critical that careful monitoring of hemodynamic status is done in the intraoperative and perioperative periods, with meticulous attention to fluid balance and electrolytes, to minimize acute cardiovascular complications.

Sampathkumar et al. reported the rare development of dilated cardiomyopathy in the early post-transplant period (seven of 754 patients); cardiac function subsequently improved [302]. Studies by Torregrosa et al. have shown that the structural and functional abnormalities of cirrhotic cardiomyopathy typically resolve by 9–12 months post OLT [300,303].

Post-reperfusion syndrome (PRS) is another complication that can occur in the immediate postoperative period. It was initially described by Aggarwal et al. in 1987 [304], and is defined as a decrease in mean arterial pressure of at least 30% and a decrease in heart rate for 1 min within the first 5 min after reperfusion. Associated hemodynamic findings include elevation of pulmonary artery, central venous, and pulmonary capillary wedge pressure, and a decrease in systemic vascular resistance [305]. This syndrome is incompletely understood, and is attributed to postoperative metabolic abnormalities, such as metabolic acidosis and hyperkalemia, and to vasoactive substances released from the allograft. PRS occurs in approximately 30% of liver transplant recipients [306]. A review by Hilmi et al. of 338 patients transplanted at the University of Pittsburg from 2002 to 2003 found that patients with more severe PRS were sicker, required longer intensive care unit and hospital stays, and were more likely to be retransplanted. However, these patients did not have a decrease in 5-year survival [307].

Other cardiac complications that can occur postoperatively include atrial and ventricular arrhythmias, MI, air embolism [308], intracardiac and pulmonary embolism [309], and cardiac arrest [294,310]. Fouad et al. reported on 324 OLT patients over 40 years of age who were transplanted between 2002 and 2007. Twenty-three patients suffered an intraoperative cardiovascular event, including two patients who died of PRS. Of twenty-one patients who died in the first 6 months, five (23%) deaths resulted from direct cardiac causes, making CVD the leading cause of death after surgery in this study [294]. Other studies have estimated cardiovascular complications to account for 7–15% of deaths post transplant [296,311].

Long-term cardiovascular death and coronary heart disease

It is now common for patients to die of CVD after liver transplantation [294]. Although early reports found CVDs to be an unusual cause of death [312–314], recent data have demonstrated cardiovascular deaths to be an increasingly important problem. Abbasoglu et al. prospectively collected data on 1174 OLT patients transplanted from 1985 to 1995, and found that 17% of patients who had survived for $>$ 1 year died of CVD or stroke at a mean of almost 5 years post transplant [315]. CVD was reported to cause death in 8.3% of 4000 consecutive patients reported by Kashyap et al. from the University of Pittsburg, with a mean follow-up of 9.4 years [316]. Pruthi et al. reported CVD as the cause of death in 21% of 299 adult patients transplanted between 1984 and 1977 who survived for at least 3 years post liver transplant; most had previously recognized coronary disease or known cardiac risk factors [317]. Rabkin et al. reported that 13% of late mortality unrelated to recurrent liver disease resulted from cardiovascular causes among 459 patients transplanted between 1991 and 2000 [318]. Johnston et al. studied 110 consecutive OLT patients and found a 2.56 relative risk for cardiovascular death compared to an age-matched population; 16% of the patients died of CVD over the 3.9-year median follow-up period [295]. Albeldawi et al. reported cardiovascular events in 775 patients transplanted at the Cleveland Clinic from 1996 to 2008; 83 patients (10.7%) died from cardiovascular-related causes over the 40-month mean follow-up period, the third most

common cause of death after liver failure and infection [319]. Watt et al. have recently published a detailed study of 798 patients from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) database transplanted at multiple centers between 1990 and 1994, and followed for a median of 10 years [320]; almost 20% of deaths not related to hepatic disease were due to cardiovascular causes [320]. Diabetes, hypertension, renal insufficiency, and patient age were important factors associated with death beyond 1 and 5 years post transplant [320]. A review of these studies reveals that, along with hepatic disease, infection, and malignancy, CVD causes a significant proportion of deaths after liver transplantation, and is an increasingly important problem.

Coronary artery disease (CAD) has been reported to have a high prevalence in patients referred for liver transplant, but is often unrecognized. Carey et al. studied 37 consecutive patients over 50 years of age who were referred for OLT and underwent coronary arteriography at the Cleveland Clinic [321]. Although 30 patients had no history of heart disease, 10 patients (27%) had moderate or severe CAD and six patients (16.2%) severe CAD; diabetes was found to be the most significant risk factor. More recently, Tiukinhoy-Laing et al. reported cardiac catheterization results from 174 consecutive end-stage liver disease (ESLD) patients over the age of 45 years who were referred for OLT between 2000 and 2003 [322]. Risk factors were common in this patient group; half of patients had hypertension or diabetes and one-third were current or previous smokers. Sixty percent of patients had angiographic coronary lesions; 26% had previously unknown coronary disease that was moderate or severe. Patients with known CHD have significantly increased morbidity and mortality after OLT [323,324]. The increase in mortality is well demonstrated by Yoo and Thuluvath's analysis of 18 044 patients recorded in the UNOS database undergoing OLT between 1988 and 2001, in whom the absence or presence of CAD and diabetes was recorded prior to transplant (Figure 88.7). A history of CAD or diabetes were both independent risk factors for an approximately 40% lower 5-year survival; patients with both conditions demonstrated a 60% survival decrease [324].

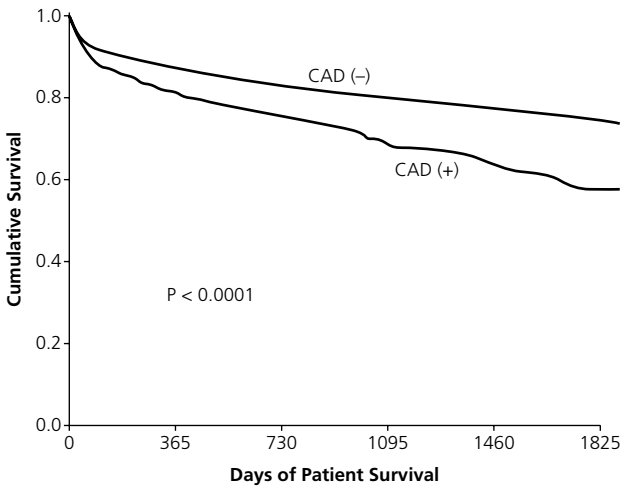

Figure 88.7. Kaplan–Meier patient survival with and without coronary heart disease (CHD) after liver transplant. (Reproduced from Yoo et al. [324], with permission of Wolters Kluwer Health).

Cardiac events post transplant

Despite routine screening of transplant candidates for CVD, cardiovascular events after liver transplantation are common [319].

In Johnston et al.'s study [295] of 110 patients assessed at a median of 3.9 years post OLT, 25 ischemic and 32 non-ischemic cardiovascular events occurred (early postoperative events were excluded). The relative risk of ischemic cardiac events was 3.07 compared with the expected risk in a matched population. Mazuelos et al. found 9.4% of 170 OLT patients experienced cardiovascular complications after 5 years of follow-up [53]. In the Albeldawi et al. study, 83 patients experienced one or more cardiovascular events over the 40-month follow-up, with a calculated cumulative event rate of 13.5% over 5 years. Multivariate analysis identified independent risk factors that included post-transplant metabolic syndrome (PTMS), age, male gender, and post-transplant diabetes and hypertension. MMF was also found to be a risk factor, but was felt to be a surrogate for renal failure as it was used when CNIs resulted in side -effects requiring a significant reduction in dose [319]. Laryea et al. reported on 118 patients in the Canadian Transplant Atlantic Registry receiving liver transplants between 1998 and 2004 [325]. Over 58 months of follow-up, 25 major vascular events occurred, including acute coronary syndrome, MI, transient ischemic attack (TIA), and stroke. Significantly more events (21 vs. 4; $P = 0.003$) occurred in patients with PTMS. Laish et al. reviewed the files of 248 patients undergoing liver transplant from 1991 to 2007, and reported similar results. Twenty-three patients experienced major vascular events over the 6.2 years of follow-up; these were significantly more common in patients with PTMS (17 vs. 6, $P = 0.027$) [326]. A recent report from the Mayo Clinic sought to define cardiac risk in 230 adults receiving transplants between 1998 and 2001 and followed for a median of 8.2 years; 59 cardiac events occurred, including clinically documented CAD (angina or angiographically significant stenosis), CHF, CVA, peripheral vascular disease, or cardiovascular death [293]. Although multiple risk factors were prevalent among this population, multivariate analysis confirmed pretransplant diabetes, prior smoking history, and prior cardiac disease as independent risk factors. In addition, elevated troponin I levels predicted the observed cardiovascular events, possibly reflecting underlying cardiac microvascular disease or unrecognized left ventricular dysfunction.

Cardiovascular risk factors

Cardiac risk factors are common in patients after OLT. Single center observational studies of relatively small numbers of patients, as reviewed above, may be too underpowered to confirm factors in liver transplant patients that are known to be associated with cardiac risk in the general population. The Framingham risk factors of hypertension and hyperlipidemia, in addition to diabetes and obesity, may be present before transplant, but are increasingly common after OLT. It can be presumed that they play an important role in causing cardiovascular morbidity and mortality in liver transplant recipients.

The high prevalence of cardiac risk factors in this population is in large part due to exposure to immunosuppressive medications. CNIs and corticosteroids play important roles in increasing blood pressure and blood glucose, as well as resulting in dyslipidemia (see above section on Mechanisms of the adverse cardiovascular effects of immunosuppressive medications); sirolimus may also contribute

Table 88.8. Risk factors for cardiovascular disease in liver transplant patients

- Patient age
- Male gender
- Diabetes pre or post transplant
- Hypertension pre or post transplant
- Dyslipidemia
- Obesity
- Post-transplant metabolic syndrome
- Non-alcoholic fatty liver disease
- Smoking
- Pretransplant cardiovascular disease
- Chronic kidney disease
- Troponin I

Modified from Watt et al. [320], with permission from John Wiley and Sons.

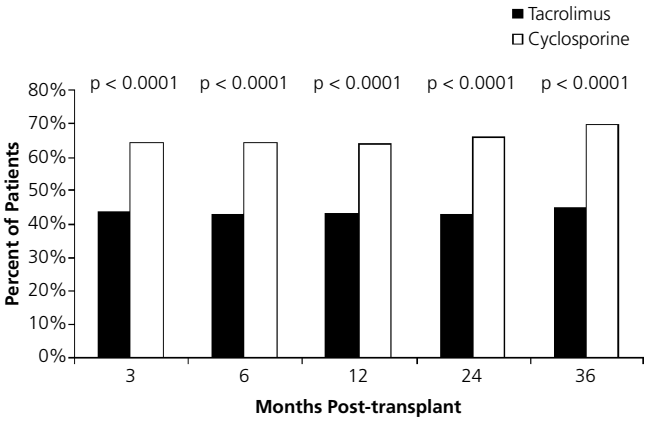

Figure 88.8. Comparison of the proportion of patients in the tacrolimus and cyclosporine treatment groups requiring antihypertensive agents, 3–36 months following liver transplant. (Reproduced from Lucey et al. [332], reproduced with permission from John Wiley & Sons.)

to cardiac risk. Cigarette smoking at the time of listing increases cardiac mortality [327]. Other contributors to the risk of CVD include patient age, a history of pre-existing cardiac disease prior to transplant, CKD, and non-alcoholic fatty liver disease (NAFLD) (Table 88.8) [328]. A significant number of patients with multiple risk factors exhibit the metabolic syndrome, which is becoming increasingly common after liver transplantation [329]. The next section will expand on the role of selected important risk factors in liver transplant patients.

Hypertension

Hypertension is common in liver transplant recipients; estimates of the incidence range from about 30% to 80% of patients [330]. The use of CNIs appears to play an important role in de-novo hypertension; this incidence is demonstrably higher in patients treated with CyA than with tacrolimus (Figure 88.8) [331,332]. The use of corticosteroids also contributes to hypertension. Studies of OLT without steroids, and of late steroid withdrawal, demonstrate improved blood pressure [333,334].

Other factors contributing to the high incidence of hypertension include obesity (see below) and the development of CKD, which is common after liver transplantation, attaining a cumulative incidence of approximately 20% after 5 years [335].

There are no specific evidence-based practice guidelines for the management of hypertension in liver transplant recipients. A target blood pressure of 130/80 mmHg has been suggested by recent reviews [330,336,337]. As with hypertension in the general population, changes in lifestyle, including weight reduction, exercise, and decreased dietary sodium, are recommended, but if not successful institution of antihypertensive medication is required. Liver transplant recipients may be successfully treated with a variety of agents; there are no large studies in this population on which to base therapy, although recommendations for treating hypertension in the general population and in renal transplant recipients may be used as a guide [213,249]. Calcium channel blockers are recommended based on their excellent safety profile and on the theoretical advantage that they may counteract the vasoconstriction that occurs with CNIs [338,339]. It is recommended that the non-dihydropyridine calcium channel blockers, diltiazem and verapamil, be used with caution, as they raise CNI levels by competing with cytochrome P450 3A4. Beta-blockers are preferred for patients with CHD, and are also well tolerated by most patients. ACE inhibitors and ARBs have been recommended for patients with CKD and with proteinuria. A small randomized study of nifedipine versus carvedilol in patients with de-novo hypertension after OLT found that carvedilol was better tolerated; both drugs lowered blood pressure to target levels by themselves in only a minority of patients [340]. Indeed, many patients will require a combination of blood pressure medications. Consideration may be given to changing immunosuppressive medication in selected patients. Corticosteroids can be reduced or eliminated. Patients on CyA can change to tacrolimus, or a regimen can be instituted based on MMF or sirolimus.

Hyperlipidemia

Although ESLD patients often have low serum cholesterol, due to impaired hepatic cholesterol synthesis and esterification, hyperlipidemia commonly occurs after liver transplantation [341]. About half of OLT recipients have hyperlipidemia, although this fraction may be decreasing over time [342]. For instance, Laryea et al. studied 118 liver transplant patients who had been followed for at least 18 months; 46% developed dyslipidemia with 45% having abnormal triglycerides [325]. In Johnston et al.'s report of 110 patients studied in 1999, and followed for a median of 3.9 years, 44% had raised serum cholesterol, 52% a low HDL, and 8% elevated triglycerides [295]. Neal et al. reviewed 181 patients receiving OLT from 1994 to 1999 with a median follow-up of 54 months [343]. Hypercholesterolemia was present in 16% of patients prior to and 60% after transplant, and the percentage of patients with elevated triglycerides increased from 10% to 40%. Mixed hyperlipidemia is most commonly seen post transplant, but isolated elevation of cholesterol or triglycerides may also be seen.

As with hypertension, immunosuppressive medications play a major role in abnormal lipid metabolism. CNIs, sirolimus, and corticosteroids all result in dyslipidemia; the mechanisms by which this occurs are discussed in detail above. Diet, obesity, and diabetes also contribute to lipid abnormalities (see below). CyA has been stated to cause more dyslipidemia than tacrolimus after OLT, and switching from CyA to tacrolimus has resulted in decreases in cholesterol [344]. However, recent studies reported different findings; one study comparing these two drugs found similar levels of total cholesterol and triglycerides 1 year post transplant in patients without baseline lipid abnormalities [345]; this observation has been confirmed [346].

Dietary modification is not usually sufficient to normalize plasma lipids, and lipid-lowering medication is usually required. Statin

drugs are safe and effective agents after OLT, and are well tolerated with a low incidence of adverse effects [347]. A variety of statins have been used safely in transplant patients. It is probably wise to start with a reduced dose, such as atorvastatin 20 mg/day or pravastatin 20 mg/day, both of which have been studied in liver transplant patients [348,349]. The drug interaction between statins and CNIs is discussed above. There are no evidence-based guidelines to provide guidance on choosing a threshold cholesterol level for treatment. We recommend that liver transplant patients be considered at high risk for CVD, and that LDL cholesterol be kept below 100 mg/dL (2.6 mmol/L). Ezetimibe may be added safely if the LDL target is not achieved with a statin or statin drugs are not tolerated [350]. Fish oil and fibric acid derivatives can be used to treat triglyceride elevations and are generally safe.

Of interest, liver transplantation has been used to treat homozygous familial hypercholesterolemia, including preemptively before the development of severe CVD [204].

Diabetes

Diabetes is becoming increasingly common after liver transplant. The prevalence is about 15% prior to and 30–40% post transplant [351]. Earlier studies reported a prevalence of 13–27% [352], but more recent studies have found a much higher prevalence. For instance, Larea et al. reported an increase in diabetes from 13% to 61%, and Laish et al. an increase from 14.4% before to 39.6% after liver transplantation [325,326]. This increase in prevalence may be explained in part by the use of a lower cut-off fasting blood sugar of 126 mg/dL to define diabetes established by the ADA (Table 88.7) [325,352,353]. Risk factors for developing NODAT in liver transplant patients include patient age over 50 years, male gender [354], BMI over 30 kg/m^2 [355], use of tacrolimus as compared to CyA [355], and infection with CMV [356]. HCV infection plays an important role as a risk factor for developing NODAT in liver and kidney transplant recipients [259,355], and has also been linked to type 2 diabetes in non-transplant patients [355,357]. Hepatic infection with HCV leads to insulin resistance [358,359]; the virus can also infect pancreatic β-cells that could potentially lead to a decrease in insulin production [360]. In both liver and kidney transplant patients, HCV seems to cause NODAT most commonly in patients taking tacrolimus compared with CyA [259,355]. Denervation of the transplanted liver itself may result in insulin resistance and contribute to NODAT [361]. As reviewed above, the occurrence of diabetes in liver transplant patients is strongly associated with CHD, cardiovascular events, and cardiac death [293,319–321].

As with other transplant patients, treatment of diabetes after liver transplant is similar to that in the general population [351]. It should be noted that the liver metabolizes most sulfonylurea and thiazolidinedione drugs; these agents should be used carefully and preferably in patients with normal and stable graft function [362]. Steroid dosage should be minimized [330]; in selected patients, conversion from tacrolimus to CyA or to a CNI-free regimen may be considered to improve glucose tolerance [363,364].

Obesity and the metabolic syndrome

PTMS is becoming an increasing problem among liver transplant recipients. We have already discussed many of the individual components of the MS, including hypertriglyceridemia, low LDL, hypertension, and impaired glucose tolerance above (Table 88.2) [351]. Abdominal obesity is also prevalent among adults in general and liver transplant patients in particular. The presence of ascites can make the assessment of central obesity difficult [351], thus some programs use a BMI above 30 kg/m^2 as an additional criterion for MS [325]. Obesity occurs in over one-third of ESLD patients and non-alcoholic steatohepatitis (NASH) is continuing to increase as a cause of ESLD requiring liver transplant. Overall the MS has been reported to occur in 5–29% of patients prior to liver transplantation, and has a prevalence of 43–58% in liver transplant recipients [365]. PTMS has been demonstrated to be an important risk factor for major vascular and cardiac events after liver transplantation, although an increase in cardiac mortality has not yet been shown [319,325,326]. PTMS is managed by addressing the individual components that contribute to cardiac risk. In addition to diet and exercise, the use of orlistat has been found to be a safe adjunct for weight loss in liver transplant patients [366].

Summary

Transplant patients suffer the same cardiovascular ills as the general population, but are encumbered by the augmentation of these conditions by their primary diseases and the acquired effects of their immunosuppressive drug regimens. As general transplant outcomes have improved and patients have enjoyed longer post-transplant lives, cardiovascular-related conditions now represent the most significant threat to long-term outcome. As such, all transplant physicians should diligently address modifiable risk factors and seek to mitigate acquired risks to avoid premature morbidity and mortality due to CVD. This should go hand in hand with the more recognized plans for immunosuppressive drug management.

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CHAPTER 89

Monitoring for HLA Antibody after Organ Transplantation

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Introduction

A peripheral biomarker capable of informing the state of an allograft, particularly as it relates to the adequacy of immunosuppression, is much needed for all organ transplants, particularly those like the heart for which a useful functional surrogate is not available. Although many biomarkers exist and some have been tested to a degree, the presence of donor specific antibody (DSA) is the most widely published and best validated. Indeed, DSA monitoring is increasingly recognized as a critical part of the postoperative management of all allograft recipients. While DSA monitoring is known to relate well to clinical outcome, it nevertheless has substantial nuances that the clinician should be aware of in order to follow a transplant recipient with appropriate rigor. This chapter will examine the existing strategies for following DSA post transplant, and outline their interpretation and ability to yield useful information about the post-transplant immune status of a solid organ recipient. Specific information about the tests used for DSA detection and operation of a histocompatibility lab are covered in Chapter 36.

Rationale

There are many reasons to identify clinically informative biomarkers of rejection; they include detection of subclinical rejection, differentiating rejection from conditions resulting from over immunosuppression, and identifying patients whose immune suppression can be minimized or eliminated. There is little doubt that the presence of DSA post transplant is a harbinger of an inferior outcome compared to individuals devoid of DSA. This holds true for renal, cardiac, lung, and possibly liver allografts, as will be discussed below [1–4]. If monitoring for HLA DSA is to be performed, there are several questions that must be addressed:

- When and how often is monitoring needed?
- What method of monitoring DSA is most informative?
- How can it be determined when and if the presence of DSA predicts poor outcome and conversely, when and if the absence of DSA indicates an immune tolerant state?
- What should be done with the information obtained?

DSA and transplant outcomes

Renal transplants

In a multicenter retrospective study involving 1329 recipients of deceased donor renal transplants, Terasaki et al. reported that the 4-year graft survival was only 58% among recipients with de-novo HLA antibody but 81% among recipients without HLA antibody [5]. The hazard ratio for graft loss in recipients with HLA antibody was 3.3. While these results were intriguing, there were several limitations to this study, including incomplete classification of rejection and reasons for graft loss, which included death with a functioning graft. Nonetheless, these data supported the earlier speculation that there was a significant association between HLA DSA and graft rejection [6], and suggested the clinical relevance of de-novo HLA antibody even though its incidence was <20% on average. Subsequently, Lee et al. [7] showed that de-novo DSA detected within the first year after transplantation was more deleterious than DSA detected after the first post-transplant year. Similar findings were described by Li et al. [8] who reported a 5-year graft survival rate of 96% among HLA antibody-negative recipients of living donor transplants compared to a 69% 5-year graft survival rate in recipients who developed de-novo HLA DSA. In a subsequent and longer term study by Lee et al. [9] of renal transplant recipients, the 5-, 10-, and 15-year graft survival rates for those in whom de-novo DSA was never detected were 85%, 75%, and 56%, respectively, and in recipients who developed de-novo HLA DSA, were 65%, 53%, and 28%, respectively. These differences were highly significant. Lachmann et al. [1] went on to show that even late producers of de-novo DSA, i.e. >5 years post transplant, had decreased long-term graft survival (49% vs. 83% in DSA-negative patients; $P < .0001$). In addition to DSA alone, Lachmann et al.'s data also indicated that a combination of DSA and graft function, as measured by glomerular filtration rate (GFR), could better identify patients with decreased long-term allograft survival. DSA alone, although associated with decreased long-term survival, was not as significant as DSA in conjunction with a decreasing GFR. Interestingly, Lachmann et al. also showed a small decrease in graft survival among individuals who developed non-DSA HLA antibodies. This is an interesting observation and may have several explanations. Since these individuals were only assessed at a single time point post transplant, it is possible that DSA could have been adsorbed to the graft and not yet detected in the serum of the recipient. The HLA antibody that was detected may be part of a broader immune response to related HLA specificities; i.e. cross-reactive groups. Lastly, non-DSA HLA antibodies could be a surrogate marker for other, non-HLA DSAs. There is a growing body of literature that indicates other non-HLA DSAs may play a role in graft failure in the absence of HLA DSA [10,11]. Future

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strategies for post-transplant monitoring will most likely need to include such assays.

In earlier studies by Terasaki and Ozawa [12], an association between serum creatinine, HLA DSA, and outcome was established. For recipients with de-novo DSA and a serum creatinine of <2.0 mg/dL, the 2-year graft survival was not significantly different from that in individuals with no DSA. However, for DSA-positive recipients whose creatinine values were >2.0 mg/dL or >3.0 mg/dL, the graft failure rate rose significantly to 17.9% and 16.3%, respectively.

Thus, the combined data of Terasaki and Lachmann shows that DSA alone is important, but not as significant as the combination of DSA plus a functional parameter, e.g. serum creatinine or GFR. These data also suggest that not all DSAs are immediately deleterious. It is well known that some patients with DSA maintain good long-term allograft function. The challenge, then, is for investigators to understand the mechanisms that lead to early graft failure in one DSA-positive group yet appear to be innocuous in another.

To address some of these issues, investigators have also used additional parameters such as C4d deposition [13], soluble CD30 [14], and the extent of transplant glomerulopathy (TG) to supplement HLA DSA detection [15]. For example, Sis et al. [15] showed that 73% of patients with TG exhibited evidence of antibody-mediated damage to their renal allograft. Similarly, Eng et al. [16] reported that, among 61 post-transplant cases with biopsy-proven TG, >50% had evidence of class I and/or class II HLA DSA. In that study, TG with DSA was associated with significantly decreased graft survival, but TG in the absence of DSA was not associated with decreased graft survival. Clearly, multiple mechanisms are in play and the association between TG and DSA requires further study.

Other studies have focused on the fine specificity of the DSA as an additional biomarker. In a series of 586 renal transplant recipients with a median post-transplant follow-up of 5.9 years, Kobayashi et al. [17] observed that antibodies against HLA-DRB1* were most closely associated with chronic antibody-mediated rejection (CAMR), at least when they were present in moderate to high concentrations. In contrast, antibodies to HLA-DQB1* were not as tightly associated with CAMR as antibodies against DRB. These intriguing data suggest there may be some importance to the immune target of the DSA response. For example, certain targets may not be expressed on the vascular endothelium or be expressed at a significantly lower concentration than other targets. Hence, binding of antibody may not occur or may not be of sufficient density to result in significant pathology. In a contrasting study by Wiebe et al. [18], patients with antibody-mediated acute renal allograft dysfunction (DSA positive with rapid rise in serum creatinine) or antibody-mediated indolent graft dysfunction (DSA positive with slow rise in serum creatinine) showed a wide distribution of different HLA antibody specificities, e.g. both class I and class II. While there was a greater percentage of class II antibodies (DRB and DQB) than class I antibodies, the latter were clearly associated with dysfunction. The contrast to the findings of Kobayashi et al.'s study [17] was that Wiebe found a greater number of affected patients with HLA-DQB1* antibody although antibodies against DRB1, DRB3, 4, 5, and DPB1 were also observed. The presence of de-novo DSA was also associated with decreased HLA-DRB matching, non-compliance, and the presence of an earlier acute rejection episode. The median 10-year survival between de-novo DSA-positive versus DSA-negative groups was 57% versus 96% ($P < .0001$). Interestingly, there was a similar distribution of HLA specificities among DSA-positive patients with stable graft function, reiterating the notion that the detection of de-novo DSA alone may be incomplete.

As previously mentioned, DSA alone may not be a robust predictor of imminent graft deterioration. Yet, it is striking that in virtually every study listed above, independent and statistically significant associations with poor graft survival were seen when de-novo HLA DSA was present post transplant. Such data suggest that the clinical manifestations of rejection result from a multi-hit pathological process. HLA DSA is clearly an important component but not necessarily a stand-alone biomarker. Future studies will need to focus on the clinical, immunological, and/or physiological factors that lead to graft dysfunction in the presence of DSA [18,19].

Cardiac transplants

In studies by Smith et al., 224 cardiac transplant recipients were followed for up to 13 years for the production of de-novo DSA [20]. Compared to recipients who did not make DSA, those with either transient DSA or persistent DSA had significantly reduced survival (hazard ratio 4.351; $P < 0.001$;). In that study, the investigators also looked at the impact of complement fixing antibodies. To their admitted surprise, the ability of the DSA to fix complement was not associated with an increased risk compared to the presence of DSA alone. However, there was a trend, although not a statistically significant one, for patients with complement fixing antibodies to have a greater risk for developing coronary artery disease. In similar studies in cardiac transplant recipients by Nath et al., the presence of de-novo DSA was associated with both AMR and coronary artery disease [21]. Smith et al.'s study also implicated antibodies against MHC class I-related chain A (MICA) antibodies along with HLA DSA. In most instances, HLA DSA preceded the appearance of MICA. Whether MICA alone is an independent biomarker of poor outcome in cardiac or renal transplantation is controversial [22–25].

Lung transplants

The importance of monitoring for DSA in lung transplant recipients has also been studied, albeit not to the same extent as in renal or cardiac transplantation [26]. This is in part because the mechanisms of antibody-mediated damage to the lung are less well understood and there is no clear histological picture that is diagnostic for antibody-mediated allograft rejection [27,28]. Rather, AMR tends to present as broncheolitis obliterans syndrome (BOS), a progressive airway obstructing process and a major cause of death following lung transplantation. Staining of biopsies for C4d or C3d has been reported as a marker for AMR in lung transplantation [29]; however, the staining process is challenging [27] and not routinely performed by all transplant centers. Nonetheless, there are studies that have identified post-transplant HLA DSA, with or without complement deposition, as a risk factor for BOS [26]. In earlier work by Girnita et al. [30], it was suggested that the combination of DSA, vascular C4d deposition, and the presence of soluble C4d in bronchoalveolar lavage fluids along with lung dysfunction, constituted a diagnostic set consistent with AMR in lung transplant recipients. Hachem et al. [31], reported that HLA DSA in the post-transplant period was quite common. Among 116 lung transplant recipients with a mean follow-up of 1.67 years, 65 recipients (56%) developed de-novo HLA DSA. In that study, some recipients were

treated in an attempt to reduce the quantity of DSA. The incidence of BOS in patients whose DSA was reduced following treatment was similar to that in patients without DSA and statistically lower than in non-treated DSA-positive individuals. In short, these data suggest the early and frequent monitoring for DSA combined with aggressive therapies to reduce DSA can reduce the incidence of BOS and, at least theoretically, increase graft and patient survival. In a retrospective study by Saini et al. of 42 lung transplant recipients [32], the relationship between alloimmunity and autoimmunity was investigated. A clear association between the appearance of HLA DSA, autoantibodies, and BOS was observed. Similar to previous studies, the incidence of HLA DSA was 42.7%. Among the HLA DSA-positive recipients, approximately 30% developed antibodies to K-α1 tubulin and collagen V self-antigens. Based on these findings, the authors proposed that the production of HLA DSA led to vascular damage that permitted the production of autoantibodies ultimately contributing to the pathology of BOS. Since HLA DSA preceded the development of autoantibodies and BOS, strategies to monitor for and reduce HLA DSA may be effective in reducing the incidence of BOS.

Liver transplants

A new and growing concern has been the role of post-transplant DSA in liver transplantation [33,34]. Once believed to be of little clinical importance [35,36], HLA DSA in liver recipients has now become a topic of extreme interest. Post-transplant DSA monitoring in association with C4d deposition is being used to help differentiate between rejection and recurrent hepatitis C. More importantly, two studies have shown that post-transplant production of DSA is associated with poor outcomes in ABO identical/compatible liver transplants [4,37]. In a study by Musat et al. [4], 17 of 43 (40%) liver transplant recipients produced DSA post transplant in association with diffuse portal C4d deposition. This group had a statistically higher incidence of acute cellular rejection and steroid-resistant rejection. Three of these patients required therapeutic DSA reduction therapy to salvage graft function. As was the case in renal transplantation, AMR in liver transplantation may be fully reversible with appropriate therapy [4,38]. Thus, for liver transplantation, the production of DSA post transplant appears to be significant and can be associated with poor outcomes.

Biological properties of DSA

One aspect of DSA that is beginning to be studied in detail is whether the intrinsic biological properties of the antibody make a difference to transplant outcomes. Deleterious effects of post-transplant DSA can be observed in renal, cardiac, lung, and most recently, liver transplantation. Several studies have evaluated the pretransplant immunoglobulin isotype and complement fixing capability of DSA and their relationship to graft allograft outcome. However, the results to date are divided, with some studies showing an association with rejection/graft loss and others showing no clinical effect [39–42]. In a recent study of 1016 patients, the presence of C1q-positive DSA in the post-transplant period was associated with an increased incidence of antibody-mediated rejection and renal allograft loss. Although C1q positivity was correlated with post-transplant outcomes, as a pre-transplant predictor of outcome is was not informative. Hence, the enthusiasm for this specific assay should be tempered [43,44]. Nonetheless, it is intriguing to speculate on the importance of the functional properties of DSA related to clinical outcomes. Clearly, more work is needed in this area.

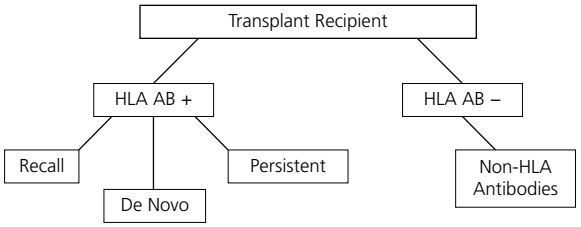

Figure 89.1. Potential classification of patients in the postoperative period. Post-transplant patients can be classified as having a donor specific antibody (HLA Ab positive) or not (HLA Ab negative). For individuals classified as HLA Ab positive, there are at least three subcategories. *Recall*, patients who are DSA negative at the time of transplant but have a significant history of sensitizing events, e.g. pregnancy or prior transplant; *persistent*, patients with DSA at the time of transplant; *de novo*, DSA-negative patients without a history of significant sensitizing events. For patients initially classified as DSA negative and who do not produce HLA antibodies post transplant, there is still the possibility for the production of non-HLA DSAs.

Strategies for post-transplant DSA monitoring

Before embarking on a strategy for post-transplant monitoring, it is important to understand and classify the types of patients who will be transplanted [45]. Depending upon the patients' histories at the time of transplant, the approach to monitoring may vary considerably. Figure 89.1 outlines some of the potentially divergent patient populations that could be encountered. While the majority of patients will be low risk (i.e. no DSA) at the time of transplant, changes in clinical practice at many centers have resulted in the transplantation of higher risk patients, with high risk defined as the presence of DSA at the time of transplant. These are patients who either underwent desensitization protocols to reduce/remove DSA just prior to transplant or were transplanted across a positive cross-match or transplanted with a DSA but a negative cross-match. Therefore, post-transplant DSA-positive patients can be divided into three groups:

- Group 1 patients have DSA prior to transplant, and were either desensitized to a negative cross-match or transplanted across a positive cross-match. Such patients could be classified as "persistent" antibody.
- Group 2 patients are DSA and cross-match negative at the time of transplant yet have a known history of DSA. For this group, the appearance of post-transplant DSA may be considered a recall or anamnestic response rather than true de-novo antibody production.
- Group 3 patients are those who truly lack DSA, both historically and at the time of transplant, yet make DSA after transplantation. This group could be considered true *de-novo* DSA.

Importantly, monitoring strategies for each of these groups may differ significantly. Presensitized and/or DSA-positive patients are at increased risk for early AMR and may benefit from early and more frequent monitoring after transplantation. Alternatively, patients in group 3 who have a low likelihood of developing a DSA post transplant may only require infrequent monitoring via a simple HLA antibody screening method. If screening results become positive, subsequent testing can be performed to confirm the presence of DSA.

Once DSA is confirmed several important questions then arise:

- Is there a benefit to more frequent DSA monitoring?
- If signs of dysfunction are present, is treatment helpful?
- If there is no graft dysfunction, is treatment warranted?

These questions await a definitive answer.

Patients with DSA at the time of transplantation

Compared to group 3 patients, groups 1 and 2 patients present a greater challenge and may require unique and individualized approaches [2,46,47]. For patients with DSA at the time of transplant (groups 1 and 2), sensitive testing should be performed that clearly identifies the DSA and perhaps attempts to quantify how much antibody is present as well as assessing the DSA response after the transplant [48]. Both of these aspects may be beneficial in planning initial therapies as well as monitoring patient progress post transplant. Identification of the DSA can be challenging since most of these recipients will possess antibody to non–donor-specific HLA antigens. First, one must ensure that the antigens of interest (DSAs) are present on the bead panel. This assessment must be made at the antigen level, but increasingly allele-specific HLA antibodies are being recognized [49]. Failure to make this distinction may result in either a false-positive or false-negative assessment of DSA. Next, one should consider if there are any factors that inhibit the detection of DSA. Immunoglobulin M (IgM) and complement components have been shown to interfere with the detection of immunoglobulin G (IgG) HLA antibodies in the single antigen bead assay [50–52]. It may be advantageous to remove these factors prior to testing. Specific treatments such at dithiothreitol (DTT), heat inactivation, or hypotonic treatments have been shown to be quite successful in removing the interfering factors and permitting the detection of HLA antibody [50–52]. Lastly, the frequency of testing must be taken into consideration. For high-risk patients, frequent monitoring soon after transplant may be useful in signaling the onset of a recall response. For low-risk patients, the frequency may be higher in the early years post transplant and then becomes significantly less in subsequent years. Importantly, frequency of monitoring is crucial when antirejection therapies are utilized. Some antirejection therapies can interfere with the detection of HLA antibody, even when solid-phase assays are used. For example, high-dose intravenous immunoglobulin (IVIG; 2 g/kg) can significantly interfere with the ability to detect an HLA antibody. Therefore, it is recommended that approximately 30 days pass between the administration of high-dose IVIG and solid phase testing. Rabbit antithymocyte globulin and other polyclonal antibody preparations are other therapeutic agents that can interfere with the detection of HLA antibody. These agents contain many different antibody specificities, including anti-HLA and anti-beta2 microglobulin [53]. As such, when present at high blood levels they can interfere with the solid phase assays. It is critical that the testing laboratory be aware of the post-transplant therapies administered to each patient.

In addition to clearly identifying the presence of a DSA, quantification of HLA DSA is highly desirable. Unfortunately, as recently reported following a Food and Drug Administration (FDA) workshop on antibody-mediated rejection [54], the current assays are not designed for quantification, and determining antibody amounts by median fluorescence intensity (MFI) levels is not reliable. A number of factors can influence the apparent quantity of antibody present. First, a patient's HLA antibodies represent a polyclonal response. This means that the observed HLA specificity is a culmination of the overall binding of many different immunoglobulin molecules. For most patients, it is not known how many different HLA epitopes are recognized by a single polyclonal serum or how many unique antibodies may be present in that serum. What is recorded is the "big picture" of the immune response to HLA. Such differences can clearly alter how a test "measures" the amount of DSA present. For example, in the current configuration of one manufacturer's class I single antigen, HLA antibody detection product, there are 31 individual beads that express the Bw4 epitope, yet only one bead for antigens such as HLA-B7 or HLA-B8. If one individual possesses a specified quantity of a Bw6 antibody and a second patient the exact same quantity of a B7 antibody, the profiles observed in the solid phase testing would be quite different. For the second patient, all of the B7 antibody would be deposited onto a single bead. In contrast, for the Bw6-bearing individual, the antibody would be divided among the 31 distinct beads. Hence, the measured fluorescence intensity, on a per bead basis, would be ~3% of the fluorescence measured on the B7 bead.

Second, if a patient has made antibodies against more than one distinct donor-specific HLA antigen, the cumulative fluorescence value for all DSAs may be more informative than the fluorescence values of the individual bead/DSA.

The above are just two examples of how challenging the interpretation of a DSA may be. It also highlights the importance of the technical aspects of the testing and how they can affect the perception of antibody quantity and strength.

Methods to detect HLA DSA

A more detailed discussion of the methods for detecting HLA alloantibodies is provided in Chapter 36. Current state-of-the-art approaches now utilize solid phase methods to identify the presence of HLA antibodies as well as determine the fine specificity of the antibody. In combination with molecular methods for HLA typing, the ability to unambiguously confirm or refute the presence of a DSA is better than in any past era. Personalized approaches to DSA assessments that utilize the HLA antigens isolated directly from the specific donor as targets for the assay [55–58] are under development and there utility awaits further studies.

Summary

The development of post-transplant HLA DSA is an important biomarker that is closely aligned with graft injury, graft dysfunction, and accelerated graft loss. Although monitoring for DSA is not uniformly predictive of impending graft failure, the presence of DSA clearly portends a worse outcome compared to that in individuals without DSA. When combined with other biomarkers (invasive and/or non-invasive), the clinical correlation with graft failure increases significantly. The observation that many recipients exhibit normal graft function in the face of demonstrable DSA suggest that there are DSAs that are not deleterious or that all of pieces of the puzzle have not yet been identified. Additional studies delineating differences in the biological activity of DSA combined with information obtained from other "-omic" evaluations may ultimately provide the complete picture. Lastly, it is unknown whether early identification of DSA or other biomarkers of rejection will translate into effective therapeutic interventions. Some groups have shown that successful reduction in DSA after transplant can lead to improved allograft survival [59–62], but the numbers are small and there has been no long-term follow-up.

However, under any circumstances, post-transplant monitoring for HLA DSA is an important tool for the transplant clinician.

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CHAPTER 90

Biomarkers and Alloimmune Monitoring after Organ Transplantation

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Introduction

Infections, malignancies, and cardiovascular and metabolic complications constitute major causes of death after organ transplantation. These complications are caused and/or negatively influenced by chronic exposure to immunosuppressive agents. Given the narrow therapeutic window of immunosuppressive drugs and the marked interindividual variability of transplant recipients, the search for tools to tailor immunosuppressive therapy to the individual needs of transplant recipients has been an ongoing research priority in transplantation. Despite the interest and clinical relevance of this topic, monitoring of immunosuppressive therapies in the clinic continues to be performed almost exclusively by employing pharmacokinetic markers. This practice persists even though currently employed pharmacokinetic guidelines have never been properly validated in long-term clinical trials. Furthermore, pharmacokinetic characteristics of immunosuppressive drugs do not accurately reflect their biological (or pharmacodynamic) effects [1,2] and cannot be employed to estimate the real need for immunosuppression in a given individual recipient.

The recipient's immune status and donor-specific immune reactivity have a large impact on transplantation outcomes. The use of immune monitoring tools to assess the optimal level of immunosuppression for a transplant recipient is therefore intuitively appealing. Recent advances in cellular and molecular analytical technologies have allowed the identification of many biomarkers with potential relevance to applied immunology and transplantation. The development of these markers holds promise to improve patient care by facilitating personalized medicine in transplantation. Furthermore, measuring biomarkers within clinical trials can provide valuable mechanistic insight into the cellular and molecular basis of clinical responses. Unfortunately, the implementation of these biomarkers in clinical transplantation is not straightforward and faces various scientific, technological, financial, and regulatory challenges (Figure 90.1). As a consequence, although a plethora of biomarker studies have been performed and many articles have been published in the field of organ transplantation, their validation and standardization is still in its infancy. Thus, to date only two biomarker-based diagnostic tests have gained regulatory approval and have reached the market [3–6]. Even in these two cases, the two commercialized tests have not yet greatly influenced routine clinical practice. Clearly, the promise of biomarker-based diagnostics and personalized medicine in organ transplantation is still far from being a reality in clinical practice. It is important to note that the detection of donor-specific alloantibody is a clear indicator of uncontrolled alloimmunity and can be considered a biomarker of sorts. It is covered separately in Chapter 89.

Identification and evaluation of biomarkers relevant to transplantation

A *biomarker* is defined by the Biomarkers Definitions Working Group as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [7]. In transplantation, biomarkers have been developed to monitor therapeutic interventions and as diagnostic and prognostic tools. A biomarker that can be employed as a substitute for a clinical endpoint is known as a *surrogate endpoint*. Characterization of a biomarker as a surrogate endpoint requires demonstration within clinical trials and/or epidemiological studies that the biomarker is capable of predicting clinical benefit with accuracy and reproducibility [7]. General considerations in biomarker discovery are covered here. A more in-depth coverage of statistical and trial design issues relevant to this discussion is given in Chapter 134.

The first step in the biomarker development process is the definition of the clinical question to be addressed and the population to be targeted (Figure 90.1) [8–10]. The technology to be employed, the sample type (blood, allograft tissue, urine, etc.), and the most appropriate gold standard against which the test will be compared also need to be selected. A biomarker discovery phase followed by one or more validation phases are then conducted. In the discovery phase a high-throughput "-omics" technology is often employed in order to simultaneously evaluate a large panel of potential biomarker candidates. During the discovery phase, the diagnostic/prognostic performance of candidate biomarkers needs to be assessed. The minimum performance threshold depends on the nature of the available gold standard and on the intended use of the test. The sample size estimation for subsequent validation studies in independent cohorts of patients can be established based on these assessments. Often the initial validation is conducted on

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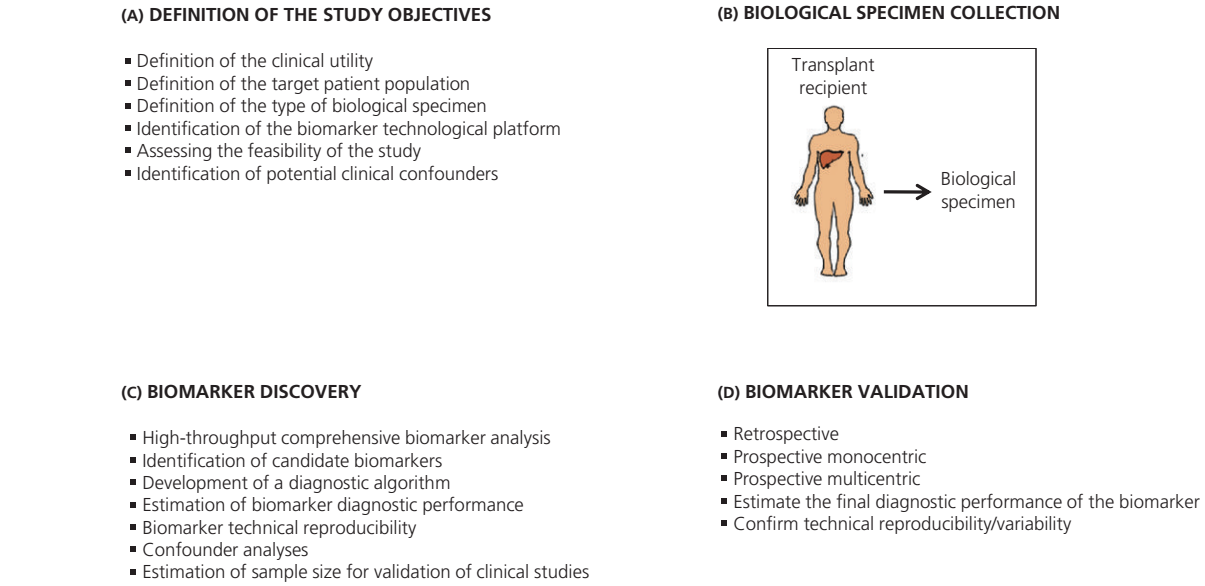

Figure 90.1. Strategy for the development of biomarkers in transplantation. The first step includes a careful definition of the clinical question to be addressed, the target population, type of sample and analytical platform, and overall study design. The key aspects are then the discovery of candidate biomarkers and their validation.

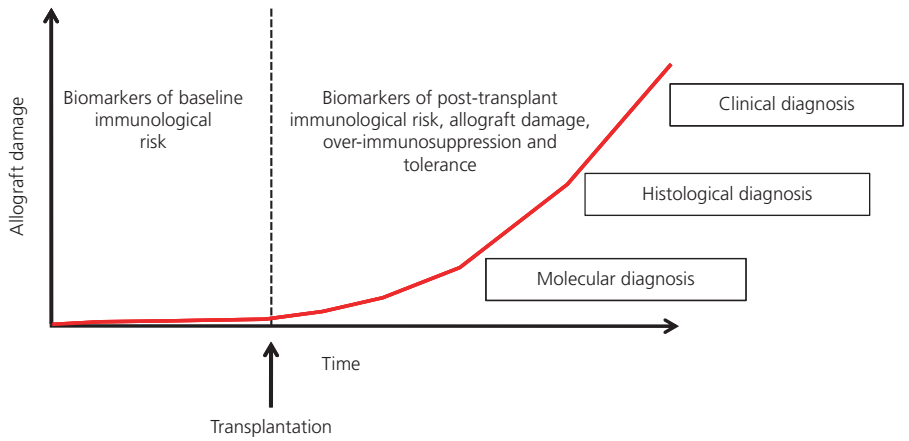

Figure 90.2. Application of immunomonitoring biomarkers in transplantation. The way biomarkers are employed in transplantation evolves with time. Before transplantation the emphasis is on assessing the baseline immunological risk of transplant recipients. After transplantation the goals are to determine the risk of graft rejection and/or graft damage and to detect graft damage as early as possible. The assumption here is that molecular markers of graft damage precede histological changes and clinical manifestations. (Adapted from Anglicheau et al. [210] and Roedder et al. [9].)

retrospective samples. This should be followed by prospective validation studies, ideally within a multicenter setting. It is important to make sure that the cohorts of patients employed for biomarker validation faithfully represent the target population on which the test will be employed. The influence of clinical and technical confounders needs to be carefully investigated. If a high-throughput platform is employed in the discovery phase, a technological transfer into a platform more amenable to widespread clinical application needs to be implemented within the validation phase.

In interventional clinical trials involving transplant recipients, the use of endpoints such as recipient survival and/or irreversible graft failure has become impractical. Given the good short-term results achieved in clinical organ transplantation, the use of these endpoints results in the need to enroll unrealistically large sample sizes and to conduct very long-term recipient follow-up. The identification of adequate surrogate endpoints is therefore of paramount importance in transplantation in order to expedite the evaluation of novel therapies. The main surrogate endpoints currently employed in routine clinical practice are either conventional markers of allograft dysfunction (e.g. serum creatinine, glomerular filtration rate, liver function tests) or pathological changes derived from allograft biopsies. These markers fail to anticipate the initiation of allograft tissue destruction and therefore provide little predictive information [11] (Figure 90.2).

Furthermore, they are not always linked to long-term outcomes and do not offer information on the recipient's overall immune status. Given the clinical complexity of transplant recipients, a combination of different biomarkers derived from a variety of different platforms is likely to be required to capture the full effect of medical interventions.

In transplantation, immune-related biomarkers have been developed in an attempt to address four major aspects relevant to transplant patients:

- to identify whether the recipient is presensitized to the donor before transplantation and therefore likely to develop rejection;
- to detect inflammatory changes related to rejection before clinically apparent graft damage has taken place;
- to predict the success of immunosuppressive drug minimization or withdrawal strategies;
- to assess the net state of immunosuppression in order to identify over-immunosuppressed recipients likely to develop opportunistic infections or malignancies.

Principles of cellular alloimmune monitoring

Recipient-to-donor sensitization has been recognized as an immunological barrier to successful transplant outcomes since the initial stages of transplantation medicine. Despite the well-established central role of T cells in allograft rejection, only the assessment of humoral sensitization by means of alloantibody measurement is currently employed in routine clinical practice. This is covered in detail in Chapter 89. In contrast to alloantibody measurements, which are currently a fundamental decision-making tool in clinical transplantation, none of the tests assessing cellular alloimmune effector mechanisms has been widely implemented in the clinic. This is attributable to the heterogeneity of T-cell subsets and the complexity of their immune responses (see Chapter 5 for detailed information relevant to the discussion of T-cell mediated immunity). The T-cell receptor complex recognizes donor antigens by two distinct pathways: (1) the direct pathway, where donor peptides bound to donor major histocompatibility complexes (MHCs) on the surface of donor antigen-presenting cells (APCs) are recognized by recipient T cells; and (2) the indirect pathway, where donor peptides bound to recipient MHCs on recipient APCs are recognized by recipient T cells. Extensive studies [12–15] revealed a dominance of the direct pathway during the first months after transplantation, when professional donor APCs are still present, whereas the indirect pathway has been associated with chronic immune-mediated graft injury [16–18]. More recently, a novel pathway, called the third or semi-direct antigen presentation pathway, has been identified in experimental animal models, in which recipient APCs acquire donor MHC–peptide complexes and thus are capable of simultaneously priming recipient T cells via direct and indirect allorecognition pathways [19].

Upon MHC–peptide–T-cell receptor complex binding and appropriate costimulation, naïve T cells become activated. This results in intracellular production of adenosine triphosphate (ATP), calcium influx, and protein phosphorylation, all of which have been used as biomarkers to quantify T-cell activation. Primed naïve T cells subsequently proliferate, synthesize cytokines, and differentiate into effector T cells, a process that takes several days. Some effector T cells eventually differentiate into memory T cells. In contrast to naïve T cells, rechallenged memory T cells have a lower activation threshold, need less costimulation, and produce effector cytokines within hours instead of days. It is now well understood that donor reactive $CD4^+$ T cells take important cues from the fine texture of inflammation in which they recognize donor antigens. Depending on the local cytokine milieu in which lymphocyte antigen activation takes place, naïve $CD4^+$ T-helper cells can commit to a variety of cytopathic and/or immunoregulatory phenotypes [20]. Thus, when activation occurs in a microenvironment rich in interleukin (IL)-12 (usually produced by activated mature dendritic cells), $CD4^+$ T cells commit to a tissue-destructive interferon (IFN)-γ-producing Th1 program. In contrast, $CD4^+$ T cells activated in an IL-4 rich milieu differentiate into the IL-4- and IL-5-producing Th2 cells. In the absence of proinflammatory cytokines, tumor growth factor (TGF)-β directs $CD4^+$ T-cell commitment into the $Foxp3^+$-induced regulatory T-cell phenotype. In direct contrast, TGF-β expression accompanied by IL-6 and/or IL-21 expression precludes commitment into the transplant-protective regulatory T-cell phenotype. Instead, such antigen-reactive $CD4^+$ T cells commit to the highly cytopathic IL-17-producing (Th17) T-cell phenotype [20–23].

Until recently, it was thought that antigen-activated T-helper cells became either Th1 or Th2 terminally differentiated cells and that these T-cell subsets caused opposing Th1-dependent cytopathic rejection or Th2-dependent cytoprotective effects. The Th1/Th2 paradigm is incorrect insofar as both Th1 and Th2 can orchestrate graft rejection [24,25], and that regulatory T cells, and not Th2 cells, are crucial to inhibition of cytopathic allospecific immune responses [26–28]. Recent discoveries have also revealed that neither Th17 nor regulatory T cells are terminally differentiated phenotypes and that these two cell subtypes are in fact closely interlinked and exhibit remarkable plasticity [29]. The current paradigm is that the clinical outcome of the allograft response following withdrawal of therapy, rejection or tolerance, is determined by the relative balance between cytopathic Th1 and Th17 $CD4^+$ T cells versus rejection blocking cytoprotective regulatory T cells, and that this balance is critically dependent on the fine texture of inflammation within the microenvironment in which T-cell activation takes place.

Cellular assays developed to assess alloimmune responses rely on the characterization of lymphocyte subpopulations and/or their repertoire, the measurement of events related to T-cell activation (e.g. intracellular markers of activation, proliferation, cytokine production, cytotoxicity), or the detection of markers of allograft damage. Furthermore, with the advent of high-throughput "multi-omics" technologies (transcriptomics, proteomics, metabolomics, etc.), systems biology approaches have been undertaken in an attempt to capture within a single read-out both the effects of immune cells and the response of the allograft to them.

Alloantigen-specific assays

In contrast to chronic immune-mediated conditions in which the identity of the causal antigens is unknown, in transplantation access to donor material allows for the assessment of *alloantigen-specific cellular immune responses*. These assays should, in principle, provide a more accurate individualized estimate of the actual risk of graft rejection. Furthermore, they might be capable of identifying patients who have developed allograft tolerance. On the other hand, these techniques are expensive, labor-intensive, and difficult to standardize. Classic alloantigen-specific cellular assays assess proliferation, cytotoxicity, and/or cytokine production, and include mixed leukocyte reaction (MLR) assays, cytotoxic T-lymphocyte precursor (CTLp) determination, and limiting dilution analyses

(LDAs). While MLR assays have been shown to have little predictive value in transplantation [30], CTLp determination and LDAs are more informative and reproducible as measurements of effector T-cell functions, such as proliferation and cytokine production, that correlate with alloreactivity [31–33]. The utility of these measurements, however, has been mostly shown in the context of bone marrow transplantation, where CTLp frequency is a good predictor of graft-versus-host disease [34,35]. In contrast, in solid organ transplantation, contradictory data have been reported and the clinical utility of these tests is less clear [36–38]. A more recent alloantigen-specific assay that is more amenable to routine clinical use is the enzyme-linked immunosorbent spot (ELISPOT) assay [32,36,39,40]. In this assay, frequencies of cytokine-secreting naïve or effector/memory recipient T cells are quantified after in -vitro stimulation with donor antigens, usually blood, spleen, or lymph node mononuclear leukocytes. If analyzed after a short stimulation period of <24 h, only memory/effector T cells are detected, thus providing an estimate of antidonor T-cell sensitization. The assay has been used to quantify IL-2, IFN-γ, IL-10, or granzyme B-producing T cells. Limitations are the impossibility to simultaneously analyze different lymphocyte subsets and/or cytokines, although new two-color assays are in development. Recent research efforts have focused on the standardization of this technique in order to increase its reliability [41–44].

Multiparameter flow cytometry has the advantage of allowing simultaneous analysis of multiple cell phenotypic markers. Furthermore, with recent technological refinements, it can also provide information about intracellular signaling pathways, cytokine release, and antigen specificity. Flow cytometric analysis of T-cell cytokine production by combined intracellular and cell surface marker staining has been developed as an alternative technique to assess donor-specific T-cell responses [45,46]. However, this assay has a lower sensitivity for the enumeration of cytokine-producing lymphocytes than ELISPOT [39]. Flow cytometry can also be employed to assess T-cell mitotic activity after donor antigen stimulation on the basis of carboxyfluorescein succinimidyl ester (CFSE) labeling, but this has shown little predictive value in kidney transplantation [36].

Non–alloantigen-specific assays

These do not require the availability of donor material, which might be problematic, particularly in the case of deceased donors. Furthermore, these techniques are often easier to standardize and/or commercialize for routine clinical application than functional antigen-specific assays. In addition, by providing information on the net state of immunosuppression (i.e. general immune competence), they may serve to predict not only graft immunological outcomes but also complications related to over-immunosuppression.

The immunophenotypic characterization of circulating lymphocytes by flow cytometry has been widely employed in an attempt to identify biomarkers relevant to transplantation. Numerous observations employing flow cytometry have shown that patients undergoing acute rejection display increased levels of circulating activated and/or effector memory T-cell subsets (as assessed by cell surface markers such as CD45RA, CCR7, CD62L, CD69, or CD38) [47–52]. The problem with these assays has been their lack of specificity, given that similar levels of cell surface activation marker expression are observed in other inflammatory conditions. As an example, renal transplant patients with cytomegalovirus (CMV) infection showed increased levels of activation markers on $CD8^+$ T cells, correlating with CMV viremia but not with acute rejection [40,53,54]. The quantification of graft- or leukocyte-derived gene transcripts in biological specimens of transplant recipients using quantitative polymerase chain reaction (qPCR) [55,56] has been one of the most informative non–antigen-specific approaches. Changes in transcript levels reflecting activation of leukocytes and/or non-immune cells can be used to analyze the quality and magnitude of the immune response in all possible compartments such as peripheral blood, graft tissue, and fluids draining the graft.

The recent discovery of small regulatory RNA molecules (microRNAs; miRNAs) has resulted in the availability of a new set of potential diagnostic and prognostic biomarkers relevant to transplantation. The activation of immune cells can also be estimated by analyzing immune cell-derived soluble products such as CD30, CXC-ligands, and receptor chemokines [57–62]. A different approach has been the analysis of the specific patterns of T-cell receptor repertoire usage associated with the development of tolerance and/or graft rejection [63–65]. Another interesting non–antigen-specific approach for measuring the overall T-cell functional capacity consists of the quantification of whole blood $CD4^+$ T-cell intracellular adenosine triphosphate (iATP) content after phytohemaglutinin (PHA) stimulation. This assay has been approved by the Food and Drug Administration (FDA) for the determination of ATP content and is available commercially (ImmuKnow® assay; Cylex Inc., Columbia, MD, USA). However, it has yet to be validated as a useful guide for clinical practice.

Given the relevance of regulatory $CD4^+CD25^+Foxp3^+$ T cells (Tregs) in the prevention and/or development of human autoimmune diseases and their critical role in many experimental animal models of transplantation [66,67], the quantification of this T-cell subset in blood and/or allograft infiltrates has gained substantial attention. In contrast to rodents, however, activated human T cells can also transiently express the intracellular transcription factor Foxp3 [68–70]. For this reason, the use in flow cytometry analysis of cell surface CD25 and intracellular Foxp3 labeling is not an accurate method to quantify Tregs in humans. The addition of the CD127 marker can partially address this limitation, given that Tregs are typically CD127low while CD127high T cells tend to be activated lymphocytes [71,72]. However, the most reliable method of identifying human Tregs is to assess the methylation state of a conserved region in the first intron of the *Foxp3* gene, known as the Treg-specific demethylated region (TSDR). This can be quantitatively assessed in sorted T cells, whole blood, and even paraffin-embedded graft biopsies [73–75]. Differences in circulating Tregs between transplant recipients exhibiting chronic graft damage and allograft tolerance, or between recipients receiving different immunosuppressive regimens, have been noted [76–81]. Furthermore, high levels of Foxp3 transcripts both in urine and within the graft have been reported in kidney patients undergoing acute allograft rejection [56,82–85]. The clinical relevance of these observations and their unambiguous association with clinical outcomes are, however, still unclear.

Pretransplantation biomarkers: assessment of the baseline immunological risk of transplant recipients

Detection of donor HLA-specific antibodies in the recipient's serum is a widely employed strategy to identify sensitized transplant recipients at risk of humoral rejection, and is extensively covered in

Chapter 89. We will focus here on novel technologies that allow the measurement of T-cell sensitization and/or activation, and that have shown potential clinical relevance.

IFN-γ ELISPOT assay

Heeger et al. [32,36,39,40] were the first to describe IFN-γ production by recipient T cells in response to donor cells in a 24-h ELISPOT assay as constituting a measurement of primed donor-specific immunity, correlating with the risk of acute cellular rejection after kidney transplantation. Thereafter, additional studies by different groups confirmed the relevant association between heightened pretransplant IFN-γ-producing donor-reactive T cells and early kidney allograft acute rejection [43,44,86,87]. Importantly, these studies recognized that a significant proportion of patients who exhibited pretransplant cellular sensitization had low panel reactive antibody (PRA) scores. Early severe rejection and graft loss were observed in several of these patients, even those under potent immunosuppression with calcineurin inhibitor (CNI) drugs [86,87]. Another study revealed that pretransplant IFN-γ ELISPOT reactivity correlated with cumulative duration of dialysis treatment [88], suggesting that increased cellular sensitization could contribute to the lower graft survival rates observed in long-term dialysis patients.

The panel reactive T-cell (PRT) assay was developed in order to evaluate cellular sensitization before transplantation when donor cells are not available [89,90]. The PRT assay measures IFN-γ ELISPOT responses against a panel of stimulator cells with the most common HLA antigens. Notably, PRT responses were shown to be independent of PRA scores [91]. Furthermore, PRT reactivity correlated with previous transplants, HLA mismatches, and female gender, and was able to predict post-transplant acute rejection and 6-month renal function better than PRA [91]. To provide a renewable source of donor stimulator cells for ELISPOT and PRT experiments, Zand et al. [92] described the use of CD40L-stimulated $CD19^+$ donor-type B cells. Thus, the IFN-γ ELISPOT assay constitutes one of the most promising tests available to assess the pretransplant immunological risk of an individual kidney recipient. The usefulness of the test in transplant recipients grafted with other organs remains to be explored.

Soluble CD30

The CD30 molecule belongs to the tumor necrosis factor receptor (TNF-R) superfamily. In activated T cells the membrane-bound CD30 molecule is proteolytically cleaved, thereby generating a soluble form (sCD30), which can be measured in serum [93,94]. Healthy humans tend to exhibit low CD30 serum levels, whereas increased sCD30 serum concentrations are detected in several inflammatory situations [95]. In transplantation it was hypothesized that sCD30 could reflect the pretransplant activation status of the T cells and thereby allow identification of high immunological risk recipients [93,96]. Indeed, several studies showed that either pre- or post-transplantation sCD30 levels predicted the development of acute kidney allograft rejection independently of PRA [57–59]. However, Altermann et al. detected a high interindividual variability in serum sCD30 levels of potential kidney recipients. Thus, when analyzed over time in kidney patients awaiting transplantation, substantial fluctuations were noted [95], hampering its implementation as a pretransplant risk-stratification marker. Furthermore, additional studies revealed that although low sCD30 pretransplant levels identified patients at low risk of developing acute cellular or humoral rejection, accurate identification of high-risk patients was not possible [97,98]. The accuracy of sCD30 as a predictive transplantation biomarker was shown to be further diminished in patients receiving induction therapy [58,99] or in those undergoing acute viral or bacterial infection [100].

Chemokine and chemokine receptor serum levels

IFN-γ-induced CXC chemokine ligands 9 (CXCL9; Mig) and 10 (CXCL10; interferon γ-inducible protein-10) attract CXC chemokine receptor 3 (CXCR3)-positive T cells into the graft. Some studies suggested that high pretransplant serum CXCL10 and CXCL9 levels were predictive of renal graft loss and early acute rejection of cardiac and renal allografts [60,61]. However, these observations were not corroborated in a subsequent study [62] in which pretransplant CXCL9 and CXCL10 serum levels had suboptimal prognostic value in recipients treated with either alemtuzumab or basiliximab induction treatment, likely due to depletion or inactivation of $CXCR3^+$ T cells.

ImmuKnow® assay

The goal of this assay, which measures whole blood $CD4^+$ T-cell iATP content after phytohemaglutinin stimulation, is to provide a reflection of the recipient's general immune status rather than a measurement of allograft-specific T-cell reactivity. The assay has been used to quantify immune responses before and after lung, small bowel, liver, and kidney transplantation [101]. Uncontrolled data have been employed to correlate iATP levels with infectious risk, with exceptionally low iATP levels having loose correlation with adverse outcomes [102,103]. However, at present, there are no prospectively controlled data supporting the use of this tool for immunosuppressive management.

Post-transplantation biomarkers: acute allograft rejection and chronic allograft injury

IFN-γ ELISPOT assay

In kidney transplantation a number of studies have employed IFN-γ ELISPOT monitoring to investigate the influence on clinical outcomes of blood alloreactive T-cell numbers [42–44,86,88,104–106]. Hricik et al. [86] first described that post-transplant directly-primed antidonor and third party responses positively correlated with 6- and 12-month serum creatinine levels. Because of its high sensitivity, the IFN-γ ELISPOT assay has also been used to measure alloreactive memory/effector T-cell responses primed by the indirect allorecognition pathway. Kidney recipients with indirect alloreactivity showed poorer graft function than those without [104–106]. Nonetheless, directly primed memory/effector T cells had superior long-term prognostic value, at least when assessed by IFN-γ ELISPOT [106]. ELISPOT and/or flow cytometry/intracellular staining technologies have also been employed to quantify frequencies of antiviral T-cell responses after transplantation. High frequencies of viral-specific T cells directed against overlapping peptide pools covering LT and VP1 BK virus (BKV) antigens correlated with control of BKV replication [107–109]. Similarly, the number of IFN-γ-producing T cells reacting against overlapping peptide pools covering CMV IE-1 protein positively correlated with protection from CMV disease and improved graft function in heart–lung and kidney transplant patients [110,111].

Chemokine and chemokine receptor serum levels

In liver transplantation CXCL10 serum levels were correlated with early liver graft fibrosis due to hepatitis C virus recurrence [112]. In addition, different reports have shown an association between high post-transplant urinary levels of CXCL10 and increased incidence of acute rejection, subclinical rejection and even BKV infection in kidney transplantation [113–115].

mRNA and miRNA expression in graft tissue samples

Studies aimed at the use of gene expression markers to identify acute rejection episodes were first undertaken in the context of kidney transplantation in the early 1990s. The group of T.B. Strom first demonstrated that during acute kidney graft rejection there was a marked increase in intragraft expression of cytotoxic T lymphocyte (CTL) transcripts such as granzyme B and perforin [116–120]. In subsequent studies, differences in CTL transcript composition were shown to discriminate between therapy-resistant and -sensitive acute rejection episodes [121], and between clinical and subclinical rejection [122]. A more comprehensive transcriptional profiling approach employing microarrays revealed different immunological and histological subgroups of rejection with different clinical outcomes [123–127]. Similar studies conducted in liver, heart, and pancreas allografts identified specific mRNA expression signatures associated with acute rejection and with histological and clinical outcomes [128–130]. The quantification of miRNAs in graft tissue samples has also been shown to be useful in discriminating between stable patients and patients with variable degrees of intragraft inflammation [131–133]. Anglicheau et al. [134] were the first to report on miRNA profiling as a tool to predict the status of human renal allografts. Expression of a set of miRNAs, including miR-142-5p, miR-155, and miR-223, was significantly altered in biopsies of patients with acute rejection [134]. Additional studies from other groups identified miRNA signatures associated with renal or intestinal acute cellular rejection [135,136] or with outcome after liver transplantation for hepatocellular carcinoma [137,138].

mRNA and miRNA expression in peripheral blood

In order to avoid invasive monitoring of immune responses following transplantation, researchers have studied the diagnostic potential of mRNA expression in other compartments such as peripheral blood [139]. The first studies conducted on peripheral blood mononuclear cells isolated from rejecting and non-rejecting kidney recipients reported that rejection was also associated with an increase in CTL transcripts such as perforin and granzyme B [140–142]. These transcripts were detected at the time of and even prior to clinically apparent acute rejection [140–142]. With the development of whole blood stabilizing reagents, such analysis was performed in a more standardized manner, which resulted in the identification of additional transcriptional markers of rejection, such as the costimulatory genes *CD154* and *ICOS* [143]. Toag-1 expression, originally identified as a rejection-related biomarker in experimental animal models of kidney, heart, and liver transplantation [139], also correlated with graft function and acute/chronic rejection when measured in whole blood from human transplant recipients before and after kidney transplantation [144,145]. In heart transplantation the analysis in peripheral blood mononuclear cells of an 11-transcript set has been commercialized as a diagnostic test to non-invasively identify heart recipients with a high likelihood of exhibiting moderate-to-severe rejection [3,4,146]. This FDA-approved test (Allomap, XDx) was designed to reduce the need to conduct surveillance endomyocardial biopsies. Accordingly, it exhibits a very high negative predictive value but very low positive predictive value. As described in detail below, analysis of peripheral blood mRNA expression can also be used to identify "operationally" tolerant transplant patients, who have stable graft function despite being off all immunosuppressive drugs. The use of blood as the source for mRNA, while highly desirable due to its accessibility, might be associated with technical problems. When peripheral blood mononuclear cells are employed, Ficoll isolation may cause significant interlaboratory variability. Although whole blood mRNA stabilization associated with globin mRNA reduction and strict sample handling protocols can address this problem, the differences in transcript levels observed when comparing different groups of transplant recipients tend to be weaker than those observed at the graft itself [139,147]. This could compromise the reproducibility of predictive expression models. The influence of blood cell subset heterogeneity [148], still not well studied in the context of transplantation, can also be a source of variability. Clearly the use of "deconvoluting" bioinformatic algorithms needs to be further pursued [149]. Alternatively, transcriptional analyses of isolated peripheral blood mononuclear cell subsets, a strategy that has proven useful in autoimmune diseases [150], should also be explored as a way to generate robust predictive signatures. Interestingly, miRNAs are very stable in serum, which makes serum analyses of miRNA levels a suitable diagnostic tool for monitoring immune responses in transplant patients. Indeed, Farid et al. recently showed that hepatocyte-derived miRNAs can serve as serum biomarkers of hepatocyte injury and rejection after liver transplantation [151]. It remains to be shown whether serum miRNA levels are also markers of rejection and/or tolerance in other transplantation settings.

mRNA and miRNA expression in fluids draining the graft

The analysis of fluids draining the graft, such as urine, bronchoalveolar lavage (BAL), and bile for kidney, lung, and liver transplantation, respectively, allow the study of local immune responses. Taking advantage of the direct communication between the kidney allograft epithelium and the outside world via the urine, several investigators have studied urinary chemokines and cytokines to non-invasively detect acute rejection. Given the fundamental relationship between invasion of the renal tubular epithelium by activated lymphocytes and the diagnosis of acute rejection, urinary biomarkers have been conceptually attractive. Suthanthiran et al. were the first to describe the possibility of a non-invasive diagnosis of renal allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine [55].

In keeping with assessment of the interferon-γ axis in serum samples as described above, increased transcript and protein urinary levels of CXCL9 and CXCL10 (IP-10) and their receptors were associated with impaired short- and long-term graft function, and correlated with T-cell mediated inflammatory events in the kidney, including acute rejection and BKV nephropathy [114, 115,155,156]. Many other groups have confirmed the usefulness of these markers not only to diagnose rejection but also to predict long-term graft function [56,152–154]. In lung transplantation,

similar transcriptional analyses have been performed on BAL fluids. In this setting Madsen et al. showed that BAL, but not blood, transcript levels of CTLA-4, Foxp3, and granzymeB were elevated during acute rejection [157].

Urinary miRNA signatures have also been proposed as biomarkers of rejection in kidney transplantation [158]. In this setting, miR-10b and miR-210 were down-regulated and miR-10a up-regulated in urine samples of patients with acute rejection, and expression levels of miR-210 predicted long-term graft function as determined by glomerular filtration rate decline 1 year after transplantation [158].

Biomarkers of over-immunosuppression

Transplant recipients require frequent monitoring of immune responses to avoid both under-immunosuppression resulting in rejection and a state of global non-specific over-immunosuppression that may lead to life-threatening complications [145]. Unfortunately, it is currently not possible to determine the "adequate" level of immunosuppression for an individual transplant recipient. Similarly, no consensus has been reached on the definition of "over-immunosuppression," other than by the development of its most common manifestations, namely opportunistic infections and malignancies [159]. Examples of biomarkers reflecting the general state of adaptive and/or innate immune competence and their relevance for transplantation medicine will be discussed here.

Assessing the functional capacity of T cells

The ImmuKnow® assay was designed as a standardized test to measure lymphocyte reactivity in immune-suppressed patients in order to discriminate between under- and over-immunosuppression [101,160,161]. In several studies, high iATP levels were weakly associated with occurrence of acute rejections [101], whereas patients displaying very low iATP levels had an apparent increased risk of developing post-transplant infectious complications and higher short-term mortality [162,163]. A single time point analysis of iATP levels, however, was subsequently shown to be inaccurate in the prediction of opportunistic infections or acute rejection, indicating that the test should be conducted and interpreted longitudinally with sequential sampling [164–168]. More recent results showed that iATP levels are influenced by patient age and immunosuppressive regimen, with transplant recipients under 12 years old showing lower immune response levels [167,169–171]. A recent study also reported that reduced ImmuKnow® levels are very common during the first 6 months after kidney transplantation and not necessarily associated with dangerous over-immunosuppression [172], whereas low 12-month levels are associated with BKV infection. Overall, when analyzed longitudinally, the ImmuKnow® assay has been envisioned as a useful tool to stratify patients into low- and high-risk groups and to guide the tailoring of immunosuppression. However, a number of critical questions regarding the clinical applicability of the assay remain unanswered (e.g. What is the ideal immune response for recipients of different organs at different time points? What is the influence of HLA mismatch, type of organ, or immunosuppressive therapy?). Thus, published data should be interpreted with caution until large prospective studies demonstrate that adjustment of immunosuppressive therapies on the basis of ImmuKnow® read-outs results in improved clinical outcomes.

Serum levels of complement pathway mediators

Complement is one of the major inflammatory mediators involved in transplantation [173–176]. Apart from the classical and alternative complement pathways, the mannose-binding lectin (MBL) pathway also plays an important role in transplantation; the binding of MBL to injured tissue amplifies the inflammatory response. In some studies, pretransplant MBL plasma levels have been shown to predict graft outcome. Berger et al. showed that low pretransplant MBL serum levels are associated with increased patient and graft survival after kidney [177] or simultaneous pancreas–kidney transplantation [178]. In addition, plasma MBL levels increase after lung transplantation, and in this setting, persistently elevated MBL levels are associated with poor long-term outcomes [179]. Plasma MBL concentration has also been associated with the development of infections. In kidney transplantation, post-transplant plasma MBL deficiency (<500 ng/mL) increases the risk of CMV disease [180,181]. Pretransplant plasma MBL levels, in contrast, do not correlate with CMV disease [182,183], while high levels have been shown to protect against urinary tract infections and urosepsis following simultaneous kidney–pancreas transplantation [183]. Regarding BKV infection in kidney transplantation, post-transplant plasma MBL levels do not appear to influence the development of BKV viremia [184]. Overall, data indicate that measuring plasma MBL concentration may be useful to identify over- or under-immunosuppression, although the capacity of MBL levels to predict clinical outcomes is variable depending on the type of viral infection and on whether this marker is analyzed before or after transplantation.

HLA-DR expression on monocytes

Another approach to assess over- or under-immunosuppression is to evaluate the expression level of the MHC class II molecule HLA-DR on circulating monocytes of transplant patients [185,186]. MHC class II expression on APCs such as monocytes is tightly regulated. Inflammatory mediators such as IFN-γ and granulocyte macrophage-colony stimulating factor (GM-CSF) increase MHC class II monocyte expression [187–189], whereas anti-inflammatory mediators such as IL-10, TGF-ß, stress hormones, immunosuppressive drugs such as steroids, and regulatory T cells decrease MHC class II levels [190–195]. Thus, studying the HLA-DR expression per circulating monocyte can reveal the individual balance between systemic inflammatory and anti-inflammatory mediators. Upon major surgery, trauma, or systemic infections, circulating monocytes develop a state of functional unresponsiveness or desensitization characterized by low MHC class II expression and impaired antigen presentation capacity [196,197]. Furthermore, low HLA-DR expression on monocytes is associated with increased risk of infection after surgery or trauma [198]. After solid organ transplantation, persistently low HLA-DR expression on circulating monocytes precedes bacterial or fungal sepsis [185,198–200]. Thus, measurement of monocytic HLA-DR levels could be useful to identify severe over-immunosuppression and to assess the risk of infection. This may be especially important in transplantation, now that more T-cell–depleting agents and biologics targeting memory T cells are entering the clinic. Similarly, this parameter could be a useful safety biomarker to identify global immunosuppression in clinical trials incorporating cellular therapies such as adoptively transferred polyclonal regulatory T cells. To determine the clinical utility of these safety biomarkers, they need to be included in future clinical trials. In order to allow patient stratification into low- and high-risk groups, standardized flow cytometric analyses with

acceptable variances (<30%) between different labs or different flow cytometers have to be established. This has been achieved with commercially-developed BD Quantibrite HLA-DR/Monocyte, which allows quantification of HLA-DR molecules per monocyte based on provided standards. Interlaboratory comparisons of this test in multicenter clinical trials have achieved very low co-efficients of variation (4–25%) [201].

Biomarkers of graft accommodation and allograft tolerance

Liver and kidney transplant recipients can occasionally discontinue all immunosuppressive drugs without undergoing rejection. These selected recipients who maintain stable graft function in the absence of clinically significant detrimental immune responses and immune deficits are conventionally known as "operationally" tolerant. Despite being a very small subgroup of transplant recipients, operationally tolerant recipients have received substantial attention from the transplantation research community. Operationally tolerant patients might exhibit a signature of tolerance that could potentially be useful to select recipients amenable to drug minimization or withdrawal. Furthermore, elucidation of the molecular pathways associated with the operational tolerance phenotype could provide novel targets for therapy. These studies have focused mostly on the analysis of peripheral blood samples and employed flow cytometry immunophenotyping and transcriptional profiling technologies. In addition, in liver recipients molecular analyses of graft tissue have also been conducted.

The first studies performed to identify biomarkers of tolerance in liver transplantation employed flow cytometry immunophenotyping and/or gene expression profiling on blood samples [79, 80,202–204]. In these retrospective cross-sectional studies, operationally tolerant recipients (stable graft function off immunosuppression for various periods of time) were compared with recipients under maintenance immunosuppression considered to be non-tolerant (although the absence of tolerance had not been formally demonstrated in all of these cases by a prior attempt at immunosuppression withdrawal). Mazariegos et al. [202] quantified the relative frequency of monocytoid (mDC) and plasmacytoid (pDC) peripheral blood dendritic cell precursors in samples from six operationally tolerant recipients (0.8–7.9 years after complete immunosuppression cessation), 23 recipients undergoing prospective drug weaning (but still on minimal immunosuppression at the time of blood collection), and 11 recipients on conventional maintenance immunosuppression (in whom drug weaning had failed or had never been attempted). In both tolerant recipients and those receiving minimal immunosuppression the pDC/mDC ratio was significantly increased as compared with recipients on maintenance immunosuppression or with healthy individuals. Li et al. [79] assessed the frequency of peripheral blood B cells natural killer (NK) cells, and several T-cell subsets from 12 tolerant (2–6 years after complete immunosuppression cessation) and 19 potentially non-tolerant (receiving maintenance immunosuppression) pediatric living donor liver transplant recipients. Tolerant recipients exhibited: (1) increased numbers of B cells and $CD4^+CD25^+$ T cells; (2) decreased numbers of NK cells; and (3) an alteration in tolerant recipients of the normal distribution of the two major peripheral blood γδ T-cell subsets (namely δ1 and δ2), with an expansion of δ1 T cells and an increase in the δ1/δ2 T-cell ratio. Martínez-Llordella et al. [80] were the first to employ whole-genome Affymetrix microarrays to comprehensively analyze the blood molecular patterns associated with the tolerance phenotype. Blood samples from tolerant recipients were enriched in transcripts related to γδT cells and NK cells. Operationally tolerant and non-tolerant recipients also differed in the distribution of circulating γδ T-cell subsets and in the frequency of $CD4^+CD25^+Foxp3^+$ T cells. This cell subset was increased in tolerant patients, albeit no differences in $Foxp3^+$ transcript levels between tolerant and non-tolerant recipients were noted.

Martínez-Llordella et al. subsequently expanded their initial observations to a larger cohort of patients (28 operationally tolerant and 33 non-tolerant recipients) [203]. The tolerance-related expression dataset was enriched in NK- and γδ T-cell–related transcripts and significantly correlated with the frequency of circulating NK and γδ (but not $CD4^+CD25^+Foxp3^+$) cells [203]. Significant transcriptional differences were also observed in magnetically sorted peripheral blood mononuclear cell (PBMC) subsets from tolerant and non-tolerant recipients. Furthermore, three predictive blood gene expression signatures (containing two to seven genes) were identified.

Böhne et al. recently reported the results of the first prospective immunosuppression withdrawal trial in liver transplant recipients that included blood and liver tissue transcriptional biomarker analyses [147]. In this study 75 of the 98 liver recipients completed the trial, 57 of whom rejected while 41 were successfully weaned. Sequential blood and/or liver tissue samples from 75 recipients were analyzed using whole-genome microarrays and qPCR. The enrichment in PBMC NK-related transcripts described by Martínez-Llordella et al. [203] was already present at enrolment (i.e. before the discontinuation of immunosuppressive drugs was initiated), thus confirming the results of previous retrospective studies. In contrast, no differences in Foxp3 transcript levels or $CD4^+Foxp3^+$ T cells were noted between tolerant and non-tolerant individuals at enrolment. Transcriptional differences between tolerant and non-tolerant recipients were also identified at the graft itself. The intragraft gene expression profile, however, was mainly enriched in genes involved in iron homeostasis, and showed no overlap with the PBMC-derived expression markers. These markers were found to be independent of the type of immunosuppression and all other clinical parameters associated with the success of drug withdrawal. In agreement with the blood results, at enrollment no differences between tolerant and non-tolerant patients in intragraft $CD4^+Foxp3^+$ T cells or Foxp3 mRNA levels were detected. Importantly, in a side-by-side comparison, liver tissue-derived transcriptional signatures were found to be more accurate and reproducible at predicting the success of drug withdrawal than PBMC-derived signatures [147].

Together these studies suggest that operationally tolerant liver recipients have unique blood and liver tissue gene expression patterns that could be useful to discriminate between tolerant and non-tolerant recipients. NK cells and related transcripts appear to be the most robust blood markers of operational tolerance, as they are already present in the blood of tolerant liver recipients before immunosuppressive drugs are discontinued. The role of $CD4^+CD25^+Foxp3^+$ T cells is less clear due to the confounding effects of pharmacological immunosuppression. Importantly, blood cellular and transcriptional markers do not appear to reflect the nature of intragraft immune responses. Furthermore, liver tissue-derived biomarkers are more accurate than blood-related markers at predicting the success of drug withdrawal strategies.

Brouard et al [205] performed the first molecular profiling analysis of operationally tolerant kidney recipients. The study included

17 operationally tolerant recipients (i.e. stable graft function without immunosuppression for at least 2 years), 22 chronic rejectors, 10 patients on corticosteroid monotherapy, 12 stable patients on standard immunosuppressive treatment, and 14 recipients with acute rejection. A custom immunology-focused cDNA microarray (Lymphochip) was employed to analyze blood samples from a training group of five operationally tolerant recipients, 11 chronic rejectors, and eight age-matched healthy subjects. A set of 49 genes was able to differentiate operationally tolerant patients from healthy subjects, and chronic rejectors were identified. On first analysis [205] the tolerance-related expression pattern was found to be enriched in T-cell–related genes and in genes involved in cell cycle regulation, but a subsequent reanalysis of the same dataset revealed a significant enrichment in B-cell–related transcripts [206,207]. Operationally tolerant kidney recipients exhibited an increased frequency and absolute number of circulating B cells with a potentially inhibitory profile [207].

A significant contribution of circulating B cells to the blood gene expression profiles noted in operationally tolerant kidney recipients was also described by the investigators of two large multicenter studies [208,209]. Newell et al. reported the results of the Immune Tolerance Network (ITN) US multicenter study, in which 25 operationally tolerant kidney patients (i.e. stable graft function without immunosuppression for at least 1 year) and 33 stable kidney recipients on triple immunosuppression were analyzed. Whole blood Affymetrix microarray experiments were performed on samples collected from a training set of 19 operationally tolerant recipients, 27 immunosuppressed recipients, and 12 healthy controls. Twenty-two of the 30 genes were differentially expressed by more than two-fold between tolerant and immunosuppressed recipients and were B-cell specific. These differences were not observed when operationally tolerant patients were compared to healthy controls. Clearer evidence of the association between B cells and operational tolerance was provided by the demonstration that CD20 transcript levels were increased in urine samples from tolerant recipients as compared with both immunosuppressed patients and healthy individuals. The Indices of Tolerance/RISET (Reprogramming the Immune System for Establishment of Tolerance) Consortium European multicenter study [209] evaluated whole blood samples from 11 operationally tolerant patients, 11 stable recipients under corticosteroid monotherapy, 10 patients on immunosuppressive treatment without CNIs, 30 patients under CNI-based treatment, nine patients with chronic rejection, and 19 healthy controls. Operationally tolerant patients exhibited a higher number of circulating B and NK cells compared to healthy controls and all other study groups. The expansion in the number of B cells was mainly due to an increase in the number of transitional and naïve B-cell subsets. Operationally tolerant patients expressed a unique set of genes not shared by the remaining comparison groups and enriched in B-cell–related pathways. In this study Foxp3 transcription per se was not significantly increased in tolerant patients, but a combinational analysis calculating the ratio of Foxp3 to MAN1A1 transcription could discriminate between drug-free patients, stable kidney recipients, and recipients undergoing chronic rejection. These results could be also reproduced in patient samples provided by the ITN study.

These studies have a number of methodological limitations. In kidney transplantation the specificity for tolerance of the gene signatures identified in the retrospective cross-sectional studies described above is difficult to establish. The main problem derives from the lack of appropriate comparators. Indeed, there is no such thing as an adequate control group for allograft kidney recipients who exhibit stable graft function despite receiving no immunosuppressive drugs. The absence of biopsies from tolerated organs is another potential problem. First, a "minimal" form of subclinical rejection cannot be excluded. Second, whether analyses on peripheral blood samples constitute a good surrogate of the immune responses taking place within the graft is unclear. The stability and prognostic value of the kidney tolerance-related signatures is also unknown and will need to be determined in long-term longitudinal studies. In liver transplantation the availability of a prospective immunosuppression withdrawal trial [147] incorporating protocol liver biopsies partially addresses some of the methodological limitations of kidney studies. However, the cohort of liver recipients from whom the predictive gene signatures were derived is still relatively small. Therefore, the robustness and stability of these signatures in large independent validation cohorts cannot be taken for granted. Furthermore, the long-term outcome and overall clinical benefit of drug withdrawal in liver transplantation is still unknown.

Summary

There is general consensus that individual patients come to transplantation with widely variable immune competence, and that their needs for immunosuppression, both in terms of amounts and types, are similarly varied. At present, there are no prospectively validated ways to stratify patients based on risk for rejection or immunosuppression-related complications, and only general associations between emerging biomarker assays and patient outcomes exist. As such, immune management remains largely driven by reactions to complications rather than pre-emptive actions. Nevertheless, the marked improvement in the collective mechanistic understanding of the biology of rejection that has characterized the past two decades is now informing the design of mechanistically driven monitoring tools. Their development and validation will play a major role in the improvement of transplantation, and their deployment will undoubtedly be a part of the transplant clinician's future armamentarium for patient care.

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CHAPTER 91

Protective Immune Competence after Organ Transplantation

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Introduction

Advances in immunosuppressive regimens and in multidisciplinary clinical care have significantly improved short-term outcomes in transplant recipients. However, improvements in long-term outcomes have plateaued due to complications of immunosuppressive medications, including metabolic dysregulation, infections, and malignancies [1,2]. The types of post-transplant infections and their epidemiology are continuously changing due to newer immunosuppressive regimens, expanding donor and recipient criteria, improved longevity of recipients, and mounting ability to provide prophylaxis, administer vaccinations, and monitor the immune status of transplant patients [3]. Thus, the field of transplant infectious disease continues to evolve along with the shifting patterns of morbidity associated with traditional and novel pathogens. The dynamic nature of transplant infectious diseases is outlined in Chapters 92–94.

Achieving the optimal balance of immune modulation has been constrained by the inability to characterize the net state of immunosuppression for the individual patient. Further complicating the assessment of protective immune competence is the recognition that one's immune system addresses the challenge of every pathogen differently. Therefore, the equilibrium between alloimmunity and protective immunity in a transplant patient is more than a dichotomous balance of simple scale; rather it is more akin to a web of interacting influences.

Ideally, a simple, non-invasively obtained biomarker or set of biomarkers linked to a particular clinical outcome would allow for targeted, individualized treatment plans for transplant recipients. Dinavahi and Heeger have reviewed the diverse methods and early results produced in this promising quest [4]. Much of this work has focused on the characterization of lymphocytes populations and functions relevant to alloimmunity. The methods employed have included ELISPOT, flow cytometry, antibody detection, gene expression, and "omics" assays. These methods and their application are discussed in detail as they relate to alloimmunity in Chapters 13 and 90. More recently, the field has begun to extrapolate these techniques and their data to the assessment of protective immunity. However, the translation of data from these assays to broader clinical contexts has suffered from each assay's variability and lack of standardization. This chapter will introduce methods proposed for the assessment of a patient's protective immune competence, and discuss their translation into broader clinical use.

Protective immunity

Earlier chapters have addressed thoroughly the biology of the immune system (see Chapters 2 and 5–7) as it faces exposure to the allograft and the therapeutic methods used to modulate these responses (notably, see Chapters 17, 98, and 101). However, these largely non-specific immune modulations also significantly thwart the recipient's immune system from appropriately identifying and controlling pathogens. Thus, it is critical to understand, and thus briefly recount here, the interplay between components of protective immunity and alloimmunity [5,6].

Primary immune responses

The innate immune system serves as the first line of defense against primary infection, and subsequently potentiates the adaptive immune system to produce an effective immune response. Natural killer (NK) cells and dendritic cells (DCs) comprise the primary cellular components of the innate immune system. NK cells can have direct cytotoxic effects on infected cells and also secrete cytokines, such as interferon-gamma (IFN-γ), which activate and coordinate the adaptive components. DCs recognize foreign pathogens through pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), mannan binding lectin receptors, integrins, NOD-like receptors, and subsequently present antigens to the adaptive immune system [7]. PRRs are not specific for any particular pathogen but are specifically non-self. Depending on the type of pathogens and the context in which the innate immune system encounters them (e.g. which TLR is activated), these cells express cytokines and costimulatory molecules to induce proinflammatory or tolerogenic immune responses [5,8]. Potentiated by cells of the innate immune system, the antigen-specific T and B cells of the adaptive immune system differentiate into effector cells, disseminate throughout the organism, and eventually contract in number after further differentiation into memory cells. For more in depth discussions of this topic, see Chapter 7.

Immunologic memory

Immunologic memory encompassed memory B cells, antibody secreting plasma cells (ASCs), antibodies, and memory CD4 and CD8 T cells. Antibodies bind directly to pathogens or foreign proteins, and may neutralize or facilitate opsonization of pathogens to aid in controlling an infection. Memory B cells provide the ability to respond to these pathogenic challenges by rapidly differentiating

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into ASCs upon reactivation to increase antibody titers. The memory T cells provide rapid cellular response by means of rapidly proliferating, secreting cytokines, and expressing cytotoxic enzymes to aid in clearance of the infection.

The mechanisms by which memory responses are maintained are being explored to date. The most effective means to maintain B-cell memory is repeated exposures to the antigen. However, even in the absence of antigenic exposures, memory B cells can persist for decades; vaccine-specific antibodies and memory B cells can be detected over 50 years after vaccination with the smallpox vaccine [9]. For antigen-specific T cells, after the effector response, ~90% are eliminated during the contraction phase. The majority of the remaining cells are converted into long-lived memory T cells [5]. Recent data have demonstrated that memory T cells are actually a heterogeneous set of populations that play distinct roles in future pathogen challenges. The type of infection, the underlying genetics, and the concurrent disease processes may significantly influence the relative frequencies of these pathogen-specific subsets [10]. Importantly, emerging research in cellular effects of immunosuppressive drugs seems to indicate that each agent may inhibit and enhance the development of these memory populations differently [11,12]. A thorough discussion of memory as it relates to the spectrum of alloimmunity is found in Chapter 9.

Immune competence prior to transplantation

Immune deviations prior to transplant

Chronic kidney disease (CKD) creates chronic inflammatory states that contribute to the morbidities faced by patients with end-stage renal diseases [13]. Studies have shown that patients with CKD have increased expression of inflammatory biomarkers, such as C-reactive protein, and increased levels of circulating proinflammatory cytokines [13–15]. Kimmel et al. demonstrated that the increased levels proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin (IL)-1 correlated to mortality in dialysis patients, with a likely association with increased infections in these patients [15]. These data would suggest that in certain patients with CKD significant cellular immune dysfunction is involved in the progression of their diseases.

Patients with chronic liver disease develop immunologic impairments related to the hepatic dysfunction and resultant loss of functional mucosal immunity [16]. Patients with cirrhosis develop diminished intestinal barriers, likely due to alterations in intracellular channels and cell integrity, leading to increased translocation of pathogens through the intestinal lining into the mesenteric and lymphatic circulatory systems [17,18]. The resultant bacterial endotoxins and antimicrobial responses lead to systematic increases in inflammatory cytokines [19]. One of the most significant ramifications of bacterial translocation is the increased concentration of TNF, which is a vital component to hepatocyte injury response and usually promotes the proliferation of hepatocytes [20]. However, in the cirrhotic liver, the exaggerated levels of TNF induce increased secretion of IL-1, IL-6, and IL-8, and activation of downstream kinases and proteases, leading to dysregulation of the adaptive immune system [19].

A large proportion of the immune dysfunction in cirrhotic patients occurs within the cellular immune system. In particular, chronic hepatic dysfunction leads to dysfunction of neutrophils, monocytes, and macrophages, and reduced opsonization against pathogens [21–25]. Probably the most significant deficit seen is within the reticuloendothelial system (RES), which constitutes the primary defense system against pathogens in the blood, and approximately 90% of these immune cells are located in the liver [26]. Patients with a decreased RES activity are at higher risk of developing bacterial infections than patients with a normal RES [27]. As the hepatic dysfunction worsens, the RES function continues to be lost, and this is largely responsible for the higher rate of infections experienced in progressive cirrhosis [28].

Thus, the underlying pathology contributing to the patient's need for organ transplantation often leads to significant deficits in protective immune functions, which can contribute to infectious diseases morbidity in the post-transplant period.

Pretransplant assessments of protective immunity

During the evaluation process, potential transplant recipients undergo extensive assessment of infection risks and exposures, which is discussed by organ system in detail in Chapters 28–33. In general, most transplant centers evaluate serologies for previous pathogen exposures, including cytomegalovirus (CMV), Epstein–Barr virus (EBV), human immunodeficiency virus (HIV), and the hepatitis viruses [29]. While positive serologies for a pathogen imply some degree of protective immunity, they do not correlate to adequate protection post transplant [30]. These screening tests also allow for the development of vaccination strategies to boost protective immunity. While most vaccines have not been sufficiently studied in the maintenance of protection post transplant, the limited data and expert opinion indicate increased efficacy of vaccination prior to transplant when possible, and that it may boost immunity sufficiently to expand the donor pool for the recipient, as in the case of hepatitis B [31,32]. Completing these assessments and vaccinations provides a better characterization of protective immune competence and what challenges the net state of immunosuppression will bring for the patient.

Assessment of the "net state" of protective immunity

The concept of a transplant patient's "net state of immunosuppression," which encompasses all the factors that contribute to the risk for infections, is important to understanding the approaches to monitoring and caring for these patients [3,33,34]. Classically, this state has been influenced by the immunosuppressive regimens used, as well as disease-specific immune deficits (as described above), bone marrow function (e.g. ability to maintain appropriate leukocyte counts), perioperative complications, allograft/organ dysfunction, metabolic derangements, exposures to antimicrobials, and concurrent infections [3,34]. This concept has been very useful for ascribing epidemiologic risks for groups of patients with similar combinations of these factors. However, due to the difficulties in quantifying these factors, and thereby an inability to specifically quantify the net state of immunosuppression, clinicians have struggled to provide individual-level risk assessments in a patient. Recently, new monitoring tools have been introduced that are enabling more individualized assessments of post-transplant risks.

Transplantation immune cell function assay

In the last decade, the transplant community has seen great effort, if perhaps not great progress, in the development of biomarkers and immunologic assays to help guide clinical decision-making and aid in individualizing the care of transplant recipients. The first and only commercially available test to directly address the global immune system responsiveness is the ImmuKnow® assay (Cylex Inc., Columbia, MD, USA). This immune function assay, also

known as the Transplantation Immune Cell Function Assay (ICFA), measures the in-vitro production of ATP by $CD4^+$ T cells in response to stimulation by a mitogen [35]. As ATP is the primary energy source for all cells, including lymphocytes, and more immunosuppressant agents modulate T-cell functions, the assay is designed to quantify responses of T-helper cells as a surrogate marker for global cell mediated immune functional capacity [35]. The ICFA is Food and Drug Administration (FDA) cleared for assessing ATP content in cells from populations undergoing immunosuppressive therapy for solid organ transplantation, and thus reports immune responses based on the ATP detected: "low" ($\leq$225 ng/mL), "moderate" (226–524 ng/mL), or "strong" ($\geq$525 ng/mL). The test has not been approved to specifically assess a patient's need for immunosuppression but no validated guidelines exist for its use.

Several studies have examined this test as a predictor of cellular rejection and/or of infection. In general, no prospective randomized data are available for the use of this assay and as such, its utility remains unvalidated. Certainly, no utility has been demonstrated with regard to anticipating rejection in any setting. The data for this assay for assessing risk for infections are, however, more intriguing. Kowalski et al. published a multicenter retrospective analysis of relative risk for rejection in 504 solid organ transplant recipients (heart, kidney, kidney–pancreas, liver, and small bowel), who received prospective observational assessments with the ICFA at various times post transplant [35]. Recipients with immune response values of 25 ng/mL of ATP were 12 times more likely (95% CI, 4–36) to develop infections than recipients with stronger responses. Interestingly, this group's data demonstrated an intersection of the odds ratio curves for rejection and infection at 280 ng/mL of ATP, suggesting this value as the target immunologic zone for solid organ recipients [35]. Several subsequent studies have shown an association of low ICFA values with higher risk of infections in general [36,37], as well specific infections: BK virus (BKV) [38,39], CMV, EBV [40,41], post-transplant hepatitis C virus (HCV) [42–44], and invasive fungal infections [45]. However, the translation of these data to common use has been limited by the small numbers of patients, heterogeneity of immunosuppression, single center enrollment, and importantly, the wide variety of target immunologic zones conveying risk of infections. Two studies have suggested that patient ethnicity may affect the ICFA values when correcting for other clinical and demographic factors [46,47]. Furthermore, many of the studies showing a correlation of the ICFA values with infectious disease risks do not demonstrate a strong association of higher ICFA values with cellular rejection [38,48–50]. Therefore, a single ICFA value for a single patient does not produce high predictive value for risk of either infection or rejection events in the immediate period of time [47,51]. Thus, several targeted studies, and a recent meta-analysis of the studies of ICFA, have not shown clear efficacy of the assay in the general monitoring of transplant recipients [52–54].

An interesting aspect of utilizing the ICFA is to monitor the response after reduction of immunosuppression in order to control infections [55,56] and malignancies [57]. Gautam et al. utilized the ICFA to titrate the reduction of immunosuppression, in addition to antivirals, in 12 patients with a variety of post-transplant viral infections without any associated rejection [55]. However, while the mean ICFA was 56.8 ng/mL of ATP during infection and 194.5 ng/mL after reduction of immunosuppression, the ICFA value ranges overlapped significantly; 3–178 ng/mL and 53–409, respectively [55]. Lee et al. compared EBV viral loads to ICFA values in 18 pediatric liver transplant recipients, and in a subset of three patients with consistently high EBV viral loads, to determine the utility of ICFA values in titrating reductions in immunosuppression [56]. None of these patients developed post-transplant lymphoproliferative diseases (PTLDs); however, while all had increases in ICFA values, only two of three demonstrated reductions in EBV viral loads [56]. These studies are limited by very small numbers, but the approach shows promise in individualizing the reduction of immunosuppression in difficult-to-treat infections where T helper function is critical.

In summary, the role of the ICFA assay in post-transplant patient monitoring remains to be defined. It is conceivable that risk thresholds and appropriate monitoring schemes will vary by patient age and ethnicity, transplanted organ, and immunosuppressive regimen, particularly when lymphocyte counts are altered through depletional induction or rescue strategies. In addition, clinical trials comparing ICFA monitoring to other conventional monitoring are needed to fully understand the utility of this assay for patient risk assessment.

Monitoring immunoglobulins

As the importance of antibody mediated rejection has emerged in the past decade and a half, the study of memory B cells, antibody secreting cells, and antibodies in transplantation has grown concurrently. While checking the titers of pathogen-specific serologies does not predict post-transplant protection, several studies have implied that immunoglobulin levels in general may help predict protective immune competence. The first large study to examine the contribution of net state of protective immunity was that by Goldfarb et al. of 67 lung transplant recipients [58]. This group demonstrated a significant incidence of post-transplant hypogammaglobulinemia (70%) and severe hypogammaglobulinemia (37%), defined as IgG levels of $<$400 mg/dL in these lung transplant patients [58]. Infections, including bacterial, viral, and fungal, were significantly more common in patients with hypogammaglobulinemia ($P = 0.006$ for total infections) [58]. Severe hypogammaglobulinemia was further associated with invasive aspergillosis (incidence of 44%) and with patient mortality [58]. Yamani et al. reported on 111 consecutive heart transplant recipients who had immunoglobulin G (IgG) level monitoring at baseline and 3 and 6 months post transplant, finding that 10% of their cohort developed severe hypogammaglobulinemia, defined as an IgG of $<$350 mg/dL, after transplant [59]. Patients with severe hypogammaglobulinemia were found to be at increased risk of opportunistic infections compared to patients with an IgG of $>$350 mg/dL, with an odds ratio of 22.8 [59]. These patients were diagnosed with a variety of bacterial, viral, and fungal infections, without a dominant syndrome [59]. They also found that post-transplant hypogammaglobulinemia was highly associated with treatment of rejection, adding another mechanism by which rejection can induce risk of infections [59]. Similar conclusions were drawn from a study of 41 heart transplant recipients by Sarmiento et al., with the additional finding of pretransplant IgG, and in particular pretransplant IgG1, being associated with an increased risk of opportunistic infections [60]. This study also showed a preponderance of CMV disease in patients with hypogammaglobulinemia. After adjustment for clinical predictive variables, decreased values of post-transplant IgG remained significant predictors [60]. This same group attempted to extend these data to liver transplantation in a report of 46 consecutive liver transplant recipients from their center [61]. However, contrary to the heart transplant studies, in this study, pretransplant IgG

hypergammaglobulinemia and IgA hypergammaglobulinemia, with relative risks of 2.78 and 2.77, respectively, were associated with post-transplant infections [61]. Post-transplant immunoglobulin levels were no different between patients with or free from infections [61]. These findings were attributed to the immune stimulation of chronic liver disease, as described above, with resultant increasing antibody levels with increased severity of liver disease [61,62]. Recently, Fernández-Ruiz et al. analyzed the impact of hypogammaglobulinemia on infectious disease risk in 226 kidney transplant recipients, in whom serum immunoglobulin levels were prospectively quantified at baseline, 1 month and 6 months. The prevalence of IgG hypogammaglobulinemia in this cohort was 52% and 31% at 1 and 6 months, respectively, and was associated with higher incidences of infections generally, and specifically with bacterial infections [63]. These data did not indicate an association of CMV or other opportunistic viral infections with hypogammaglobulinemia within the first 6 months [63].

These studies provide preliminary evidence that monitoring of pre- and post-transplant immunoglobulin levels may serve a valuable role in predicting a transplant recipient's risk for infection. Alterations in immunoglobulin levels pretransplant seem to be linked with significant voids in protective immunity, while post-transplant hypogammaglobulinemia appears to extend the risk for infections. It is intuitive that alterations of humoral immunity may be linked to bacterial infections, given the dependence on neutralization, opsonization, and complement activation for clearance of these infections [63]. The lack of association CMV disease with hypogammaglobulinemia in many of these studies is not surprising given the primacy of T-cell immunity for the control of most manifestations of this virus. Again, the available data may not be generalizable for global use in transplantation. Most studies were single center and many were retrospective analyses. The definition of hypogammaglobulinemia and assays used to assess it varied across these studies. Also, these groups used different immunosuppressive regimens, infectious diseases diagnostics, and follow-up durations, and monitored for different infections. Given these variations, the association between acquired hypo- or hyper-gammaglobulinemia and risk for infections may not be causal, as many infections induce immune dysregulation leading to altered B-cell functions. However, given the ready availability and low cost of these assays at most transplant hospitals, the serial monitoring of serum immunoglobulin levels in well-designed multicenter studies, in particular in trials of new immunosuppressive agents, should be warranted.

Molecular monitoring of pathogens as surrogates for net state of immunosuppression

Molecular monitoring for pathogens, particularly quantitative polymerase chain reaction (PCR) assays for viral pathogens, has become commonplace in transplant centers. Many experts believe that these pathogen monitoring tools may be used as markers of the net state of protective immune competence [64]. This idea emerged primarily from the experiences in monitoring for polyoma BKV for the prevention of BK nephropathy. After the original descriptions of BKV inciting a significant incidence of tubular nephritis and nephropathy in kidney allografts and that BKV titer correlates with risk of polyoma virus associated nephropathy (PVAN), PCR monitoring for BKV replication in kidney transplant recipients has become increasingly prevalent [65–67]. Much of this experience demonstrated the possible utility of reduction of immunosuppression in response to BKV viremia [68–70]. Ginevri et al. published the first report of specific reductions in immunosuppression in response to BKV viremia as pre-emptive intervention to prevent PVAN in 62 pediatric kidney transplant recipients [71]. This cohort was prospectively monitored with quantitative BKV PCR from blood and urine samples. This group's four stage process for reducing immunosuppression demonstrated clearance of viremia in all patients without any associated incidence of allograft rejection [71]. BKV-specific ELISPOT assays were used to monitor the viral-specific T-cell responses and demonstrated an association of poor responses with risk for BKV viremia. (The ELISPOT assay is described in Chapter 13). Importantly, it was shown that the pre-emptive reduction of immunosuppression improved viral-specific responses, and these improved responses were associated with viral clearance [71]. Several studies have further demonstrated the pre-emptive reductions in immunosuppression in kidney transplant patients with BKV viremia are associated with viral clearance, prevention of PVAN, low incidence of subsequent rejection, and good long-term renal function [67,72–76]. Thus, in renal transplant patients who develop BKV viremia, the BKV PCR assay provides a good assessment of the net state of immunosuppression and can be used to guide the balance of protective immunity and alloimmunity.

More recently, many have speculated that EBV monitoring may inform the assessment of the net state of immunosuppression applicable to all solid organ transplantation groups. To address the utility of EBV PCR in this manner, Ahya et al. conducted a retrospective analysis in 31 lung transplant patients who were followed with serial plasma and whole blood assays for up to 2 years after transplantation [77]. Patients with detectable EBV viral loads had a significantly lower incidence of grade 2 or higher rejection compared to recipients without detectable EBV (45% vs. 83% respectively, with an odds ratio of 0.17) [77]. Importantly, having detectable EBV was not associated with risk of other infections (odds ratio = 1.6) [77]. Doesch et al. published similar data from a study evaluating the utility of EBV PCR as a prognostic tool for detecting the development of PTLD in 172 stable adult heart transplant recipients [78]. Thirty-six (21%) patients had sustained detectable EBV, which did not correlate to the risk of PTLD, but was associated with level of immunosuppression [78]. A study of 41 pediatric heart transplant recipients also found a strong relationship between the type and amount of immunosuppression and increased EBV viral load [79]. Bakker et al. demonstrated the ability to exploit EBV monitoring to guide immunosuppression in a prospective evaluation of 75 lung transplant recipients [80]. Patients at this center received EBV viral load monitoring at least twice a year, and at least monthly after a positive test, and when demonstrating a rising slope, their immunosuppression was reduced [80]. Of the 26 (35%) patients who developed detectable EBV, immunosuppression could be reduced in 19 and led to a rapid and sustained decrease in EBV viral load without associated rejection or bronchiolitis obliterans [80].

These data provide the very real possibility of using the viral PCR testing already employed in monitoring patients for viral activity as a measure of the net state of immunosuppression. One of the current limitations to broadly translating these data is the variety of assays being employed and their broad spectrums of sensitivity and dynamic ranges [81–83]. The variability of some PCR assays is being addressed with the introduction of World Health Organization (WHO) standards for CMV and EBV. However, the highly variable rates of pathogen exposure and the unique biology of each organ transplant type make the application of a single PCR assay difficult. Some experts have suggested that the simultaneous quan-

tification of multiple viruses would improve specificity of viral monitoring as a surrogate for assessing the level of immunosuppression [64]. Given the ready availability of PCR assays, the diminishing costs of the technology, and improved reproducibility with international standards, the utility of viral PCR assays, either in single or multiplex format, for the assessment of immunosuppression should be evaluated further in large clinical studies.

Assessment of pathogen-specific protective immunity

The assessments of immunosuppression and global protective immunity described above provide insights into the individual level of risk for infectious diseases in general and could influence clinical care tremendously. However, it is well observed that select patients have difficulty eliminating specific infections, while successfully controlling other pathogens. Thus, the understanding of the net state of immunosuppression lacks the specificity of informing the clinician on which pathogen the patient is at risk of. There is growing research into assessments of immunity to specific pathogens and evidence that these data can convey specific estimation of risks to those pathogens. In addition, the ability to monitor for parameters of effective immune responses may aid in determining the type and duration of monitoring and treatment of infectious diseases.

Immunologic testing

It has become well understood that monitoring of pathogen-specific antibody titers in immunosuppressed patients does not sufficiently establish a quantitative risk for disease from that pathogen, particularly for pathogens, such as viruses, that require T-cell help and/or cytotoxic activity for control [84,85]. Optimal T-cell activation, which is the primary target of most immunosuppressive drugs, requires a delicate network of signals and eventually results in the formation of immunologic memory [86]. The memory T-cell compartment is comprised of subsets of both CD4 and CD8 T cells that rapidly proliferate, secrete inflammatory cytokines, and kill infected cells upon infection to eliminate the pathogen. Recent technologic developments in measurements pathogen-specific immunity, including pathogen–peptide/major histocompatibility complex (MHC) multimers, peptide proliferation assays, and cellular cytokine assays by ELISPOT and flow cytometry, have allowed quantification, characterization, and monitoring of pathogen-specific T cells [87–90]. MHC multimers, usually tetramers, detect $CD8^+$ or $CD4^+$ T-cell responses to a single HLA-restricted epitope from a viral protein. Several studies in organ transplantation have utilized viral multimers, including those for CMV, EBV, and BKV, to detect and track pathogen-specific T-cell populations post transplant. These assays consistently show that transplant patients on immunosuppression are able to generate and expand viral-specific T cells, and the kinetics often correlate to the viremic course [91–93]. These data, however, indicated that the enumeration of pathogen-specific T cells alone does not predict the level of protection for the patient due in part to the lack of data as to the functional quality and proliferative ability of these cells.

An effective anamnestic T-cell response is marked by rapid cytokine secretion. The mixture of cytokines secreted by T cells influences the character of this pathogen-specific response; for example, Th1 cytokines (TNF, IFN-γ, IL-2) are important for immune responses against intracellular pathogens, such as EBV and CMV, whereas Th2 cytokines (IL-4 and IL-10) are crucial for generating immune responses to extracellular infections. Measures of these characteristic responses are proving useful for predicting protection in transplant recipients, particularly against CMV, which has been well reviewed by Egli et al. [90]. These assays usually involve in-vitro stimulation of lymphocytes from whole blood or from PBMCs with a single or combination of peptides, an overlapping peptide pool, or whole lysate of a particular virus. HLA-specific epitopes stimulate $CD8^+$ and/or $CD4^+$ T cells to produce cytokines, which are then detected by a flow cytometer, ELISPOT, or enzyme-linked immunosorbent assay (ELISA) (Figure 91.1). As would be expected, baseline functional immunity correlates with serostatus [93]. Data from several studies indicated that the ability to produce cytokines, usually IFN-γ, with in-vitro stimulation with viral antigens correlates with protection against viral replication and viral diseases [94–98]. However, these responses may not be predictive in all populations. Transplant recipients at high risk of CMV infection, as defined by CMV donor seropositive–recipient seronegative pairing, seem to be capable of developing a diverse population of CMV-specific T cells, but these responses do not predict freedom from CMV disease [99,100]. The lack of clearance of CMV viremia with antiviral therapy may be associated with deficiencies of a functional specific T-cell response and not antiviral resistance [93]. Another utility of measurements of these responses may be the determination of antiviral treatment [90]. For example, patients who are being treated with antivirals and develop improved viral-specific T-cell function may be able to stop treatment earlier than similar patients without functional responses. The ability of these data to predict risk of infection and ability to control infection are immensely interesting, yet need to be evaluated in larger studies.

These assays for the enumeration and functional characterization of pathogen-specific immunity have tremendous research potential, but the feasibility of moving them into the clinical setting is limited [90]. These assays require specialized equipment, expensive and temperamental reagents, and significant technical expertise and time. Most importantly, these assays lack standardization across laboratories, which in turns inhibits the ability to create reference ranges or categorical values. However, a recently developed assay, QuantiFERON-CMV® (Cellestis Ltd., Valencia, CA, USA), addresses many of these limitations for CMV immune monitoring. For this assay, whole blood is cultured overnight with a combination of 21 CMV peptides from several different CMV proteins and specific for 20 HLA class I haplotypes. Two other tubes of whole blood are simultaneously incubated for 24 h with no antigens (negative control) or with phytohemagglutinin (PHA; positive mitogen control) [101]. IFN-γ production from each tube is quantified by an ELISA. Results are positive when the CMV peptide response minus the negative control response is $\geq$0.2 IU/mL of IFN-γ [101]. The test is very sensitive and specific in detecting CMV immunity in healthy controls [101]. The predictive value of this test for CMV disease has been variable. In a study of 297 samples from 39 lung transplant recipients drawn at the time of a bronchoscopy, QuantiFERON® responses did not predict for episodes of CMV reactivation in lung transplants, but the group did not evaluate viremia [102]. In a study better designed to predict viremia, Kumar et al. evaluated this test at baseline, 1, 2, and 3 months post transplant in 108 lung, kidney, liver, and kidney–pancreas recipients from a single center [103]. CMV immunity was detected in 35.2% of patients, though a lower cut-off of 0.1 IU/mL of IFN-γ was used in this study [103]. CMV disease occurred in 5.3% patients with detectable CMV responses compared to 22.9% of those with a negative response ($P = 0.038$) [103]. Thus, a positive result was highly predictive of freedom from disease, but a negative test was not predictive of developing CMV. Lisboa et al. followed this with a prospective study of QuantiFERON-CMV® values in 37

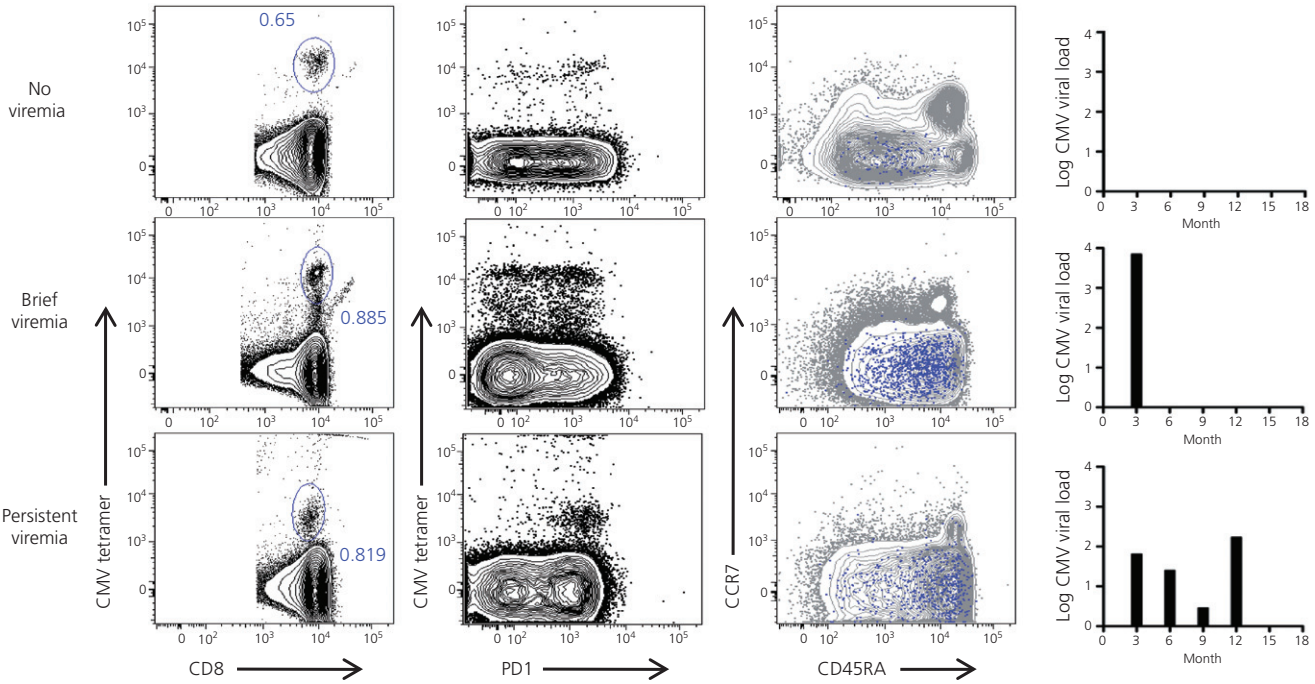

Figure 91.1. Example of integrating cellular phenotyping by flow cytometry, enumeration of viral-specific T cells by MHC tetramers, and virologic monitoring by PCR. Data from three patients are displayed, one health control without viremia and two transplant patients, at month 18. The second column demonstrates duration of cytomegalovirus (CMV) viremia is associated with increased expression of PD-1 on CMV-specific $CD8^+$ T cells. The third column displays CMV tetramer stain cells overlaying bulk $CD8^+$ T cells and demonstrates the skewing of both $CD8^+$ T cells in general and CMV-specific $CD8^+$ T cells with persistent CMV viremia.

transplant recipients who developed low-level viremia [104]. In patients with a positive test at onset, the frequency of spontaneous viral clearance was 92.3% compared to 45.5% in those with a negative test ($P = 0.004$) [104]. These data demonstrate in limited studies that this method may have a clinically useful predictive value in determining the risk of developing CMV viremia and the ability to control low-level viremia without antiviral medications. However, this test is limited by the frequency of indeterminate results, occurring when both the mitogen and the CMV results are <0.2 IU/mL, of up to 30% in transplant patients [102,103]. Furthermore, the positive threshold of 0.2 IU/mL has not been well validated in solid organ transplant recipients [90]. Yet, the ease of performing the assay and a determined interpretation has allowed some centers to utilize this test in clinical monitoring. It is expected more assays of a similar nature will be available for study in transplant patients and these and the ongoing study will inform the transplant center on their utility in the coming years.

Summary

The introduction of newer immunosuppressive regimens and better clinical monitoring tools has improved transplant patient survival steadily over the last three decades. However, the long-term survival of patients is still restricted by the morbidity of infections, which in some patient groups exceeds that of acute rejection [105]. While the models of the net state of immunosuppression and pathogen-specific protective immunity have been understood, the ability to explicitly quantify these concepts and translate them to patient-specific risk is still elusive. It has been understood that every type of allograft conveys unique risks of infectious diseases based on anatomy and level of exposures to external and non-sterile internal environments. More recently, data from clinical trials have indicated that specific immunosuppressive medications incur unique infectious risk. Examples of agent-specific modulation of risk are a proposed association between mTOR inhibition and a decreased risk of CMV infections [106–109], and the emergence of the increased incidence of EBV-related PTLD in clinical studies of costimulation blockade [110] These trials and mechanistic data indicate each agent and regimen specifically modulates generation, maintenance, and quality of protection immunity [11,12]. Therefore, it is highly plausible that the assessments of protective immune competence and the probability of infections will require stratification by age, ethnicity, organ type, and immunosuppressive regimen. Despite the complex nature of the transplant patient's state of protective immunity, if the recent data presented in this chapter are an indication, the field is burgeoning into an era that provides the clinician with the tools for patient-specific predictive assessments of protective immune competence.

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CHAPTER 92

Donor-derived Infections after Organ Transplantation

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Introduction

Transplant recipients are known to be at risk for numerous infectious diseases (see Chapters 93 and 94). While their immunocompromised state (a result of therapeutic immunosuppression and the influence of their underlying organ failure) clearly contributes to this risk in a manner similar to in other states of immunodeficiency, transplant recipients have unique risks related to the transplanted organ itself. Indeed, transplantation itself breaches the mechanical infectious disease barriers that effectively exclude many pathogens, and in introducing an organ with disparate major histocompatibility molecules, perturbs a major means of immune surveillance. This chapter will provide an overview of donor-derived infections and guidelines for their diagnosis, treatment, and avoidance.

General considerations

Donor-derived infections are a significant source of morbidity and mortality following solid organ transplantation. The actual incidence of these infections is unknown, in part due to the diversity of the infections, the absence of required reporting for anticipated transmissible infections, and the failure to recognize and/or report unanticipated donor-derived events. In data from US surveys of infections reported to the Organ Procurement and Transplantation Network (OPTN), <1% of transplants were associated with donor-derived infection [1,2]. Since most reports stem from deceased donors, it is unknown whether this accurately reflects the incidence of transmissible infections from living donors, which is presumed to be lower. Of note, this estimate does not include common expected potentially transmissible infections such as cytomegalovirus (CMV) or Epstein–Barr virus (EBV); inclusion of these infections would increase the overall incidence of donor-derived infections.

Donor-derived infections are a well-recognized risk of transplantation, and in some cases predonation identification of infection has led to the implementation of prophylactic strategies to mitigate the impact of pathogen transmission. Other infections, including geographically restricted mycoses and tuberculosis, may not be anticipated, and thus available prophylactic strategies are not immediately implemented following transplantation; a result is the development of symptomatic infection. Recent reporting has focused on the occurrence of infections that are more difficult to diagnose and have limited or no treatment or prophylactic options.

Donor-derived infections have been attributed to diverse pathogens, and bacteria, viruses, fungi, protozoa/parasites, and prions have all been transmitted via organ transplantation [1–4]. The etiologies of donor-derived infections vary with donor geography and demographics (Table 92.1). Given the greater geographic diversity of donor populations and the more frequent use of expanded criteria donors (see Chapter 53) in the US, there is increased potential for unexpected infections with geographically restricted organisms related to donor demographics [5].

Although the changing demography of the donor population has had a significant impact on outcomes in patients with donor-derived infection, other factors have also been important. These include the choice of immunosuppressive agents, improved diagnostics both for screening and treatment, and the availability of prophylactic antimicrobials. In many cases, not all recipients may be equally affected by a transmission event [1,3]. This may reflect the specific pathogen, variable infection of different donor organs by a given organism, recipient immunity, and the impact of specific immunosuppressive regimens or antimicrobial interventions.

The timing of donor-derived infections is variable and related to the specific pathogen, the use of antimicrobial prophylaxis, and the choice of immunosuppression (see Chapter 94). Bacterial infections typically occur in the first month after transplant; other pathogens may present later [4]. Prophylaxis may alter the timing of symptomatic infection, e.g. CMV typically presents following suspension of prophylaxis [6].

There are several clues that should alert providers to the possibility of donor-derived infection, especially when considering infections presenting in the first few months after transplant. These include the development of unanticipated infection with unusual or unexpected organisms in a recipient for whom there is no other explanation or the development of the same infection in multiple recipients from the same donor. Additionally, the finding of infection in a donor, either pre- or post-mortem, should alert providers to the potential for transmissible infection to recipients.

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Table 92.1. Major pathogens reported to cause donor-derived infections in transplant recipients*

Organism	Distribution	Comment
Bacteria		
Pyogens (e.g. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, etc.)	Worldwide	
Treponema pallidum	Worldwide	
Mycobacterium tuberculosis	Worldwide	Most common in Africa, South-East Asia, India, Pakistan, Afghanistan, and Russia; also in donors with history of incarceration, homelessness, alcohol abuse, history of untreated tuberculosis or tuberculosis contact
Fungi		
Candida	Worldwide	Perforated abdominal viscus
Histoplasmosis	Ohio and Mississippi river valleys in the US; South America, Asia, and Africa	
Coccidioides	South-western US, northern Mexico, Central and South America	
Aspergillus	Worldwide	Association with immunosuppression in donors
Cryptococcus	Worldwide	Association with immunosuppression in donors, including cirrhosis
Parasites/protozoa		
Toxoplasmosis	Worldwide	More common in Latin America, Sub Saharan Africa, parts of Europe
Trypanosoma cruzi	Latin America	
Strongyloides stercoralis	Worldwide	Most common in tropical, subtropical, and warm temperate regions
Viruses		
Cytomegalovirus	Worldwide	
Epstein–Barr virus	Worldwide	
Hepatitis B virus	Worldwide	Most common in South-East Asia and Africa
Hepatitis C virus	Worldwide	Most common in injection drug users, commercial sex workers and other high-risk sexual behaviors, transfusion prior to 1994
Human immunodeficiency virus (HIV)	Worldwide	
Human T-cell lymphotropic virus 1 (HTLV 1)	Worldwide	Asia, Caribbean, South America, Oceania, and West Africa
West Nile virus	Worldwide	Seasonal with incidence based on prevalence of infection in mosquito vector

*Additional organisms transmitted by organ donors include other bacteria (*Bbrucella*, *Bartonella*, non-tuberculous mycobacteria); fungi (*Scedosporium*, *Prototheca*), parasites/protozoa (*Plasmodium*, *Balamuthia*), viruses (other herpesviruses, parvovirus, rabies, lymphocytic choriomeningitis virus), and prions.

Screening

Optimal screening of donors should begin with obtaining a medical and social history and physical exam, and include laboratory testing for common transmissible infections [7] (Table 92.2). As donor organs are often retrieved from remote hospitals, the recipient clinician should become skilled in eliciting details from the procuring center regarding the history and physical exam relevant to the donor's potential for infectious agent transmission. Since donor transmissions may result from either latent or active infections in the donor, screening should evaluate both. In the US, the OPTN has established a minimum standard for donor screening. This includes serologic testing for human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV and HCV), syphilis, CMV, and EBV, and blood and urine cultures [8]. Additional recommended screening tests include toxoplasmosis antibody (especially for cardiac donors), herpes simplex and varicella antibodies, and human T-cell lymphotropic virus 1 and 2 (HTLV 1 and 2) antibody [7]. Donor medical and social history and geography may be important clues to additional potential donor infections, and dictate the need for additional testing (Table 92.2). In some cases, the results of these assessments will exclude a donor from donation because the risk to the recipient exceeds an acceptable threshold, but in other instances test results may dictate prophylactic interventions for the recipient.

Regardless of the test, it is important to consider the potential limitations of test accuracy due to hemodilution [7,8]. Donors who require massive volume repletion with blood products, colloids, or crystalloids may have false-negative test results; consequently, whenever possible pretransfusion samples should be used for donor screening [7–9]. If the only available specimens are post-transfusion samples, recommendations for calculating the impact of hemodilution are outlined in a guidance document from the Food and Drug Administration (FDA) [10].

Determining the ideal donor testing platforms for specific infections varies with the pathogen and test availability. It is notable that although organ donors are frequently tissue donors, testing recommendations differ for organ and tissue donors, in part due to both the availability of different assays and the impact of false-positive test results on donor utilization. In the US, tests used for routine organ donor screening must be approved by the FDA [8]. Serologic testing is most useful for determining the presence of past infections that may reactivate in the recipient after transplant (e.g. CMV). Nucleic acid amplification testing (NAT) will identify active infections prior to the development of an antibody response and has been most commonly used for the identification of HIV, HCV, and HBV in donors with recent infections. The time between infection and detection of nucleic acid and antibody varies with the pathogen and with the test. One study estimated that time to identification of infection in tissue donors was 7 days by NAT versus 22 and 70 days by antibody testing for HIV and HCV, respectively; thus, infection is identified at a significantly earlier time using NAT [11] (Table 92.3). Newer antigen antibody tests may identify HIV infection within 3–5 days of NAT positivity, and may be another option for earlier identification of infection; currently, these tests are not licensed for organ donor screening in the US [12]. "Window-period" infections (i.e. those that occur prior to the development of a positive laboratory test) remain an uncommon source of donor-derived infection. NAT testing may be most useful for identification of window-period infection for HCV as it increases the test yield ten-fold [13,14]. Whether NAT testing should become the standard for organ donor screening for HIV, HBV, and HCV is controversial; there has been concern regarding the potential for

Table 92.2. Recommended donor testing for major transmissible pathogens

Disease	Primary test*	Additional tests	Deceased donor	Living donor
Cytomegalovirus (CMV)	CMV IgG*		Yes	Yes
Human immunodeficiency virus (HIV)	Anti-HIV 1 and 2*	NAT	Yes.	Yes
		Antigen testing (should not replace serology)	NAT for donors at increased risk for HIV	NAT if new risk factors close to transplant
Hepatitis B virus (HBV)	HBsAg, anti-HBcAb*	HBV DNA (should not replace serology)	Yes	Yes
			NAT for donors at increased risk for HBV	Check NAT if new risk factors close to transplant
Hepatitis C virus (HCV)	Anti-HCV*	NAT (should not replace serology)	Yes	Yes
			NAT for increased risk donors, possibly universal	Check NAT if new risk factors close to transplant
Syphilis	RPR or VDRL*		Yes	Yes
Epstein–Barr virus (EBV)	EBV antibody tests*		Yes	Yes
Bacteria	Blood and urine cultures*	Respiratory cultures	Yes	No
Additional Testing based on risk				
Human T-cell lymphotropic virus 1 and 2 (HTLV 1 and 2)	Serology	NAT		
West Nile virus	Serology	NAT		
Toxoplasma	Serology		Heart donors	
Trypanosoma cruzi	Serology (EIA, IFA)	Peripheral blood smear, NAT		
Strongyloides stercoralis	Serology	Stool ova and parasites		
Leishmania	Serology			
Plasmodium	Peripheral blood smear	Antigen detection		
Schistosomiasis	Urine and stool ova and parasites	Serology	Kidney donors	Kidney donors
Mycobacterium tuberculosis	Skin testing with purified protein derivative (PPD)	Interferon-γ release assay	No	Yes
Coccidioides	Serology (immunodiffusion, complement fixation)	Antigen testing		
Histoplasmosis	Serology (immunodiffusion, complement fixation)	Urine and serum antigen		

*Required by US Organ Procurement and Transplantation Network
NAT, nucleic acid amplification testing; HBsAg, hepatitis B surface antigen; HBcAB, hepatitis B core antibody; HBSAB, hepatitis B surface antibody; EIA, enzyme-linked immunoassay; IFA, indirect immunofluorescence assay; RPR, rapid plasma reagin; VRDL, Viral and Rickettsial Disease Laboratory.

Table 92.3. Impact of window period on detection of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in potential organ donors*

		Window period* serology	Estimated risk of undetected infection during serology window period*	Window period* nucleic acid test	Estimated risk of undetected infection during nucleic acid test window period*
HIV	Normal risk	22	1.72 (0.63–4.40)	7	0.55 (0.2–1.3
	Increased risk~	22	8.54 (1.52–23.99)	7	2.72 (0.49–7.44)
HCV	Normal risk	70	3.65 (1.69–8.30)	7	1.16 (0.53–2.61)
	Increased risk (as defined in [19])	70	19.91 (10.75–31.93)	7	1.99 (1.09–3.23)

Adapted from Ellingson et al. [13], with permission from John Wiley & Sons Ltd.
*Window period is defined as the time between when the virus reaches a transmissible level to the time when it is detectable by standard laboratory test (serology or NAT).

organ donor loss due to false-positive NAT and unavailability of testing in a timely and reliable fashion by all Organ Procurement Organizations (OPOs). Consequently, a consensus conference convened in 2009 recommended that NAT be reserved for donors perceived to be at increased risk for infection with HIV, HCV, or HBV [15].

Recipient evaluation

Since infections after transplant may occur due to reactivation of latent recipient infection or to de-novo infection of the recipient from the donor, a key to identification of donor-derived infection is screening of the recipient prior to transplant. Some donors with known infections may be more appropriate for recipients with a prior history of the same infection (e.g. HCV). Pretransplant recipient screening also allows for immunization against potentially transmissible infections, notably HBV, thereby potentially increasing the donor pool. Standard recipient screening for HIV, HBV, and HCV is mandated by OPTN policy in the US [16] Additional studies that should be considered include CMV antibody, HTLV 1 and 2 antibody, VDRL or rapid plasma reagin (RPR) (to screen for syphilis), EBV antibody (either to viral capsid antigen or EBNA), and toxoplasmosis antibody [17]. Recipients with potential geographic exposure to specific parasites/protozoa, such as *Trypanosoma cruzi* and *Strongyloides*, or fungi, including *Coccidioides immitis* and *Histoplasma capsulatum* may benefit from serologic screening for those organisms [17].

Limitations to determining donor-derived infections

Despite increased attention to the identification of donor-derived infections, there are a number of limitations that affect recognition of these infections [18]. Initial donor evaluation includes review of medical and social history, something that may be suboptimal in deceased donation due to its provision by individuals who may not

Table 92.4. Key organisms transmitted by donors with undiagnosed meningoencephalitis and/or altered mental status*

- Viruses:
  - Lymphocytic choriomeningitis virus
  - West Nile virus
  - Rabies
- Bacteria:
  - *Mycobacterium tuberculosis*
- Fungi:
  - *Coccidioides*
  - *Cryptococcus*
  - *Aspergillus*
- Parasites/protozoa:
  - *Balamuthia*

*Excludes donors with positive cultures on routine screening.

be fully aware of the donor history. This history may affect the choice of tests that are obtained as part of the donor evaluation. Laboratory screening may also be limited as serologic testing is dependent on adequate time for the development of an antibody response and NAT is not available for all pathogens and at all times. Both serologic and NAT testing have the potential for false positives and negatives; hemodilution may further confound the test accuracy. Moreover, there are some pathogens for which there are no standard screening assays. A particular challenge has been the identification of transmissible infections in donors presenting with altered mental status of unclear etiology; these donors should be carefully evaluated for infection and excluded from donation if there is likelihood that undiagnosed infection may have contributed to the donor's demise (Table 92.4).

Consent Issues

Transmission of infection is a significant potential risk of transplantation and recipients should be aware of this prior to transplant. Consequently, OPTN policy mandates that recipients must be notified if the donor has a medical condition that may be transmissible to them [16]. If the donor has medical or social risk factors that have been associated with increased risk for HIV, HBV, or HCV based on US Public Health System guidelines, or if the testing for these infections was only performed on hemodiluted blood, the recipient must be specifically informed regarding the potential risk for transmission of HIV, HBV, and HCV [19]. Recipients should be notified that donor screening is never comprehensive; some organisms cannot be anticipated and there are limitations to all available tests. In some cases, donor infections may be identified following transplant and recipients should also be informed of this possibility.

Reporting of donor-derived infections

Globally there has been increased attention to the reporting of donor-derived infections. In the US, all unexpected proven or potential transmissions must be reported to the OPTN Patient Safety System. These cases are then reviewed by the Ad Hoc Disease Transmission Advisory Committee, which determines the likelihood of donor transmission, using a set of standardized definitions [2,16]. This reporting allows the notification of centers caring for other potentially infected recipients, thereby permitting interventions for prevention and/or treatment as appropriate. A World Health Organization (WHO) initiative, Project Notify, has developed a similar set of standardized definitions to encourage reporting of donor-derived transmission events with the hope of developing a worldwide database of donor-derived infections to improve the safe use of donors [18].

Pathogen-specific issues

Bacterial infections

Donor-derived bacterial infections are uncommon but can cause significant morbidity and mortality in the organ transplant recipient, particularly when they result in endovascular disease, infection of anastomotic sites, or disseminated infection. Bacterial cultures in donors are routinely obtained and frequently positive, although these often reflect colonization or involvement of sites with minimal risk of transmission to recipients [20]. Bacteria isolated from the bloodstream may have a greater potential for transmission and blood cultures have been noted to be positive in approximately 15% of donors [20,21]. Recipient infection may occur through bloodstream transmission from bacteremic donors, infection or contamination of the donor organs, or contamination of preservation fluids. Deceased donors often have multiple risk factors for bacterial infection or colonization prior to organ donation, including endotracheal intubation, intravascular devices, urinary catheters, and prolonged stays in intensive care units [22–24].

The significance of a positive donor culture depends on the site of the culture, the organ to be transplanted, and the specific organism. At times, differentiation of true infection from contamination or colonization can be difficult. In the case of blood cultures, isolation of organisms such as coagulase-negative staphylococci, *Corynebacterium* spp., *Propionibacterium* spp., and *Bacillus* spp. typically reflect contamination. Isolation of the same organism (even typical contaminants) from multiple blood cultures suggests the presence of donor infection.

Donor-derived bacterial infection usually occurs within the first 7–14 days after transplant, but it may be delayed due to the use of prophylactic antibiotics and partial treatment. Additionally, an infection with a common hospital or community organism may not be recognized as donor derived. Confirmation of donor-derived infection requires molecular typing to prove an infection is caused by the same strain in both the donor and recipient; finding the same organism in multiple recipients from the same donor also suggests donor derivation of infection [2].

A number of retrospective analyses have attempted to quantify the risk to the recipient when organs from infected donors are used [20,25,26]. In a retrospective review of all donor bacterial cultures (including true infections, colonizers, and preservation fluid cultures), 8.8% of 3322 donors were identified as "infected" [20]. The greatest number of positive cultures reflected lung colonization and lung infection, followed by bloodstream, urinary tract, central nervous system, abdomen, wound, and preservation fluid cultures. Despite the high incidence of donor infections, donor-to-recipient transmission was documented in only 1.71% of recipients and the majority of post-transplant bacterial infections occurred in those individuals whose donors had negative cultures [20]. Studies suggest that bacteremic donors do not transmit infection regardless of whether the positive cultures are appreciated prior to transplant, provided recipients receive standard postoperative surgical prophylaxis, and antibiotics are adjusted and treatment extended to cover donor cultures as appropriate [25,26]. Furthermore, overall recipient and graft survival does not appear to be compromised when organs from donors with bacterial infection are used [20,22,23,25–28]. Thus, organs from bacteremic donors with antibiotic-susceptible

organisms may be safely used if the transplanted organ is not involved and antibiotics are tailored to the sensitivity of the organism and administered for 7–14 days postoperatively.

Donors with bacterial meningitis may also be considered acceptable. A number of small studies involving donors with *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Hemophilus influenzae*, among other organisms, have shown favorable recipient outcomes and no evidence of transmission [28–30].. Whether donors with bacterial meningitis due to bacteria with a higher risk of metastatic seeding, such as listeria, salmonella, tuberculosis, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, can be used is not known, but it has been postulated that these donors may be at greater risk for pathogen transmission and thus may not be used as safely [31].

Lungs are unique in that they are usually colonized or sometimes infected at the time of procurement [32].. Bacteria have been transmitted from donor lungs to recipients; however, positive donor Gram stains are not predictive of early postoperative pneumonias [33]. In one retrospective analysis, despite the presence of colonization or infection in >50% of donors, transmission only occurred in approximately 15% of recipients of donors with positive cultures [34].

Information regarding outcomes for specific donor-derived bacterial infections is usually limited to case reports. *S. aureus* and *P. aeruginosa* have been more frequently reported to cause anastomotic dehiscence and significant morbidity and mortality in the recently transplanted patient. However, despite multiple reports of *S. aureus* bacteremia in donors, transmissions have been uncommon [20,25]. Transmission events have been more frequently noted with *P. aeruginosa*, and a number of case reports and small series of donor-derived *Pseudomonas* infection have been reported with significant morbidity and mortality related to vascular dehiscence and allograft loss [35–38]. While this high morbidity and mortality likely reflects some reporting bias, particular care must be taken with donors infected with *Pseudomonas*, especially those with bacteremia, and early antimicrobial therapy should be administered to recipients [6]. Donors with multidrug-resistant bacteria may pose a greater risk to recipients due to the failure of standard antimicrobial prophylaxis to cover these organisms. Adverse outcomes have been noted when multidrug-resistant organisms have been transmitted; however, at this time there are no specific recommendations for exclusion of these donors [39–41].

Tuberculosis

Mycobacterium tuberculosis has been increasingly recognized as a cause of donor-derived infection. This infection has been concentrated in areas of the developing world where organ transplant is not often offered. However, changing migration patterns, globalization, and expansion of transplant programs to parts of the world that have higher incidences of tuberculosis (TB) have increased the likelihood that organ donors may be infected with latent TB or undiagnosed active TB. Early studies in the US and Europe estimated that 0.35–6.5% of transplant recipients develop TB and of these 4% are likely donor-derived infections; this is likely an underestimate [42]. Between 2005 and 2009, 22 cases of TB in organ donors were reported to the US OPTN; 10 recipients had confirmed transmissions and there were two related deaths [1,2]. Lung transplant recipients are at highest risk given the predilection of TB for the lungs; however, all organs can be involved in the setting of disseminated TB [43,44].

Donor-derived TB may be difficult to recognize in recipients due to the increased occurrence of disseminated and extrapulmonary disease and the low index of suspicion for both active and latent donor infection [44,45]. Donor risk factors for TB, including residence in areas where TB is endemic, social risk factors (e.g. homelessness and incarceration), and suspicious medical histories (e.g. persistent or recurrent unexplained pneumonias), were often present in reported cases of donor-derived infection and may be clues to which donors require more intensive scrutiny [43,44]. Unexplained meningoencephalitis in a donor has also been related to TB [44]. Even in cases with a high index of suspicion for TB, diagnosing TB in the donor can be difficult. Screening donors for TB relies on donor infection and exposure history and assessment of risk factors; both may be incomplete at the time of organ procurement. Physical examination and imaging [chest radiography or computed tomography (CT) scanning] may be helpful. Acid fast bacilli (AFB) smears of sputum and tissue may identify active disease either premortem or soon after transplant, but must be specifically requested as they are not part of routine donor evaluations. Definitive confirmation requires cultures, which will take longer (up to 6–8 weeks with standard techniques). Molecular methods for mycobacterial culture and identification should be used if possible to reduce the time to diagnosis.

Guidelines for screening for TB have been developed by a consensus conference sponsored by the American Society of Transplantation, the Canadian Society of Transplantation, and the Transplantation Society [46]. These guidelines have recognized the difficulties of standardizing screening on an international scale due to the geographic differences in TB prevalence worldwide. Regardless of geography, these guidelines and others recommend exclusion of donors with active TB due to poor recipient outcomes [17,45]. It is less clear how donors with latent TB should be managed. Currently, there is no automatic exclusion from donation; instead the risks and benefits of the transplant must be weighed [17]. Identifying which donors should be screened for latent TB and how donors with latent TB should be managed, especially in areas where TB is common in both donor and recipient populations, is also controversial. Identification of latent disease is particularly challenging in the deceased donor as there are currently no tests that have been validated in this setting. In low-prevalence areas especially, living donor candidates should be screened for latent TB using history, examination, and either tuberculin skin testing or interferon-γ release assays [17,45]. Chemoprophylaxis should be considered for living donors, assuming this can be completed prior to donation. If donors are found to have untreated latent TB, previously uninfected recipients should be given standard chemoprophylaxis to prevent donor-derived infection, although rifamycins should be avoided due to drug interactions with immunosuppressive medications [47]. Donors with a history of active TB in the preceding 1–2 years are at higher risk for relapse and this increases the risk for transmission of TB to the recipient. Careful consideration must be given to the risks and benefits of transplantation utilizing organs from these donors, and chemoprophylaxis, based on known drug susceptibilities, should be strongly considered [17]. When active TB is discovered in a donor after donation, infectious disease consultation should be sought to determine the optimal management of recipients.

Syphilis

Serologic testing for syphilis is standard practice for all solid organ donors. The results may not be available until after transplantation; nevertheless, positive serologies or a history of prior treatment for syphilis is not a contraindication to donation as treatment is

available. The estimated risk of donor-derived syphilis is 0.15% [48]. Immunosuppressive regimens in the recipient may attenuate and delay the serologic responses and these cannot be relied upon to diagnose recipient disease. Early treatment of recipient and donor if possible has been successful in preventing the development of clinical disease [49–52]. The long-term outcome of untreated donor-derived syphilis is unclear, but potentially could result in accelerated disease. Recipients of organs from donors with active or latent syphilis should be treated according to standard guidelines and then monitored postoperatively for both clinical and serologic signs of response to treatment [7,53].

Viral infections

Viral infections are commonly transmitted from organ donors to recipients. Many infections can be anticipated, such as CMV and EBV, and therefore are not included in most surveys of donor-derived infections. Details regarding these infections are given in Chapter 94 and the focus here is unanticipated donor-derived viral infections.

Donor-derived viral infections present unique challenges, particularly with regard to predonation diagnosis. In some cases, these infections may be asymptomatic or mistaken for other conditions or may stem from blood transfusions administered to the donor in the predonation phase; thus, routine diagnostic assays may be inadequate to identify the infection prior to transplant. Moreover, even when they are identified either prior to or following transplant, therapeutic options are often limited or unavailable.

Donor viral infection should be considered when donors present with fevers without a clear-cut etiology. In particular, donors with unexplained altered mental status should be suspected of having viral meningitis and/or encephalitis. Lymphocytic choriomeningitis virus, West Nile virus (WNV), and rabies have all been transmitted by donors with these presentations [54–56]. A detailed history (including exposure to rodents and insects) should be elicited, physical exam performed, and imaging of the brain either with a contrast CT scan or magnetic resonance imaging (MRI) and lumbar puncture should be considered when the clinical suspicion is high. In the absence of a specific diagnosis of a treatable infection, these donors should be deferred from donation [17].

Because many donor viral infections are latent, standard screening may not detect potentially transmissible infections. Routine donor screening includes testing for HIV, HBV, HCV, and EBV [17]. Whether or not all donors should be screened for either HTLV or WNV is controversial. In some cases, false-positive NAT for WNV may result in unnecessary donor exclusion [57]. Consequently, although tissue banks routinely screen for WNV, this is not part of the standard organ donor evaluation. In the US, testing for HTLV 1 and 2 is no longer a requirement due to the low incidence of HTLV 1, the occurrence of false-positive tests in a low prevalence population, and the discontinuation of the FDA-approved test for this virus [58].

Human immunodeficiency virus

HIV is one of the most feared donor-derived infections, although the actual incidence of donor transmission of HIV is miniscule. In the US, donors with HIV are excluded from donation and donor screening for HIV became a requirement in 1985 [59]. This has greatly reduced the number of donor-transmitted infections; however, sporadic cases of transmission from both deceased and living donors have occurred in the US and in Europe [60–66]. Recipient outcomes have been variable, with the development of acquired immune deficiency syndrome (AIDS), allograft loss, and death in the earlier cases especially. In part, poor outcomes likely reflect the impact of co-morbidities, including HCV, limited antiretroviral options, and immunosuppressive therapies, as carefully selected HIV-infected kidney donors have been successfully used in a population of HIV-infected recipients in South Africa [67].

The more recent unanticipated transmission events have sparked a controversy regarding optimal evaluation of donors for HIV infection. Deceased donor screening should consist of a careful assessment of the donor's risk for HIV infection by reviewing both medical and social histories and laboratory testing for HIV. Mandated screening relies on serologic testing [8]. As previously noted, NAT has the potential to identify donors with more recent infection, by decreasing the window period for infection detection [11] (Table 92.3). Because two recent transmissions were related to window-period infection, greater consideration has been given to mandating NAT testing [63,64]. A 2008 survey of US OPOs revealed that almost half voluntarily use NAT testing for HIV screening in potential donors [68]. NAT testing cannot fully replace serology for HIV; however, as HIV-infected individuals on highly active antiretroviral therapy (HAART) may have undetectable virus, false-negative testing results if serology is not performed.

Screening of live donors enables a more thorough assessment of risk factors, and where potentially risky behavior is present, allows for risk reduction counseling and repeat serologic testing for identification of potential window-period infection. If there is a significant delay between evaluation and donation, repeat assessment of risks and testing should be considered.

It is difficult to estimate the incidence of undetected HIV infection in potential donors. One extrapolation from blood donor data suggests a prevalence of HIV in normal-risk donors of 0.10% and 0.50% for high-risk donors [13] The estimated incidence of undetectable infection by serologic screening was 1 in 50 000 for normal-risk donors and 1 in 11 000 in high-risk donors. Optimal screening for HIV should consider both the risk of donor transmission and the potential for organ donor loss due to false-positive or unavailability of NAT testing. US OPTN policy requires that all potential recipients are informed of and give specific consent for transplantation of organs from donors who are designated at increased risk for HIV [8]. Guidelines suggest that NAT screening be reserved for increased-risk donors and that recipients of these donor organs be tested 1, 3, and 12 months post transplant to exclude donor-derived HIV transmission [15].

Hepatitis B virus

Despite well-documented transmission risks, donors with hepatitis B have been considered an important potential source for expanding the donor pool, especially in areas such as South-East Asia and parts of Europe where the prevalence of HBV exceeds that in the US [7,69,70]. In most cases, the risk of donor-derived infection may be predicted based on the results of serologic testing. The highest risk of transmission is associated with HBV surface antigen- and/or DNA-positive donors who can transmit infections regardless of organ; however, donors with isolated core antibody positivity have also transmitted infection [71,72]. Additional factors affecting transmission and outcomes include the organ transplanted, recipient immunity, prophylaxis, and immunosuppression [7,72–78].

Standard testing for HBV in organ donors generally includes hepatitis B surface antigen, surface antibody, and core antibody [17]. Additional tests may include hepatitis B core IgM and hepatitis B DNA assays, although these are typically reserved for specific

donors considered to be a higher risk for infection and donors with isolated hepatitis B core positivity, as these tests may identify window-period donors, who are at higher risk for HBV transmission [17]. Data specific to the organ donor population regarding the utility of DNA testing is not available. However, blood donor screening suggests that DNA positivity may be found in 1.6% of core antibody-positive individuals and 1 in 410 540 blood donations (the majority of whom had evidence of prior vaccination) [79,80]. Notably, DNA levels were typically low and in at least one study, only 36% of surface antigen-positive individuals had detectable DNA; thus, only screening organ donors with HBV DNA may fail to identify donors with HBV [79–81]. To date, there has been only one case report identifying transmission of HBV from a multiorgan donor with negative serology and low level DNA positivity to three of five non-immune recipients [82]. Testing for HBV delta has not been standard and it is not known whether donors with this virus may pose a greater risk to recipients.

Currently, because of the high risk of transmission, it is recommended that donors with hepatitis B surface antigen, core IgM, and/or DNA positivity be excluded from consideration, although there may be exceptions in life-threatening situations [7]. Donors who are positive for surface and/or core antibody may be considered in certain circumstances, which are outlined below [7].

Liver transplant recipients are at the greatest risk for the development of de-novo hepatitis B from donors who are positive for HBV core antibody, with rates as high as 33–100% in the absence of prophylaxis [72]. Transmission risk is substantially lower for recipients of other core antibody-positive organs and long-term patient and graft survival are comparable for these recipients [70,73,83–88]. One key factor that has been associated with reduced transmission is the presence of pre-existing immunity. Prior infection, as evidenced by the presence of both surface and core antibody in the recipient, conveys greater protection than vaccination [73,76,87]. Nevertheless, pretransplant hepatitis B vaccination has been an important preventive strategy and is recommended to increase the safety of core antibody-positive donors [7]. Because the level of pre-existing antibody may affect transmission, booster dosing may be indicated when levels decline below 200 mIU/mL [75,78].

Antivirals and immunoglobulin also play an important role in preventing transmission from core antibody-positive donors, especially for liver recipients, dramatically reducing the rates of transmission and symptomatic disease [72,74,76,77,89]. The optimal prophylactic strategy for recipients has not been determined; many centers combine early hepatitis B immunoglobulin with prolonged antiviral administration (typically lamivudine) for liver recipients [90]. At this time, there are no specific recommendations for prophylaxis in non-hepatic recipients.

Recognition of donor-derived hepatitis B may be difficult, especially since the onset of infection may be delayed for as long as 60 months [72]. The average time to de-novo infection probably reflects recipient immune status, with an average time to evident infection of 14.7 months in naïve liver recipients and 23.7 months in recipients with both surface antibody and core antibody positivity [76]. Because of this, it is recommended that recipients of potentially infectious organs (especially liver recipients) be screened every 3 months for 5 years with hepatitis B surface antigen, core and surface antibody, and DNA testing [72].

Hepatitis C virus

Transmission of HCV from donor to recipient has been well-recognized for many years [91–93]. In contrast to HBV, HCV is efficiently transmitted regardless of the organ transplanted, with 50–93% of HCV-negative recipients acquiring HCV from their donors [92,94–97]. A number of factors may affect the likelihood of transmission, especially to non-liver recipients. These include the presence or absence and level of donor viremia [97]. Not all antibody-positive donors are viremic, and donors without detectable viremia appear less likely to transmit HCV if the liver is not used [98]. The method of organ preservation may also affect transmission; pulsatile perfusion has been reported to decrease the HCV viral load, thereby reducing the risk of transmission via kidney transplantation [99].

Recipients of HCV-positive organs have had significant complications following transplant; however, outcomes vary based on recipient HCV status and the organ transplanted. The best outcomes have been reported in HCV-infected liver recipients. In these recipients, adjusted short- and long-term patient survival and graft survival have been similar regardless of whether or not the donor was infected with HCV [100–104]. However, organs from HCV-infected donors have been associated with earlier recurrence of HCV, a factor that has been associated with patient death and graft loss [103]. Retrospective analyses of the US Kidney Data System and the OPTN databases noted reduced survival with HCV-positive kidney donors, regardless of recipient age; furthermore, deaths due to liver disease and infection were more common in recipients of HCV-positive grafts [105,106]. When HCV-positive recipients were considered, outcomes with HCV-positive and -negative donors were similar [107,108]. Because using HCV-positive kidneys decreases waiting time on transplant lists, this may improve survival in some dialysis patients [106,109]. Several studies have demonstrated that recipients of HCV-positive cardiac allografts have decreased early and long-term survival when compared with HCV-negative allografts regardless of recipient age or HCV status [110,111]. Recipients of HCV-positive hearts experienced increased vasculopathy and advanced liver disease. Consequently, it is recommended that HCV-positive donor organs (especially kidney and liver grafts) be reserved for HCV-positive recipients or those in life-threatening situations [112].

Routine donor screening for HCV includes antibody testing. NAT significantly increases detection of window-period infections and should be considered for donors at increased risk for HCV [15]. The highest yield for NAT may be in donors with a history of intravenous drug use, commercial sex workers, and others with high-risk sexual behaviors [14]. Donor genotypes are not routinely obtained and there are no specific recommendations regarding matching of donor and recipient HCV genotype. Whether the donor or recipient genotype prevails following transplant may be genotype dependent rather than reflective of the HCV source; genotype 1 has more commonly prevailed [71,90,113].

West Nile virus

In recent years, there has been greater concern regarding donor-derived WNV infection. This reflects the increasing prevalence of WNV in donor communities as well as enhanced recognition of donors as a potential source of infection. Nevertheless, the actual number of reported donor-derived transmissions remains small. Also, the true transmission rate is unknown, since infection is commonly asymptomatic and donor screening is not routinely performed. Moreover, although symptomatic infection has been described in the majority of recipients of infected donor organs, there is the possibility that recipient infection may not be recognized due to asymptomatic or atypical presentations.

Donors may transmit infection regardless of the presence or absence of symptomatic infection in the donor [56,114,115]. Blood products may also be a source of WNV infection and in some cases, donor-derived transmission occurred due to the administration of infected blood products to the donor, further confounding the ability to recognize donor infection [116,117]. The most severe consequences of donor-derived infection are neuroinvasive disease and death; asymptomatic transmission has also been reported [56,114,116,117]. There have also been cases in whom donor infection was identified without evidence of transmission to any recipients. Factors affecting transmission risk are not known. There does not appear to be a propensity for any single donor organ to transmit infection. It is likely that recipient immunosuppression has an impact on outcomes and there are reports of improved outcomes with immunoglobulin administration to at-risk recipients [56,115].

Whether all potential donors should be screened for WNV prior to organ procurement is controversial. Although testing for WNV is routinely performed in blood and tissue donors in the US, screening of organ donors is not required. Some European centers routinely screen for WNV [118]. Screening tests include serology and NAT. A single screening test is probably insufficient, since neither antibody nor NAT alone will identify all potentially affected donors [114]. The geographic and seasonal variability of infection, which can change on a year-to-year basis, further complicate screening. At this time, routine screening has not been demonstrated to be cost-effective and at least one large-scale screening initiative in Alberta, Canada, failed to identify any cases between 2003 and 2005 [119]. Moreover, a medical decision analysis performed based on prevalence data for 2002 demonstrated a potential loss of 452 life years related to false-positive testing [57]. At this time there is insufficient data to recommend routine screening of all donors; however, obtaining NAT and antibody may be warranted if the prevalence of WNV in the community is high. Because WNV may be associated with neuroinvasive disease, donors with undiagnosed febrile illnesses associated with altered mental status and/or new limb weakness should be excluded from donation.

If a donor is found to have infection, based on anecdotal evidence, some investigators advocate administration of hyperimmune globulin to recipients prior to the development of infection [56]. There is insufficient evidence to recommend this routinely at this time. Further investigation regarding optimal donor identification and recipient management is warranted.

Arenaviruses including lymphocytic choriomeningitis virus

Donor-derived arenavirus infection is a rare occurrence following organ transplantation, albeit one associated with a high fatality rate [55,120,121]. Lymphocytic choriomeningitis virus has been most frequently described; in at least one case a novel arenavirus has also been implicated in donor-derived infection. Donor-derived arenaviruses are difficult to recognize prior to donation since the majority of donor infections are asymptomatic; only one donor was reported to have a febrile illness [120–122]. Routine screening of donors for arenaviruses is not indicated.

Recipient infections have been characterized by hepatocellular necrosis, cytopenias, and encephalitis, and may present as early as 3–4 days following transplant, although the majority occur approximately 2–3 weeks postoperatively. Deaths occurred within 2 months of transplant [120–122]. Based on a single case report, ribavirin may improve outcomes when administered early after the onset of symptoms [120]. Currently, there is insufficient evidence to routinely recommend ribavirin for recipients suspected of acquiring donor-derived arenavirus infection.

Human T lymphotropic virus 1 and 2

HTLV 1 and 2 are retroviruses that are routinely considered jointly due to the testing methodology and despite the differences in their epidemiology and disease association. HTLV 1 is endemic in Asia, the Caribbean, parts of South America, West Africa, and Oceania, with lower prevalence in North America and Europe. It has been implicated in the development of acute T-cell leukemia and lymphoma, and HTLV 1-associated myelopathy (HAM) and tropical spastic paraparesis (TSP). In the US, HTLV 2, which is endemic in American Indians and Western and Central Africa, is predominantly seen in intravenous drug users and sexual contacts of infected individuals. It has yet to be associated with a specific disease entity. Confirmed donor-derived transmissions of HTLV 1 and 2 are rare, even in endemic countries, and it is likely that many transmission events are not associated with the development of symptomatic disease or impaired transplant outcomes [58,123–127]. When symptomatic transmissions have occurred, recipients have typically developed neurologic symptoms (TSP/HAM) within 2 years of transplant [125].

Testing donors for the presence of these viruses is controversial and currently a requirement in select geographic locales only [58,128]. In part this reflects the low positive predictive value of the currently available tests [58]. Standard serology for HTLV 1 and 2 involves performance of an enzyme-linked immunosorbent assay (ELISA), which does not distinguish between the two viruses. Although highly sensitive, it is not specific, increasing the potential for false-positive results. Confirmatory testing with Western blot or NAT is not routinely available within the time frame of deceased donor management. There is significant potential for false-positive screening serology with estimates of 167–227 potential organs lost annually [58]. In 2010, the FDA-licensed test for donor screening became unavailable in the US, prompting a reassessment of the requirement for HTLV screening. Based on data demonstrating an extremely low prevalence of confirmed positive screening tests, the absence of significant impairment of recipient outcomes, and the potential for donor loss, the OPTN abandoned the requirement for donor screening in 2010. In Spain, HTLV screening is restricted to donors from endemic areas only [128].

Donor-derived fungal infections

Fungal infections are a significant cause of morbidity and mortality following transplantation; donor-transmitted infections are rare. Nevertheless, there have been significant reports of donor-derived fungal infections with subsequent allograft loss and death [1]. Donor-transmitted infections may result from latent donor infection, donor bloodstream infection at the time of procurement, unrecognized disseminated infection, or through contamination of preservation fluid. Proving donor derivation may be especially challenging for some fungal infections as it can be difficult to exclude the presence of latent endemic mycoses in recipients who may share geographic risk factors with their donors.

Candidiasis

Donor-derived *Candida* infections are an uncommon but important cause of allograft loss and death in transplant recipients. Abdominal organ and kidney recipients are at the highest risk of donor-derived infection, most likely related to bowel colonization with *Candida* and subsequent seeding of the allograft [129]. The

actual incidence of these infections is not known; a multicenter study in France estimated an incidence of one infection per 1000 renal allografts [129].

The sources of *Candida* are diverse and include the bloodstream, urinary tract, and direct contamination of the allograft from preservation fluid contamination or soilage related to abdominal trauma and bowel disruption [20,129–132]. The pancreas is particularly vulnerable to direct infection due to the presence of *Candida* in duodenal contents; in some cases, organ contamination has been attributed to incision of the duodenum during back-bench preparation [133,134]. Donor-derived *Candida* infections are less frequently reported in heart and lung transplantation [135]. *Candida* is a common oropharyngeal tract colonizer; consequently, donor respiratory cultures are often positive but the significance of this is unknown. Infection of the anastomosis is the primary concern; however, in at least one study this did not occur despite positive donor cultures, possibly related to the routine administration of nebulized amphotericin B [34,135]. Donor-derived cardiac infections with *Candida* have not been routinely described.

Determining the significance of *Candida* in preservation fluid culture is a particularly difficult issue and the true incidence of *Candida* infection in this setting is unknown. The risk for infection when the preservation fluid is negative appears to be low, at least in the setting of renal transplantation [136,137]. In kidney recipients, the rates of positive preservation fluid cultures range from 5% to 23%, with 2–10% attributed to fungi [133]. *Candida* has been isolated in approximately 4% of preservation fluid samples from liver recipients [22,138,139].

Donor-derived *Candida* infections have been most clearly characterized in kidney recipients who typically present within the first months after transplantation [129]. Complications of this infection are diverse and include infected perinephric collections, urinomas, and pyelonephritis in renal transplant recipients, and candidemia, surgical site infections, and involvement of vascular structures in all recipients [129–131,140]. The most significant complications in other abdominal organ transplants involve infection of vascular structures resulting in anastomotic rupture, arteritis, and mycotic aneurysm, all of which may lead to catastrophic intraperitoneal hemorrhage with graft loss or death [129,130,134].

To prevent adverse outcomes of donor-derived *Candida* infection, prophylactic strategies for recipients of donors with *Candida* infection and colonization are used and have been most clearly defined for kidney recipients. When *Candida* is detected in preservation fluid or there is evidence of viscous perforation, expert opinion recommends administration of 2 weeks of empirical antifungal therapy combined with imaging of the graft at baseline and at day 7. If no graft site infection is found, antifungal therapy may be discontinued after 2 weeks [141]. Recipients who develop symptoms, signs, or microbiologic evidence of infection should have imaging repeated and antifungal therapy prolonged. Because the liver, pancreas, and small bowel are particularly susceptible to contamination by bowel flora, some centers recommend amphotericin B instillation into the donor duodenum during procurement [134]. Other centers may choose to start prophylactic antifungals irrespective of cultures. Given the concerns for arteritis and graft loss, imaging should be considered when evaluating positive pancreatic donor cultures. There is no evidence-based data for recommending management of positive donor cultures in this setting. In cardiothoracic recipients, evidence for management of positive donor cultures, including preservation fluid, is scant. Lung recipients routinely receive antifungals as part of peritransplant management due to the high rate of fungal infections in these recipients and because donor status is not the only factor considered. When lung donors are colonized with *Candida*, systemic antifungals and nebulized amphotericin have been used alone and in combination by some centers [34,135]. The duration of prophylaxis is not defined but may be prolonged, depending on the appearance of the anastomosis. *Candida* in preservation fluid cultures from heart donors does not appear to be associated with recipient infection and is not an indication for prophylaxis [142].

Although some donors with *Candida* infections have been successfully used, based on the potential for adverse outcomes, donors with untreated candidemia and pyelonephritis should not be routinely used [20,25,143]. The risk of transmission when donors have candiduria is unknown, although transmission to non-renal recipients is unlikely in the setting of isolated candiduria.

If donor-derived *Candida* infection is suspected, therapy should be based on the species and known antifungal sensitivity, when available. Fluconazole is generally the preferred antifungal, as it is well absorbed and attains high levels in the urine; the other azoles do not achieve high levels in the urinary tract and thus should be avoided if the urinary tract is involved. Because of their extensive metabolism and poor penetration into the urinary tract, echinocandins should be reserved for non-albicans infections (which may be resistant to fluconazole) and for extrarenal and renal parenchymal disease [144]. Amphotericin B deoxycholate is active against most forms of *Candida*; however, its use is limited by the potential for renal toxicity and the lipid formulation does not achieve appreciable levels in the renal parenchyma or urine, making it a less preferred option, especially for infections involving renal allografts. Recommendations for surgical management of cases vary from transplant nephrectomies for all to a more conservative approach of immediate antifungal therapy and close monitoring [130,131,145]. In general, drainage of renal abscesses or perinephric collections should be considered and the presence of arteritis or aneurysm should prompt evaluation for possible nephrectomy.

Cryptococcal infections

Cryptococcus is a ubiquitous encapsulated yeast that is a rare cause of donor-derived infection. Infection occurs following inhalation of the organism. It is typically asymptomatic in the normal host and can persist in latent form primarily in the lungs, providing the potential for donor-derived infection in the absence of symptoms [146].

The vast majority of cryptococcal infections in transplant recipients are not donor derived, instead representing reactivation of latent recipient infection [147,148]. Although uncommon, donor-derived cryptococcal disease was reported as early as 1971 and there have been additional reports since then [149,150]. Differentiating donor- from recipient-derived infection can be difficult; however, a donor source should be suspected if the donor presents with an undiagnosed neurologic disorder or meningoencephalitis [150]. Potentially immunosuppressed donors, e.g. those with a history of corticosteroid use, other organ dysfunction such as end-stage renal or liver disease, sarcoidosis, or rheumatologic disorders, are at increased risk for cryptococcal infection and therefore should be more closely scrutinized, especially if they present with undiagnosed neurologic disorders or granulomatous pulmonary disease. Although the majority of the cases are related to deceased donors, living donors have also been reported to transmit *Cryptococcus* [151].

Recognition of cryptococcal infection in donors may be challenging and it is easy to miss the diagnosis prior to donation. In donors with abnormal neurologic findings, sampling serum and cerebrospinal fluid (CSF) for cryptococcal antigen may be informative. Obtaining donor serum for cryptococcal antigen is helpful if positive, but does not exclude the diagnosis if negative, especially if findings are isolated to the lungs. In those cases, sampling of donor tissue or fluid for microbiology and histopathology is required to confirm the diagnosis. Assessment of living donors with evidence of granulomatous disease on lung imaging should include a thorough investigation to exclude *Cryptococcus* prior to donation [151]. Although finding cryptococcal infection in the first month following transplant is a possible sign of donor derivation, it is important to remember that some recipients, especially liver recipients, may have been at increased risk for this infection prior to transplant, which further confounds source attribution.

Donors with suspected or confirmed cryptococcal infection should not be routinely used for donation because of the risk of severe disease in the recipient. However, if the lung is the only donor organ known to be involved, the risk for non-lung recipients is undefined, as pulmonary infection may exist without dissemination. If donor infection is identified after the transplant has occurred, recipients should receive prophylactic fluconazole. Based on a case report of a successful transplant from a donor with asymptomatic pulmonary disease, a 3-month course has been proposed to be effective, although there are no large-scale trials to confirm this recommendation [151]. Treatment of donor-derived cryptococcal disease should follow published guidelines with support from infectious diseases experts [152].

Aspergillosis

Aspergillus is ubiquitous in the environment. It is the second most common fungal infection post transplant, but is an uncommon cause of donor-derived infection [153]. The majority of donor-derived cases occur in lung transplant recipients where the greatest concern is the potential for infection along suture lines and vascular invasion. In one study of lung transplant recipients and their donors, five of 197 donors were found to be colonized with *Aspergillus fumigatus.* Despite nebulized amphotericin B prophylaxis, donor-to-host transmission of infection was documented in three of the five cases, with the development of tracheobronchitis in two recipients and fatal mediastinitis in one [34].

Identification of *Aspergillus* prior to donation may be difficult as it is rarely suspected. Extreme caution should be taken when evaluating donors who are themselves immunocompromised as they are at increased risk for this infection. This is particularly true in cases where potential donors have died of unexplained neurologic disease. Two case reports of donor-derived *Aspergillus* infections have been described where the donors were solid organ transplant recipient themselves whose deaths were attributed to cerebral hemorrhage [154,155]. Because of the potential for adverse outcomes, including allograft loss, donors with confirmed or suspected aspergillosis should be excluded from donation.

When donors are suspected or found to have aspergillosis following donation, optimal preventive measures should include administration of systemic antifungals to the recipients (e.g. azoles such as voriconazole, echinocandins, or amphotericin-based compounds). There are no guidelines to direct either the specific choice of antifungal or the duration of prophylaxis. Many lung transplant centers routinely use prophylactic antifungals against *Aspergillus* in the postoperative period until donor respiratory cultures and/or recipient bronchoscopy show no evidence of infection or colonization. This pre-emptive strategy may avert many potential cases of donor-derived infection.

Coccidioidomycosis

Coccidioidomycosis is endemic in areas of the south-western US, northern Mexico, and parts of Central and South America. Infection is acquired by inhalation and leads to primary pulmonary infection, which is typically asymptomatic; endospores may then spread hematogenously to distant sites [156]. The majority of *Coccidioides* infections are likely due to reactivation of latent disease [157,158]. The incidence of donor-derived infection is unknown but it is thought to be rare, although likely under-recognized. Both living and deceased donors have transmitted infection both in the presence and absence of donor symptoms [159–162]. The incidence of *Coccidioides* infection in donors will depend on donor exposure to locations where this infection is endemic. In one center located in an endemic region, positive serologies for this infection were detected in 2.1% of asymptomatic living donors [163].

Donor-derived *Coccidioides* has resulted in significant morbidity and mortality in transplant recipients, with deaths due to disseminated infection, severe pneumonia, and central nervous system infection in >50% of organ recipients [162]. Although lung recipients may be at greatest risk due to the predominance of infection in the lungs, diverse organ recipients have been affected and it is likely that outcomes are most affected by the degree of T-cell dysfunction and timing of targeted antifungal therapy [161,164]. Presentation of donor-derived infection frequently occurs within the first month following transplantation, especially when donors have unrecognized active infection, although later onset may occur, especially in the setting of reduced immunosuppression and/or prophylactic antifungals. Early intervention with prophylactic antifungals, including fluconazole or itraconazole, may prevent development of recipient infection [163].

Because of the potential impact on recipient outcomes with early antimicrobial intervention, it is imperative that donor infection be identified as early as possible. Donor-derived *Coccidioides* infection should be considered in deceased donors presenting with undiagnosed neurologic syndromes and/or pulmonary infiltrates or nodules, especially if the donor has spent significant time in an endemic area [160,162]. Unfortunately, recognition of *Coccidioides* infection in a donor may be complicated by the absence of symptoms at the time of donation and limited history of residence and/or travel to an endemic area. Diagnosis may be further confounded by the variable availability, sensitivity, and specificity of different serologic tests and the difficulties isolating the organism in culture. There are a number of serologic tests available for detecting antibody responses against specific coccidioidal antigens. These include complement fixation, enzyme-linked immunoassays (EIAs), tube precipitin, and immunodiffusion assays for the detection of antibody. Antigen tests, polymerase chain reaction (PCR), and culture may detect active infection, although these are less likely to be available in the organ donor setting. Donor screening should include multiple assays as the sensitivity and specificity of the available tests may be variable [163]. Serologic tests may be useful for screening both deceased and living donors for past infection, but there is a potential lag in antibody responses following acute illness, especially in the setting of immunosuppressive medications; thus, none of them can be relied upon to make a diagnosis in the acute setting [163,165,166]. Antigen tests may play a role when serologic

testing is negative [165]. In some cases histopathologic examination may be required to diagnose active infection.

Although donors with active *Coccidioides* should be excluded from donation, a history of past infection is not an absolute exclusion and living donors with a history of coccidioidomycosis and no evidence of active infection have been successfully used for kidney and liver donation [163]. Recipients of organs from donors with quiescent infection should receive prophylaxis with fluconazole or itraconazole. Those already on an azole for prophylaxis against other fungi generally do not require the addition of fluconazole [167]. At this time, the optimal dose and duration of antifungal prophylaxis for recipients of donors with quiescent infection is unknown. Regimens as short as 1 month with doses as low as 100 mg of fluconazole have been used, although the majority of experts recommend 6–12 months of prophylaxis with higher doses of fluconazole [157,163]. Because development of disease may be affected by the extent of donor infection, the organ received, and the level of immunosuppression, determination of optimal prophylaxis should be tailored to individual recipient circumstances and infectious diseases experts should be consulted.

Histoplasmosis

Histoplasma capsulatum is ubiquitous in the soil in diverse regions worldwide, including the Americas, Asia, and Africa; in the US, this infection is most commonly observed in the Ohio and Mississippi river valleys. In endemic areas, up to 75% of residents show evidence of prior infection, although the vast majority are asymptomatic [168]. Histoplasmosis occurs in 0.1–0.5% of transplant recipients in endemic areas and the vast majority of these infections are presumed to be due to reactivation of prior disease or acquisition of new infection; infection in the first post-transplant month may be donor derived [168–170]..

The true incidence of donor-derived histoplasmosis is unknown, but is likely extremely low, even in hyperendemic areas [171]. One estimate of donor-derived histoplasmosis in endemic areas is approximately 1 in 10 000 transplants [172]. When a donor is found to have histoplasmosis, not all recipients are equally affected; the majority of reports have involved renal recipients. In a large retrospective study from an endemic area, approximately 0.1% of recipients (all lung) were found to have evidence of histoplasmosis in donor lymph nodes [170]. No cases of donor-derived histoplasmosis were noted, possibly due to the use of routine antifungal prophylaxis.

There are few published cases of donor-derived histoplasmosis [173–177].. Although recipients with donor-derived infection generally present within 2–3 weeks of transplant, presentation as late as 36 weeks has been reported. Non-specific fever and allograft dysfunction are the most common manifestations of infection, followed by pancytopenia and weight loss.

Given the low incidence of donor-derived histoplasmosis and relatively high prevalence of prior exposure in healthy individuals, routine testing of all donors is not warranted; however, it is important to identify donors who are actively infected as they are more likely to transmit infection [141]. Diagnosis of histoplasmosis in donors requires a multifaceted approach, including antigen detection in urine and serum, antibody testing (by immunodiffusion and complement fixation), and culture and histopathologic examination of involved tissues. In order to exclude active histoplasmosis, donors with unexplained pneumonias from endemic areas should undergo specific testing. Because the liver and spleen are common sites of involvement in disseminated infection, these organs should be included in the histopathologic evaluation. Evaluation of living donors from endemic areas should include specific information about past histoplasmosis and unexplained pneumonia, followed by imaging studies and serologic and antigen testing. Expert opinion suggests that active disease in living donors should be treated for at least 6 weeks prior to donation [141]. Because antibody production may be delayed for up to 3 months and reduced in immunosuppressed hosts, antigen detection may be preferable in cases of early infection and immunosuppression [178].

There are no large-scale trials to dictate management of donors with suspected or confirmed infection. Expert opinion recommends that if the donor infection is confirmed by either culture or antigen testing, then 1 year of treatment of the recipient is recommended [141]. If histopathology alone is consistent with infection and/or donor complement fixation titers are $>$1:32 or if H and/or M precipitins are present, these experts recommend a 3–6-month course of itraconazole [141]. In unconfirmed cases with lower titer antibodies, close monitoring with serum and urine antigen may be helpful for detection of early infection in recipients [179].

Parasitic disease

With the exception of toxoplasmosis, most donor-derived parasitic infections are restricted to specific geographic regions and/or donor risk profiles. They are thought to be uncommon even in those regions where the infections are endemic. In many cases, it is especially difficult to attribute post-transplant infection to the donor organ because the recipient and donor may share similar exposure histories. However, given the greater diversity of donors in regions where these infections are not endemic, there have been sporadic case reports of donor-derived parasitic infections involving deceased and living donors. Some examples have included donor-derived trypanosomiasis, strongyloidiasis, schistosomiasis, malaria, filariasis, and clonorchis [180–189]. Because of the predilection for involvement of different organs by the various parasites, transmission risk varies based on the pathogen. For example, donor-derived schistosomiasis and clonorchis infection are both more likely in liver transplantation, whereas *Trypanosoma cruzi* infection is associated with heart transplantation [180,181,188]. In some cases, living donor screening by serology, review of peripheral blood smears, and ova and parasite examination of stool, urine, and tissue has allowed for donor treatment prior to donation to prevent transmission [180,189,190]. Because many of the tests required for diagnosis of parasitic infections are not routinely available within the time frame of deceased donor evaluation, predonation diagnosis is often not possible. Donor travel and residential history should be part of routine donor evaluation and peripheral blood smears and tissue biopsies should be considered for febrile donors from areas where these organisms are endemic. Donor eosinophilia may be an additional clue to the presence of parasitic infections and should prompt serologic evaluation for specific organisms and prophylactic antimicrobials to avert reactivation of donor disease (Table 92.2). In the absence of documented donor organ infection, there are no recommendations for avoidance of deceased donors who are "high risk" based on exposure history alone.

Toxoplasmosis

Toxoplasma gondii, the etiologic agent of toxoplasmosis, is found worldwide. Infection is most common in patients from endemic areas such as the tropical areas of Latin America and sub-Saharan Africa and European countries such as France where prevalence approaches 90% [191]. Rates in the US are lower with estimates of

10–40% [192,193]. Primary sources of infection in the US include ingestion of raw and undercooked meats, and exposure to soil and cat feces [194]. Acute infection after transplant may result from donor-to-recipient transmission, reactivation of quiescent infection, and rarely, newly acquired infection after transplant.

Because of the predilection of the organism for musculoskeletal tissue, cardiac transplantation bears the highest risk for donor transmission, although donor transmission via other organs has been reported [195]. It is estimated that seronegative cardiac transplant recipients have a 50–75% risk of developing symptomatic infection from a seropositive donor without prophylaxis [196,197]. Donor transmission in the seronegative recipient is the most common cause of disease acquisition in all recipients; in cardiac transplant recipients, donor transmission accounts for approximately 60% of disease [198].

Toxoplasmosis after solid organ transplant can result in severe disease, often with a fulminant course. Presentation may be non-specific and in the absence of prophylaxis, usually occurs within the first 3 months after transplant [195,199]. Myocarditis, pneumonitis, cerebral abscesses and meningitis, chorioretinitis, hepatitis, lymphadenitis, and disseminated disease have all been described [195,198,199].

Donor toxoplasmosis is typically latent and easily identified using standard serologic methods; however, this is not currently mandated even for heart recipients in the US where the infection is presumed to be low in prevalence. This practice has been supported by a Canadian study which demonstrated both low seroprevalence in donors and recipients and minimal acquisition of new infection; possibly related to the standard use of prophylaxis [200]. Screening may be more cost-effective in areas of the world with higher prevalence of infection.

Prevention of symptomatic infection can be accomplished by administration of either trimethoprim sulfamethoxazole (TMP-SMX) or pyrimethamine and should be considered, especially for the highest risk recipients (seronegative recipients of seropositive donor organs) [192,199,201]. The optimal dose and duration of TMP-SMX is unknown. Most of the earlier studies used a dose of 160 mg of TMP/800 mg of SMX three times per week [201,202]. Current guidelines support the use of daily single strength TMP-SMX as an acceptable alternative to the three times weekly dosing regimen [192,203]. For patients unable to receive TMP-SMX, alternative regimens may include pyrimethamine, atovaquone, or dapsone. Symptomatic infection has been noted rarely after discontinuation of prophylaxis [199,203].

Serodiagnosis may demonstrate asymptomatic transmission by around 7.5 $\pm$ 2 weeks post transplant, but given the blunted immune responses post transplant, seroconversion alone should not be relied upon to rule out the acquisition of new infection [198].. Active infection often involves the myocardium in heart recipients and ring-enhancing lesions in the brain in any recipient. Diagnosis may be confirmed by either histopathologic examination of involved tissue, detection of anti-*Toxoplasma* antibodies in the CSF, or the presence of DNA on PCR testing of CSF or bronchoalveolar lavage [192]. Pyrimethamine in combination with sulfadiazine is the preferred first-line treatment; alternatively, sulfadiazine may be substituted with clindamycin, clarithromycin, azithromycin, or atovaquone [192].

Trypanosoma cruzi (Chagas disease)

Trypanosoma cruzi, the etiologic agent of Chagas disease, is most commonly found in Latin America and is typically associated with prolonged periods of asymptomatic infection, during which time *T. cruzi* may be transmitted by blood transfusion and solid organ transplantation. In the US over 300 000 persons are thought to be infected with imported *T. cruzi*, with the highest prevalence recorded in Florida and California [204]. Due to immigration from Latin America, it has also been identified as a potential problem in Europe, Japan, and other countries [205]. Because donor epidemiologic information may not be readily available and local prevalence may be low, diagnosis is often delayed in areas of low prevalence.

Donor-derived infection most commonly involves heart transplant recipients, although it has also been reported following kidney transplantation; it often results in significant morbidity and mortality [206–208]. Presentation of donor-derived *T. cruzi* is usually non-specific with fevers and malaise. Acute infection may mimic transplant rejection, especially in the setting of cardiac transplant [206–208].

Donor screening for *T. cruzi* relies on detection of antibody to *T. cruzi* antigens, most commonly by EIA or indirect immunofluorescence antibody testing; radioimmune precipitation assays may also be used [209]. Due to low sensitivity and specificity of any single test, it is recommended that two tests be used for donor screening [209,210]. Screening for *T. cruzi* is routine in solid organ donors in endemic areas, but is not routinely recommended in low prevalence areas [209,211]. However, current US recommendations encourage the screening of donors from Latin America [209].

Because transmission of *T. cruzi* from infected donors is not universal, affected donors of organs other than the heart may be considered for donation [209,211]. When donors with Chagas infection are used, there is no recommendation for routine prophylactic antimicrobials, due to the limited transmission risk, cost, and toxicity of the medications. Instead, close monitoring with both serologic and buffy coat examination with Giemsa staining and PCR is preferred [209]. Cardiac transplantation from affected donors should be avoided due to the higher transmission rates, and extreme caution or avoidance of transplant of other organs is advised where high immunosuppression is required [209]. Suspicion of *T. cruzi* donor-derived infection should prompt immediate contact with the Centers for Disease Control and Prevention (CDC) for confirmatory testing and antiparasitic therapy if indicated [209].

Summary

Although donor-derived infections cannot always be anticipated or avoided, careful attention to donor evaluation with directed prophylactic interventions may avert significant morbidity and mortality from these infections. It is important that centers and OPOs be aware of the potential for this occurrence. If donor-derived infection is suspected, prompt communication to OPOs and the OPTN (or other regulatory bodies as dictated by government authorities) is critical to ensure that those caring for the other recipients of the donor's organs may intervene to prevent further adverse consequences of unintended transmission events.

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CHAPTER 93

Perioperative Infections after Organ Transplantation

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Introduction

Infectious complications are an important cause of morbidity and mortality in recipients of solid organ transplantation (SOT). The timing of specific infections is generally predictable regardless of the type of organ transplanted, with most clinically important infections occurring within the first 180 days post transplant. Individual pathogens and infectious syndromes tend to present at stereotypical times after transplantation (see Figure 94.1). However, the timing of presentation of certain pathogens, e.g. cytomegalovirus (CMV), can be affected by the use of prophylactic strategies, augmentation in immunosuppression, or need for additional surgery. Over time, we have learned to categorize infectious complications according to which of three major time intervals the patients present in: (1) early (0–30 days after transplantation); (2) intermediate (30–180 days); and (3) late (>180 days). However, some infectious syndromes, e.g. catheter-associated bloodstream infections, can occur throughout the post-transplant course whenever risk factors predisposing to them are present. While some exceptions to these patterns may occur, these divisions provide a useful framework for the approach to a potentially infected patient after SOT and serve as a guide to differential diagnosis. This chapter will focus on postoperative infections occurring within the first 30 days following the organ transplant procedure. We will also address common preventative strategies applied during this period. Additional information on infectious complications is given in Chapters 92 and 94.

We define perioperative infections as those occurring up to day 30 after SOT. Infections developing during this time period are frequently associated with the presence of pre-existing conditions in the recipient or are attributable to complications of the transplant or subsequent surgeries. Additionally, some of these infections may be derived from the organ donor (see Chapter 92). In general, bacteria or yeast are the most frequent pathogens recovered during this time period [1,2]. Fifty percent or more of all bacterial infections that develop after transplantation occur during the early post-transplant period [1,2]. Superficial or deep surgical site infections are the most common infectious syndromes observed during this period. Technical difficulties, particularly those resulting in anastomotic stenosis or leak, are important risk factors for the development of invasive infection in the first month after most types of organ transplantation. Given the relationship between the majority of early infections and the surgical site, it is not surprising that the organ undergoing transplantation is the most important determinant of the location of infection, especially during this time period [3]. The chest, abdomen, and urinary tract are the most common sites of infection after thoracic, liver, and kidney transplantation, respectively. Explanations for these site-specific infections include local ischemic injury and bleeding, as well as potential contamination during the transplant surgical procedure [4].

Predisposing factors

The primary factors predisposing to infection during the early time period after organ transplantation can be categorized into those present in the recipient before transplant and those secondary to intraoperative and post-transplant events.

Pretransplant factors

The underlying illnesses causing organ failure are important risk factors for the development of infection during the early time period after SOT. For example, a history of cystic fibrosis predisposes to early pseudomonal and fungal infections after lung transplantation; colonization with *Clostridium difficile* is also common in this population, predisposing them to disease due to this pathogen after transplantation [5]. Diabetes mellitus is a risk factor for the development of post-transplant urinary tract infection (UTI) [6]. A history of palliative surgery before transplantation increases the technical difficulty of the transplant procedure, enhancing the risk of developing a post-transplant infection [7]. The severity of disease at the time of transplantation tends to correlate with the risk of postoperative morbidity and mortality [8]. For example, liver transplant recipients with higher Model of End-stage Liver Disease (MELD) scores are at increased risk for postoperative infectious complications [9]. Similarly, long-standing malnutrition predisposes to infections before and after transplantation. While correction of nutritional deficits with parenteral alimentation may help to alleviate these risks, its use is associated with the risk of catheter-associated infection, which may present in the perioperative time period. Finally, admission to the intensive care unit (ICU) and requirement for mechanical ventilation while awaiting transplantation increases the risk of colonization and infection with hospital-acquired pathogens, many of which have multidrug resistance and cause disease in the first month but also beyond the early time period.

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Age is an important determinant of both susceptibility to certain pathogens and severity of infection after transplantation. While age-related differences in the type and range of manifestations associated with individual pathogens may be less notable in the first month after SOT, these can still be important for some early infections. Young children undergoing transplantation experience greater disease severity with certain viruses, such as respiratory syncytial virus (RSV) or parainfluenza virus, compared to older children or adult recipients [10,11]. This is particularly true early after transplant when the recipient is more highly immune suppressed and not fully recovered from the transplant surgery [10,11]. Decreased psoas muscle size, a marker of frailty in older adults, is associated with increased mortality in liver transplant recipients, and we have observed an increased incidence of early bacterial and fungal infections in recipients with preoperative markers of frailty [12]. Older recipient age may increase the risk of early infection after heart transplantation [13].

Transplant recipients are at risk for acquiring pathogens from their donors who have active or latent infections at the time of organ harvesting. Many of the donor-derived infections, including bacteria-associated systemic infection in the donor [14], West Nile virus, *Strongyloides*, and *Cryptococcus neoformans* to name a few, tend to present shortly after transplant during the early time period. A more complete discussion of donor-derived infection can be found in Chapter 92.

Intraoperative factors

Intraoperative factors unique to each SOT procedure can predispose to infectious complications. For example, the type of biliary reconstruction used in liver transplantation influences the likelihood of developing an infectious complication [15]. Similarly, the type of drainage chosen after pancreas transplant is also associated with a differential risk of infection [16,17]. Surgical events during the operation also alter the risk of infection. Injury to the phrenic, vagal, or recurrent laryngeal nerves during surgery affect pulmonary toilet, predisposing a lung transplant recipient to pneumonia [18]. Ischemic injury to the allograft during the transplant procedure reduces its viability and increases the risk of infection. Additional factors, including prolonged operative time, contamination of the operative field, and bleeding at or near surgical sites, also increase the risk of postoperative infections. Specific aspects of the surgical procedures are covered by organ type in Chapters 55–64.

Post-transplant factors

Technical problems manifesting after transplantation are amongst the most important risk factors for perioperative infectious complications. Thrombosis of the hepatic artery predisposes to hepatic abscesses, infected biloma, and bloodstream infection (BSI) after liver transplantation [19]; vesicoureteral reflux predisposes to graft pyelonephritis in renal transplant recipients [20,21]; and mediastinal bleeding requiring re-exploration predisposes to mediastinitis and BSI in thoracic transplant recipients [2].

The prolonged use of indwelling cannulas is a significant risk factor for infection after transplantation. Central venous catheters are a risk for BSI, urethral catheters predispose to UTI, the use of a cannula in an obstructed biliary tract predisposes to cholangitis, and prolonged endotracheal intubation is associated with pneumonia. While the presence of an indwelling cannula is associated with infection at any time post transplant, their use typically is greatest during the early post-transplant period. Accordingly, this is a very important cause of perioperative infections.

Nosocomial exposures constitute another important group of post-transplant risk factors. All transplant recipients are at risk for developing infection with transfusion-associated pathogens, though these infections tend to be associated with relatively long incubation periods, leading to clinical presentation beyond the first month post transplant. Children and to a lesser extent adults undergoing transplantation during the winter months may be exposed nosocomially to common community-acquired viruses associated with annual seasonal epidemics, e.g. respiratory syncytial virus (RSV) and influenza. Infection early after transplant has been shown to be associated with an increased risk for morbidity and possibly mortality, especially in young pediatric transplant recipients [10,11]. The presence in the hospital of areas of heavy contamination with pathogenic fungi, such as *Aspergillus* spp., increases the risk of invasive fungal disease in these patients. Finally, nosocomial transmission of multiply resistant bacteria predisposes to infection with these pathogens.

No discussion of postoperative risk factors would be complete without including immunosuppression. While less important in the early post-transplant period, immunosuppression remains the major risk factor for infection following transplantation. Immunosuppressive regimens are continually evolving in an attempt to achieve more specific control of rejection with the least impairment of immune function. However, all immunosuppressive regimens interfere with host defenses. Treatment of episodes of rejection exacerbates this risk. The use of antilymphocyte preparations or other biologic agents is associated with an enhanced risk of infection [15,22,23]. As newer immunosuppressive agents (particularly the increasingly diverse number of new biologic agents) are introduced, clinicians must be aware of and alert for the changes in infectious manifestations and profiles seen in SOT recipients [24,25].

Early postoperative infections

Infectious complications occurring during the early post-transplant period can generally be divided into two major categories: those affecting all SOT recipients and those associated with the individual transplant procedures (Table 93.1).

Infections common to most organ transplants

The vast majority of early postoperative infections are caused by bacterial pathogens, though infections due to *Candida* and some viral pathogens also occur in the early time period [26]. Early postoperative infectious complications may manifest as systemic disease associated with sepsis and bloodstream involvement or may present with localized signs and symptoms with or without fever. Early infections are frequently a direct complication of the surgical procedure and can be categorized as being either "superficial" or "deep." "Superficial" infections that can develop in any SOT recipient affect the skin and/or fascia at the surgical site and are indistinguishable from wound infections occurring after any surgical procedure. Accordingly, their management is similar to that used for surgical site infections in patients undergoing other types of surgeries. "Deep" infections typically occur at or near the site of the transplanted organ and are usually related to anastomotic issues or other technical problems complicating the transplant surgery. Because of the differences in technical aspects and locations associated with each of the organ transplant procedures, infectious syndromes associated with deep infections vary by the type of organ transplantation the patient undergoes (see below). In this section,

Table 93.1. Risk factors and preventative strategies for early infections by organ type

	Predisposing factor	Specific infection	Preventative intervention
Kidney	Urinary catheter/stent	UTI	Decreased duration of catheterization/ stenting
	Diabetes mellitus [6]		
	Vesicoureteral reflux [20,21]		Antibacterial prophylaxis
	Female gender [57]		Ureteroneocystostomy
Pancreas	Duodenal stump	Intra-abdominal infection ± BSI	
	Anastomotic leak	UTI	Enteric drainage
	Bladder drainage [16,17]		
Liver	Anastomotic leak	Infected biloma	T-tube use may increase risk (late infections)
	Hepatic artery thrombosis [19]	Liver abscess/cholangitis	
	Renal failure/prolonged intraoperative time/*Candida* colonization/high transfusion requirement/choledochojejunostomy [74,75]	± BSI Candidemia	Serial ultrasound and early intervention Antifungal prophylaxis
	Above + graft dysfunction/prolonged ICU [15,78,79,112]	Aspergillosis	Mold active prophylaxis
Lung	Cystic fibrosis/operative nerve injury/prolonged mechanical ventilation	Pneumonia	
	Ischemia	Fungal anastomotic site infection	Fungal prophylaxis
	Donor or recipient colonization [112]	Aspergillosis	Mold active prophylaxis
Heart	Prolonged mechanical ventilation	Pneumonia	
	Postoperative circulatory support	BSI	
	Reoperation/bleeding/VAD [2,89]	Mediastinitis	
Small Intestine	Use of mesh	Intra-abdominal	
	Re-exploration	Infection	
	Disruption of mucosal barrier (harvest injury or rejection)	BSI	

UTI, urinary tract infection; BSI, blood stream infection; VAD, ventricular assist device.

we highlight details of selected perioperative infectious issues occurring regardless of transplant type.

Catheter-related infections

Catheter-related infections are an important cause of bacterial, and to a lesser extent fungal, infections, potentially affecting all SOT recipients in the perioperative period. The use of central venous catheters (CVCs) is associated with the development of central line-associated bloodstream infection (CLABSI). The risk of developing a CLABSI persists as long as the central line is in place. While many SOT recipients may require the presence of a CVC only for the first week or two after transplant, some patients need a CVC for longer periods of time due to requirements for hyperalimentation, ongoing hemodialysis, limited alternative vascular access, and/or ongoing clinical instability. While the use of long-term catheters, e.g. Broviac, or implanted ports may reduce the risk of CLABSI, these patients remain at increased risk for the development of this complication until the line is removed. In general, the management and treatment of CLABSI in SOT recipients is similar to that for other patients developing this complication. Guidelines published by the Infectious Diseases Society of America provide appropriate recommendations (including duration and ability to treat without removal of the affected line) based upon both recovered pathogen and the clinical condition of the patient [27,28].

Clostridium difficile colitis

C.difficile colitis (CDAD) is an important potential complication affecting all SOT recipients. *C. difficile* is the most common infectious cause of diarrhea following SOT in adults. In general, the rate of CDAD in SOT recipients appears to be higher than that observed in other hospitalized patients [29]. Recent studies demonstrate a rate of about 5%, which represents a significant increase in the rate of this complication in the past decade due to the emergence of the NAP1 strain [29–35]. While data are more limited for pediatric SOT recipients, rates of CDAD ranging from 5% to 13% have been reported in children undergoing organ transplantation [5,36]. In many series, the median time to onset of CDAD was <30 days [29,32,34,37], though later cases clearly occur. Particularly during the early post-transplant period, traditional risk factors for CDAD such as antibiotic use, hospitalization, and acid suppression are common. In liver transplant recipients, retransplantation, higher MELD scores, intra-abdominal complications, and other systemic infections have also been associated with the development of CDAD [29]. Higher rates of colonization with *C. difficile* have been documented in patients with cystic fibrosis, predisposing to infection after lung or liver transplantation [5]. Death and severe morbidity due to CDAD are relatively uncommon in SOT recipients, occurring in <5% of CDAD patients [35,37–39]. Early recognition and treatment likely improve outcomes. Diagnostic testing for CDAD is generally indicated for patients with otherwise unexplained diarrhea in the early transplant period both because early treatment of CDAD improves outcome and because findings such as abdominal pain, fever, and leukocytosis may be masked and non-specific in immunosuppressed patients. The methodology used to test for CDAD is rapidly evolving, with the development of highly sensitive polymerase chain reaction (PCR) and antigen testing providing an improved negative predictive value compared to older enzyme immunoassay (EIA) testing. Nonetheless, in highly suggestive cases empiric treatment may be indicated even following a negative assay.

Treatment of CDAD does not differ significantly in SOT recipients as compared to other patients, and guidelines are available [40,41]. Other antibiotics should be discontinued if possible. Initiation of empiric therapy pending results of testing is recommended in more severe cases [40,41]. A summary of recommended regimens both for initial therapy and for relapse is available [40,41]. Prevention of CDAD relies on limiting the use of antimicrobial agents (both for treatment and prophylaxis), and infection control measures, including hand washing (using soap and water as alcohol-based sanitizers may not be sporocidal) and possibly the use of bleach solutions in room cleaning [41].

Infections due to multidrug resistant bacteria

Over the past decade, a significant increase in infection with multidrug resistant (MDR) organisms in hospitalized patients has occurred [42–45] and SOT recipients have been significantly

impacted by this trend [46–48]. While vancomycin-resistant enterococcus (VRE) remains a major problem, the development and licensing of antimicrobial drugs with activity against VRE has preserved treatment options for most VRE-infected patients. MDR (resistant to two or more classes of commonly used broad-spectrum antimicrobials) and extremely drug resistant (XDR; often resistant to all but tigecyline and colistin) Gram-negative organisms are an emerging clinical problem. New mechanisms of resistance and local outbreaks are constantly emerging. Accordingly, clinicians must be aware of current institutional unit antibiotic resistance patterns to appropriately select empiric therapy in critically ill post-transplant patients. Liver transplant recipients with abdominal infections and cystic fibrosis patients with postoperative pneumonia are likely at the highest risk for MDR and XDR infections. In some series, outcomes in transplant patients with MDR/XDR Gram-negative infections have been poor with mortality rates as high as 90% and higher than for controls with infection with more drug-sensitive organisms [46,47,49,50]. For XDR organisms, treatment is difficult and often associated with significant renal toxicity. Combination therapy and older antibiotics such as colistin are commonly employed [48,49,51].

Perioperative infectious complications associated with individual transplant procedures

Renal transplantation

Septicemia originating from the urinary tract, lower respiratory tract, or transplant wound accounts for most life-threatening infections in the first month after renal transplantation [21,52]. UTIs, especially pyelonephritis, are the most common infectious complication, accounting for up to 50% of episodes [53,54]. In the immediate postoperative period, UTI rates of 23–45% have been reported [55–57]. Commonly described risk factors include female gender (particularly for later UTIs), diabetes mellitus (although not in two recent studies), older age, reflux disease, acute rejection, use of ureteral stent, use of cadaveric grafts, and urological malformations of the native kidney [6,57–59]. The prolonged use of urinary catheters is likely a particularly significant risk factor in the first month after transplant [60]. While *Escherichia coli* remains the most common pathogen, *Enterococcus* spp. and other Gram-negative bacteria are commonly reported. Fungal pathogens, largely *Candida* spp., are recovered in <5% of cases. More resistant bacteria, e.g. *Pseudomonas aeuroginosa* and extended spectrum beta-lactamase (ESBL)-producing organisms, are commonly seen early in the post-transplant course, likely related to exposure to resistant hospital-acquired pathogens as well as the use of perioperative antibiotics [56,59,61]. Resistance patterns vary greatly from institution to institution; appropriate empiric selection of antibiotics for early post-operative UTI depends on knowledge of hospital- and unit-specific antibiotic resistance patterns. While immediate consequences of UTIs, e.g. need for antibiotic therapy, hospitalization, sepsis in cases of acute pyelonephritis, are obviously detrimental, controversy exists regarding the long-term outcomes on graft function and mortality resulting from UTIs [6,57–59,62]. The incidence of UTIs in kidney transplant recipients may be decreased with the use of ureteroneocystostomy and prophylactic use of daily trimethoprim-sulfamethoxazole (TMP-SMX) (see UTI prophylaxis for renal transplant recipients below) [20,63,64].

Liver transplantation

Bacterial and fungal infections are a common early problem after liver transplantation [65–68]. Intra-abdominal infection or the presence of a central venous catheter predisposes to BSI, but it can occur without an obvious source. Enteric Gram-negative organisms (which are frequently MDR) account for more than one-half of episodes. Bacterial infections involving the abdomen or surgical wound are common in most series. Post-transplant biliary tract complications and resulting infection continue to be a major problem, with anastomotic leaks and hepatic artery thrombosis the most important early problems. Biliary strictures mostly present in the later postoperative period. Recent studies describe a biliary leak rate of 4.9–18%; late leaks are often related to T-tube removal [69–72]. While in many cases of anastomotic biliary leak conservative management is successful and significant infection does not occur, many patients develop biloma infection and require prolonged and often repeated courses of antibiotics and drainage procedures. Infection with MDR organisms is common in this circumstance. Hepatic artery thrombosis—which may lead to biliary leak and hepatic necrosis with resulting abscess—is much less common, occurring in <2% of recipients [72]. In some centers, introduction of frequent surveillance Doppler studies early after transplantation to monitor development of thrombosis, coupled with the use of operative thrombectomy and thrombolysis, has essentially eliminated the development of hepatic artery thrombosis and resulting infection of the graft.

Fungal infections due to pathogens other than *Candida* are relatively uncommon in the first month after liver transplantation [26,73]. While a recent large series demonstrated that 7% of fungal infections in adult liver transplant recipients were due to *Cryptococcus neoformans*, no cases occurred prior to postoperative day 45 [73]. We have, however, observed earlier cases possibly related to subclinical infection in the recipient prior to transplant- or donor-derived infection. In contrast, *Candida* infections (typically candidemia with or without dissemination) occur more commonly in the early postoperative period. Studies in adult liver transplant recipients have identified a population at very high risk for *Candida* infections [74,75]. Risk factors include renal failure, length of operation, retransplant or reoperation for complication, colonization with *Candida* spp., high transfusion requirements, and choledochojejunostomy [74,75]. Based on these risk factors, most centers employ targeted antifungal prophylaxis in adult high-risk patients, typically for 1 month or until resolution of the risk factors (see Antifungal prophylaxis below) [75,76].

Invasive aspergillosis is rare after liver transplantation, occurring in <2% of recipients [77–79]. However, when present, a significant proportion of cases occur in the early post-transplant period. These patients have generally had difficult post-transplant courses. Risk factors for early aspergillosis include retransplantation, dialysis, graft dysfunction, and ongoing need for ICU care. Early episodes of invasive aspergillosis have occurred in children undergoing liver transplantation for cystic fibrosis [67]. Outcomes are often poor in liver transplant recipients with invasive aspergillosis [78,79].

Intestinal transplantation

An increasing number of children and adults have received intestinal transplants. Many have undergone combined transplantation of the liver and intestine or multivisceral transplant procedures. Bacterial infection occurs frequently in these patients, particularly in the first month following transplantation [1,80,81]. BSI, which can be explained in part by disruption of the mucosal barrier associated with harvest injury or rejection, is a common finding [80,81]. Coagulase-negative staphylococci, enterococci, and Gram-negative enteric bacilli account for most episodes. MDR organisms are fre-

quently seen. Candidemia can also occur in these settings, and among SOT recipients small bowel recipients are at the highest risk for fungal infection [26]. Intra-abdominal infections also occur frequently in these patients during the first month after transplantation. While this likely relates to complications of the initial transplant surgical procedures, the risk is also increased by the use of mesh to temporarily close the abdominal space when the transplanted organs are too large to allow complete surgical closure of the wound at the time of the transplant. Additional risk of intra-abdominal infection is associated with the potential need for recurrent exploratory laparotomies in this patient population. The treatment of intra-abdominal infection is similar to that used for abdominal space infections in immune competent patients. Where appropriate, drainage of collections and abscesses (intraoperatively or by interventional radiology) is recommended. Empiric antibiotics should cover enteric Gram-negative bacilli and enterococci. A prior history of infection or colonization with MDR bacteria may warrant use of agents known to cover these pathogens pending availability of cultures and sensitivities. This is particularly true in patients presenting with septic shock or other signs of significant clinical instability. Accordingly, agents such as carbapenems (for resistant Gram-negative bacilli) or linezolid (for vancomycin-resistant enterococci) are frequently initiated in this patient population. However, where possible, treatment choices should be narrowed once culture and susceptibility results are available.

Heart transplantation

Infections are an important cause of morbidity and mortality in the perioperative period following heart transplantation. Rates of infection during this time period as high as 22% have been reported [82], and infections accounted for approximately 11–18% of very early deaths after cardiac transplantation in other series [82–86]. Common sites of infection during the early time period include the lungs, bloodstream, sternum, and urinary tract [13,82,83]. The need for postoperative circulatory support, e.g. extracorporeal membrane oxygenation, intra-aortic balloon pumps, left ventricular assist devices (LVADs), increases the risk for line or device-related infection [87]. Infection of the lower respiratory tract (including both pneumonia and lung abscess) occurs at a high rate in most series of heart transplantations [13,83,88]. Due to high rates of morbidity and mortality, mediastinitis is an important early infection following heart transplantation. The reported incidence of mediastinitis in heart transplant recipients is 2.4–3.8% [83,89]. The most common pathogens associated with mediastinitis include *Staphylococcus aureus* and Gram-negative enteric bacilli. Recent studies have suggested a potential enhanced risk for the development of sternal wound infections and mediastinitis in heart transplant recipients receiving mammalian target of rapamycin (mTOR) inhibitors [82,89]. Additional risk factors beyond those seen in the non-transplant population include requirement for re-exploration of the chest, age at transplantation, duration of mechanical ventilation, and the use of ventricular assist devices prior to transplantation [89]. The diagnosis of mediastinitis may be more challenging compared to patients undergoing non-transplant thoracic surgery due to a decreased likelihood of manifesting fever, leukocytosis, or local redness. The presence of chest pain in disproportion to that expected from the sternotomy is an important diagnostic clue. The finding of mediastinal air or fluid collection on computed tomography (CT) scan can further support diagnosis with confirmation made by aspiration or surgical exploration. Once confirmed, management should include aggressive surgical drainage and debridement with excision of necrotic tissue. In addition, appropriate antimicrobial therapy should be provided.

Children who undergo heart transplantation at a young age are at increased risk for invasive infection due to *Streptococcus pneumoniae* disease [90]. Infants undergoing heart transplantation represent a unique population and have increased risk of serious bacterial or fungal disease. Backer et al. reported that over 20% of infants who underwent heart transplantation had serious bacterial infection [91]. Similarly, high rates of bacterial or fungal infection were noted in infants younger than 6 months of age who underwent heart transplantation in Pittsburgh. Further, adult patients may develop early postoperative infection (often disseminated) with invasive molds such as *Aspergillus* spp. [83,92].

Ventricular assist devices, which are used with increasing frequency as a bridge to transplantation [84], are associated with postoperative infections [13]. These devices may be chronically infected preoperatively and chronic suppression of these infections with antibiotics may lead to the presence of MDR organisms. Further, the large tract sites remaining after removal may be colonized with these organisms, resulting in difficult-to-treat deep space infections.

Lung and heart–lung transplantation

Infection accounts for 10–20% of deaths occurring in the first 30 postoperative days in both adult and pediatric lung and heart–lung transplant recipients [93]. Recipients of lung transplantation are at high risk for developing bacterial infection of the respiratory tract. Pneumonia, the most important infectious complication, is difficult to diagnose definitively because differentiation between chronic colonization and lower respiratory tract infection can be problematic. Gram-negative pathogens (often MDR organisms) and *S. aureus* can be recovered in the presence or absence of disease. Radiographic abnormalities are present almost universally in patients with pneumonia or graft rejection [94–96]. Therefore, bronchoalveolar lavage (BAL) or transbronchial biopsy is often required to help distinguish between causes.

Patients undergoing lung transplantation because of cystic fibrosis have a high rate of infectious complications. They are usually colonized with *Pseudomonas* or *Aspergillus spp*. Colonization with *Burkholderia cenocepacia* (formerly *B. cepacia genomovar* III) has been associated with excessive mortality [97–99]. BSI due to organisms present before transplantation is common after lung transplantation in patients with cystic fibrosis [100,101]. Because of the importance and difficulty in treating these bacterial complications, protocols at most transplant centers include thorough evaluation of the microbial flora of candidates prior to transplantation. This should include antibiotic synergy testing and evaluation of isolates of *B. cepacia* complex species at reference laboratories.

Fungal infection frequently complicates lung transplantation and can present early after surgery, occasionally at the anastomotic site leading to later airway complications [102,103]. Among all SOT recipients, lung recipients are at the highest risk of invasive *Aspergillus* and are the only SOT group in which infection with *Aspergillus* is more common than infection with *Candida* spp. [26]. Nonetheless, early *Candida* infections, including candidemia, deep surgical site infections, and anastomotic site infections, do occur. Early fungal infections are more likely to occur in recipients undergoing lung transplantation for cystic fibrosis who are previously colonized with *Aspergillus*. However, early infection can also occur due to the presence of occult or latent fungal disease in the donor lungs. This is particularly true for the endemic mycoses, e.g. histoplasmosis

and coccidiomycosis. The relatively high rate and clinical consequence of early fungal disease in lung transplant recipients has prompted many transplant centers to institute antifungal prophylaxis with agents with activity against *Aspergillus* [104].

Pancreas transplantation

Pancreas transplants are performed almost exclusively in adult patients with poorly controlled diabetes mellitus; thus, one risk for infection is already present. In patients who also have end-stage renal disease, the procedure may be performed simultaneously with a kidney transplant, or the procedures may be done sequentially. Pancreas or renal–pancreas transplantation has a higher early postoperative infection rate than kidney-alone transplants, likely related to technical factors, e.g. contaminated donor duodenal stump, graft thrombosis leading to necrosis and infection, anastomotic leak, increased need for immunosuppression, and higher rejection rates. One series reported a 16% rate of sepsis from bloodstream infection in the first 45 days after transplant, with intra-abdominal infection the most common source [105]. Improved surgical technique and treatment to reduce the rate of thrombosis may result in a decreased incidence of intra-abdominal infection [106]. In one registry, 1.2–1.4% of recipients died from infection, making infection the second-leading cause of death after cardiovascular disease [107]. Drainage of the pancreas may be enteric using donor duodenal stump or the exocrine pancreas may be drained into the bladder; patients with bladder drainage experience very high rates of UTI [16,17]. Pancreas recipients are at higher risk for fungal infections than kidney alone recipients, likely related to enteric leaks and UTI, with a 4% rate versus 1.3% [26].

Prevention of early postoperative infections

A variety of prophylactic strategies are generally implemented in the early postoperative period. While some of these focus on the prevention of perioperative and early infectious complications, many of the strategies that are initiated shortly after transplant will be maintained for a prolonged period of time and aim to prevent infectious complications later in the post-transplant period. General considerations for prophylaxis as well as specific circumstances felt to justify universal or targeted prophylaxis for a particular organism or class of organisms in selected high-risk organ recipients are discussed below (Table 93.2).

Perioperative antibacterial prophylaxis

Despite more than 40 years of clinical experience with organ transplantation, little data exist to inform the appropriate antibacterial perioperative prophylaxis for patients undergoing organ transplantation. General principles for perioperative prophylaxis for

Table 93.2. Preventative strategy for non-viral infections by organ type and selected infection

	Wound infection	UTI prophylaxis	Antifungal prophylaxis	PCP prophylaxis[8]	Toxoplasmosis
Kidney	Skin flora[1]	Most centers use TMP-SMX for 1–3 months post transplant	None	4–12 months Additional course of 2–3 months after high-risk events[2]	None
Kidney–pancreas or pancreas alone	Skin flora[1] Enteric flora[3]	None for pancreas alone	None	4–12 months Additional course of 2–3 months after high-risk events[2]	None
Liver	Skin flora[1] Enteric flora[3]	None	Adult high risk recipients[4] fluconazole for 1 month or until resolution of risk factor Mold active agent if ultrahigh risk[5]	4–12 months Additional course of 2–3 months after high-risk events[2]	None
Lung	Skin flora[1] Respiratory flora[6] Extended (14 days) treatment of pathogens recovered from donor or recipient lungs	None	Most centers employ universal mold active prophylaxis[7]	Minimum 12 months; lifelong prophylaxis likely justified	None
Heart	Skin flora[1] (some centers include MRSA coverage if recipient colonized) Respiratory flora[6] Additional coverage (based on sensitivity data) if known LVAD colonization or positive cultures	None	None	4–12 months Additional course of 2–3 months after high-risk events[2]	Donor positive/ recipient negative IgG serology[9]
Small intestine	Skin flora[1] Enteric flora[3]	None	None	Minimum 12 months; lifelong prophylaxis likely justified	None

[1] Commonly used antibiotics for skin flora prophylaxis include cefazolin and clindamycin.
[2] Cytomegalovirus, cell-depleting therapy for rejection, prolonged high-dose corticosteroids.
[3] Commonly used antibiotics that cover enteric organisms (Gram negative + anaerobes) include piperacillin/tazobactam, ampicillin/tazobactam, and cefoxitin, and these provide skin flora prophylaxis as well.
[4] Renal failure, length of operation, retransplant for reoperation for complication, colonization with *Candida* spp., high transfusion requirements, and choledochojejunostomy high-risk events.
[5] Risk factors above combined with graft failure, hemodialysis, prolonged intensive care unit stay.
[6] Commonly used antibiotics that cover respiratory pathogens include cefepime and piperacillin/tazobactam and these provide skin flora prophylaxis as well.
[7] Agents with activity against molds include echinocandins, voriconazole, and posaconazole.
[8] Some experts recommend lifelong PCP prophylaxis.
[9] TMP-SMX alone or dapsone or atovaquone usually combined with pyrimethamine and leucovorin.
UTI, urinary tract infection; PCP, *Pneumocystis jiroveci* (formerly *carinii*); TMP-SMX, trimethoprim-sulfamethoxazole; MRSA, methicillin-resistant *Staphylococcus aureus*; LVAD, left ventricular assist device.

standard surgical procedures have been combined with specific risk factors and circumstances associated with enhanced risk of infection for specific organ transplant recipients. One survey of 61 European liver transplant centers found general agreement in the duration of prophylaxis (median 3 days), but identified broad variability in the choice of perioperative antibacterial prophylaxis [76]. Strategies ranged from relatively narrow spectrum beta-lactams to combined therapy utilizing advanced-generation beta-lactams (e.g. third- or fourth-generation cephalosporin or piperacillin/tazobactam) in combination with vancomycin. In the absence of definitive data, perioperative prophylactic regimens should aim to cover skin organisms (for all organ transplant procedures) as well as enteric Gram-negative and anaerobic bacteria and enterococci for liver, pancreas, and intestine transplant procedures. Knowledge of pre-existing colonization with methicillin-resistant *Staphylococcus aureus* (MRSA), VRE, and MDR Gram-negative bacteria may lead to individualization of perioperative prophylactic regimens.

Several special circumstances have led to broadly accepted recommendations for more prolonged and specific prophylaxis. Many centers cover respiratory pathogens in heart transplant recipients, and will treat for 1–2 weeks after transplant if known LVAD colonization or infection or cultures of LVAD tracts are positive for resistant organisms postoperatively. For lung transplant recipients, the presence of bacterial or fungal pathogens recovered from donor BAL cultures typically prompts a 2-week course of antibacterial agents based upon the susceptibility of the recovered organisms. Additional circumstances in which the agent used for prophylaxis might be modified or prolonged include the donor being bacteremic or having infection within the graft or central nervous system.

Urinary tract infection prophylaxis for renal transplant recipients

Many centers use antibacterial prophylaxis to prevent UTI in the first 1–12 months after transplantation. A recent meta-analysis of studies conducted in the 1980s and 1990s found that antibiotic prophylaxis reduced the rate of sepsis, bacteremia, and bacteriuria, but had no effect on long-term graft outcomes [64]. To some degree, the widespread use of *Pneumocystis jiroveci* prophylaxis with TMP-SMX by >80% of US transplant centers for 3–6 months post-transplant render this question mute [108]. More recent evidence regarding increasing antimicrobial resistance (particularly to TMP-SMX among urinary isolates) of patients on prophylaxis [61] limits the efficacy of this agent and may reduce enthusiasm for extended courses of UTI prophylaxis. The use of broader-spectrum agents, e.g. fluorquinolones, is limited by more severe consequences of resistance, e.g. no available oral agents to treat UTI, as well as other adverse events and superinfections.

Antifungal prophylaxis

As discussed above, risk factors for invasive fungal infections after SOT have been described, and due to the serious nature of these infections many centers employ antifungal prophylaxis for high-risk groups. A reduction in mortality has not been demonstrated, and drug interactions and the emergence of resistance may limit the benefit of antifungal prophylaxis. The patients most commonly targeted for antifungal prophylaxis are lung and high-risk liver recipients, as discussed below.

Liver transplantation

Adult liver transplant recipients with certain risk factors (see above) are at high risk for *Candida* infection in the early postoperative period [74,75]. A number of randomized studies using either varying doses of fluconazole or itraconazole have demonstrated a reduction in fungal infections, although no reduction in overall mortality [109–111]. Due to decreased drug interactions with calcineurin inhibitors (CNIs) and easier drug delivery, fluconazole is generally preferred to itraconazole. While a 400-mg daily dose of fluconazole may be superior, many centers employ a lower dose (100 mg daily) with success. Fluconazole prophylaxis increases the rate of fluconazole-resistant *Candida* spp. [73], and about 50% of *Candida* spp. observed after liver transplantation are non-albicans [26,73]. As non-albicans species are more likely to be azole resistant, interest in using an echinocandin that is active against most non-albicans species has increased and a trial in high-risk patients is ongoing. Some experts suggest prophylaxis with a mold-active agent in critically ill recipients (retransplantation, prolonged ICU stays, graft dysfunction, hemodialysis) at higher risk for early invasive aspergillosis [75,112], although drug interactions, hepatotoxicity, drug delivery issues, and other adverse events may limit the use of drugs such as voriconazole or posaconazole in the early postoperative period. Of note, antifungal prophylaxis has not been recommended for pediatric liver transplant recipients. This may be due to differences in frequency and outcome of *Candida* infections in children undergoing liver transplantation.

Lung transplantation

Among SOT recipients, lung recipients are at the highest risk of invasive fungal infection; invasive pulmonary aspergillosis (IPA) is the most common fungal infection in this group [26]. Because of the frequency and severity of IPA, most centers employ some form of prophylaxis [104]. Randomized placebo-controlled data, however, are not available to confirm the efficacy of prophylaxis. Case series demonstrate varying results, and firm recommendations cannot be made [112–115]. Options for prophylaxis include inhaled amphotericin products, itraconazole, or voriconazole [112]. Many centers treat for 3–6 months or base duration on negative fungal cultures obtained at routine follow-up bronchoscopies [104]. Some centers have noted an increased rate of hepatotoxicity [113,115] as well as emerging adverse events, e.g. painful periostitis [116] and increased risk of skin cancer, with prolonged voriconazole use. These concerns, combined with drug interactions between azoles and CNIs, have increased interest in the use of inhaled amphotericin products. Other centers attempt to target prophylaxis at patients with additional risk factors for IPA, e.g. *Aspergillus* colonization, cell-depleting induction therapy, CMV infection [112,113]. A multicenter, randomized study is required to clarify the optimal approach to antifungal prophylaxis in this high-risk population.

Pneumocystis jiroveci prophylaxis

Prior to the routine use of prophylaxis, *Pneumocystis jiroveci* (formerly *carinii*) pneumonia (PCP) rates from 1% to 88% were reported in SOT recipients, with the highest rates observed in recipients of chest organs and during the first postoperative year [117]. Accordingly, both American and European guidelines recommend PCP prophylaxis for the first 4–12 months after SOT [118,119]. However, some experts have recommended the use of indefinite prophylaxis as the risk never returns to that of the non-immunosuppressed patient. In particular, because of the higher risk noted in lung transplant and small bowel transplant recipients, many centers employ life-long prophylaxis in these populations. Given that >85% of US transplant centers currently use PCP prophylaxis for at least 3 months after transplantation [108], the

incidence of PCP on current immunosuppression protocols is difficult to ascertain. Nonetheless, a number of more recent reports suggest that with limited or no prophylaxis, rates of PCP may be lower than expected in lower risk, e.g. renal transplant, recipients and call into question the need for universal prophylaxis [120–125]. Interestingly, some of these reports describe outbreaks of PCP likely due to human-to-human transmission after long periods with very low PCP rates [121–125]. One possibility is that the high rates of PCP noted in earlier studies reflected measurement during an outbreak. In any case, most centers do restart (or continue) prophylaxis for 2–3 months after clinical events that create higher risk of PCP, e.g. CMV infection, rejection treatment with cell-depleting agents, prolonged high doses of corticosteroids. PCP prophylaxis with TMP-SMX is highly effective, with most trials showing no cases of PCP in treated patients [126]. TMP-SMX can be administered as one double-strength tablet daily or thrice weekly. A dose of 5 mg/kg/day (trimethoprim component) is used with the same frequency for pediatric SOT recipients. Some centers prefer to use a single-strength tablet daily; dosing should be adjusted based on renal function. Other benefits of TMP-SMX include possible protection against uncommon but significant infections such at *Nocardiosis* and *Toxoplasmosis*. Patients intolerant of TMP-SMX may be treated with dapose 50–100 mg daily, atovaquone 1500 mg daily, or inhaled pentamidine 300 mg monthly. Weight-based dosing of these agents is recommended for pediatric SOT recipients. For dapsone, the dose would be 2 mg/kg given daily (maximum dose 100 mg/day) or 4 mg/kg given once per week (maximum dose 200 mg). For atovaquone, the dose varies by age. Children aged 4–24 months should receive 45 mg/kg/day once daily (maximum dose: 1500 mg/day), while those aged >24 months should be treated with 30 mg/kg/day once daily (maximum dose 1500 mg/day). The dosing frequency of inhaled pentamidine for children is every 3–4 weeks, with children <5 years of age receiving a dose of 150 mg while those >5 years receive the adult dose of 300 mg.

Toxoplasmosis prophylaxis

Toxoplasma gondii is a protozoa that establishes latent infection in approximately 10–20% of North Americans, and has a much higher prevalence in other parts of the world. The most important problem in the SOT population is activation of latent infection in the myocardium of seropositive heart donors transplanted into seronegative recipients. Thus, all heart donors and recipients should undergo serological testing for *T. gondii* to determine if the high-risk situation (donor positive, recipient negative) is present. Routine screening is not necessary for non-cardiac donors and recipients. TMP-SMX at the doses described above in the PCP section appears to be effective at preventing toxoplasmosis, although some centers prefer daily dosing for high-risk recipients [127,128]. Patients unable to tolerate TMP-SMX may be treated with dapsone or atovaquone, possibly combined with pyrimethamine and folinic acid; some centers use atovaquone alone. Any of these regimens will provide PCP prophylaxis as well. Duration of prophylaxis is unclear; many centers treat for 6 months to 1 year, but some maintain lifelong prophylaxis.

Antiviral prophylaxis

Although clinical disease due to CMV, EBV, BK virus (BKV), and hepatitis B (HBV) and hepatitis C virus (HCV) does not typically manifest until later after SOT, strategies for the prevention of infection and disease attributable to these viral pathogens are typically initiated in the perioperative time period. Specific considerations for each virus are discussed below.

Cytomegalovirus

For CMV, one of two major strategies is usually initiated. Universal chemoprophylaxis provides antiviral therapy with ganciclovir or valganciclovir to all "at-risk" transplant recipients for a specified period of time. Current recommendations tend to favor the use of oral valganciclovir for a 3–6-month period of time, though some centers have opted for even longer courses of treatment, particularly for lung transplant recipients [129–131]. Pre-emptive therapy is based on the performance of serial measurements of viral load in the blood to detect early subclinical infection to inform initiation of pre-emptive antiviral treatment. It is recommended that serial viral load measurements be obtained weekly for a defined period (e.g. 6 months) following transplantation [129,131]. Once initiated, antiviral treatment is usually continued until the CMV load becomes non-detectable. The pre-emptive strategy limits exposure to ganciclovir or valganciclovir to those with proven evidence of subclinical infection. However, the requirement that SOT recipients undergo weekly surveillance screening of their blood brings logistical challenges and costs to this strategy. Further, this strategy may not be effective in patients at high risk for CMV disease with rapid doubling times (e.g. donor-positive, recipient-negative chest organ recipients). A third strategy, known as the "hybrid approach," has evolved, particularly for pediatric centers where oral valganciclovir has only recently become available [131]. The hybrid strategy typically combines a short course of antiviral chemoprophylaxis (typically 2–4 weeks) for patients at increased risk for CMV infection and disease, followed by surveillance viral load measurements for a designated period of time. A more detailed description of the relative benefits of each of these approaches is given in Chapter 94.

Epstein–Barr virus

Despite increasing efforts and attention, definitive evidence confirming the efficacy of strategies for the prevention of EBV disease and post-transplant lymphoproliferative disease (PTLD) are lacking. With the exception of a small number of prospective, randomized trials, evidence supporting EBV prevention strategies derives from retrospective clinical series of variable quality and design. The efficacy of chemoprophylaxis with acyclovir or ganciclovir is unclear. Retrospective studies in support of this approach have lacked appropriate controls and have been confounded by changes in immunosuppression regimens over time. The use of intravenous immunoglobulin was evaluated in one multicenter randomized clinical trial. Although a trend towards benefit was seen, the study did not achieve adequate enrollment to demonstrate a statistically significant difference between the two groups [132]. The preventive strategy currently favored by most experts uses serial monitoring of EBV load in the peripheral blood to inform pre-emptive reduction in immunosuppression [132]. Data on the pre-emptive use of rituximab for patients with rising EBV loads is also emerging [133]. Studies evaluating the efficacy of this approach are generally limited by their retrospective design. In general, monitoring is only recommended for patients who are EBV seronegative prior to transplant as those who are EBV seropositive prior to transplant only rarely develop EBV disease after transplant. However, this is not true of intestinal transplant recipients where EBV seropositivity (at least in pediatric patients) is not protective against development of disease. The prevention of EBV disease and PTLD is discussed in greater detail in Chapter 96.

BK virus

Attention and effort have also focused on the prevention of BKV-associated nephropathy (BKVAN) in kidney transplant recipients. In general, the use of surveillance measurement of BKV load to identify patients at risk of progression to BKVAN is supported for kidney recipients [134]. Measurement of BKV load in urine is attractive as it is non-invasive. However, in general, the presence of an elevated urine load does not predict progression to BKVAN. Accordingly, pre-emptive interventions usually require identification of an elevated BKV load in the blood. Current consensus favors the reduction of immunosuppression in response to persistent elevated loads [134]. While interest in the potential role of cidofovir, leflunomide, intravenous immunoglobulin, and ciprofloxacin for the treatment of subclinical BKV infection or disease exists, studies are limited and most experts are skeptical that any of these are efficacious.

Hepatitis B and C virus

Without preventative strategies, HBV commonly recurs in infected liver transplant recipients and may progress rapidly in the setting of immunosuppression. Prior to the availability of effective preventative strategies, recurrent HBV resulted in significantly poorer survival for patients transplanted for HBV as compared to those transplanted for another indication [135]. Before transplant, potential recipients with active hepatitis B should be treated as per current guidelines and lower viral loads at the time of transplant may reduce the risk or recurrence [136,137]. After transplant, combination therapy with hepatitis B immunoglobulin (HBIg) and antivirals has been shown to prevent recurrent hepatitis B in most recipients and is superior to either HBIg alone or lamivudine alone [137–141]. While most studies have used lamivudine as the antiviral agent, lamivudine resistance may develop and thus the use of combination therapy or antivirals with reduced potential for resistance is preferred [137]. Future research is likely to better define the efficacy of various antivirals or antiviral combinations. Most centers employ lifelong antiviral prophylaxis; shorter courses of HBIg, e.g. 1–6 months, may be adequate, particularly in patients with low or undetectable hepatitis viral loads at the time of transplant and no history of resistance [137,142]. Similar preventative strategies are commonly employed to prevent HBV transmission from HBV-infected donors into non-infected recipients. From the perioperative standpoint, it is important to vaccinate seronegative recipients preoperatively to reduce their risk of acquiring HBV at the time of transplantation. Donor-derived HBV is discussed in further detail in Chapter 92.

HCV recurs in most infected liver transplant recipients, and no widely accepted strategy for prevention or prophylaxis exists [137]. The development and licensure of more effective and easier-to-tolerate HCV therapies provides hope for improvements in treatment or prevention of recurrent HCV in the future.

Summary

Transplant recipients are subject to numerous infectious illnesses in the perioperative period. These relate closely to the recipient's co-morbid conditions at transplant and the type of transplant received. Some infections are rather typical, mimicking those seen in the non-transplant surgical setting, but the immunosuppressed state inherent in transplantation and exposure to a donor who may be an infectious vector can predispose the transplant recipient to unique opportunistic infections that require prompt recognition and management.

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CHAPTER 94

Late Infectious Disease after Organ Transplantation

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Introduction

The risk of infection following solid organ transplantation (SOT) has increased with the availability of new immunosuppressive agents and the prolonged duration of allograft and patient survival. As such, treatment of transplant-associated infections requires intervention not only with effective antimicrobial agents, but also specific consideration of the burden of immunosuppression imposed for prophylaxis against allograft rejection. This chapter will provide an understanding of the dynamic relationship between the intensity of immunosuppression and new prophylactic strategies, coupled with advances in microbiologic assays, and in doing so, provide the transplant clinician with the tools needed to prevent and to treat most opportunistic infections.

In considering the transplant patient generically, a few central concepts are useful in the assessment of infectious risk:

1 Recognition of infection is more difficult in transplant recipients than in individuals with normal immune function given the diminished signs and symptoms of infection and the array of non-infectious etiologies of fever, e.g. graft rejection and drug toxicity [1,2].
2 The spectrum of potential pathogens is broad; multiple pathogens may be present at the same time, e.g. viral and molds. Improved microbiologic assays are available to guide therapy for many common pathogens [3].
3 The risk for infection is related to the nature and intensity of immunosuppression used to prevent graft rejection [1,2].
4 Infection progresses rapidly in the immunocompromised host, increasing the importance of preventing infection.
5 Rapid and specific diagnoses are needed to guide antimicrobial therapies and to limit avoidable drug interactions and toxicities. *Invasive diagnostic procedures* are often required.

Currently, available assays can help guide the optimal dosing or combination of immunosuppressive medications so as to prevent graft rejection while avoiding infections, but no assay exists to specifically indicate which immunosuppressive approach is required for an individual patient. Significant clinical management and follow-up remain paramount in navigating the transplant recipient at risk for infectious illness.

Risk for infection and the timeline of infection

The risk of infection in the transplant recipient is largely determined by two closely linked factors:

1 The *epidemiologic exposures* of the patient and the organ donor, including those unrecognized by the patient or distant in time (Table 94.1) [2,4].
2 The patient's *"net state of immunosuppression,"* including all factors contributing to the risk for infection (Table 94.2) [2].

It is easiest to consider these factors as semi-quantitative measures that evolve over time. Thus, the measure of risk is comparable to measuring the area under the curve of a formula relating epidemiology and immune function: At higher levels of immunosuppression over time, infection occurs at lower levels of infectious "exposure" or with organisms of lower degrees of native virulence or "invasiveness." With lower levels of immunosuppression, infection is less common and drug toxicities are less frequent, but graft rejection is more prevalent. Specific immune deficits, and thus specific immunosuppressive regimens, predispose to infection with specific types of organisms, e.g. T-lymphocyte depletion activates latent cytomegalovirus (CMV) and allows viral replication to occur, while corticosteroids predispose to *Pneumocystis* infection [5]. Successful preventative strategies (prophylaxis or pre-emptive therapies) reduce the burden of potential pathogens or the ability to cause invasive infection, and allow greater intensity of immunosuppression. Thus, preventative strategies must be linked to the nature of immunosuppression and other risk factors for infection. Additional information on strategies for infectious disease prophylaxis can be found in Chapter 99.

Epidemiologic exposures

Exposures can be divided into four overlapping categories: donor-derived (see Chapter 92) or pre-existing recipient-derived infections, and community or nosocomially-derived exposures. These have been discussed in some detail in previous chapters. Some salient features will be emphasized here. Any infection in the transplant recipient that is not eradicated prior to initiation of immunosuppression may re-emerge later in the recipient's course.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 94.1. Significant epidemiologic exposures relevant to transplantation

- Donor-derived
- Viral:
  - Herpes group (Cytomegalovirus, Epstein–Barr virus, human herpesviruses 6, 7, and 8, herpes simplex, varicella zoster virus)
  - Hepatitis viruses (notably HBV and HCV)
  - Retroviruses (human immunodeficiency virus, human T-lymphotropic virus 1 and -2)
  - Others: West Nile virus, lymphocytic choriomeningitis virus, rabies
- Bacteria:
  - Gram-positive and Gram-negative bacteria (*Staphylococcus* spp., *Pseudomonas* spp., *Enterobacteriaceae*)
  - Mycobacteria (tuberculosis and non-tuberculous)
  - Nocardia species
- Fungi:
  - *Candida* species
  - *Aspergillus* species
  - Endemic fungi (*Cryptococcus neoformans*)
  - Geographic fungi (*Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Paracoccidioides* spp.)
- Parasites:
  - *Toxoplasma gondii*
  - *Trypanosoma cruzi*
  - *Strongylodes stercoralis*
  - *Leishmania* spp.
  - *Balamuthia* spp.
- Nosocomial exposures:
  - Methicillin-resistant staphylococci
  - Vancomycin-resistant enterococci (also linezolid and quinupristin–dalfopristin resistance)
  - Multidrug resistant Gram-negative bacilli
  - *Aspergillus* species
  - *Candida* non-*albicans* strains
- Community exposures:
  - Food and water borne (*Listeria monocytogenes*, *Salmonella* spp., *Cryptosporidium* spp., hepatitis A, *Campylobacter* spp.)
  - Respiratory viruses (respiratory syncytial virus, Influenza, parainfluenza, adenovirus, metapneumovirus)
  - Common viruses, often with exposure to children (coxsackie, parvovirus)
  - Other viruses (polyomavirus, papillomavirus)
  - Chikungunya virus
  - Atypical respiratory pathogens (*Legionella* spp., *Mycoplasma* spp., *Chlamydia*)
  - Geographic fungi and *Cryptococcus*, *Pneumocystis jiroveci*
- Parasites (often distant):
  - *Strongyloides stercoralis*
  - *Leishmania* spp.
  - *Toxoplasma gondii*
  - *Trypanosoma cruzi*
  - *Naegleria* spp.

Table 94.2. Factors contributing to the "net state of immunosuppression"

- Immunosuppressive therapy: type, temporal sequence, intensity
- Prior therapies (chemotherapy or antimicrobials)
- Mucocutaneous barrier integrity (catheters, lines, drains)
- Neutropenia, lymphopenia (often drug induced)
- Underlying immune deficiency (e.g. hypogammaglobulinemia from proteinuria, systemic lupus, complement deficiencies)
- Metabolic conditions: uremia, malnutrition, diabetes, alcoholism/cirrhosis
- Viral Infection (cytomegalovirus, hepatitis B and C, respiratory syncytial virus)

Recipient and donor-derived infections

Donor and recipient screening (see Chapter 92) are critical to the development of post-transplant preventative strategies [6,7]. The strategies selected will vary by patient; these might include avoiding corticosteroids in patients infected with hepatitis B virus (HBV), pretreatment with isoniazid in individuals with latent tuberculosis or ivermectin in patients from areas endemic for *Strongyloides stercoralis*, vaccination of seronegative recipients, e.g. varicella zoster virus (VZV), or empiric antifungal therapy in recipients of organs from donors with candiduria. Antiviral strategies for cytomegalovirus (CMV) or Epstein–Barr virus (EBV) are based on an individual assessment of risk for all transplant recipients based on serologic assays (discussed below).

Some donor-derived infections, e.g. *S. stercoralis*, tuberculosis or histoplasmosis, may manifest well beyond the peritransplant period. Similarly, colonization of donor lungs, e.g. *Burkholderia, Aspergillus* or *Cryptococcus* spp., or of the recipient's urinary or biliary tracts, e.g. with vancomycin-resistant enterococcus (VRE), may cause disease only in the presence of other immunomodulatory events such a treatment of graft rejection or concomitant viral infection. Recipient-derived exposures also generally reflect latent infections or colonization activated in the setting of immunosuppression and/or technical defects. The patient with incompletely treated pneumonia or peritonitis is likely to suffer a relapse when no longer receiving appropriate antimicrobial agents.

Community exposures

Recent and remote exposures may reactivate long after transplantation. Travel, new hobbies, young children, or work may provide exposures to contaminated food or water ingestion (*Listeria, Cryptosporidium*), soil (*Aspergillus or Nocardia*), birds (*Cryptococcus*), and geographically restricted systemic mycoses (*Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides* spp., and *Histoplasma capsulatum*), or respiratory virus infection with the risk for bacterial or fungal superinfections.

Nosocomial Exposures

Nosocomial infections are of increasing importance in transplant recipients (see Chapter 93). In part, this reflects the burden of organisms with significant antimicrobial resistance in medical centers. As patients wait longer for organs, they clinically deteriorate and have often undergone multiple courses of antimicrobial therapy and greater exposure to antimicrobial-resistant nosocomial pathogens. Among the common pathogens are VRE, methicillin-resistant *Staphylococcus aureus* (MRSA), fluconazole-resistant *Candida* spp., *Aspergillus* spp., and highly resistant Gram-negative bacteria [8]. Respiratory viral infections may be acquired from medical staff and should be considered among the causes of fever and respiratory decompensation among immunocompromised individuals.

Net state of immunosuppression

The net state of immunosuppression is a conceptual measure of all factors contributing to the patient's risk for infection (Table 94.2) [2]. Among these are:

- Specific immunosuppressive therapy, including dose, duration, and sequence of agents—an "area under the curve" of immunosuppression.
- Technical problems in the transplant procedure, resulting in leaks (blood, lymph, urine) and fluid collections, devitalized tissue, poor wound healing, and surgical drainage catheters.
- Prolonged airway intubation.
- Use of broad-spectrum antibiotics.
- Post-transplant renal, hepatic, pulmonary, or cardiac dysfunction or diabetes.
- Prolonged use of urinary, vascular access, or dialysis catheters or surgical drains, or other breaks in skin or mucosal defenses.
- Infection with one of the immunomodulating viruses, including CMV, EBV, HBV or hepatitis C virus (HCV), or human immunodeficiency virus (HIV). Note that most viruses, including

Table 94.3. Immunosuppression and infection: Common associations

- Antilymphocyte globulins (lytic depletion) and alloimmune response:
  - T lymphocytes: activation of latent (herpes)viruses, fever, cytokines
  - B lymphocytes: encapsulated bacteria
- Plasmapheresis: encapsulated bacteria
- Costimulatory blockade: unknown so far; possible increased risk for Epstein–Barr virus/ post-transplantation lymphoproliferative disease
- Corticosteroids: bacteria, *Pneumocystis jiroveci* (formerly *carinii*) pneumonia, hepatitis B, possibly hepatitis C
- Azathioprine: neutropenia, possibly papillomavirus
- Mycophenolate mofetil (MMF): early bacterial infection, B cells, late cytomegalovirus
- Calcineurin inhibitors: enhanced viral replication (absence of immunity), gingival infection, intracellular pathogens
- mTOR inhibitors:
  - Poor wound healing
  - Excess infections in combination with other agents
  - Idiosyncratic interstitial pneumonitis

community-acquired respiratory viruses, have local effects that predispose to superinfection.
- Malnutrition.
- Neutropenia, which may be related to medications such as mycophenolate mofetil (MMF), azathioprine, ganciclovir, and valganciclovir.

The impact of various combinations of immunosuppressive agents on immune function in an individual patient is often not predictable. The assessment of "immunity" to specific pathogens, e.g. cellular immune function or serum antibody levels against specific organisms, is a relatively recent set of research technologies. Serologic assays determine past exposures to various pathogens but are poorly predictive of the efficacy of the immune response to specific pathogens in the immunosuppressed host. Measures of "global" immune function remain relatively crude [9]. Individual drugs are associated with increased risk for certain infections (Table 94.3) [5]; combinations of agents may enhance this risk or cause toxicity, e.g. nephrotoxicity. Few data exist, for example, on the rate of immune reconstitution against infection after T- or B-lymphocyte depletion or treatment with other antibody preparations, e.g. antibodies to tumor necrosis factor and costimulatory blockade. The effects of organ dysfunction, e.g. cirrhosis and renal dysfunction, on systemic immune function resist quantification.

Among important "immune defects" are breaches in cutaneous barriers or innate immune function. It was noted that the combination of "sticky" organisms such as VRE or *S. aureus* from colonized skin with in-dwelling catheters or drains leads to increased risk for bacteremia. These effects are amplified by neutropenia caused by drug effects [ganciclovir, MMF, azathioprine, allopurinol, trimethoprim-sulfamethoxazole (TMP-SMX), and others]. Surgical, invasive radiologic, or gastrointestinal procedures employed to repair technical complications such as bile leaks or biliary or ureteric strictures, risk dissemination of organisms from those sites with distant seeding and abscess formation in joints or ischemic regions of grafts.

Timetable of infection

As immunosuppressive regimens became more standardized, the most common infections are observed in a relatively predictable pattern depending on the time elapsed since transplantation (Figure 94.1). This is a reflection of changing risk factors over time: surgery/ hospitalization, immunosuppression, acute and chronic rejection, emergence of latent infections, and exposures to novel community infections [1]. The pattern of infection changes with alterations in the immunosuppressive regimen (side-effects, intensification for graft rejection), intercurrent viral infection, neutropenia (drug toxicity), graft dysfunction, or significant epidemiologic exposures (travel or food). The pattern also will be changed by the use of prophylactic antimicrobial agents in that infections will appear later than "normal" when delayed by effective prophylaxis. This observation suggests that a reduction in the net state of immunosuppression is desirable over time so that infection is less likely when prophylaxis is discontinued [10,11].

The timeline reflects three overlapping periods of risk for infection: (1) the perioperative period, approximately 0–30 days after transplantation (see Chapter 93); (2) the period 1–6 months after transplantation (depending on the taper of immunosuppression and the specific type, dosing, and effect of antilymphocyte "induction," which may persist); and (3) the period beyond 6-–12 months after transplantation. These periods reflect the changing major risk factors associated with infection: (1) surgery and technical complications; (2) intensive immunosuppression with viral activation; and (3) community-acquired exposures with the return of normal activities.

The timeline may be used in a variety of ways: (1) to establish a differential diagnosis for the transplant patient suspected of having infection; (2) as a clue to the presence of an excessive environmental hazard for the individual, either within the hospital or in the community; and (3) as a guide to the design of preventative antimicrobial strategies. Infections occurring outside the usual period or with unusual severity suggest the presence of an excessive epidemiologic hazard or of excessive immunosuppression [1]. The *prevention* of infection must be linked to the *risk* for infection at various times after transplantation. Routine preventative strategies from the Massachusetts General Hospital are outlined in Tables 94.4 and 94.5. It should be noted that such strategies serve only to delay the onset of infection in the face of epidemiologic pressure. The use of antibiotic prophylaxis, vaccines, and behavioral modifications, e.g. routine hand washing or advice against digging in gardens without masks, may only result in a "shift to the right" of the infection timeline unless the intensity of immunosuppression is reduced or immunity develops.

First phase post transplantation (0–30 days)

The types of infection during the first month after transplantation are outlined in Chapter 93. These are often related to complications of the transplant surgery, infections present in the donor or recipient prior to transplantation, or nosocomial infections. These infections, unless eradicated, are a common source of difficulties later in the patient's course. Recurrent infections are most often related to technical issues (strictures, leaks, graft injury) that are often difficult to resolve. The risk for infection may be reduced with attention to early removal of lines and drains, discontinuation of unneeded medications including antimicrobial agents, and meticulous wound care. Notable for their absence in the first month after transplantation are opportunistic infections, even though the daily doses of immunosuppressive drugs are at their highest during this time. The implication of this observation is that sustained administration of immunosuppressive agents is generally required to allow organisms of little native virulence to cause invasive disease. Thus, early post-transplant *Pneumocystis* pneumonia is seen in patients treated with corticosteroids, e.g. for autoimmune disorders, in advance of transplantation in the absence of prophylaxis. Early *Aspergillus* infection is the result of colonization prior to surgery or to excessive

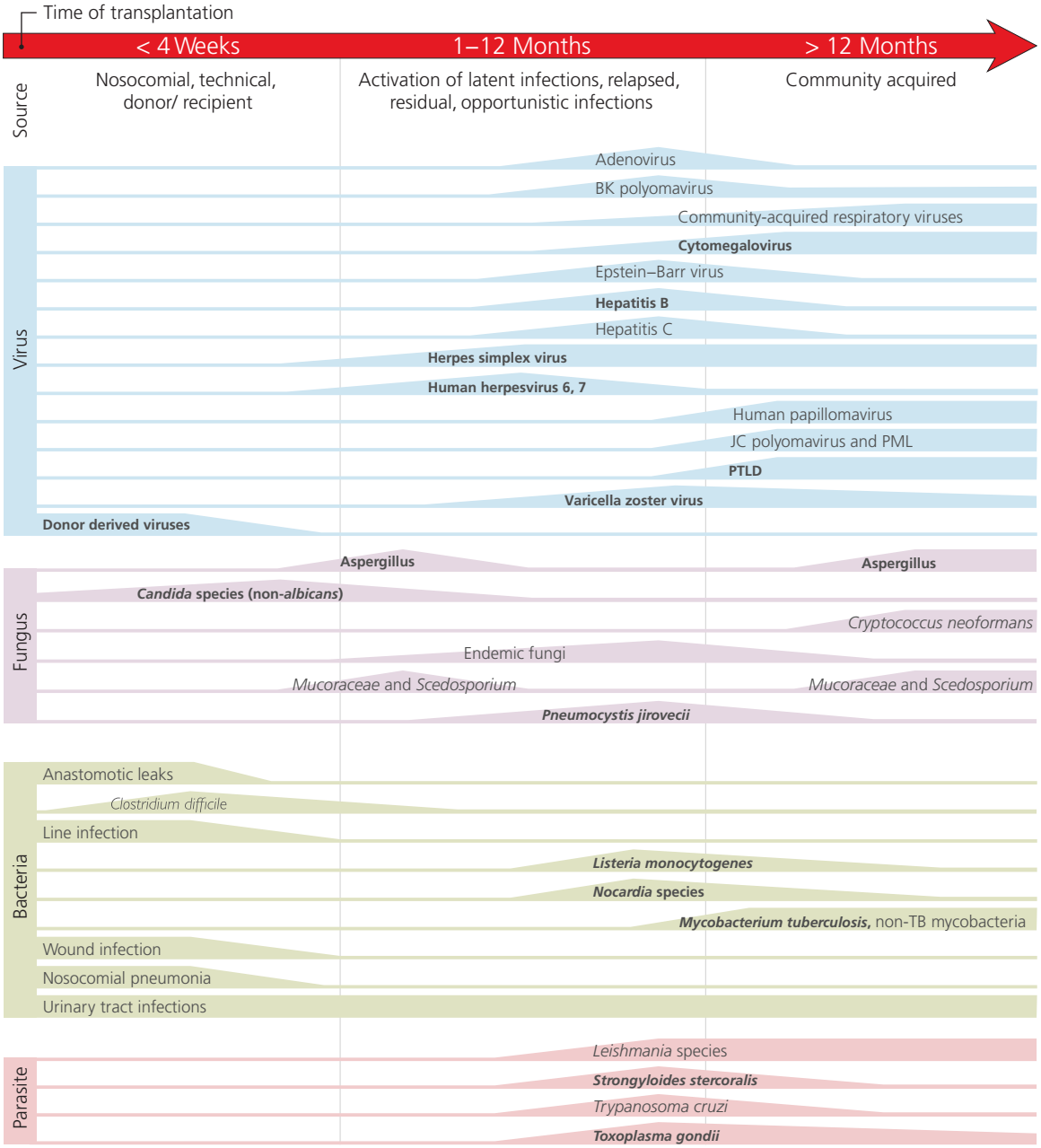

Figure 94.1. Timeline of infections following organ transplantation. The risk for infection following organ transplantation varies in a relatively standard pattern with the net state of immunosuppression and the epidemiology of infectious exposures. The potential pathogens for which the risk is modified by prophylaxis, including vaccinations and antimicrobial agents, are depicted in bold. Individual risk is modified by events such as the treatment of graft rejection or malignancy. Thickness of line indicates relative risk. Bold type indicates infections potentially preventable by prophylaxis. May be delayed until until prophylaxis is discontinued. Modified from Fishman [1] and Fisham and Rubin [2].
PML, progressive multifocal leukoencephalopathy, PTLD, post-transplantation lymphoproliferative disease.

environmental exposure in the hospital— in areas of construction or faulty air handling ducts, or in highly trafficked areas. Additional treatment of donor-derived and perioperative infections can be found in Chapters 92 and 93.

Second phase post transplantation (1–6 months)

There are multiple causes of febrile syndromes in the transplant recipient 1–6 months after transplantation. The routine use of anti-CMV therapy and TMP-SMX prophylaxis has altered the pattern of post-transplant infections. TMP-SMX eliminates *Pneumocystis jiroveci* (formerly *carinii*) pneumonia (PCP) and, given daily, reduces the incidence of urinary tract infection (UTI) and urosepsis, *Listeria monocytogenes* meningitis, some *Nocardia* spp. infections, and *Toxoplasma gondii* [12]. Notably, agents that may be substituted for TMP-SMX in the sulfa-allergic patient do not provide this benefit of prophylactic effects against these other

organisms. Effective anti-CMV prophylaxis should prevent most CMV infections (and those due to most herpesviruses) for the duration of therapy (discussed below). Thus, the differential diagnosis of infectious syndromes in this period includes:

- Graft rejection, particularly in calcineurin inhibitor (CNI)-sparing immunosuppressive regimens.
- Lingering infection from the perisurgical period, including relapsed *Clostridium difficile* colitis, inadequately treated pneumonia, or infection related to technical problems, e.g. pleural effusion, urine leak, cholangitis due to biliary ischemia, lymphocele, infected hematoma, airway necrosis, and dehiscence in lung transplant recipients.
- Nosocomial infections remaining from hospitalization.
- Viral infections including CMV, herpes simplex virus (HSV), shingles (localized or disseminated zoster due to VZV), human herpesvirus (HHV) 6 or 7, BK polyomavirus, EBV, relapsed hepatitis (HBV and HCV), and the community-acquired respiratory viruses [adenovirus, influenza, parainfluenza, respiratory syncytial virus (RSV), and metapneumovirus]. In transplant recipients, viral infection is generally more persistent and more often invasive than in normal hosts.
- Opportunistic infection due to *P. jiroveci*, *L. monocytogenes*, *T. gondii*, *Nocardia* spp., *Aspergillus* spp., and other agents. The specific opportunistic infections that occur reflect the specific immunosuppressive regimen used and the presence or absence of immunomodulating viral infection.

The herpesviruses are common and produce life-long infection for which the marker is seropositivity. The herpesviruses are prominent in transplantation due to the role of T-cell immune function in the control of these infections and the efficiency of transmission with allografts from seropositive donors. The herpesviruses tend, like other chronic viral infections, to produce systemic immunosuppression and yet, via cytokine production and other mechanisms, may predispose to graft rejection [13,14].

Table 94.4. Prophylaxis for *Pneumocystis jiroveci* pneumonia (PCP)

Background
Low dose trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis (in adults: one single-strength tablet/day orally) is well tolerated and essentially eradicates *Pneumocystis* infection from this patient population. Lower doses (3 days/week) prevent PCP but may not prevent other infections such as urinary tract infection, including those due to susceptible *Nocardia* and *Listeria*, toxoplasmosis, and a variety of gastrointestinal and pulmonary infections

Regimen
One single-strength TMP-SMX tablet (containing 80 mg of trimethoprim, 400 mg of sulfamethoxazole) p.o. q.h.s. for a minimum of 4–6 months post transplant. Patients infected with cytomegalovirus, with chronic rejection, recurrent infections, and most lung, liver, and heart recipients may be maintained on life-long prophylaxis

Alternative regimen
For patients proven not to tolerate TMP-SMX, alternative regimens include:

- A combination of atovaquone 1500 mg p.o. with meals once daily plus levofloxacin (or equivalent fluoroquinolone without antianaerobic spectrum) 250 mg once daily
- Pentamidine (300 mg IV or inhaled q 3–4 weeks)
- Dapsone (100 mg p.o. q.d to b.i.w.) ± pyrimethamine

Each of these agents has toxicities that must be considered, including hemolysis in glucose-6-phosphate-deficient hosts with dapsone. None of these alternative programs offers the same broad protection as TMP-SMX.

Table 94.5. Prophylaxis for herpes group viruses*

Background
The human herpes viruses are among the most important causes of infectious disease morbidity and mortality in the transplant recipient. Preventative regimens are determined by the clinical risk, the major determinants of which are the past experience of donor and recipient with the virus (as defined by the presence or absence of circulating antibody prior to transplant) and the nature of the immunosuppressive therapy.

CMV Universal Antiviral Prophylaxis

Cytomegalovirus (CMV) serologic status ± antilymphocyte globulin induction therapy (ALG)	Prophylaxis	Monitoring (antigenemia)
D+/R– no ALG	Intravenous ganciclovir 5 mg/kg IV (loading dose) then p.o. valganciclovir (900 mg/day corrected for renal function) or p.o. ganciclovir (3 g/day)† × 3 months	Monthly for 6 months after discontinuation of therapy*
D+ or R+ with ALG	Intravenous ganciclovir 5 mg/kg IV for first dose then either per renal function to discharge or switch to p.o. valganciclovir (900 mg/day corrected for renal function) or po ganciclovir (3 g/day)† × 6 months	Monthly for 6 months after discontinuation of therapy*
D–/R+ no ALG	Oral valganciclovir (900 mg/day corrected for renal function) × 3 months	Symptoms only
D–/R–	Oral famciclovir 500 mg p.o. q.d. × 3–4 months (or valacyclovir 500 mg b.i.d. or acyclovir 400 mg t.i.d) Use of CMV-negative or leukocyte-filtered blood	Symptoms, fever/neutropenia
Status unknown with ALG	Intravenous ganciclovir 5 mg/kg IV for first dose and q.d. (corrected for renal function) until sero-status determined	As above

Neutropenia: The dose of antiviral therapies are *not*, in general, reduced for neutropenia.
*ALG: antilymphocyte antibodies include any lytic, lymphocyte-depleting antibody preparations.
†Valacyclovir (8 g/day) has been used as an alternative agent in renal transplant recipients.

Pre-emptive therapy
Pre-emptive therapy requires a carefully organized monitoring program and patient compliance. Either a molecular CMV viral load test or a pp65 antigenemia assay may be used for monitoring. Monitoring should be performed once weekly after transplantation for 12–24 weeks. Infections indicated by positive assays are treated with either oral valganciclovir (900 mgb.i.d.) or intravenous ganciclovir (5 mg/kg b.i.d.). Full doses are used for loading after which dosing is corrected for renal function. Therapy is continued until viremia is undetectable.

Mixed prophylaxis
Many centers prefer universal prophylaxis for highest risk recipients (D+/R– or R+ with lymphocyte depletion) and pre-emptive therapy for other groups.

Background
The human herpes viruses are among the most important causes of infectious disease morbidity and mortality in the transplant recipient. Preventative regimens are determined by the clinical risk, the major determinants of which are the past experience of donor and recipient with the virus (as defined by the presence or absence of circulating antibody prior to transplant) and the nature of the immunosuppressive therapy.
CMV Universal Antiviral Prophylaxis

In this period, the stage is also set for the emergence of a subgroup of patients—the "chronic ne'er do wells," individuals who require higher than average immunosuppression to maintain graft function or who have prolonged, untreated viral infections and other opportunistic infections, which predicts long-term susceptibility to many other infections (discussed in the Third phase below) [2]. Such individuals may merit prolonged (life-long) prophylaxis (antibacterial and/or antiviral) and careful clinical and laboratory monitoring to prevent life-threatening infection.

Third phase post transplantation (>6–12 months)

Transplant recipients who are >6 months past the procedure can be divided into three groups in terms of infection risk. First, the majority of transplant recipients who have had a technically good procedure with satisfactory allograft function will have reduced maintenance immunosuppression and lower risk for reactivation of latent infections. These patients have community-acquired respiratory viruses as their major risk, which may mask or predispose them to more serious opportunistic infections. Occasionally, such patients will develop primary CMV infection (socially acquired) or infections related to underlying diseases, e.g. skin infections in diabetics.

Second, some recipients will develop relapsing viral infections with their outcome dependent upon the availability of effective antimicrobial therapy. The major challenges in this group include CMV, EBV, notably with antiviral resistance, persistent BK polyomavirus infection, HCV in liver recipients, and papillomavirus (skin warts). In the era in which there were few therapeutic options, the challenges in this group also included HBV and HIV. Emerging agents for HCV may alter the outcome of this infection also. These infections are associated with:

- end-organ damage, e.g. BK polyomavirus nephropathy, cryoglobulinemia, or cirrhosis from HCV;
- malignancy, e.g. post-transplantation lymphoproliferative disease (PTLD) due to EBV, and skin or anogenital cancer due to papillomaviruses;
- graft rejection may be provoked by these infections and also by attempts to reduce the intensity of immunosuppression to combat these infections.

The third group of patients has less than satisfactory allograft function due to poor technical outcomes, a poor quality allograft, or chronic graft rejection (cellular or humoral). Many of these patients also will suffer renal dysfunction due to the effects of chronic CNI toxicity. They also may suffer chronic viral infection, as described above. Thus, these recipients are over-immunosuppressed relative to the threshold for viral activation and also suffer inadequate graft function. These individuals are often identifiable early in the post-transplant course due to delayed or poor initial graft function, complications (bleeding, leaks, drug interactions, intensive care requirements), or recurrent disease. This subgroup of transplant recipients is often termed the "chronic ne'er do wells," who are at highest risk for opportunistic infection with pathogens such as *P. jiroveci*, *L. monocytogenes*, *Nocardia asteroides*, *Aspergillus* spp., and *Cryptococcus neoformans* [2]. Organisms more often associated with the chronic immune dysfunction of acquired immune deficiency syndrome (AIDS) (*Bartonella, Rhodococcus, Cryptosporidium,* and *Microsporidium* spp.) and invasive fungal pathogens (*Scedosporium*, *Zygomycetes*, the *Dematiaceae*, or pigmented molds) should also be considered in this group. Minimal clinical signs or symptoms merit careful evaluation in this group of "high-risk" individuals. This group may benefit from life-long TMP-SMX prophylaxis and, in the appropriate situation, life-long antifungal prophylaxis.

Selected infections and syndromes of importance to transplantation

General approaches

The spectrum of infection in the immunocompromised host is quite broad. Given the potential toxicity of antimicrobial agents and the need for rapid interruption of infection, *early and specific diagnosis* is essential in this population. Advances in diagnostic modalities, including advanced imaging and molecular microbiologic techniques, greatly assist in this process. However, the need for invasive diagnostic approaches cannot be over emphasized. While these tools carry some risk, the failure to achieve a specific diagnosis often necessitates broad, empiric therapy without clear endpoints for therapy. Initial, often empiric, therapy will target a broad range of potential pathogens with rapid narrowing of the antimicrobial spectrum as data become available.

A central consideration for the patient with an "infectious syndrome" is whether to reduce the intensity of immunosuppression as a part of therapy, risking graft rejection or an immune reconstitution syndrome (IRIS) [15]. Conversely, treatment of infection as if graft rejection were present may exacerbate infection. Given that infection and rejection are linked, such distinctions may be difficult. For example, recurrent HCV infection may increase the risk for liver graft rejection. Pneumonia, including aspiration, community-acquired respiratory viruses, CMV, and bacterial infections, will increase the rate of obliterative bronchiolitis in lung recipients [16–18]. CMV also contributes to cardiac and renal allograft rejection [19,20]. In practice, it is often possible to decrease the intensity of immunosuppression in the face of significant infection; however, as the patient improves, rejection may occur. The selection of the specific reduction in immunosuppression may depend upon the organisms isolated. Similarly, reversal of some immune deficits (neutropenia, hypogammaglobulinemia) may be possible with adjunctive therapies (colony stimulating factors or antibody) [21]. Co-infection with virus (CMV) is also an important consideration and merits additional therapy.

Cytomegalovirus

CMV remains an important pathogen in transplant recipients despite the availability of effective antiviral therapies [22,23]. CMV infection has multiple manifestations depending on the site of infection and the nature of the host response. The reservoir for latent infection appears to include cells of the monocyte lineage, but viral replication may occur in multiple differentiated cell types, including fibroblasts, epithelial and endothelial cells, and other parenchymal cells.

The manifestations of CMV infection have been traditionally termed "direct" and "indirect" effects [13]. More accurate terms might be "viremic/cytopathic" effects and "cellular/immunologic" effects. The common direct effects or clinical syndromes include:

- "CMV syndrome": fever and neutropenia syndrome with variable features of infectious mononucleosis, including hepatitis, nephritis, lymphadenitis, leukopenia, and/or thrombocytopenia;
- pneumonitis;
- gastrointestinal invasion with esophagitis, colitis, gastritis, ulcers, bleeding, or perforation;
- hepatitis, pancreatitis, myocarditis, or chorioretinitis;
- meningoencephalitis;

- hemolytic-uremic syndrome or microangiopathic thrombosis.

With the exception of chorioretinitis, the direct clinical manifestations of CMV infection usually occur 1 –6 months after transplantation in the absence of prophylaxis. Viremia and symptomatic infections are rare during effective antiviral prophylaxis [24] and so may be delayed until after cessation of prophylaxis and/or following intensification of immunosuppression, e.g. for rejection [25]. Chorioretinitis may occur at low levels of viral replication and generally occurs later in the transplant course. This is relatively uncommon in transplant recipients, in contrast to individuals with AIDS in whom retinitis is a major complication of CMV infection [26].

The clinical manifestations of the cellular and immunologic effects of CMV infection (discussed below) are the result of suppression of a variety of host defense mechanisms, predisposing to secondary invasion by such pathogens as *P. jiroveci*, *Candida*, and *Aspergillus* spp., and some bacterial infections. CMV infection also contributes to the risk for graft rejection, PTLD, HHV6 and HHV7 infection, and increased risk for death.

Patterns of transmission

Transmission of CMV in the transplant recipient occurs in one of three patterns: primary infection, reactivation infection, and superinfection.

Primary infection

The greatest risk for infection is in the setting of primary CMV infection, which occurs when seronegative individuals receive grafts from latently infected, seropositive donors [donor seropositive, recipient seronegative (D+R–)], with subsequent reactivation of the virus and systemic dissemination after transplantation [17,27]. Over 50% of these patients become viremic in the absence of prophylaxis, often without symptoms. Similarly, over 50% will become viremic after the cessation of antiviral prophylaxis, frequently (25–35%) with symptomatic disease. Primary CMV infection may also occur in seronegative individuals after transfusion or sexual contacts in the community. This disease may be severe. The greatest viral burden is thought to be in the allograft, notably as the source of infection in the D+/R–combination. The allograft is regarded as a privileged site for viral replication because the MHC-restricted, virus-specific, cytotoxic T cells have a decreased ability to eliminate virally infected cells in the presence of MHC mismatch between donor and recipient.

Reactivation infection

In reactivation infection, seropositive individuals reactivate endogenous virus after transplantation [donor seropositive or seronegative (D+ or D–), recipient seropositive (R+)]. When conventional immunosuppressive therapy is used without antilymphocyte antibody "induction" treatment, approximately 10–15% experience direct infectious disease syndromes with a higher rate with the use of induction antilymphocyte therapy or antilymphocyte therapy for the treatment of graft rejection. Up to 50% of these individuals are viremic, often without symptoms.

Superinfection

Virus derived from the donor may be reactivated in the setting of an allograft from a seropositive donor transplanted into a seropositive recipient (D+R+). Blood transfusions, even if leukocyte reduced, have a low rate (~4%) of transmission of CMV infection. Some centers use seronegative blood for seronegative recipients, while others use leukocyte-filtered blood. This observation gains importance in patients requiring significant transfusion in the perioperative setting.

Pathogenesis of infection

CMV activation occurs as the result of multiple factors, including the intensity of immunosuppression, notably pulsed-dose corticosteroids; the amount of virus in the graft; the use of lytic, T-cell depleting therapies; co-infections, notably with HHV6 and HHV7 with inflammation and fever (via proinflammatory cytokines including tumor necrosis factor (TNF)-α); and graft rejection (possibly via inflammation as well as injury to infected endothelial cells). The risk for viral activation is in the setting of intensified immunosuppression for graft rejection treated with antilymphocyte antibodies. The alloimmune response, while systemic and associated with proinflammatory cytokines and chemokines, most often targets the graft ,which is often the site of greatest viral load. While CMV activation may increase the risk for rejection, rejection will also increase the risk for viral replication. Thus, in an interesting study, Reinke et al. showed that 17 of 21 patients for whom biopsy revealed evidence of "late acute rejection" demonstrated a response to antiviral therapy [28]. Further, Lowance et al. demonstrated that the prevention of CMV infection also resulted in a lower incidence of graft rejection [20].

Cellular and immunologic effects

Seroconversion is a marker for the development of host immunity and correlates well with the presence of a cellular immune response. Control of CMV infection is largely via MHC-restricted, virus-specific, cytotoxic T-lymphocyte response ($CD8^+$ cells) [29–32]. $CD4^+$ lymphocytes play an important role in the maintenance of the cellular immune response and may directly kill some human CMV (HCMV)-infected cells [33]. The precise role of $\gamma\delta$T-cells remains to be defined [34,35]. Neutralizing antibody responses appear to be important clinically [36]. HCMV glycoprotein B (gB) is involved in cellular attachment and penetration by CMV and is a major target of neutralizing antibodies and a major component of recent HCMV vaccines [37]. HCMV interacts with Toll-like receptors (TLR9 and TLR3) to activate inflammatory cytokine pathways and costimulatory pathways [38]. Macrophages, dendritic cells, and natural killer (NK) cells participate in the innate immune response to CMV.

The cellular and immunologic effects of CMV (indirect effects) may be as important to the immunocompromised host as is invasive viral infection [30,39]. The mechanisms for these effects are complex and relate to viral strategies to evade the host's antiviral responses to allow HCMV-infected antigen-presenting cells (APCs) to travel throughout the host to spread virus [39–41]. HCMV infection blocks the differentiation of APCs from monocyte precursors, impairs APC migration, and blunts antigen presentation. HCMV gene products, e.g. UL18, inhibit NK cell activation by MHC class I molecules and suppress activating signals for cytotoxicity [42,43]. HCMV interferes with the normal $CD8^+$ T-lymphocyte MHC class I processing and presentation pathways to prevent recognition. Multiple homologs of human gene products (transmembrane G-protein-coupled, chemokine receptors, TNF receptor, regulators of complement activation) inhibit the recruitment of inflammatory cells and the killing of infected cells. CMV-encoded antiapoptotic gene products delay apoptosis of infected cells [44]. An HCMV-encoded Fc-gamma receptor may obscure viral antigens and thus protect infected cells from antibody-mediated cellular cytotoxicity [45]. These features alter many aspects of the innate and adaptive

immune response, predisposing to opportunistic infection when coupled with exogenous immunosuppression used to prevent graft rejection [30].

The bidirectional linkage between CMV infection or disease and graft rejection and injury has been observed in multiple clinical trials, including in cardiac, lung, liver, and kidney transplant patients [19,20,39,46]. Both symptomatic disease and asymptomatic infection stimulate alloimmune responses, as demonstrated in clinical trials with intensive, universal prophylaxis for CMV infection. One of the clearest links is the effect of TNF-α [47] during fever or rejection; it activates intracellular NF-κB. NF-κB translocates to the cell nucleus and controls the expression of the CMV major immediate-early (IE) promoter/enhancer required for the expression of CMV *IE* genes encoding the transcriptional regulatory proteins necessary for viral replication [48]. TNF-α and other inflammatory cytokines appear to contribute to cellular graft rejection. CMV also contributes to graft fibrosis, possibly by increasing elaboration of profibrotic and vasculopathic growth factors, such as tumor growth factor (TGF)-β, platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), vascular endothelial growth factor, and adhesion molecules, including intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 [49–57]. The profibrotic effect of CMV infection likely amplifies the effects of the immune and inflammatory injury that accompany alloimmune graft rejection [58,59]. Endothelial injury allowing adhesion of platelets to the basement membrane of blood vessels with release of PDGF is coupled with increased matrix metalloproteinase (MMP)-2 activity and reduced matrix gene expression, and results in increased smooth muscle cell migration, promoting vascular neointimal hyperplasia [60–62]. CMV-encoded inhibitors of cell death mediate reduction in CMV-infected smooth muscle cell apoptosis. Increased production of interleukin (IL)-8 increases systemic inflammation with activation of neutrophils, macrophages, and endothelial cells.

Diagnosis

Clinical management of CMV has evolved rapidly over the past 20 years with the advent of sensitive and quantitative assays for the diagnosis and management of CMV infections [63–65]. Serologic tests are useful prior to transplantation to predict risk, but are of little value after transplantation in defining clinical disease [this includes measurements of anti-CMV immunoglobulin M (IgM) levels]. Should a patient seroconvert to CMV, this is evidence that the patient has been exposed to CMV and has developed some degree of immunity [66]. However, seroconversion in transplantation is generally delayed and thus, not useful for clinical diagnosis. In the past, CMV cell cultures, often coupled with immunofluorescent detection, were useful but relatively too slow and insensitive for clinical utility [64]. Further, positive CMV cultures (or shell vial cultures) derived from respiratory secretions or urine of immunocompromised hosts is of little diagnostic value; many patients secrete CMV in the absence of invasive disease. The demonstration of CMV inclusions or a positive immunostain on tissue histology in the setting of a compatible clinical presentation is the "gold standard" for diagnosis of tissue-invasive CMV disease [23,67]. Biopsy-proven immunohistologic diagnosis remains essential in the setting of possible gastrointestinal disease. In transplant recipients with ulcerative colonic, gastric lesions, diffuse erythema, or bleeding, CMV must be considered. These individuals will often have low or undetectable viremia [23,68] and intracellular inclusions are uncommonly seen histologically.

The intensity of CMV infection has been linked to the risk for infection in transplant recipients. Thus, the gold standard for the management of CMV infections in transplantation has become a quantitative assay. Two types of quantitative assays have been developed: the molecular assays for viral DNA or RNA and antigen detection assays. Either assay can be used in management. The antigenemia assay is a labor-intensive, semi-quantitative fluorescent or immunoperoxidase-based assay in which circulating neutrophils are stained for CMV early antigen (pp65), which is taken up non-specifically as a measure of the total viral burden in the body [64,69,70]. The molecular assays [direct quantitative DNA polymerase chain reaction (PCR), hybrid capture, amplification assays] are highly specific and sensitive for the detection of viremia [63,64]. Most commonly used assays include plasma-based PCR testing and the whole blood hybrid capture assay, noting that whole blood and plasma-based assays cannot be directly compared as whole blood assays often yield higher viral loads [64,65,71,72]. World Health Organization (WHO) standards exist for the standardization of laboratory CMV testing [65]. Viral loads in the CMV syndrome are variable. The highest viral loads are often associated with tissue-invasive disease, with the lowest in asymptomatic CMV infection or in gastrointestinal or central nervous system (CNS) disease [23,73,74]. In these syndromes, the CMV assays are often *negative* and invasive (biopsy) diagnosis may be needed.

Assays play a central role in the prevention and treatment of CMV (Table 94.5). The schedule for screening is linked to the risk for infection. Thus, in the high-risk patient (D+/R– or R+ with antithymocyte globulins) after the completion of prophylaxis, biweekly or monthly screening may be performed to assure the absence of infection for an additional 3–6 months [23,75]. This combined prophylactic and pre-emptive strategy is common, but practices vary widely between centers and patient populations. There are few comparative data to guide practice, although consensus guidelines have been published [23,75]. In the patient being treated for CMV infection, the assays provide an endpoint (zero positivity) for therapy and the initiation of prophylaxis.

Prevention

Prevention of CMV infection must be individualized for immunosuppressive regimens and the patient. Two strategies are commonly used: (1) universal prophylaxis and (2) pre-emptive therapy [23,76]. Universal prophylaxis involves giving antiviral therapy to all "at-risk" patients beginning at or immediately post transplant for a defined time period. In pre-emptive therapy, quantitative assays are used to monitor patients at predefined intervals to detect early disease. Positive assays result in therapy. Pre-emptive therapy incurs extra costs for monitoring and coordination of outpatient care while reducing the cost of drugs and the inherent toxicities of drug exposure [77]. Prophylaxis has the possible advantage of preventing not only CMV infection during the period of greatest risk, but also diminishing infections due to HSV, VZV, HHV6, HHV7, and EBV [27]. Further, the indirect effects of CMV, i.e. graft rejection, opportunistic infections, and mortality, are also reduced by routine, universal prophylaxis [27]. In practice neither strategy is perfect. Both breakthrough disease and ganciclovir resistance have been observed in both approaches [78–80]. Increasingly, "late" disease has been observed after the completion of prophylaxis [81,82]. The rate of late disease varies, but is thought to be as high as 17–37% in D+/R– recipients. The IMPACT study has confirmed the prolongation of prophylaxis to 200 days (vs. 100 days) in D+/R– renal recipients [11].

Given the risk for invasive infection, heart, liver, pancreas, and kidney transplant patients at risk for primary infection (CMV D+/R–) are generally given prophylaxis for 3–6 months after transplantation (Table 94.5) [23,75]. Many centers utilize 6 months of prophylaxis in CMV D+/R– or R+ patients receiving antilymphocyte antibodies [11]. Lung transplant recipients appear to benefit from prolonged (12 months) prophylaxis, as shown in a recent randomized trial of 3 versus 12 months of valganciclovir after lung transplantation [10]. Other groups are candidates for pre-emptive therapy *if* an appropriate monitoring system is in place and patient compliance is adequate [23,75]. The routine addition of CMV hyperimmune globulins on a monthly basis for 3–6 months has been used in some cardiac and lung transplantation programs [23,75,83,84].

Treatment

The standard of care for treating CMV disease is generally 3 weeks of therapy with intravenous ganciclovir (5 mg/kg twice daily, with dosage adjustments for renal dysfunction) or valganciclovir (900 mg p.o. twice daily corrected for renal function) [23,75]. In a recent clinical trial, oral valganciclovir was shown to be non-inferior to intravenous ganciclovir for the treatment of patients with mild-to-moderately severe CMV disease with modest viral loads [85]. This trial may have excluded patients with high viral loads and invasive gastrointestinal disease. Clearance of viremia within 21 days in both arms was approximately 50% [85]. In patients slow to respond clinically or virologically to therapy and who are seronegative, the addition of 3 months of CMV hyperimmune globulin in seronegative individuals (150 mg/kg/dose IV monthly) is used in some centers. Hypogammaglobulinemia merits correction during therapy [86]. Relapse does occur, primarily in those not treated beyond the achievement of a negative quantitative assay [85] and in some of those with gastrointestinal disease treated with an oral regimen. In practice, it is reasonable to initiate therapy with intravenous ganciclovir, monitor weekly to assure a response, and treat until monitoring is negative. Such patients may benefit from 2– 4 months of oral valganciclovir (900 mg daily based on creatinine clearance) administered as secondary prophylaxis after the completion of intravenous therapy [87,88]. This approach has resulted in rare symptomatic relapses and has been associated uncommonly with the emergence of antiviral resistance. It may be worth measuring the creatinine clearance to assure adequate dosing.

Ganciclovir resistance in CMV is generally uncommon with some higher rates reported in lung transplant recipients [78]. The risk for resistance is greatest in D+/R– recipients with higher viral loads, inadequate dosing of prophylactic or therapeutic ganciclovir, more intensive immunosuppression including antilymphocyte antibody induction, and prolonged antiviral prophylaxis [78,79]. Clinically, the patient's viral load or clinical syndrome fails to respond to appropriate therapy. Genetic resistance testing is useful in managing resistant CMV infection; mutations in the viral *UL97* (thymidine kinase) or *UL54* (DNA polymerase) genes can confer ganciclovir resistance [89]. Some of the common mutations in the *UL97* gene respond to higher doses of intravenous ganciclovir [90]. Combined mutations (*UL97* and *UL54*) may manifest high-level resistance to ganciclovir. Alternative therapies are available in intravenous form only. These include foscarnet and cidofovir [78]. Foscarnet is active against many ganciclovir-resistant strains of CMV, although it is associated with marked magnesium and potassium wasting, seizures (notably with CNI therapy), and some renal toxicity. Cidofovir often incurs significant nephrotoxicity and ocular toxicity. Combination therapy (ganciclovir and foscarnet) may be useful as is the addition of hyperimmune globulins [91]. Most centers try to reduce overall immunosuppression during the course of therapy. *UL54* mutations may cause resistance to foscarnet and to cidofovir depending on the nature of the mutation [89]. Multiple courses of antiviral therapy may be needed to cure resistant CMV infection. Given the toxicity of available medications, several investigational drugs are under study that may alter recommended therapies for antiviral-resistant CMV in the future [92–94].

Post-transplant lymphoproliferative disorder and Epstein–Barr virus

PTLD is a spectrum of syndromes characterized by lymphocyte proliferation from benign, polyclonal conditions to malignant, monoclonal lymphomas [95–98]. The majority of PTLD is associated with infection by EBV with B lymphocytes as the primary target. In immunosuppressed transplant recipients, primary EBV infection (and relapses in the absence of antiviral immunity) causes a mononucleosis-type syndrome, generally presenting as a B-cell lymphocytosis with or without lymphadenopathy or pharyngitis. Meningitis, hepatitis, and pancreatitis may also be observed. Remitting–relapsing EBV infection is common in children and may reflect the interplay between evolving antiviral immunity and immunosuppression. This syndrome should suggest relative over-immunosuppression. The most clearly defined risk factor for PTLD is primary EBV infection that increases the risk for PTLD by 10–76 fold [95]. PTLD may occur, however, in the absence of EBV infection or in seropositive patients.

Post-transplant non-Hodgkin's lymphoma (NHL) is a common complication of SOT. Lymphomas comprise up to 15% of tumors among adult transplant recipients (51% in children) with mortality of 40–60%. Compared with the general population, PTLD has increased extranodal involvement, poor response to conventional therapies, and poor outcomes. The spectrum of disease ranges from benign, polyclonal B-cell infectious mononucleosis-like disease to malignant, monoclonal lymphoma. The majority is of B-cell origin. T-cell, NK-cell and null-cell tumors occur in up to 37% of some adult series and are more often monomorphic and carry a worse prognosis. Other negative prognostic indicators include CNS disease, disease in multiple body sites, EBV-negative PTLD, disease of recipient origin, and the presence of mutations in proto-oncogenes or tumor suppressor genes [95,99–101].

The diagnosis of PTLD is complicated by the presence of EBV-negative PTLD or the presence of T-cell PTLD within allografts, which is easily confused with graft rejection or other viral infections. EBV-negative PTLD is more common late (>1–2 years) after transplantation in adults [95].

In addition to primary EBV infection, risk factors for PTLD include CMV donor–recipient serostatus mismatch, T-cell depletion therapies, younger age in children and older age in adults, and the overall intensity of immunosuppression [95]. PTLD can present at any time post transplantation, with primary infection common in the first 12 weeks post transplantation. Some risk is associated with the specific organ transplanted, although this may reflect the burden of virus, the presence of co-infections, and the intensity of immunosuppression. Thus, intestinal transplants in children carry a 32% incidence of PTLD, while the incidence in heart, lung, liver, and pancreas transplants is lower (3–12%) and in kidney transplants is even lower (1–2%) [95].

The clinical presentations of EBV-associated PTLD vary:

- Unexplained fever (fever of unknown origin).
- A mononucleosis-type syndrome, with fever and malaise, with or without pharyngitis or tonsillitis (often diagnosed incidentally in tonsillectomy specimens). Often no lymphadenopathy is observed.
- Gastrointestinal bleeding, obstruction, and perforation.
- abdominal mass lesions (most often with abdominal transplantation).
- Infiltrative disease of the allograft.
- Hepatocellular or pancreatic dysfunction.
- Isolated CNS disease or meningitis.
- Pulmonary infiltrative lesion.

Diagnosis

Serologic testing is not useful for the diagnosis of acute EBV infection or PTLD in transplantation. However, seronegative recipients of seropositive organs (EBV D+/R–) should be monitored for EBV infection and symptoms consistent with such infection. Any episodes of "rejection" should have biopsy tissues evaluated for evidence of PTLD. Quantitative EBV viral load testing is extremely useful in the diagnosis and management of EBV-positive PTLD [102–104]. These assays have high sensitivity but lower specificity. Some centers prefer assays using unfractionated whole blood rather than plasma samples for EBV viral load surveillance [105]. Serial assays are more useful in an individual patient than specified viral load measurements [102]. These assays are not standardized and cannot be directly compared between centers [105]. WHO standards for laboratory testing can be used for proficiency testing. In general, tissue diagnosis is essential for the diagnosis and management of PTLD. Tissues should be assessed by expert pathologists given the potential for confusion of infiltrative lesions with other processes [100]. Tissue or circulating lymphocytes should be tested using RNA in-situ hybridization for EBV-encoded small nuclear RNA (EBER) as a more sensitive assay than hybridization targeting viral DNA [106]. This is generally supplemented with immunohistochemistry for EBV latent antigens EBNA-1, EBNA-2, and LMP-1 [107,108]. These can be supplemented with studies of lymphocyte antibody clonality, oncogenes, tumor suppressor genes or chromosomes, therapeutic target markers (expression of CD20, cytotoxic T-cell epitopes), and donor or recipient origin.

Management

Clinical management depends on the stage of disease. In the polyclonal form, particularly in children, re-establishment of immune function through reduction of immunosuppression may suffice to cause PTLD to regress [95]. At this stage, it is possible that antiviral therapy might have some utility given the viremia and role of EBV as an immunosuppressive agent [103,109]. Given the role of CMV as a co-factor to PTLD, it is reasonable to use ganciclovir or valganciclovir as a prophylactic agent in EBV viremic individuals. Definitive data are limited regarding the efficacy of such therapy. With the progression of disease to extranodal and monoclonal malignant forms, reduction in immunosuppression may be useful, but alternate therapies are often required. Combinations of anti-B-cell therapy (anti-CD20, rituximab), chemotherapy (CHOP), and/or adoptive immunotherapy with stimulated T cells have been utilized [95,110,111]. The duration of response is often disappointing. In transplantation, the failure to regress with significant reductions in immunosuppression may suggest the need to sacrifice the allograft for patient survival. Rejection is uncommon in the face of active viremia, but some deaths have been associated with allograft failure after withdrawal of immunosuppression during treatment of malignancy [96]. CNS PTLD generally requires irradiation for resolution [111]. Biopsies are required to establish the diagnosis in the absence of extraneural disease.

Polyomaviruses

Polyomaviruses have been identified in transplant recipients in association with nephropathy and ureteral obstruction (BK virus) and in association with demyelinating disease of the brain (JC virus) similar to that in AIDS [112]. Adult seroprevalence is 65–90%. BK virus appears to achieve latency in renal tubular epithelial cells. JC virus has also been isolated from renal tissues but appears to have preferred tropism for neural tissues. Reactivation occurs with immunodeficiency and suppression and tissue injury, e.g. ischemia–reperfusion.

BK polyomavirus infection

BK virus (BKV) is associated with a range of clinical syndromes in immunocompromised hosts: viruria and viremia, ureteral ulceration and stenosis, and hemorrhagic cystitis [112,113]. Active infection of renal allografts has also been associated with progressive loss of graft function in some individuals and is termed "BK nephropathy" or polyomavirus-associated nephropathy (PyVAN or PVAN). Despite viral replication and viruria, PVAN is rarely recognized in recipients of non-renal organs, suggesting renal injury, e.g. ischemia–reperfusion, or other processes specific to renal transplantation are needed to cause nephropathy. Uncommonly, BKV may also cause pneumonitis, hemophagocytic syndrome, encephalitis, or polyomavirus-associated multifocal leukoencephalopathy (PVML, most often associated with JC virus) [114,115].

The clinical presentation of disease usually includes detection of cells in the urine in the absence of bacterial or fungal infection, reflecting shedding of infected tubular and ureteric epithelial cells. These cells contain sheets of virus and are detected by urine cytology as "decoy cells" [116,117]. In most cases, in the absence of screening, such cells are not detected and the patient presents with diminished renal allograft function or, less commonly, with ureteric stenosis and obstruction. In such patients, the etiologies of decreased renal function must be carefully evaluated, e.g. mechanical obstruction, drug toxicity, pyelonephritis, rejection, thrombosis, and recurrent disease, and choices must be made between *increasing* immunosuppression to treat suspected graft rejection or *reducing* immunosuppression to allow the immune system to control infection. Patients with BK nephropathy treated with increased immunosuppression have a high incidence of graft loss [112,118]. Reduced immunosuppression may stabilize renal allograft function [119] but risks graft rejection. Polyoma-associated nephropathy manifested by characteristic histologic features and renal dysfunction is found in about 1–8% of transplant patients [112].

Approximately 30–50% of renal recipients with high-level viruria can be expected to progress to PVAN. Risk factors may include ischemia–reperfusion injury, lack of BKV-specific immunity, overall intensity of immunosuppression including use of tacrolimus-based regimens, HLA mismatches, acute rejection including pulsed-dose steroids, and T-cell depletion for treatment of rejection [112]. The role of specific immunosuppressive agents has not been confirmed [120].

Screening, prevention, and diagnosis

BKV infection is often asymptomatic. Tubular epithelial cell damage and inflammation are reflected in a slowly rising serum creatinine and this is the first clinical sign of PVAN. Most centers have developed screening programs to document early disease [118,120,121]. The use of urine cytology to detect the presence of infected decoy cells in the urine has approximately 100% sensitivity for BKV infection but a low (29%) predictive value. Decoy cells are useful in screening but cannot establish a firm diagnosis [117]. Urine BKV can be detected by electron microscopy; urine BKV (DNA) loads of >7 log gEq/mL or BKV VP1 mRNA of >6 log copies/ng total urine RNA are useful diagnostically. Hirsch et al. showed that patients with BK nephropathy have a statistically significantly higher plasma viral load (>7700 BKV copies/mL of plasma; $P < 0.001$; 50% positive predictive value, 100% negative predictive value) when compared to patients without such disease [122]. A high serum viral load coupled with an elevated serum creatinine is generally considered a basis for reduction in immunosuppression, but diagnosis should be made by demonstration of BKV cytopathic changes in the renal allograft and by immunohistology for BKV proteins, or by in-situ hybridization for BKV nucleic acids in a renal biopsy [112]. PVAN is characterized by intranuclear polyomavirus inclusion bodies in tubular epithelial and/or glomerular cells. Renal biopsies will generally demonstrate cytopathic changes in renal epithelial cells, with the gradual evolution of cellular infiltration consistent with the diagnosis of interstitial nephritis. Fibrosis is often prominent, occasionally with calcification. For immunohistochemistry, cross-reacting antibodies against the large T antigen of the Simian virus 40 or antibodies against BKV VP1 or agnoprotein have been used [117]. PVAN is often focal and results in false-negative biopsies, in some cases necessitating rebiopsy in suspected cases. Graft rejection may accompany PVAN, and complicates both diagnosis and management. There is a semi-quantitative scoring system for the histologic changes of PVAN [117].

Recommendations regarding screening for BKV infection vary, but generally suggest testing once every 3 months during the first 2 years after transplantation, and at least annually for years 2–5 [112,118]. A urinary test for BKV (cytology for decoy cells or urine BKV loads of >7 log gEq/mL) is adequate for screening; if negative the risk for PVAN is quite low. Patients with high urinary BKV loads should be tested for plasma BKV DNA load [118]. Some centers screen using plasma BKV DNA loads. For patients with plasma BKV viral DNA loads of >4 log10 gEq/mL on duplicate testing 2–3 weeks apart, a presumptive diagnosis of PVAN should be made and immunosuppression reduced (see below). If screening is performed by plasma viral load, the interval between screening assays should be reduced to monthly for the first 6 months post transplant. This reflects reduced time before permanent renal injury in patients with viremia compared with urinary excretion. For patients with a rise in serum creatinine and positive BKV screening, a renal biopsy should be obtained [112].

Treatment

There is no accepted treatment for PVAN other than reduction in the intensity of immunosuppression [112,119,123]. It is possible to monitor the response to such maneuvers using plasma viral load measurements. Despite controversy, it is reasonable to reduce dosing of both CNIs and antimetabolites in a step-wise fashion while monitoring BKV plasma loads. Given the toxicity of CNIs for tubular cells, the role of injury in the activation of BKV, as well as the need for anti-BK T-cell activity, these agents should be included in initial reductions. General targets include tacrolimus trough levels of <6 ng/mL, cyclosporine trough levels of <150 ng/mL, sirolimus trough levels of <6 ng/mL, and/or MMF daily dose equivalents of ≤1000 mg. Regardless of the approach, renal function (at least one to two times per week), drug levels, and viral loads (alternate weeks), must be monitored carefully during reductions [112]. Rebiopsy may be needed for poor responses.

The use of adjunctive antiviral therapies remains controversial. Some centers advocate the use of cidofovir [124] in low doses (0.25–1 mg/kg every 2 weeks) for BK nephropathy. Of note, significant renal toxicity may be observed with this agent, especially when used in combination with the CNIs. Some data exist to support the use of alternative therapies in carefully selected individuals. These include leflunomide, an immunosuppressive agent with some antiviral activity for BKV, intravenous immunoglobulin, and fluoroquinolone antimicrobials [125,126]. Prospective clinical trial data and Food and Drug Administration (FDA) approval are lacking for these agents for this indication. These agents are best used in consultation with experienced transplant infectious disease specialists.

Retransplantation has been successful in PVAN patients with failed allografts, possibly as a reflection of immunity developing subsequent to reduction in immunosuppression [127,128]. Most centers allow retransplantation after immunosuppression has been discontinued for some period (6 months) and BKV is undetectable in blood and low in urine. In the future, measurements of BKV-specific cellular immunity after discontinuation of immunosuppression may help to determine the optimal time for retransplantation [129]. Surgical removal of the allograft does not protect against future BKV infection or PVAN, but may be needed if immunosuppression cannot be reduced (double transplants, allosensitization) and/or elevated viral loads persist.

JC virus

Infection of the CNS by JC polyomavirus has been observed uncommonly in transplant recipient as progressive multifocal leukoencephalopathy (PML) [130]. This infection may present with focal neurologic deficits or seizures, as well as with more slowly progressive neurologic lesions, and may progress to death following extensive demyelination. PML may be confused with calcineurin neurotoxicity; both may respond to a reduction in drug levels. No proven therapies exist for PML, although a reduction of immunosuppression is commonly employed, based on the analogy to immune reconstitution in AIDS patients with PML.

Fungal infections

Transplant recipients are at risk for opportunistic infection with a variety of fungal agents, the most important of which are *Candida* spp., *Aspergillus* spp., *C. neoformans,* and the endemic mycoses or geographic fungi [131–134]. With prolonged survival and epidemiologic exposures, many additional species have been observed [135].

Candida species

The most common invasive fungal pathogen in transplant recipients is *Candida*, with *C. albicans, C. tropicalis*, and *C. glabrata* accounting for approximately 90% of these infections [134]. While the exact distribution of *Candida* infections and the incidence of fluconazole resistance varies by institution, most centers report an increased detection of non-*albicans Candida* species in transplant recipients. Mucocutaneous candidal infection, e.g. oral thrush,

esophageal infection, cutaneous infection at intertriginous sites, and candidal vaginitis, occurs particularly in the setting of poorly controlled diabetes with immunosuppression, as a result of poor oral hygiene, and with broad-spectrum antibacterial therapy. These infections are usually treatable through correction of the underlying metabolic abnormality and topical therapy with clotrimazole or nystatin. A special problem in renal transplant recipients is candiduria, which is often asymptomatic [136,137]. Particularly in individuals with poor bladder function, obstructing fungal balls can develop at the ureteropelvic junction, resulting in obstructive uropathy, ascending pyelonephritis, and the possibility of systemic dissemination.

Candidemia is most common as a nosocomial infection in the early post-transplant setting in association with surgery, vascular access catheters, surgical drains, bladder catheters, antimicrobial use, peripheral hyperalimentation, and diabetes [134,138]. However, in liver recipients with biliary and anastamotic issues, candidemia may be observed as the result of antibacterial therapy for persistent cholangitis. In such liver transplant recipients with a choledochojejunostomy, the risk for *Candida* infection is increased over duct-to-duct anastomoses. Bile leaks and hematomas may be superinfected with *Candida* species. Pancreas transplant recipients have a high rate of operative infection due to yeasts with fluconazole prophylaxis generally employed [139]. Longer term, enteric drainage is associated with a greater risk of invasive candidiasis than is bladder drainage. Other factors associated with candidemia include kidney and liver allograft dysfunction, intensive care unit (ICU) stays, large volume blood transfusions, re-exploration surgery after abdominal transplantation, graft pancreatitis, parenteral hyperalimentation, colonization, and broad-spectrum antimicrobial therapy [134] Infections are generally diagnosed based on blood cultures. Positive blood cultures merit therapy in all cases in addition to removal of foreign bodies, including vascular access catheters. Delay in therapy is associated with significant mortality. All *Candida* isolates from sterile sites should be tested for susceptibility to antimicrobial therapy to assure successful responses as fluconazole-resistant species such as *C. glabrata* and *C. krusei* are increasingly common [140]. A retinal examination should be obtained to exclude retinitis. It should be noted that pulmonary candidiasis is very rare, while colonization of the pharynx is common. The sole exception to this rule is the tracheal anastomosis of lung recipients where direct visualization is possible [141]. *Candida* spp. may also superinfect esophageal lesions due to HSV or CMV. Vascular anastomotic infections may lead to the development of mycotic aneurysms with risk for rupture. These events have, in some studies, been associated with contamination of graft preservation fluid with *Candida* spp. [142]; distinction of contamination of preservation fluid from surgical contamination at the time of procurement is often difficult.

Aspergillus species

Invasive aspergillosis is a medical emergency in the transplant recipient. The portal of entry is generally the lungs and sinuses. Metastatic spread to the CNS is common and may define the clinical presentation. Early in the course of transplantation, when most *Aspergillus* infections are observed, CNS involvement in fungal infection is most often due to *Aspergillus* spp.; >1 year after transplantation, other fungi (zygomycetes, dematiaceous fungi) are increasingly prominent [143,144]. The incidence of invasive aspergillosis varies from 1% to 15% depending on the organ transplanted [145]. General risk factors include colonization, CMV infection, neutropenia, graft rejection with therapy, ICU stays, renal dysfunction, and requirement for dialysis [133]. In addition organ-specific risk factors include re-exploration (liver and lung), HCV infection, retransplantation (liver), tracheal ischemia, and acquired hypogammaglobulinemia (lung) [146]. Special considerations include the role of *Aspergillus* in infection of tracheal anastomoses in lung transplantation [141] and in patients suffering neutropenia due to viral infections, drug toxicities, or following combined hematopoietic stem cell and solid organ transplantation.

Early diagnosis is essential to a successful outcome—often requiring biopsy. Detection of *Aspergillus* in airway samples carries a high risk for invasive infection in heart, lung, and liver recipients. The utility of the galactomannan test for the early diagnosis of invasive aspergillosis has not been as great as for neutropenic individuals [147]. The use of the galactomannan assay for the diagnosis of invasive aspergillosis in transplant recipients has been most useful in bronchoalveolar lavage samples [148]. Tissue or culture diagnosis of *Aspergillus* is essential given that other molds, with differing susceptibility patterns to antifungal agents, may also infect this population. Each of the effective antifungal agents has limitations. Liposomal amphotericin carries some risk of nephrotoxicity in association with CNI therapy; voriconazole and other azoles have significant interactions that increase serum levels of CNIs and sirolimus; the echinocandins are approved but static for molds, may carry some hepatotoxicity, and have no activity against the zygomycetes and *Cryptococcus* spp.

Central nervous system infection and *Cryptococcus neoformans*

CNS infection in the transplant recipient is a medical emergency. The spectrum of causative organisms is broad and the outcome of infection is often related to the rapidity of treatment. Many infections are metastatic to the CNS, often from the lungs. Thus, a "metastatic work-up" is a component of evaluation of CNS lesions, including those due to *Aspergillus, Cryptococcus*, and *Nocardia* spp, or *S. stercoralis*. Viral infections include CMV (nodular angiitis), herpes simplex meningoencephalitis, JC virus (PML), and VZV. Common bacterial infections include *L. monocytogenes*, mycobacteria, *Nocardia* spp., and occasionally *Salmonella* spp.. Brain abscess and epidural abscess may be observed with MRSA, penicillin-resistant *Streptococcus pneumoniae*, and quinolone-resistant streptococci, all of which are problematic therapeutically. Metastatic fungi include *Aspergillus* and *Cryptococcus*, but also spread from the sinuses (*Mucoraceae*), skin (*Dematiaceae*), and bloodstream (*Histoplasma* and *Pseudallescheria/Scedosporium, Fusarium* spp.). Parasites include *T. gondii* and *Strongyloides*.

Given the spectrum of etiologies, precise diagnosis is essential. In particular, empiric therapy must "cover" *Listeria* (ampicillin), *Cryptococcus* (fluconazole or amphotericin), and HSV (acyclovir or ganciclovir), as well as the more common forms of bacterial meningitis (pneumococcal, *Haemophilus influenzae*) and known colonizing pathogens of the lungs while awaiting data from lumbar puncture, blood cultures, and radiographic studies. Included in the differential diagnosis are non-infectious etiologies, including CNI toxicity, lymphoma, as well as metastatic cancer. Biopsy is often needed for a firm diagnosis.

Cryptococcus neoformans

Cryptococcal infection is common, but rarely seen in the transplant recipient until >6 months after transplantation and generally >16

months post transplantation [132]. In the relatively intact transplant recipient, the most common presentation of cryptococcal infection is that of an asymptomatic pulmonary nodule, often with active organisms present. In other patients, meningitis or pneumonia or skin involvement at sites of tissue injury (catheters), cellulitis, nodular or papular lesions may be observed. Cryptococcosis should be suspected in transplant recipients who present with unexplained headaches (especially when accompanied by fevers), decreased state of consciousness, failure to thrive, pneumonia, or unexplained focal skin disease (which requires biopsy for culture and pathologic evaluation) more than a year after transplantation [132]. Risk factors include exposures to bird and bat excreta, donor-derived infectious infections, and T-cell depletion. Liver recipients are at the greatest risk among transplant recipients.

Diagnosis is often achieved by serum cryptococcal antigen detection, but all such patients should have a lumbar puncture for cell counts, cultures, cerebrospinal fluid (CSF) pressure measurement, and cryptococcal antigen studies. Initial treatment response is best with lipid amphotericin and 5-flucytosine followed by high-dose fluconazole until the cryptococcal antigen is cleared from blood and CSF. Life-long suppression is recommended with fluconazole. IRIS or scarring may cause obstruction with increased CSF pressure and hydrocephalus [15].

Strongyloides stercoralis

S. stercoralis infection may activate >30 years after initial exposure with immunosuppressive therapy. Such reactivation can result in either a diarrheal illness and parasite migration with hyperinfestation syndrome (characterized by hemorrhagic enterocolitis, hemorrhagic pneumonia, or both) or disseminated infection with accompanying (usually) Gram-negative bacteremia or meningitis [149,150]. Patients from tropical areas and the south-eastern US should be screened with *Strongyloides* IgG serology prior to transplantation, and should be treated with ivermectin pre-emptively if seropositive [6].

Fever, pneumonitis, and *Pneumocystis* infection

The spectrum of potential pathogens of the lungs in transplantation is broad. Some general concepts are worth consideration. As for all infections in transplantation, invasive diagnostic techniques are often necessary for the diagnosis of pneumonia. The depressed inflammatory response of the immunocompromised transplant patient may greatly modify or delay the appearance of pulmonary lesions on radiography. As a result, computed tomography (CT) of the chest should be considered to define the anatomy of pulmonary lesions early in the course of evaluation or when the chest radiographic findings are negative or non-specific, and also to guide invasive diagnostic approaches, e.g. bronchoscopy versus needle or surgical biopsy. CT is also essential to the definition of the extent of the disease process and the possibility of multiple simultaneous processes. Focal or multifocal consolidation of acute onset on chest radiographs will quite likely be caused by bacterial infection. Similar multifocal lesions with subacute to chronic progression are more likely secondary to fungi, tuberculosis, or nocardial infections. Large nodules are usually a sign of fungal or nocardial infection or PTLD, particularly if they are subacute to chronic in onset. Subacute disease with diffuse abnormalities, either of the peribronchovascular type or miliary micronodules, are usually caused by viruses (especially CMV) or *P. jiroveci.* Additional clues can be found by examining pulmonary lesions for cavitation; cavitation suggests such necrotizing infections as those caused by fungi (*Aspergillus* or *Mucoraceae*), *Nocardia*, *Staphylococcus*, and certain Gram-negative bacilli, most commonly with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Pneumocystis jiroveci pneumonia

The risk of infection for PCP is greatest in the first 6 months after transplantation and during periods of increased immunosuppression [151]. Lung transplant recipients more than other groups retain a life-long risk for *Pneumocystis* infection [152]. Given universal prophylaxis for PCP, the onset of *Pneumocystis* pneumonia may occur beyond prophylaxis and should be suspected in individuals presenting with significant hypoxemia out of proportion to minimal or subtle chest examination and radiologic findings. The natural reservoir of infection remains unknown. Aerosol transmission of infection has been demonstrated by a number of investigators in animal models and clusters of infections have developed in clinical settings, including between HIV-infected persons and transplant recipients [153]. Activation of latent infection remains a significant factor in the incidence of disease in immunocompromised hosts. In the SOT recipient, chronic immunosuppression that includes corticosteroids is most often associated with pneumocystosis. Bolus corticosteroids, cyclosporine, or co-infection with CMV or community-acquired respiratory viral infection may also contribute to the risk for *Pneumocystis* pneumonia.

In patients not receiving TMP-SMX (or alternative drugs) as prophylaxis, most transplant centers report an incidence of *P. jiroveci* pneumonia of ~10% in the first 6 months post transplant [151]. There is a continued risk of infection in three overlapping groups of transplant recipients: (1) those who require higher than normal levels of immunosuppression for prolonged periods of time due to poor allograft function or chronic rejection; (2) those with chronic CMV infection; and (3) those undergoing treatments that increase the level of immune deficiency, such as cancer chemotherapy or neutropenia due to drug toxicity. The expected mortality due to *Pneumocystis* pneumonia is increased in patients on cyclosporine when compared to other immunocompromised hosts. The hallmark of infection due to *P. jiroveci* is the presence of marked hypoxemia, dyspnea, and cough, with a paucity of physical or radiologic findings. In the transplant recipient, *Pneumocystis* pneumonia is generally acute to subacute in development. Atypical *Pneumocystis* infection (radiographically or clinically) may be seen in patients who have co-existing pulmonary infections or who develop disease while receiving prophylaxis with second choice agents, e.g. pentamidine or atovaquone. Patients outside the usual period of greatest risk for PCP may present with indolent disease confused with heart failure. In such patients, diagnosis often has to be made by invasive procedures [151].

Rapamycin (sirolimus) toxicity may contribute to diffuse interstitial pneumonitis [154]; it is not known whether this syndrome is directly attributable to rapamycin or reflects concomitant infections such as those due to influenza, RSV, PCP, or CMV.

Diagnosis

The characteristic hypoxemia of *Pneumocystis* pneumonia produces a broad alveolar–arterial Po_2 gradient. The level of serum lactic dehydrogenase (LDH) is elevated in most patients with *Pneumocystis* pneumonia (>300 IU/mL). However, many other diffuse pulmonary processes also raise serum LDH levels. Like many of the "atypical" pneumonias (pulmonary infection without sputum production), no diagnostic pattern exists for *Pneumocystis* pneumonia

on routine chest radiography. The chest radiograph may be entirely normal or develop the classical pattern of perihilar and interstitial "ground glass" infiltrates. Microabscesses, nodules, small effusions, lymphadenopathy, asymmetry, and linear bands are common. Chest CT scans will be more sensitive to the diffuse interstitial and nodular pattern than routine radiographs. The clinical and radiologic manifestations of PCP are virtually identical to those of CMV. Indeed, the clinical challenge is to determine whether both pathogens are present. Significant extrapulmonary disease is uncommon in the transplant recipient [151].

Identification of *P. jiroveci* as a specific etiologic agent of pneumonia in an immunocompromised patient should lead to successful treatment. A distinction should be made between the diagnosis of *Pneumocystis* infection in AIDS and in non-AIDS patients. The burden of organisms in infected AIDS patients is generally greater than that in other immunocompromised hosts and non-invasive diagnosis (sputum induction) more often achieved. In general, non-invasive testing should be attempted to make the initial diagnosis, but invasive techniques should be used when clinically feasible. The diagnosis of *P. jiroveci* infection has been improved by the use of induced sputum samples and immunofluorescent monoclonal antibodies to detect the organism in clinical specimens, and molecular diagnostic assays. Antibodies bind to both cysts and trophozoites. The cyst wall can be displayed by a variety of staining techniques; of these, the Gomori methenamine–silver nitrate method (which stains organisms brown or black) is most reliable, even though it is susceptible to artifacts. Calcofluor white is also commonly used for detection of *Pneumocystis*. Sporozoites and trophozoites are stained by polychrome stains, particularly the Giemsa stain, but organisms are less easily visible to non-experts than by other methods.

Therapy

Early therapy, ideally with TMP-SMX is preferred; few transplant patients will tolerate full-dose TMP-SMX for prolonged periods of time. While the treatment dose is higher than the prophylactic dose, recommended dosing (15–20 mg/kg/day of the trimethoprim component) has not been well studied in adults with less than normal renal function. The toxicity is the result of the elevation of creatinine due to trimethoprim (competing for secretion in the kidney) and the toxicity of sulfa agents for the renal allograft. Hydration and the gradual initiation of therapy may help. Alternate therapies are less desirable, but have been used with success, including: intravenous pentamidine, atovaquone, and clindamycin with primaquine or pyrimethamine. While a reduction in the intensity of immunosuppression is generally considered a part of anti-infective therapy in transplantation, the use of short courses of adjunctive steroids with a gradual taper is useful in transplant recipients as in AIDS patients [151].

The importance of preventing *Pneumocystis* infection cannot be overemphasized. Low dose TMP-SMX is well tolerated and should be used in the absence of concrete data demonstrating true allergy or interstitial nephritis. Alternative prophylactic strategies, including dapsone, atovaquone, inhaled or intravenous pentamidine, are less effective than TMP-SMX, but useful in the patient with significant allergy to sulfa drugs. TMP-SMX is the most effective agent for prevention of infection due to *P. jiroveci*. The advantages of TMP-SMX include increased efficacy, lower cost, the availability of oral preparations, and possible protection against other organisms, including *T. gondii*, *Isospora belli*, *Cyclospora cayetanensis*, *N. asteroides*, and common urinary, respiratory, and gastrointestinal bacterial pathogens. It should be noted that alternative agents lack this spectrum of activity [151].

Summary

Transplant infectious disease has increasingly become a field characterized by a preventative approach and early therapies based on sensitive molecular diagnostic tests. Compared with the early days of transplantation, considerable advances have been made regarding prevention of CMV and other opportunistic pathogens, but infection still poses a problem for many recipients. In the future, management of transplant recipients will be improved by the availability of assays for pathogen-specific immune function, enhanced microbiologic screening of donors and recipients, data regarding host risk factors including genetic polymorphisms of receptors and immunoregulatory elements, and advances in the individualization of transplant immunosuppression.

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CHAPTER 95

General Malignancy after Organ Transplantation

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Introduction

Solid organ transplantation (SOT) has increased the life expectancy of patients suffering from numerous causes of irreversible chronic organ failure. Despite substantial improvement over the last decades in short-term allograft outcome, as a result largely of the advent of novel and more potent immunosuppressive combinations, long-term graft and patient survival has not achieved a similar improvement. Co-morbidity as a result of the permanent use of immunosuppressants is thought to reduce graft and recipient survivals. In addition to opportunistic infections and cardiovascular events, development of de-novo malignancies is a well-established major adverse outcome after SOT [1,2].

Multiple studies have estimated a general two- to 500-fold increased risk of cancer among transplanted patients as compared to the general population, with the variation dependent on the transplant setting and the specific type of malignancy considered. However, these data are not precise, mainly because of the different study methodologies and patient selection methods used. Indeed, there is significant diversity in reported studies in the literature [3–11]. It is also worth noting that histologic forms and incidences of malignancies vary depending on the geographic region, population served by the transplant center, and type of immunosuppressants used. Regardless of this variation, it is clear from the data reported from the preponderance of sources that transplant recipients have a substantially increased risk of developing cancer.

Once cancer has developed, the survival of transplant recipients is poor, and treatment options are limited by the transplant, the need for immunosuppression to sustain a life-saving organ, and co-morbidities. It is therefore important to understand the main underlying risk factors, as well as to precisely characterize those cancers that are significantly increased in the transplant population. This will inform patients of their risks, and allow a systematic, rational approach for screening and surveillance after organ transplantation. Toward this end, significant efforts are being made among the scientific community for a better characterization of the predominant risk factors, oncogenic mechanisms, and antitumor immunologic responses that may influence the advent of de-novo malignancies after SOT.

Epidemiology

It is widely accepted that chronic immunosuppression is associated with a markedly increased risk of de-novo malignancies after organ transplantation. Cancer registries for transplant populations from distinct geographic regions have allowed a better knowledge of the prevalence, incidence, and outcome of cancers in organ transplant recipients, and the recognition of the main underlying risk factors.

It is difficult to precisely ascertain, using data from small, single center studies, the incidence of most tumors or to compare their rates of occurrence with those in the general population. There have been few reports from transplant registries of the incidence of cancer [1–5]. In a recent study reported from a UK registry audit [4] comparing the incidence of malignancy in recipients of different types of organs to that in the general population, it has been shown that 10 years after transplantation, recipients have an overall incidence rate of 90 per 1000 patients. Reporting of cancer to registries is often incomplete, and as such, it is difficult to determine the extent to which registry data underestimate the true incidence of cancer [12–16]. However, in addition to immunosuppression, a variable risk of cancer that depends on both recipient age and gender has been demonstrated in cohort studies; increasing age of transplant recipients is a clear confounding factor influencing the incidence of malignancies. Specifically, the risk of developing cancer is only two-fold greater for 65-year-old patients, whereas young transplant recipients have a 15–30-fold increased risk compared with an age-matched general population.

Large-scale registry linkage studies document a widely variable cancer risk among transplant recipients. Some malignancies may arise from the loss of immunologic control of oncogenic viruses, but others are unrelated to known infections. Additional contributing factors for some cancers may include other effects of chronic immune disturbance or inflammation, underlying medical conditions, or medication toxicity. Malignancies associated with viral infections such as Epstein–Barr virus (EBV)-associated non-Hodgkin lymphomas [NHLs; post-transplant lymphoproliferative disorder (PTLD)], human herpes virus 8 (HHV 8)-associated

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Kaposi's sarcoma, some other specific malignancies such as non-melanoma skin cancer (both basal cell and squamous cell carcinomas), and lip and anal cancer are increased in SOT recipients compared with the general population [6].Some other cancers common among the general population have a higher incidence in transplant patients, e.g. colon, lung, or liver cancers, while the incidence of other malignancies, e.g. breast or prostate cancers, is similar to that in the general population (Table 95.1). The progressive increase of cancer with time of observation has been illustrated by the Australian experience. In this high-risk population of fair-skinned individuals with intense exposure to sunlight, the cumulative incidence of skin cancer, calculated by life-table analysis, increases progressively from 7% after 1 year of immunosuppression, to 45% after 11 years, to 70% after 20 years [17].

Cancer pattern variance by transplanted organ

It is of note that the incidences of different types of malignancies differ according to the organ transplanted (Table 95.1). While non-melanoma skin cancers (NMSCs), PTLD, lip and anal cancers are the most frequent malignancies after SOT, regardless of the type of organ transplanted, ranging from standardized incidence ratios (SIRs) of 7.5 to up to 61.4 as compared to the general population, other cancers have a remarkably increased risk among selected subgroups of transplant recipients such as lung, liver and kidney transplant recipients [4,18].

Reported data from kidney transplant registries have shown significantly increased SIRs for developing various malignancies after transplantation as compared to the general population. The overall SIR of all cancers in transplant recipients was 3.27 (95% CI 3.09–3.46); the increase in risk of developing cancer was less during dialysis (SIR 1.35, 95% CI 1.27–1.45) and before renal replacement treatment (1.16, 95% CI 1.08–1.25). NHL and NMSC were the most common malignancies, and colorectal and renal cell cancers (RCCs) were the most common solid organ tumors [6,19].

The reported SIR of developing any de-novo solid organ malignancy in recipients of liver transplants has been reported to be 2.7, as compared with the general population, with higher risks for colon, oropharyngeal, liver, and RCCs. In addition, cancer-related mortality ranged from 0.6% to 8% [20]. Of note, the SIR for NHL in liver transplant patients has been described as higher than that in kidney transplant recipients, with higher SIRs within the first year after transplantation and in patients between the ages of 35 and 50 years [21].

It has been reported that cardiac transplant recipients experience relatively higher rates of de-novo malignancies after transplantation than recipients of other solid organs, potentially due to the relatively higher level of immunosuppression that heart recipients receive as compared to other transplanted organ patients [22,23]. Solid organ tumors are the most common malignancies in heart transplant patients, and among these lung cancer has been reported to be highly prevalent in some studies [24,25], although this has not been confirmed in another study of 572 patients [26].

Up to 10–15% of deaths in recipients of lung transplants are the consequence of cancer during follow-up [27].

The reported incidence of de-novo malignancies in intestinal or multivisceral transplant recipients is 15%, of PTLD is 13%, and of non-lymphoid cancer is 3.2%. Children are at a significantly higher risk of PTLD, and adults are more vulnerable to non-lymphoid cancers [28]. PTLD in this transplant population seems to be strongly associated with EBV infection and graft-versus-host disease.

Table 95.1. Cancer risk categorized by standardized incidence ratio (observed/expected cases) for all solid organ transplant patients in the US, and kidney transplants in Europe (UK) and Australia/New Zealand. The incremental risk increases from bottom to top

Cancer	USRTR [18] 175 732 patients (1987–2008)	UKRA [4] 25 104 patients (1980–2007)	ANZDATA [1, 14] 12 633 patients (1980–2002)	
			Male	Female
All cancers*	2.1	2.4	2.5	3.1
NMSC	13.8	16.6	60	90
Kaposi's sarcoma	64.4	17.1	17.4	62
NHL	7.5	12.5	6.9	29.1
Kidney	4.6	7.9	14.1	14.6
Bladder	1.5	2.4	1.6	3.6
Melanoma	2.3	2.6	6.9	5.2
Cervix	1.0	2.3	4.3	5.7
Liver	1.1	2.4	4.2	4.5
Esophagus	1.5	1.8	2.4	2.8
Colorectal	1.2	1.8	1.6	2.8
Lung	1.4	1.4	2.3	3.6
Prostate	0.9	1.1	1.6	–
Breast	0.8	1.0	–	1.1

*Exclusion of non-melanocytic skin cancer.
NMSC, non-melanoma skin cancer; NHL, Non-Hodgkin lymphoma; USRTR, U.S. Scientific Registry of Transplant Recipients; UKRA, United Kingdom Registry Audit; ANZDATA, The Australia and New Zealand Dialysis and Transplant Registry.

Etiology and risk factors

A better understanding of cancer risk factors among SOT recipients logically would help to clarify the role of the immune system, infections, and other factors in the development of malignancy, and could identify opportunities to improve transplant safety. In general, it is difficult to precisely define the pathogenesis of cancer development post transplantation. While certain malignancies are associated with specific risk factors, others relate to accumulative risk factors. Regardless, it is generally accepted that a substantial portion of the excess risk of developing de-novo malignancies is due to chronic immunosuppression, with the range of cancers resembling that observed among patients infected with human immunodeficiency virus (HIV) [11]. Although HIV-related data suggest that the increased frequency of malignancies in allograft recipients results mainly from immunosuppression, this simple fact cannot explain the distinct increased risk of the different types of neoplasias in transplant recipients in comparison with the general population. It would therefore appear that in addition to immunosuppression, other risk factors, such as those associated with the primary diseases that lead patients to require transplantation, likely also play a relevant role.

There are multiple factors implicated in malignancy onset in the general population, including genetic susceptibility and carcinogenic factors such as smoking, alcohol, and intense exposure to sunlight in fair-skinned individuals. Moreover, there is an especially high likelihood for certain malignancies in patients exposed to some chronic or latent viral infections, including NHL (due to EBV), Kaposi's sarcoma (related to HHV 8), anogenital cancers [facilitated by human papillomavirus (HPV)], or liver cancer (due to hepatitis C and B viruses). The increased risk in these cases is in part due to the immunosuppressive-related loss of viral control. However, direct effects of the viruses have also been implicated. In addition, DNA damage, either by interference with normal DNA repair mechanisms, induction and promotion of cancer promoters

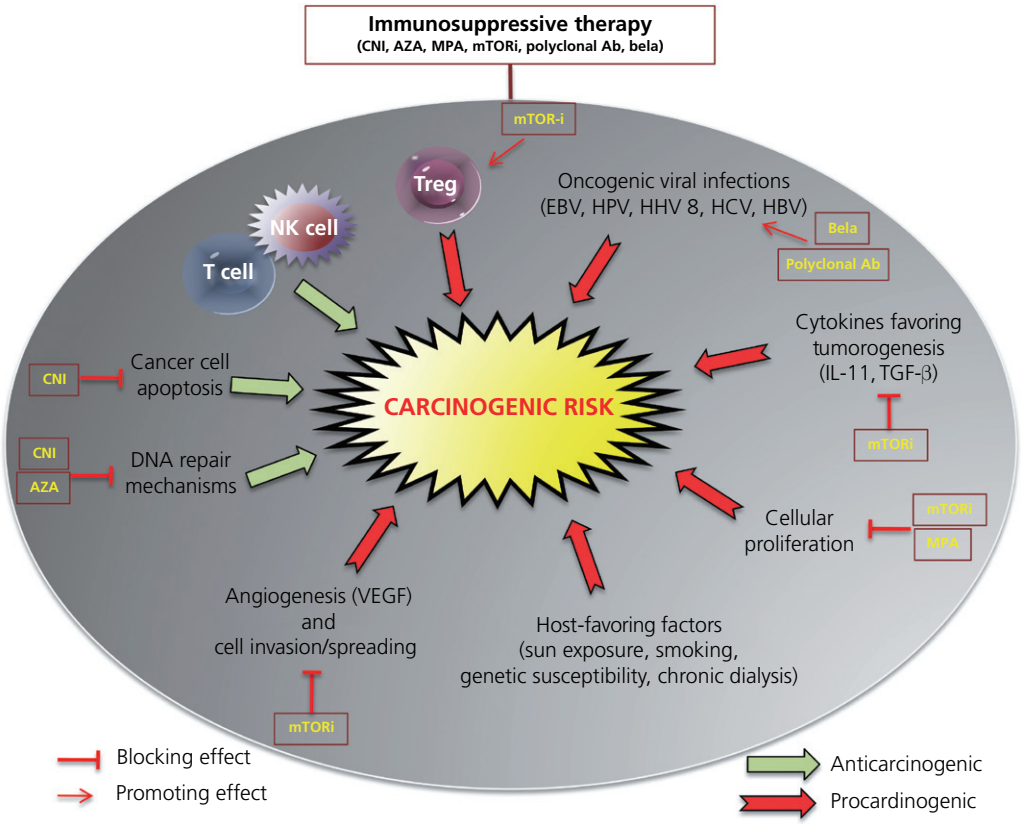

Figure 95.1. Associated mechanisms of pathogenesis in de-novo transplant malignancies.
CNI, calcineurin inhibitor, AZA, azathioprine; MPA, mycophenolic acid; mTORi, mTOR inhibitor; Ab, antibodies; bela, belatacept; EBV, Epstein–Barr virus; HPV, human papilloma virus; HHV 8, human herpes virus 8; HCV, hepatitis C virus; HBV, hepatitis B virus; IL, interleukin.

such as transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF), or by intercalation at the DNA level inhibiting splicing and eliciting codon misreads, is well described for current immunosuppressants, such as cyclosporine, tacrolimus or azathioprine. Finally, impairment of immunosurveillance, which usually prevents the growth and development of malignancies, is a generalized effect to which all transplant recipients are exposed due to chronic immunosuppressive therapy (Figure 95.1) [29,30].

Conventional and geographic risk factors

Usually the known risk factors for the general population that favor malignancy apply to transplant recipients. Among them, advanced age, tobacco, alcohol, some diets, and analgesic abuse are well-described risk factors for post-transplant malignancies [31,32].

Another important point to take into account when analyzing risk factors of post-transplant malignancy is geographic variation. For example, it is well known that in Japan, gastrointestinal malignancies (mainly liver, stomach, colon, rectum) are the most frequently observed post-transplant malignancies, and this is consistent with the generally high prevalence of gastrointestinal cancers in this country. Thus, endemic risks are superimposed on the risks imposed by the transplant experience. Similarly, among organ recipients from Australia and New Zealand, the risk of developing post-transplant skin cancer, particularly spinocellular carcinoma, is extremely high. The preferential explanation is that a fair-skinned Caucasian population is extremely exposed to excessive ultraviolet light [33]. In Europe and the US, the most frequent post-transplant malignancies are NMSC, NHL, RCC, carcinoma of the digestive system, and Kaposi's sarcoma [10,34]. In Saudi Arabia and some Mediterranean countries, where HHV 8 is more prevalent than in northern Europe [35], the most frequent malignancies are Kaposi's sarcoma, infection lymphoma (particularly in children), skin malignancy (with melanoma being more frequent in children than in adults), and anogenital cancers [36]. In South-East Asia, where hepatitis B and C infections are endemic, the frequency of liver cancer after renal transplantation is rather high [37].

Genetic factors

As in the general population, genetic factors may facilitate and promote the development of malignancies. In fact, there are some well-described diseases such as Von Hippel–Lindau disease that are associated with an intrinsically higher risk of developing RCC with an aggressive clinical course. When such patients receive a renal allograft, the frequency of RCC increases further [38]. This phenomenon may also be observed in patients with Wiskott–Aldrich syndrome or Drash syndrome in whom the risk of carcinoma is also markedly increased. In transplant recipients with these uncommon syndromes, an excessive frequency of lymphoma and Wilms' tumor has also been described [39,40]. The assumption that there might be a genetic predisposition in the genesis of post-transplant malignancies that relates to the etiology of the original organ failure is also sustained by the fact that patients with malignancies after transplantation often have more than one type of tumor [39]. In

Table 95.2. Viruses related to malignancies after organ transplantation

Viral agent	Malignancy (target organ)
Hepatitis B virus (HBV)	Hepatocellular carcinoma (liver)
Hepatitis C virus (HCV)	Hepatocellular carcinoma (liver)
Epstein–Barr virus (EBV)	Nasopharynx, Hodgkin and non-Hodgkin lymphoma
Human herpes virus 8 (HHV 8)	Kaposi's sarcoma (vascular)
Human papillomaviruses (HPV)	Cervix, penis, vulvar carcinomas
Human papillomavirus 58 (HPV 58)	Bowen's disease
Human papillomavirus 8, 19 (HPV 8, 19)	Non-melanoma skin cancer
Human papillomavirus 16, 20 (HPV 16, 20)	Skin and tonsillar carcinoma

patients with two malignancies, the most common secondary malignancy is a skin tumor [34].

Oncogenic viral agents

It has been well described that certain chronic viral infections can facilitate particular types of malignancies in transplanted patients (Table 95.2). There are several reported mechanisms by which viral agents can promote uncontrolled cell proliferation. These include viral-mediated disengagement of the machinery controlling the progress of cell cycling and division, escape of immunosurveillance by oncogenic cells carrying viral-derived particles on their surface, and viral inhibition of host cell apoptosis [41,42]. Inhibiting cellular apoptosis is of especial relevance as it is thought to be requisite for unceasing growth after transformation of the host cell by oncogenic viruses. A class of proteins, FLIPs (FADD l-like interleukin 1B converting enzyme-like protease inhibited proteins), interfere with the initiation of apoptosis through several apoptosis-reducing receptors, including CD95, TNF-R1, TRAMP/DR3, and TRAIL-R1, that presumably share common signaling pathways [43,44]. Herpes viruses such as herpes virus Saimiri (HVS) or HHV 8 encode some FLIPs. All viruses encoding vFLIPs can transform cells in vitro and are associated with tumors in susceptible hosts. HVS causes lymphoma and leukemia in susceptible primates and induces stable growth transformation of human T cells in vitro. Another important pathway for virus-induced malignancy is interference with the *p53* tumor suppressor gene, which induces cell cycle arrest or apoptosis in response to DNA damage [45]. Small DNA viruses use distinct mechanisms to counter p53. They either bind directly to p53 or inhibit p53-mediated transcriptional activation, or they promote the degradation of p53 via the ubiquitin pathway. HPV proteins E6 and E7 inhibit the *p53* gene and are thought to be involved in the induction of carcinoma. In addition, exposure of the skin to ultraviolet (UV) radiation causes DNA mutations by formation of thymidine dimmers, leading to inactivation of the *p53* gene. Malignant proliferation is thought to be a result of the failure to repair such mutations.

EBV has been frequently associated with lymphoma (both Hodgkin's and non-Hodgkin's) and HHV 8 is frequently associated with Kaposi's sarcoma [46,47], especially in those receiving monoclonal or polyclonal antibodies as induction or rescue therapy for steroid-resistant rejection [48]. Several varieties of HPV have been associated with skin, cervical, penile, or anogenital carcinomas [49]. Moreover, the polyoma BK virus, a double-stranded DNA virus that induces acute interstitial nephritis in renal transplant recipients [50], has been shown to have tumorogenic faculties by transforming cells mainly by the action of the middle T antigen [51,52].

Table 95.3. Proposed waiting times after primary malignancies before solid organ transplantation

Malignancy	Recommended pretransplant waiting time (years)
Lung	2
Colon:	
Stage I	2
Stage II or higher	5
Breast:	
Carcinoma in situ	2
Cancer	5
Prostate	2
Liver	Not recommended (except in liver transplant patients)
Bladder:	
Non-invasive	2
Invasive	5
Melanoma in situ	5
Non-melanoma skin cancer:	
Squamous cell carcinoma	2
Basal cell carcinoma	No waiting time
Multiple myeloma	Not recommended (bone marrow and kidney transplantation)
Leukemia	2
Genital cancers:	
Testicular	2
Cervical/uterine	2

Role of immunosuppression

As discussed throughout this chapter, there is substantial evidence regarding the significantly increased risk of developing malignancy after organ transplantation, suggesting that immunosuppressive therapy plays a key role in its promotion. Accordingly, it is typical for clinicians to wait between 2 and 5 years after cure of malignancy before proceeding with transplantation. This practice is predicated on the assumption that immunosuppression will accelerate residual tumor growth. General guidelines are provided in Table 95.3 and presented with the acknowledgment that the data supporting these guidelines are not particularly robust. General considerations regarding the variance of maintenance immunosuppression based on malignancy risk are described in Chapter 66. Supporting this concept, in a very illustrative population-based cohort study from Australia [6] in patients with end-stage kidney disease, it was shown that kidney transplantation was associated with a more than three-fold increased risk of cancer, whereas this increased susceptibility was not observed before transplantation. Likewise, the observation that patients with autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, or dermatomyositis develop tumors if they receive immunosuppressive therapy also supports the assertion that the action of immunosuppressive drugs is responsible in part for the increased incidence of tumors in transplant recipients [53–55]. Indeed, it seems intuitive that the overall amount of immunosuppression is a relevant factor in establishing the risk of post-transplant malignancy [56]; as already mentioned, cardiac transplant recipients, who receive a relatively higher immunosuppressive load, display higher rates of malignancies after transplantation than other solid organs recipients, who receive less intensive immunosuppression [22]. However, the relative carcinogenicity of individual immunosuppressive agents or combinations is still poorly understood, basically limited by a selection bias due to the absence of random allocation to treatments, limited recording of

drug exposures in the majority of studies, and lack of reported information regarding cancer outcomes. All of these issues hamper proper evaluation of confounding variables [2,6,57]. Nonetheless, relevant associations between diverse immunosuppressants and increased cancer susceptibility have been reported.

A clear relationship has been shown between the use of polyclonal or monoclonal lymphocyte-depleting antibodies for induction or rescue therapy and an increased incidence of PTLD (see Chapter 96). This has especially been observed among both renal and cardiac transplant recipients [58,59], but is not a relationship that holds for all agents in all malignancies [60].

Glucocorticoids have been widely used in SOT. They have been shown to have antiapoptotic and proliferation-promoting effects on carcinoma cells from different tumor types [61,62]. Although there is evidence suggesting its pro-oncogenic role among non-transplanted patients receiving glucocorticoids only, mainly favoring NMSC [63], there are few epidemiologic data showing their potential carcinogenic role after SOT.

In addition to inhibition of the calcineurin complex, calcineurin inhibitors (CNIs), including both cyclosporine A (CyA) and tacrolimus (TAC), particularly when used at higher dosages, enhance production of TGF-β and expression of VEGF. Both of these actions have been suggested to be responsible for tumor invasiveness, spread, and tumor angiogenesis [64–66]. Initial reports suggested that the introduction of CyA was followed by a higher frequency of cancer [67–69], especially among those transplant recipients on triple drug regimens of CyA, azathioprine (AZA), and corticosteroids, and those with maintained elevated doses as compared to transplant recipients receiving AZA only [22,68,70–73]. Nonetheless, in a recent study by Gallagher et al. [74], 489 recipients of first-deceased donor renal transplants were treated with either AZA and corticosteroids, CyA monotherapy, or CyA monotherapy followed by a switch to AZA and corticosteroids after 3 months. The different incidence rates of post-transplantation malignancies (non-skin and skin cancer) during a median follow-up of 20.6 years were compared. Interestingly, in the multivariate analyses, treatment allocation did not associate with development of either form of cancer; instead, general risk factors for development of cancer such as increasing age, previous smoking history, non-brown eye color, and fairer skin were the independent risk factors for carcinogenicity after kidney transplantation. Comparisons between CyA and TAC have suggested a slightly higher cumulative incidence of PTLD among TAC-treated patients, but other solid tumors actually seem to have a lower incidence in these patients compared to cyclosporine-treated patients [75–80].

In a large, 3-year follow-up, observational cohort study comparing two groups of renal transplant recipients who received or did not receive mycophenolate mofetil (MMF)-based immunosuppression from two large registries [Organ Procurement and Transplantation Network (OPTN)/United Network for Organ Sharing (UNOS) and Collaborative Transplant Study (CTS)], a trend was shown toward a lower risk of malignancy in those patients receiving MMF-based immunosuppression [81]. This potential antioncogenic effect could be explained by the observation that the inosine monophosphate dehydrogenase enzyme that is inhibited by MMF is significantly elevated in many types of malignancies [82,83].

As well as immunosuppressive effects, mammalian target of rapamycin (mTOR) inhibitors [mTORi; sirolimus (SRL) and everolimus (EVL)] have also been shown to have antiproliferative properties. This is thought to be due to their interference with cell cycle and several growth-related processes, such as signal translation, ribosome biogenesis, and transcription of many genes encoding for different metabolic and biosynthetic pathways [84]. Although mTOR complex II is partially insensitive to SRL/EVL, it may also be partially blocked by these agents, leading to inhibition of the PI3K/Akt/mTOR signaling pathway. Importantly, both upstream and downstream signaling components of mTOR, including aberrant activation of the PI3K/Akt/mTOR pathway and/or altered regulation of the cell cycle process, may frequently be altered in multiple human malignancies [85]. Furthermore, there have been reports of an antiangiogenic effect of mTORi through interference with the VEGF signaling pathway, leading to an abrogation of tumor growth and even metastatic processes as compared to CNI drugs both in vitro and in vivo [86,87]. Together, the multiple biologic effects of such immunosuppressive agents have increased interest among the transplant community in evaluating the potential for prevention of post-transplantation malignancies among solid organs recipients [88].

Several retrospective studies with short-term follow-up (between 2 and 3 years after transplantation) have compared the incidence of post-transplantation malignancies in renal transplant recipients receiving CyA- or SRL-based regimens post transplant; a slightly lower incidence of overall cancer and especially of NMSC was seen in the SRL-treated group [89]. A retrospective study of the OPTN/UNOS database [90] compared the incidence of post-transplant malignancies among kidney transplant recipients receiving only an mTORi, an mTORi in combination with a CNI, or a CNI-based regimen, with a censored follow-up of 963 days to allow comparable follow-up among the treatment groups; a significantly reduced incidence was seen of any malignancy of 0.60% for both SRL or EVL alone and for SRL/EVL plus a CNI, in comparison with 1.81% for a CNI alone. The incidence rates for de-novo solid malignancies were 0% for SRL or EVL, 0.47% for SRL/EVL in combination with a CNI, and 1.0% for a CNI alone. Multivariate analysis indicated that mTORi maintenance immunosuppression was associated with a 60% reduced risk of any post-transplant malignancy and a 55% reduced risk of solid malignancy. In a recent report evaluating conversion from a CNI to a mTORi with 24 months of follow-up in kidney transplant recipients receiving either a CNI-based regimen (either TAC or CyA) or an mTORi-based regimen (SRL), both with AZA or MMF and corticosteroids, a reduction in non-skin cancer risk in those patients switched to SRL was shown [91,92]. Another recent 4-year report from a European prospective study in kidney transplant recipients converted from CyA to SRL at 3 months after transplantation showed fewer de-novo malignancies within the SRL-treated patients (three vs. nine) [93]. However, contradictory observations have been reported by other trials or meta-analysis, showing no clear benefits of mTOR inhibition in the reduction of malignancies after transplantation [94,95].

Importantly, although a general trend toward reduced incidence of general malignancies has been reported among patients receiving a mTORi-based immunosuppressive regimen, there is a lack of prospective and randomized studies with long-term follow-up addressing the prevention of malignancies after transplantation. An interesting case report involves a hepatic recipient who experienced complete regression of three pulmonary metastases of hepatocellular carcinoma after conversion of maintenance immunosuppression from CyA and AZA to SRL and MMF [96].

Of note, the incidences of NMSCs and especially Kaposi's sarcoma in patients treated with SRL is significantly lower than those in non–SRL-treated patients. Moreover, successful regression

of Kaposi's sarcoma has been shown by switching patients from a CNI to a mTORi [97–99].

While waiting for more robust and conclusive data from prospective, randomized studies with longer-term follow-up, many transplant groups suggest that patients with malignancies prior to transplantation should receive a mTORi agent or switch to it when a cancer develops. Nonetheless, it is also generally accepted that for larger, aggressive, and metastatic malignancies, there is less likelihood of response to conversion [100].

Novel immunosuppressants continue to be developed with the aim of improving graft and patient survival, and it is generally considered that drugs with more specific sites of action will have a reduced risk of fostering malignancies. One of the most promising agents, and one that has been recently introduced for use in renal transplantation, is belatacept, a costimulatory blockade fusion protein that binds to the B7 family members CD80/86 on antigen-presenting cells. So far, there have been three prospective core studies using belatacept at two different dosages, all compared to a control group receiving CyA. In a 2.4-year median follow-up, a greater risk of PTLD [specifically located in the central nervous system (CNS)] was reported in the belatacept groups, especially in EBV-seronegative patients and with the more intensive dose [101]. No significantly increased risk of solid organ tumors has been described, although a longer follow-up is needed.

Immunosurveillance of malignancies

It is now clear that the immune system recognizes not only self versus pathogen or self versus non-self, but also the subtler differences that exist between self and transformed self. This realization prompted the hypothesis of cancer immunosurveillance, which encompasses the dual opposing functions of immunity in the cancer setting: host protection versus tumor promotion. These functions form the conceptual basis for a process that is currently known as cancer immunoediting in which the immune system is capable of either blocking tumor growth, development, and survival, or facilitating tumor outgrowth by sculpting tumor immunogenicity or by inhibiting host-protective antitumor responses [102–105]. The recognition of tumor cells in order to eliminate them is performed by the innate and the adaptive immune systems. Interestingly, the local immune environment of malignancies has been described as playing a crucial role. Galon et al. showed that the presence, location, and density of T cells and their products within colorectal tumors were a much better predictor of a cancer patient's survival than the commonly used tumor staging criteria, which are based on the size and spread of a tumor [106]. Identification of tumor antigens that are recognized by T cells from melanoma and other cancers has set the stage for developing more effective, antigen-specific immunotherapy against cancer, as well as vaccination with peptides or dendritic cells (DCs) pulsed with antigenic peptides derived from various melanoma antigens; these advances have great potential in cancer therapy [107–109]. In addition, interesting forthcoming studies will focus on the role of regulatory T cells (Tregs) as potential markers for predicting tumor outcome. In the general population with cancer, the presence of increased numbers of Tregs within the tumor and peripheral circulation has been associated with poor prognosis [110–113]. Interestingly, blocking the function of Tregs in murine tumor models resulted in strong cellular antitumor immunity and even complete tumor destruction [114–118], indicating a crucial role for Tregs in tumor immunity. Furthermore, natural killer (NK) cells have also been shown to play an important role in the host's defense against malignancy—either controlling or inhibiting the advent of cancer both in vitro and in vivo [119–121].

It seems, therefore, obvious that the antitumor immune responses in transplant recipients under immunosuppression will be significantly hampered, thereby facilitating the onset and progression of malignancies. In fact, attempts have been made to define those patients at increased risk for cancer after transplantation by measuring lymphocyte subsets. In two studies, a low CD4 count predicted those at risk for any cancer after transplantation, including squamous cell carcinoma (SCC) [122,123]. However, although predictive, CD4 T-cell count in this population had limited clinical utility. Similarly, among kidney transplant recipients with SCC, it has been recently reported that high and low numbers of peripheral $Foxp3^+$ Tregs and NK cells, respectively, are significantly associated with increased risk for new SCC development, and the ratio of CD8/Foxp3 expression within the cancer itself correlates with development of new cutaneous SCCs [124]. These recent data suggest that monitoring components of the immune system could facilitate prediction of cancer development.

Donor cancer transmission

Transmission of an undiagnosed malignancy from the donor is relatively unusual, especially from living donors because they are thoroughly evaluated before donation; however, for deceased donors there is a greater possibility and this should be considered in the differential diagnosis of malignancy after transplantation. Under immunosuppression, (micro)malignancies or even metastases from a donor could more easily disseminate after transplantation. According to data from the OPTN/UNOS, a donor-related malignancy incidence of 0.2% was reported among a total of 108 062 evaluated transplant recipients [125].

There are some cancers with a higher risk of transmission as compared to others. This is the case for melanoma or choriocarcinoma tumors, whereas RCC confined within the renal capsule without vascular invasion or CNS tumors carry a very low risk. A relevant consideration is viral-transmitted malignancies: EBV donor transmission to seronegative recipients may result in subsequent development of PTLD, especially if the recipient is receiving specific immunosuppressants such as polyclonal antibodies or belatacept.

In an interesting report, it was shown that 50% of patients receiving an allograft from a donor carrying a cancer did develop the malignancy, half of them with metastatic disease [81]. In some patients, eliminating immunosuppressive treatment led to rejection of the donor-derived malignancy without the need for further therapy. However, in most patients with donor-transmitted malignancies, in addition to reducing immunosuppression, specific antitumor therapy is also necessary. The overall mortality from donor-related malignancies is 38%, with that from transmitted tumors at 46% and that derived from de-novo tumors at 33%. Overall, deceased donor-related tumor mortality is 0.007% (eight of 108 062 recipients) [125].

Cancer types

Non-malignant and malignant skin lesions

NMSCs, both basal cell carcinomas (BCCs) and SCCs carcinomas, are the most frequent malignancies after SOT [126], accounting for the majority of skin cancers and being ten- to 200-fold more frequent than in the general population, respectively [127]. Moreover, the normal ratio of each tumor type in the general population

is reversed in transplanted patients (SCC/BCC, 4:1) [128]. Although with lower frequency than for NMSCs, SOT patients also display increased risk of other skin malignant lesions such as melanoma (3.6 times higher risk), Merkel cell carcinoma (10 times), and Kaposi's sarcoma (80 times) [129–131]. The incidence of NMSCs increases with time after transplantation. The incidence in the US and Europe at 2, 10, and 20 years after kidney transplantation has been described as 5%, 10–27%, and 40–60% [10,132,133]. These skin lesions may appear in younger individuals and although considered to be less severe than other solid malignancies, they have been described as having a much more aggressive behavior in transplant recipients than in the general population, growing more rapidly, recurring locally after resection, and capable of metastasizing much more frequently [134].

The development of NMSCs has been associated with several conditioning features such as a greater degree of immunosuppression with direct oncogenic properties, intense sun exposure, association with previous keratotic skin lesions, decrease in immunosurveillance, and interaction with some oncogenic viruses. Cumulative sun exposure is a crucial responsible carcinogen, as these lesions primarily occur in sun exposed skin areas and the incidence is clearly higher in sunnier countries. Age is another important risk factor for skin cancer, as shown by the significantly increased risk of developing skin cancer among patients older than 55 years as compared to younger recipients [135].

Furthermore, mutations of the *p53* tumor suppressor gene have been described as a common genetic disorder in skin cancers. These mutations predispose to skin patches, which in turn appear to be a risk factor for SCC [44].

As previously mentioned, HPV infection is also associated with an increased risk of skin cancer [136,137]. Immunosuppressants such as CNIs (especially CyA at maintained standard doses) [55], due to their pro-oncogenic properties related to the production of certain cytokines, may facilitate angiogenesis resulting in tumor growth and even metastasis. Similarly, due to the sensitization of skin cells to UVA-induced skin damage, AZA has also been associated with skin cancer development [138]. Conversely, there have been reports of lower malignancy rates, especially of NMSCs, in renal allograft recipients receiving SRL/EVL de novo or converted from other immunosuppressants, basically CNIs [93]. Nonetheless, there is still a lack of controlled clinical trial data to support conversion to an mTORi-based immunosuppressive regimen specifically to induce tumor regression, with the probable exception of Kaposi's sarcoma, for which conversion from a CNI to SRL enhanced regression [98,99]. It is unclear whether the Kaposi's lesions regressed due to elimination of the CNI, specific effects of SRL, or both. Controversy exists because in another report eight of 24 patients with Kaposi's sarcoma had a complete tumor response to reduction/cessation of immunosuppressive drug therapy [139].

Risk of malignant melanoma seems to be only slightly increased among SOT patients. However, the mortality from melanoma is significant. Moreover, melanoma in transplant recipients has been associated with a worse overall survival in comparison to the non-transplant population. Despite the scarcity of reported data, it seems that the thickness of the lesion determines the prognosis; patients with a malignant melanoma of <0.76 mm do as well as the general population, whereas those in whom the malignant melanoma is >0.76 mm may experience worse outcomes with increased mortality [140].

Solid cancers

Renal cell cancer and other cancers of the urinary tract

Urinary cancers are the second most common tumors in transplant recipients. The excess risk of RCC varies among different reports, ranging from five- to even 100-fold greater than in the age- and gender-matched general population [141–143]. The majority of RCCs most likely occur in the native kidneys rather than in the renal allograft. A significant increased risk is evident in patients with polycystic kidney disease.

There are no homogeneous recommendations for screening for renal cancer in the kidney transplant population. The American Society of Transplantation and the Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines for the Care of the Kidney Transplant Recipient found no evidence to support routine screening for renal cancers in recipients of kidney transplants and did not recommend screening using ultrasonography in average-risk transplant recipients [144,145], but guidelines from the European Association of Urology recommended annual screening of the native and the graft kidneys using ultrasonography for recipients at high risk [146]. Because there is no clear and precise definition of patients at risk, there are concerns regarding routine screening for renal cancers in terms of cost-effectiveness for kidney transplant recipients [147]. Nonetheless, annual surveillance of the native kidney by ultrasound is recommended and, importantly, patients with or without adult polycystic kidney disease (APKD) who have Bosniak I or II cysts should undergo renal ultrasounds twice a year [148].

Like RCC, there is also an increased incidence of developing cancers of the native urinary tract, and among these, prostate cancer is significantly increased [149]. The US Renal Data System shows a 3-year cumulative incidence of 3.1% for prostate cancer, 2.2% for RCC, 0.7% for bladder cancer, and 0.1% for testicular cancer. Moreover, patients undergoing renal transplantation for analgesic nephropathy are at high risk of developing transitional cell carcinoma of the upper urinary tract [150,151]. Current cancer screening guidelines advocate screening for prostate cancer, although a survival advantage has not been demonstrated, and there is even the potential for over-diagnosis of trivial malignancy.

Colorectal cancer

Whether or not the incidence of colorectal cancer is increased among renal transplant recipients is a controversial question. Some groups have reported increased rates, both in men and women as compared to the general population [10], but these reports have been questioned by others [152,153]. However, the risk of colorectal cancer is clearly increased in patients with primary sclerosing cholangitis receiving a liver allograft and with a relevant history of inflammatory bowel disease [154].

Anogenital cancers

There is a clear increased incidence of anal cancer among all transplant recipients. Gynecologic cancers such as cervical carcinoma in situ, vulvar, ovarian, and cervical malignancies are also significantly increased after transplantation. All of these malignancies are related to HPV 16 or HPV 18 infections, and accounted for 2–3% of all cancers in patients who underwent a transplant [155,156]. However, the role of HPV vaccination is controversial. Vaccination post transplantation might be ineffective for HPV-naïve patients because

these patients might have lower secondary responses and more rapid decline of antibodies as compared to the general population [157]. These cancers tend to be extensive or multiple, especially in women, 30% of whom have concurrent cervical cancer. Therefore, gynecologic examinations should be performed annually.

Lung and breast cancer

Lung and breast cancer are increased two-fold in SOT recipients. In two studies with a significant number of patients receiving a SOT, up to 0.3% of patients developed lung cancer, with a higher incidence in non-smokers compared with smokers in contrast to the general population, and with a rapid progression in patients who had not undergone tumor resection [158,159]. A study by Jimenez et al. [160]. showed that patients being transplanted for alcoholic cirrhosis had significantly higher rates of lung cancer than those transplanted for other causes (4.3% vs. 0.7%). This result, however, could be confounded by the fact that patients who drink alcohol are more likely to smoke cigarettes as 73.3% of de-novo lung cancer patients in this study were both heavy drinkers and intermediate or heavy smokers.

There is some controversy regarding the incidence of breast cancer in female SOT recipients. While one study in female heart transplant recipients reported a reduced incidence of breast cancer as compared to the general population [161], another study showed an increased incidence of breast cancer in women receiving a liver transplant [162]. However, a higher biologic aggressiveness of breast cancer in transplant recipients compared with the general population has been described [163]. In such cancers, adherence to the routine screening guidelines established for the general population is also appropriate for transplant recipients.

Cancer screening in solid organ transplant patients

The well-established increased risk of developing cancer after SOT has led international transplant organizations to investigate regular tumor screening in SOT patients [144,155,164–166]. However, there are important concerns regarding the specific recommendations for cancer screening among transplanted patients as the main guidelines have been completely extrapolated from data for the general population. Differences between the general population and transplanted recipients, differences in diagnostic tests, and competing risks for death from other causes such as cardiovascular disease with reduction of overall life expectancy all question the validity of these recommendations in the SOT population. Guidelines developed for the general population need to be assessed for applicability to the SOT population, balancing the benefit of screening with the costs and potential harm to the individual [167]. Additional information regarding preventative healthcare screening in transplant recipients is given in Chapter 88.

Nonetheless, several international transplantation societies have reported guidelines or recommendations for cancer screening in different types of SOT. In general, advice such as avoidance of excessive immunosuppression if possible, avoidance of repeated contact with depleting antilymphocyte antibodies, screening of donors and recipients for cancer, and the avoidance of carcinogenic factors such as persistent high sun exposure should be the initial approach to preventing the advent of de-novo malignancies. Moreover, the rising age of transplant patients together with the length of time spent on the waitlist increase the risk of malignancy escaping detection and therefore recipients with pre-existing tumors receiving transplants.

After observing the primary prevention of skin cancers with the avoidance of intensive sun exposure, use of protective clothing, and effective sunscreen, the next important practice is examination to detect skin tumors. Monthly skin self-examination and total body skin examination every 6 to 12 months by expert physicians and dermatologists are highly recommended; in high-risk patients, e.g. those who have previously been diagnosed with SCC, more frequent examinations are mandatory.

In women, yearly gynecologic examinations, and transvaginal ultrasonography in those without hysterectomy, are also strongly recommended to exclude vulvar, perineal, and uterine malignancies. As cervical cancer is a virus-related neoplasia that is more aggressive among immunocompromised patients, the European and American transplantation societies recommend annual cervical cancer screening with Pap smear and pelvic examination in all adult female renal transplant recipients [145,165].

Multicystic alteration of native kidneys in patients with chronic kidney disease is a well-known precancerous condition. Although there is no precise definition of patients at risk of RCC, ultrasonographic examination of the recipient's kidney should be performed every year, especially in those showing Bosniak type I or II cysts.

A urologic examination is indicated in patients with a history of analgesic nephropathy who develop microhematuria and in patients who have received cyclophosphamide, especially if the cumulative doses exceeded 20 g [168]. Some authors also recommend urologic examination in patients who have been treated with AZA for >10 years [169].

Periodic screening of feces for occult blood is advisable because colorectal carcinoma is more frequent after transplantation. However, recommendations for flexible sigmoidoscopy are not clear; this is currently recommended every 5 years beginning at age 50 years. Colorectal cancer after liver transplantation is more often a right-sided lesion, is more aggressive, and is associated with a higher rate of metastasis. In addition, those liver transplant recipients with primary sclerosing cholangitis and inflammatory bowel disease, who are at an increased risk for developing a colonic carcinoma after liver transplantation, require more frequent screening/surveillance with annual follow-up screening examination with colonoscopy and random colorectal mucosal biopsies for early detection [170].

For other solid organ cancers such as prostate and breast, guidelines published for screening and prevention of solid organ cancers in the general population should be strictly adhered to in transplant recipients. For breast cancer, both the American and European transplant societies recommend screening all female transplant recipients between 50 and 69 years of age at recommended intervals of between 12 and 24 month [171–173]. Women between 40 and 49 years of age can still undergo screening mammography every 1 or 2 years, but there is no evidence for or against screening in this age group [145,165]. Prostate cancer screening is a persistent topic of debate both for the general population and for SOT recipients. Nevertheless, both the American and European transplantation societies encourage annual prostate cancer screening with digital rectal examination (DRE) and prostate-specific antigen (PSA) measurement in all male renal transplant recipients who are older than 50 years and have a life expectancy of at least 10 years [145,165].

Management of de-novo malignancies after solid organ transplantation

Management of transplanted patients who have developed a de-novo malignancy is challenging and will vary depending on the type and stage of the cancer and aim of the treatment, i.e. whether it is curative or palliative. However, the same preventative recommendations that are given to the general population should always be given. These include modification of lifestyle such as following a healthy diet, regular exercise, and smoking cessation. Then, specific therapeutic strategies for the particular tumor type, either with surgery extirpation, radiotherapy, or chemotherapy, can be used as appropriate [174].

Along with the specific therapeutic management of a cancer, the general advice for immune responsive tumors is for a careful reduction or withdrawal of immunosuppression, keeping in mind the competing risks of organ failure and malignant progression. Wholesale elimination of immunosuppression for a life-sustaining organ, e.g. heart, lung, or liver, cannot be recommended, particularly for malignancies that are unlikely to have been induced by immunosuppression. However, elimination can be more freely considered for immune-responsive malignancies, e.g. EBV-driven lymphomas, or in kidney transplantation. Conversion to alternative immunosuppressive regimens based on mTORi also can be considered. However, the benefit of changing to an immunosuppressant with antiproliferative activity to overall success of treatment is still unknown. Liver transplant recipients can be maintained on minimum concentrations of prednisolone and AZA/MMF or even converted to an mTORi, as acute rejection is unusual late after transplantation, typically mild, and generally easy to reverse. In kidney transplant recipients, CNI concentrations should be reduced to a minimum and SRL/EVL seriously considered as a CNI alternative [175,176]. Management of baseline immunosuppression among other SOT recipients, such as heart, lung, or intestinal transplant recipients, should follow the same recommendations, although for these consideration needs to be given to the consequences of rejection of a vital organ.

A change to a immunosuppressive regimen with an antiproliferative effect, such as MMF and particularly mTORi, might help to decrease the incidence of graft rejection and even to regress the malignancy. This strategy should be followed especially in patients with NHL, skin cancer, Kaposi's sarcoma, or donor-derived malignancies [177–179]. Although growing evidence seems to indicate that mTORi agents have beneficial effects in terms of cancer regression and patient survival in the early stages of post-transplantation malignancies [175], long-term prospective clinical trials are needed to truly define their role in this setting.

When a patient develops a serious malignancy, minimization or even complete cessation of immunosuppression should be considered, particularly for completely HLA-matched patients. Although immune responses seem to be relatively impaired with severe malignancies due to a generalized anergy [180], graft monitoring at short intervals is necessary, particularly in the early stages after transplantation; otherwise fulminant rejection may be diagnosed too late, with the potential consequence of graft loss. The degree to which loss of the allograft is of primary concern varies based on the stage of the malignancy, its immune responsiveness, and in the terminal situation, the degree to which quality of life will be impaired should graft loss occur. In the setting of a donor-derived malignancy, some degree of allograft rejection may be required to facilitate elimination of the malignancy.

Interestingly, immunotherapy of some cancers such as melanoma [181], colorectal cancer [182], and RCC [183] is currently under investigation in non-transplant patients. Whether or not these treatment modalities will be also useful in organ transplantation needs further study.

Summary

In summary, malignancies are sufficiently common in transplant recipients to warrant their consideration in all patients receiving a solid organ allograft. Relevant risk factors or tumor development should be taken into account and systematically monitored in detail. Furthermore, the prompt detection of cancer allows for effective therapeutic decisions to be made. Given the numerous competing risks of graft failure and metastatic progression, and the related morbidity and mortality of each, the care of a patient with a post-transplant malignancy should involve a multidisciplinary team. Given the complexity of dealing with post-transplant malignancies, prevention should be a major objective of transplant physicians.

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CHAPTER 96

Post-Transplant Lymphoproliferative Disorders

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Introduction

Solid organ transplantation (SOT) is increasingly accepted as treatment for end-stage disease of the heart, kidney, lung, liver, and intestine. Recognition of the importance of post-transplant lymphoproliferative disorders (PTLDs) in recipients of SOTs has grown in parallel with the growth and success of this field. While this is particularly true for pediatric organ transplant recipients, owing in large part to their increased likelihood of Epstein–Barr virus (EBV) naïve status at the time of transplant, the development of PTLD in adults is also an important cause of morbidity and mortality [1]. Although the majority of PTLD lesions have been associated with the presence of EBV, EBV-negative PTLD also occurs [2,3]. Despite a growing understanding of the pathobiology of PTLD, much remains unresolved and its optimal management in SOT recipients remains controversial. This chapter will review the biology, pathology, epidemiology, clinical manifestations, diagnosis, and management of PTLDs, and will also discuss possible prevention of PTLDs in SOT recipients. This chapter complements Chapter 95, which covers post-transplant malignancy in general.

Historical perspectives

The association between therapeutic immunosuppression in the transplant recipient and the development of lymphoid tumors was first recognized in 1968 [4]. As surgical and immunosuppressive strategies evolved, cases of lymphoid malignancy increasingly emerged among organ transplant recipients. Despite ongoing concern for this complication, it was not until a series of seminal observations from the University of Minnesota in the early 1980s that the role of EBV in the development of PTLD was established [5,6]. With ongoing evolution and introduction of more specific and potent anti–T-cell immunosuppression, the problem of PTLD became increasingly apparent, prompting an increased focus on the cause and potential therapeutic response to this condition. Subsequent studies confirmed the central pathogenic role that EBV plays in the majority of patients undergoing organ transplantation who experience this complication. However, the availability of molecular probes for EBV also revealed that a substantial subset of cases of PTLD (especially in adults or in children long out from transplant) do not appear to demonstrate any relationship with EBV [2,3]. Regardless of the involvement of EBV, these cases emphasized the role of intact immune surveillance in the prevention of the development of neoplasms, particularly those driven by viral infection.

EBV-associated PTLDs include a spectrum of conditions that straddle the borders between infection and malignant neoplasms [1]. EBV disease in SOT recipients manifests a continuum of clinical and histopathologic findings ranging from non-specific febrile illness to PTLD, including frank lymphoma associated with various genetic alterations [1,7]. Thirty years of efforts to understand factors that determine the severity and outcome of EBV-associated disease have identified the adequacy of the host immune response and the level of extrinsic immunosuppression as major determinants. The initial observations by Starzl et al. of the potential reversibility of PTLD lesions with lowering of immunosuppression support the validity of these observations [8]. The recognition of the role of EBV in the majority of PTLD lesions raised questions as to the potential role of antiviral therapy in the treatment of this condition. Hanto et al. provided initial evidence for the use of acyclovir in the treatment of PTLD in the 1980s [5]. Subsequently, the greater potency of ganciclovir led many clinicians to use this agent in the treatment of EBV disease and PTLD [9]. However, an understanding of the disease pathogenesis of EBV-associated PTLD, particularly its relationship to viral latency (see below) rather than lytic infection in its progression, reconciles well with a lack of definitive data supporting the efficacy of antiviral therapy in either the treatment or prevention of PTLD, and raises doubt about the utility of these agents.

Work underway since the late 1980s identified an association between the presence of elevated EBV loads in the peripheral blood and the presence of EBV-associated disease in transplant recipients [10]. The growing availability of EBV load assays has led to their widespread use. Twenty years of experience with these tests has emphasized both their promise and their limitations [11]. An elevated EBV load identifies the majority of newly infected EBV-naïve patients at risk for, or presenting with, EBV-associated disease. However, a few patients experiencing this complication will not have an elevated EBV load, while others with elevated loads may not currently manifest and will not develop clinical illness. Nonetheless, the fact that EBV loads become positive and rise to high levels weeks before the development of clinical disease has led to a growing acceptance that the following of serial measurements can inform pre-emptive strategies aimed at the prevention of EBV disease in patients who are seronegative for EBV at the time of

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transplantation [1,11]. EBV load monitoring is of less clear value for the prediction of PTLD in patients who are EBV seropositive at the time of transplantation.

Biology of Epstein – Barr virus-associated post-transplant lymphoproliferative disorder

EBV is a double-stranded DNA virus of the gammaherpes family [12]. The virus is most frequently transmitted through saliva, though it can be transmitted from the donor, likely by way of passenger leukocytes. Whether through saliva or passenger leukocytes, EBV infection is primarily manifested through the infection of B lymphocytes. Infection of the B lymphocyte progresses through one of two phases. In the lytic phase, EBV actively replicates within infected B cells with subsequent production and release of infectious virions, which can spread infection to non-infected B cells. The second or latent phase is more complex [12,13]. EBV-associated latency involves several different latency gene programs. One of these, termed latency type III, is characterized by cellular activation and autonomous proliferation of the infected B cell. Affected B cells resemble antigen-activated B lymphoblasts in immunocompetent individuals, where the presence of a competent immune system and the development of EBV-specific cytotoxic T lymphocytes (CTLs) eventually control both lytic infection and the outgrowth of EBV-activated lymphoblasts. In the normal host, latency type III infection progresses first to latency type II infection, which takes place in B-cell follicles of secondary lymphoid tissue, and then to latency type I infection within resting memory B cells [13]. This progression, which is mediated in part by the development of EBV-specific T-cell immunity, involves both migration of infected B cells to secondary lymphoid tissue and sequential expression of different genes, culminating in minimal gene expression in latency type I infection in resting memory B cells despite maintenance of the EBV episome. In essence, the sequence of EBV latency programs co-evolved with the human immune system to establish life-long latent infection in a manner that typically does not result in chronic or serious disease. However, in transplant recipients, the presence of immunosuppression disrupts the development of an adequate host immune response, potentially leading to continued expansion of B cells in latency type III infection and thus manifestations of EBV disease including PTLD [12–14].

The outgrowth of latency type III lymphoblasts during primary infection in immune competent individuals is primarily controlled by the presence of EBV-specific $CD8^+$ CTLs [13]. Subsequently, seropositive immunocompetent individuals chronically devote a significant proportion of their T-cell repertoire to controlling EBV, including EBV-specific T cells specific for both lytic and latent epitopes. Less is known about the $CD4^+$ T-cell response to EBV and the role of humoral responses in protecting against PTLD in organ transplant recipients is also unclear. Although the exact pathway from infection to PTLD remains unclear, the absence of an adequate immune response to drive and contain EBV through its normal pattern of infection is clearly the key to the development of EBV-related PTLD.

Epstein–Barr virus-negative post-transplant lymphoproliferative disorder

Although the majority of PTLD lesions have been shown to be associated with EBV infection, a growing number of lesions have been found to have no evidence of EBV [2,3]. As many as 21–34% of histologically proven PTLDs are EBV negative [2,3] and this number may be increasing. At least some of these lesions have been identified as being T-cell PTLD [7,15]. EBV-negative cases typically occur later than EBV-positive cases, are often of T-cell origin, and if B cell in nature, typically demonstrate monomorphic histology. They also tend to have a worse prognosis [3].

A variety of etiologies have been considered as potential causes of EBV-negative PTLD. The possibility that unidentified infectious agents might stimulate the lymphoproliferative process has been widely discussed. Vilchez et al. found evidence of SV40 large tumor antigen sequences in two of 16 cases of PTLD, including one case of EBV-negative T-cell disease [16]. Rare cases have been associated with human herpesvirus 8 (HHV 8) infection [17]. Regardless of their etiology, evidence suggests that EBV-negative PTLDs appear to represent a distinct subset of lesions with differences in biologic behavior, therapeutic responsiveness, and prognosis [2,3].

Table 96.1. WHO classification of the post-transplant lymphoproliferative disorders

- Early lesions
  - Plasmacytic hyperplasia
  - Infectious mononucleosis-like
- Polymorphic PTLD
- Monomorphic PTLD (B and T/NK cell types)*
- Classical Hodgkin's lymphoma type PTLD

*Further classified according to the lymphoma they resemble.
PTLD, post-transplant lymphoproliferative disorders; NK, natural killer.
Data from Swerdlow et al. [7].

Pathology

PTLDs are a spectrum of lymphoid and plasmacytic proliferations occurring in the post-transplant setting. As discussed above, they are frequently, but not always, associated with EBV infection. Most PTLDs in SOT patients are of host origin, but they are of donor origin in bone marrow/stem cell transplant recipients. The classification of these disorders has evolved over the last two decades, and at times has been confusing for both clinicians and pathologists. The World Health Organization (WHO) has developed a standard classification, which is now used in most centers [7]. In the 2008 revision of the WHO classification, four main categories were defined (Table 96.1). It should be noted that individual patients may demonstrate different types of PTLD, sometimes clonally unrelated, or even of different cell origin. These may occur simultaneously or at different times. Recurrences may show evidence of progression from polymorphic lesions to B-cell or T-cell monomorphic PTLD or Hodgkin's lymphoma [15,18].

It is important to exclude the possibility of other lymphoid or plasmacytic inflammatory lesions. In the allograft, distinction from rejection must be made. In rare circumstances, PTLD and rejection can occur concurrently. PTLD remains a pathologic diagnosis, and wherever there is a high index of suspicion, biopsy should be performed. Excision biopsy or core biopsy [often computed tomography (CT) or ultrasound guided], but not cytology, are appropriate. Tissue should be handled in the same manner as for work-up of lymphoma in the immunocompetent host, and the pathologist should be informed whenever a biopsy for this diagnosis is being performed. Evaluation should include histologic sections, fresh material for immunophenotypic studies, and fresh/frozen material for potential genotypic studies. Classical cytogenetic studies may be valuable in some instances [7].

Early lesions

These are non-destructive lymphoplasmacytic proliferations that are further divided into two subgroups: plasmacytic hyperplasia and infectious mononucleosis-like PTLD. Some lesions do not fall into either category, but represent florid follicular hyperplasia, e.g. of the lingual tonsil. These lesions may cause significant symptoms, including airway obstruction, yet traditionally have not been categorized as PTLDs. This remains controversial. Early lesions may resemble reactive changes seen in lymphoid tissues in the immunocompetent host. In general, these lesions are non-clonal and cytogenetic abnormalities are unusual.

Polymorphic PTLD

This is the form most commonly seen in children and the lesions are generally positive for latent EBV, expressing non-coding EBV RNAs (EBERs) or EBV nuclear antigen (EBNA) by immunohistochemistry (Figure 96.1a,b). These are destructive lesions but do not fulfill the criteria for any of the classic lymphomas seen in the non-transplant population. The distinction from cases of infectious mononucleosis-like PTLD or monomorphic PTLD can at times be difficult. The lesions include lymphocytes of various types, sizes, and shapes, and at various stages of maturation, as well as plasma cells. There is usually a mixture of variably sized B cells and heterogeneous T cells. Transformed B cells/immunoblasts tend to be in the minority and may resemble Reed–Sternberg cells. These lesions do not clearly fulfill the criteria for one of the classic malignant lymphomas. Nonetheless, B-cell/plasma cell clones are often identified by molecular studies. *Bcl-6* mutations are reported in some polymorphic PTLDs, but abnormalities in tumor suppressor genes and oncogenes are generally not expected [19].

Monomorphic PTLD

Monomorphic PTLDs most often fulfill criteria for diffuse large B-cell lymphoma (DLBCL) (Figure 96.1c); Burkitt's lymphoma (Figure 96.1d), plasma cell neoplasia, and one of the T/natural killer (NK)-cell lymphomas recognized in immunocompetent hosts are also observed. They are categorized based on the type of lymphoma they most closely resemble in the non-transplant population. At the current time, the small B-cell lymphomas are not considered PTLD, though MALT lymphomas are observed in transplant patients.

B-cell monomorphic PTLDs are typically composed predominantly of numerous transformed lymphoid cells at one maturational stage, i.e. monomorphic. However, some degree of cellular pleomorphism is generally present. These lesions are expected to be monoclonal. The greatest frequency of EBV-negative cases is found in monomorphic PTLD. DLBCL are CD20 positive; Burkitt's lymphomas typically have a $CD20^+$, $CD10^+$, bcl-6^+, bcl-2^- phenotype. Plasma cell neoplasms usually lack B-cell markers and are $CD138^+$ and typically demonstrate light chain class restriction. Monomorphic B-cell PTLDs often demonstrate abnormalities of

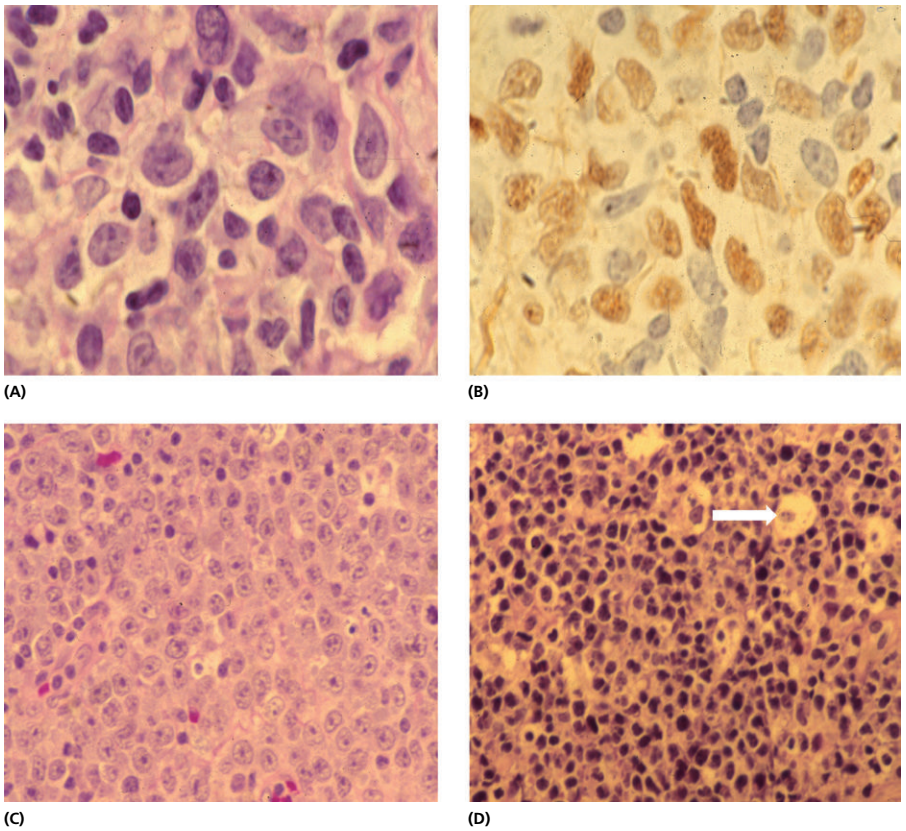

Figure 96.1. Histology of post-transplant lymphoproliferative disorders (PTLD). (A,B) Polymorphic PTLD.(A) Shows significant pleomorphism of these lesions, as well as the presence of numerous abnormal transformed cells. (B) Shows positive staining for non-coding Epstein–Barr virus (EBV) RNAs (EBERs) by immunohistochemistry, indicating the presence of latent EBV (dark brown appearance) in many cells from the lesion shown in (A). (C) Shows a monomorphic PTLD. There is a high degree of cellular monotony in this lesion. (D) Shows another monomorphic PTLD demonstrating the typical features of Burkitt's disease. The so-called starry sky appearance represents areas of macrophage ingestion of dying cells (arrow).

tumor suppressor genes (e.g. *TP53*), oncogenes (*N-RAS*), and sometimes *BCL6* mutations. Translocations such as of *MYC* are a typical feature of Burkitt's lymphomas [7]. Other cytogenetic abnormalities are relatively common.

Monomorphic T-cell PTLDs fulfill the WHO criteria for one of the T-cell neoplasms and in contrast to DLBCL, are not typically composed of monomorphic large transformed cells. Most commonly, they resemble peripheral T-cell lymphoma. The histopathology for the T-cell PTLD is very variable as is seen among T-cell lymphomas in the immunocompetent host. Because many T-cell lymphomas appear heterogeneous, confusion with reactive lesions and polymorphic PTLD may occur. Immunophenotypic studies are critical to the diagnosis. Molecular studies help identify clonal T-cell populations. Occasionally, lymphomas of NK cells are observed.

Classical Hodgkin's lymphoma

Rarely post-transplant cases of classical Hodgkin's lymphoma are observed in the transplant population. These lesions fulfill the same criteria as those for classical Hodgkin lymphoma in immunocompetent hosts. Most cases in the transplant setting fulfil the criteria for the mixed cellularity type and most are EBV positive. As mentioned earlier, cells resembling Reed–Sternberg cells are seen in many different types of PTLD including polymorphic lesions. Although some groups have described "Hodgkin-like" PTLD [20], these are not included with the classical Hodgkin's type PTLD within the WHO classification [7].

Epidemiology

The epidemiology of PTLD has recently been reviewed by Dharnidharka et al. [21]. This section focuses on the incidence/prevalence of PTLD and the risk factors for its development.

Incidence

The risk of PTLD is highest in the first year after transplantation, yet the patient remains at risk indefinitely while under iatrogenic immunosuppression [22]. The time to PTLD varies with age, organ transplanted, and donor/recipient EBV status, among other factors. There has been a trend to earlier onset with more potent immunosuppressive regimens and disease tends to be earlier in children, especially those who are EBV naïve at transplant and receive organs from EBV-positive donors [22]. Early-onset disease is more likely to be polymorphic, of B-cell origin, and EBV positive, at least in children [23].

The frequency of occurrence in any series will depend on post-operative survival and length of follow-up, as well as on case definition. Patients dying soon after transplant are "at risk" for only a very short period. Some reports have, therefore, quoted the frequency among 30-day survivors. Most reports appear to exclude so-called "early lesions" and report only destructive forms of disease. The following cumulative percentage "incidences" have been reported for pediatric recipients at 5 years post transplantation: kidney 3%, heart 5%, liver 6%, and lung 11% [21]. Among adult recipients, the 5-year cumulative risk is nearer to 1% [24], but appears to be slightly higher for both liver and lung (2–4%) [21].

More appropriate methods of analysis for time-related events with variable follow-up include incidence density or "survival analysis" techniques. The latter is particularly helpful when the hazard for disease development is continuously changing. In a recent study, the incidence and hazard for PTLD were evaluated for 3170 primary heart transplants performed between 1993 and 2009 at 35 pediatric programs [22]. PTLD was documented in 147 pediatric recipients with freedom from PTLD of 98.5% at 1 year, 94% at 5 years, and 90% at 10 years after transplantation (Figure 96.2). The overall risk for PTLD declined in the most recent transplant era (2001–2009). This is in keeping with the impression (unproven at this time) of many pediatric transplant physicians that viral load monitoring and other preventive strategies may be reducing the burden of early PTLD. However, the Pediatric Heart Transplant Study (PHTS) dataset demonstrates a worrying phenomenon [22]; the increasing hazard for late-onset PTLD in very long-term follow-up (Figure 96.2). These late PTLDs are more likely to be monomorphic in nature and include very aggressive lesions such as Burkitt's disease [25,26].

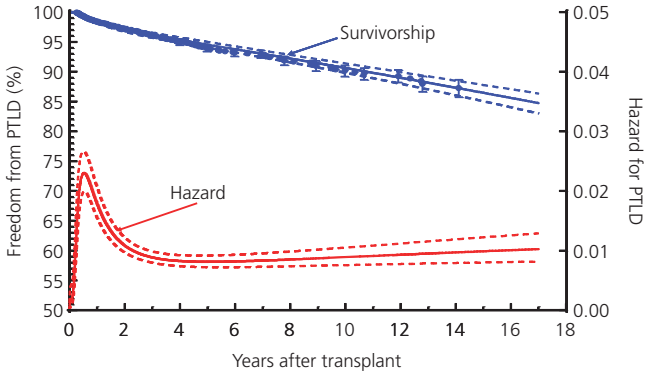

Figure 96.2. Freedom from post-transplant lymphoproliferative disorders (PTLD) in the Pediatric Heart Transplant Study (1993–2009). Note the high initial hazard for disease in the first year, followed by a second phase of slowly rising hazard late out from transplantation. (Modified from Chinnock et al. [22], with permission from John Wiley & Sons.)

Risk factors

Many different risk factors for PTLD development have been described [21]. These include infection-related factors (notably recipient and donor EBV serostatus), host-related factors such as age, primary disease-related factors, and intensity and types of immunosuppression. EBV status is probably the single strongest risk factor and has most influence in pediatric transplantation, since a much higher proportion of these recipients are naïve to this virus at the time of transplant. Positive donor EBV status was a very strong risk factor for PTLD in the seronegative recipients in the PHTS [22], but with the magnitude of the risk being dependent on recipient age at the time of transplantation. Nearly 25% of EBV-seronegative recipients aged 4–7 years at transplant with EBV-positive donors developed PTLD. In some series, cytomegalovirus (CMV) co-infection is also a risk factor [27]. Among host risk factors, beyond age, Dharnidharka et al. demonstrated that male gender and Caucasian race were risk factors among children receiving kidney transplants followed within the United Network for Organ Sharing (UNOS)/Organ Procurement and Transplantation Network (OPTN) and North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registries [28].

Many studies have focused on the impact of various immunosuppressive regimens as risk factors for PTLD. Few of these were randomized trials, and it has been very difficult to ascertain whether specific drugs are especially guilty in this regard, or whether the risk primarily relates to global intensity of immunosuppression,

especially that targeting T-cell immunity (the primary mechanism of surveillance of EBV-infected B cells; see above). PTLD was observed in the azathioprine-steroid era, but there was an increase in the number of reports that followed the introduction of cyclosporine [6]. OKT3 was also implicated as a specific risk factor by Swinnen et al. at the end of the 1980s [29]. Several reports emphasize the complexity of placing blame on specific agents. In a recent randomized trial of steroid withdrawal after pediatric kidney transplant, patients received basiliximab induction, calcineurin inhibitor (CNI), sirolimus, and corticosteroids. PTLD developed in an unacceptably high 6.9% of patients [30]. These data do not provide support for "blame" of a specific agent, but simply tell us that the total immunosuppression burden in this population was too high. Since the original report by Swinnen et al. [29], there has been concern that T-cell–depleting antibodies might be associated with increased incidence of PTLD. In an analysis of the Scientific Registry of Transplant Recipients (SRTR), Kirk et al. examined the use of depletional induction in kidney transplant recipients, demonstrating that both OKT3 and polyclonal rabbit antithymocyte globulin were associated with an increased risk of PTLD, while non-depleting induction with interleukin (IL)-2-receptor–specific agents was not, compared to patients without induction therapy [31]. Interestingly, the use of the depleting agent alemtuzumab was not associated with an increased risk of PTLD, perhaps reflecting lower levels of maintenance therapy associated with the practice patterns of this agent, e.g. steroid avoidance and lower CNI target levels, or the fact that alemtuzumab depletes both T and B cells, and thus may eliminate some of the latent EBV reservoir concomitant with the T-cell depletion. Finally, it should be noted that belatacept usage has been associated with an increased rate of PTLD development in EBV-seronegative adults after kidney transplantation, leading to specific exclusion of EBV-naïve patients [32]. Longer-term follow-up and experience in other populations will be required to understand the full implications of this observation.

Prevention of Epstein–Barr virus-associated post-transplant lymphoproliferative disorders

The importance of EBV infections in PTLD has led to an interest in the prevention of EBV disease. Potential approaches to the prevention of EBV disease can be categorized as immunoprophylaxis, chemoprophylaxis, and pre-emptive therapeutic strategies.

Immunoprophylaxis

Immunoprophylaxis can be categorized as active or passive. Active immunoprophylaxis would be accomplished through the use of an EBV vaccine. Immunization of all EBV-seronegative recipients with an effective vaccine prior to transplantation would likely dramatically reduce the risk of EBV disease and PTLD following transplantation. Unfortunately, progress in the development of an EBV vaccine has been limited, making availability of an effective vaccine unlikely in the near future [33–35]. Passive immunoprophylaxis is accomplished by providing anti-EBV antibody through the infusion of intravenous immunoglobulin (IVIG). A protective effect of IVIG against the development of EBV disease has been demonstrated in a severe combined immunodeficiency (SCID) mouse model [36,37]. These experimental data supported initiation of a multicenter, randomized, controlled trial in EBV-seronegative pediatric liver transplant recipients [38]. Although statistically significant differences were not observed, the study demonstrated a trend towards decreased rates of EBV disease and PTLD in patients receiving CMV-IVIG compared to those receiving placebo. The absence of a statistically significant effect of CMV-IVIG in this study may have been due to limitations of sample size, a lack of efficacy of the drug, or the confounding effect of pre-emptive reductions in immunosuppression based upon the presence of an elevated EBV load following introduction of polymerase chain reaction (PCR) assays in the later years of this study. Finally, the use of EBV-specific cytotoxic T lymphocytes as adoptive immunotherapy could serve as a third potential immunoprophylactic strategy. Unfortunately, although this approach has been proven to be efficacious in stem cell transplant recipients, efforts to translate these benefits to the prevention of EBV disease and PTLD in SOT recipients have not been fully realized at this time [39].

Chemoprophylaxis

Chemoprophylaxis, using antiviral agents such as acyclovir and ganciclovir, is another possible approach to the prevention of EBV disease and PTLD. Both acyclovir and ganciclovir actively inhibit lytic EBV replication in vitro, but neither agent has any effect on EBV in its latent state or on the proliferation of EBV-transformed B cells. Accordingly, if EBV disease progression is dependent upon expansion of EBV immortalized B cells independent of the lytic phase of EBV replication, the use of ganciclovir or acyclovir is unlikely to effectively prevent the development of EBV disease. Unfortunately, only limited clinical evidence is available to address the efficacy of antiviral therapy in the prevention of EBV/PTLD in humans. Published reports supporting the potential efficacy of antiviral agents have been largely retrospective or lacking in appropriate control groups [40,41]. Other reports have attempted to address the potential efficacy of antiviral therapy in the prevention of EBV disease through analyses of large registries [42,43]. Results from these two studies were contradictory, with Funch et al. concluding that antiviral therapy appeared to prevent EBV disease [42], while Opelz et al. found no protective benefit associated with the use of antiviral therapy [43].

To date, only a single randomized controlled trial has been completed evaluating the role of antiviral agents in the prevention of EBV/PTLD [44]. This randomized trial compared 2 weeks of intravenous ganciclovir alone to 2 weeks of ganciclovir followed by 50 weeks of high-dose oral acyclovir in pediatric liver transplant recipients. No difference was observed between the study arms. This suggests that the prolonged use of acyclovir did not prevent EBV/PTLD. It is possible that prolonged use of the more potent ganciclovir might have resulted in a different outcome; however, it is of interest to note that development of PTLD has been observed in patients while receiving prolonged courses of intravenous ganciclovir [45].

Viral load monitoring and pre-emptive strategies of prevention

Surveillance monitoring of EBV loads to inform pre-emptive reductions in immunosuppression has resulted in a decreased incidence of EBV disease and PTLD compared to historical controls. McDiarmid et al. reported a decreased incidence of PTLD from 10% to 5% using EBV viral load monitoring to guide the combined use of reduced immunosuppression and intravenous ganciclovir in pediatric liver transplant recipients with rising EBV loads [41]. Using decreased immunosuppression alone without ganciclovir in response to elevated EBV loads, Lee et al. noted a decline in the incidence of PTLD from 16% to 2% in a group of pediatric liver

transplant recipients when compared to historical controls [46]. Ganschow et al. also showed that lowering immunosuppression in response to results of frequent viral load monitoring was associated with low rates of PTLD in pediatric kidney transplant recipients (0.9%) [47].

More recently, Martin et al. explored the use of EBV load monitoring to inform the pre-emptive use of the anti-CD20 monoclonal antibody, rituximab, in EBV donor-positive/recipient-negative adult kidney transplant recipients who developed symptoms or persistent EBV viremia [48]. Although the authors suggested an efficacious effect of this strategy, given its small size, the relatively short period of observation prior to the use of rituximab and the concomitant reduction of immunosuppression, additional experience is needed to confirm these results. There are no randomized trials to establish the role of rituximab in PTLD prevention at this time.

Clinical presentation and diagnosis

EBV infection is associated with a wide range of disease manifestations in organ transplant recipients [1]. The spectrum of clinical disease ranges from a non-specific viral syndrome to PTLD. It is important to note that significant overlap exists in clinical manifestations of patients with EBV disease independent of whether or not they are subsequently shown to have PTLD. Similarly, patients presenting with EBV-negative PTLD may manifest clinical symptoms that are indistinguishable from EBV-associated disease.

The diagnosis of PTLD (and EBV disease) is based on clinical history and physical examination in combination with laboratory confirmation (Table 96.2). The most important factor in making this diagnosis is maintenance of a high index of suspicion at all times. In contrast to early experiences with PTLD, increased awareness and improved diagnostic tools have made presentation of PTLD in critically ill patients with disseminated disease much less common. To achieve earlier diagnosis, attention must be paid to more subtle symptoms of lethargy, malaise, weight loss, and fever, which have become more common presentations of PTLD. An additional history of vomiting and/or diarrhea (which may be guaiac positive) is suggestive of gastrointestinal involvement, which has been increasingly recognized to be a common site of disease.

Table 96.2. Diagnostic evaluation of patient with suspected post-transplant lymphoproliferative disorders

Routine	Selected patients
CBC, platelets, WBC with differential	GI endoscopy
Serum electrolytes, calcium, BUN, and creatinine	Bone scan
Liver function tests	Bone marrow biopsy
Uric acid	Brain CT/MRI
Lactate dehydrogenase	Lumbar puncture
Quantitative immunoglobulins	Screen for co-morbid infection
Serum protein electrophoresis	PET scan
EBV serologies	
EBV viral load by quantitative PCR	
Stools for occult bleeding	
Chest radiograph (AP and lateral)	
CT scan of chest/abdomen/pelvis	
Core needle or excisional biopsy of lesion(s)	

GI, gastrointestinal; WBC, white blood count; EBV, Epstein–Barr virus; AP, anteroposterior; PCR, polymerase chain reaction.

This is especially true in intestinal transplant recipients, nearly all of whom present with involvement of either the transplanted intestinal allograft or their native intestine. Intestinal hemorrhage, obstruction, and perforation may also be seen in patients with intestinal PTLD. Surprisingly, the highest risk period for the latter may be during therapy when necrosis of transmural lesions may develop.

Additional sites of involvement vary with the type of organ transplant received. Pulmonary disease is very common in cardiac recipients and is almost invariably present in lung recipients. Pulmonary presentation ranges from asymptomatic nodule(s) on a routine chest radiograph to life-threatening pulmonary dysfunction in the lung allograft. The latter may resemble lung rejection on a chest radiograph with rather diffuse consolidation without clearly defined mass lesions. This may lead to inadvertent augmentation of immunosuppression with severe consequences [49]. Involvement of the liver with EBV disease and PTLD is seen most frequently in liver transplant recipients. Disease may present as a diffuse hepatitis or with nodular lesions of the liver. Interestingly, the heart is the only organ transplant in which there is no strong tendency for the disease to involve the allograft. In most other SOTs, allograft dysfunction may be a manifestation of PTLD and may mimic acute or chronic rejection.

Other presentations include persistent sore throat, adenopathy, and cutaneous nodules, as well as seizures, headaches, and focal neurologic lesions with central nervous system (CNS) disease. PTLD is also sometimes diagnosed following elective removal of enlarged tonsils and adenoids in children. Thus, all surgically removed adenoid and tonsilar tissue should receive pathologic evaluation in the transplant recipient.

Although physical examination may not reveal specific findings, there will frequently be evidence of pallor, weight loss, peripheral adenopathy, or hepatosplenomegaly. A full physical examination is essential and should include thorough neurologic examination. Examination of the entire skin and sites of all lymph nodes is warranted. Careful examination of the oropharynx is required.

An overview of the laboratory evaluation for the diagnosis of PTLD is given in Table 96.2. Initial evaluation should include a complete blood count with white cell differential and platelets. Leukopenia, often in association with atypical lymphocytosis, and thrombocytopenia are frequent findings. Anemia is common and may be normocytic and normochromic or may demonstrate findings of iron deficiency when occult gastrointestinal bleeding is present. Rarely, evidence of hemolytic anemia may also be present. Stools should be tested for blood. End-organ function (liver, kidney) should be evaluated. Elevations in uric acid and lactate dehydrogenase are common and should also be sought on blood chemistry testing. Immunoglobulin levels may be elevated. This is particularly true of IgE, perhaps due to a Th2-predominant cytokine environment that may be present with PTLD. Some centers also routinely perform serum protein electrophoresis to look for evidence of monoclonal gammopathy. Although many patients do not demonstrate a monoclonal or oligoclonal gammopathy, if present, this provides an additional means of following the patient's response to therapy.

A variety of imaging tests are helpful in the evaluation of the transplant recipient with suspected PTLD. A chest radiograph often reveals evidence of pulmonary nodular disease and/or of mediastinal lymphadenopathy. The most informative diagnostic study is usually contrast-enhanced CT evaluation of the chest, abdomen, and pelvis. Evidence of nodal or extranodal disease will

frequently be apparent on CT at one or more sites. In the chest, small pulmonary nodules or enlarged mediastinal lymph nodes may be apparent even in the presence of a normal chest radiograph. In the abdomen, disease may be found at normal lymph node sites, within the gastrointestinal tract, or at extranodal sites, including the liver, spleen, and kidneys. Some centers routinely perform CT or magnetic resonance imaging of the brain. These studies should always be performed if there is any clinical suggestion of CNS disease.

The potential role of positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (FDG) in the diagnosis and assessment of clinical response to treatment of PTLD has gained increasing attention [50]. Potential advantages over routine CT scanning include enhanced sensitivity to detect lesions at the time of diagnosis, as well as the ability of PET scans to potentially differentiate between viable tumor and residual necrotic or fibrotic lesions on follow-up during or after completion of therapy. Additional experience is needed to determine whether routine use of PET scanning will result in improved clinical outcomes.

Other studies performed on selected patients as directed by the clinical findings are shown in Table 96.2. Upper and/or lower gastrointestinal endoscopy should be performed when there are gastrointestinal symptoms or evidence of occult gastrointestinal bleeding. Lumbar puncture should be performed when there is evidence of CNS disease. Bone marrow evaluation should be performed when there is evidence of bone marrow failure and in the presence of monomorphic disease before commencement of therapy. It is less clear that this needs to be performed in patients with early lesions or polymorphic disease (mostly pediatric patients).

Measurement of EBV viral load in the peripheral blood with PCR has been evaluated as a diagnostic tool for patients with symptomatic EBV disease including PTLD [10,11,48,51,52]. A growing experience suggests elevated EBV viral loads will be present in the vast majority of children with EBV-associated PTLD as well as many adults. This is most apparent when EBV-driven PTLD develops in the recipient who is EBV seronegative at the time of transplantation. It is important to note that the height of the EBV viral load is often comparable among patients with EBV-associated viral syndromes and those with PTLD. In addition, viral loads may be elevated in the absence of active disease, with some patients becoming chronic high load carriers after asymptomatic primary EBV infection [25]. Thus, while measurement of the EBV viral load is a useful screening procedure for suspected EBV disease, the test lacks specificity and it cannot replace histologic examination of suspected sites of involvement when the diagnosis of PTLD is contemplated. Finally, clinicians should not rely on serologic tests to make the diagnosis of PTLD as many patients will have positive EBV titers on the basis of passive immunization from blood products (or of maternal origin in infant recipients) and others may seroconvert without manifesting any clinical symptoms. In addition, the immunosuppressive agents used in transplant recipients might result in some patients having falsely negative serologic results.

Treatment

Despite a growing understanding of the pathophysiology of PTLD, its optimal management remains controversial. There is an increasing armamentarium of treatments available to the clinician, but with little evidence base to define how and when to use these treatments, or how best to combine therapies. Of note, no pivotal randomized trials of any form of therapy have been performed. The choice of therapy is often arbitrary, e.g. institutional preference, but may also be driven by predictive factors (real or perceived) such as pathologic findings, age at onset, organ transplanted, disease stage, presence or absence of EBV, co-morbid conditions, and prior rejection history.

The optimal treatment regimen for PTLD is one that rapidly eradicates the disease, does not increase the risk of allograft rejection (acute or chronic), and is simple to give, cost-effective, and associated with minimal adverse events. It is apparent that no single treatment fulfills all these criteria. Furthermore, the treatment must be geared to the individual patient, since the appropriate treatment for severe PTLD in a lung recipient early after transplant with adverse rejection profile, for example, is likely to be very different from that for a renal recipient with benign rejection history late out from transplant. These considerations underscore the enormous challenges involved in designing clinical trials for a rare disease with such clinical heterogeneity. Clinical response is also likely to depend on intrinsic characteristics of the tumor, such as rate of mitosis, presence of oncogenes, presence or absence of EBV, and the ability to be controlled by reconstitution of T-cell immune surveillance. Unfortunately, it is currently very hard to predict tumor behavior, even after extensive pathologic evaluation is completed.

Reduction of immunosuppression

In 1984, Starzl et al. reported the reversibility of PTLD by reduction of immunosuppression in cyclosporine-treated patients [8]. This strategy remains the initial therapy for most patients with early lesions or polymorphic disease in most centers. It is also used as first-line therapy in monomorphic disease by some groups (especially in children), although more often modern regimens include adjunctive therapies such as anti–B-cell monoclonal antibodies from time of diagnosis for this histology. Regardless, immunosuppressive reduction should be considered as a critical part of a response to PTLD. The goal of reduction (or cessation) of immunosuppression is to allow the host to recover natural immune surveillance and subsequently gain control over the proliferation of latent EBV-infected B cells. Reconstitution of immune surveillance may also be associated with resolution of EBV-negative PTLD, though this seems to occur with lower frequency [53]. The majority of PTLD in children (especially polymorphic lesions) will respond to reduction in immunosuppression, though with significant rates of rebound acute cellular rejection that may vary by organ [23,54]. The reported response rates of PTLD to reduction of immunosuppression among adults are highly variable, with acceptable results reported by some groups for low-risk patients [55] and very poor results by others [56,57]. These diverse outcomes may reflect differing referral patterns and patient characteristics, differences in range of pathology, e.g. proportion with polymorphic versus monomorphic disease, and perhaps differences in use of adjunctive therapies (such as antiviral agents) that are not always well described. As for children, rebound rejection rates in adults are significant and are an important cause of death after treatment of PTLD by reduced immunosuppression, especially after heart transplantation [56].

In general, most patients show evidence of clinical response within 2–4 weeks of reduction of immunosuppression, though a delayed response has been observed as long as several months in some patients. Complete responses to reduced immunosuppression

in DLBCL [53,57], post-transplant Hodgkin's disease, and other rarer PTLDs, e.g. T-cell lymphoma, are less likely, and if they do occur, are often incomplete or not durable. There is, therefore, increased reluctance to use reduction in immunosuppression as sole initial therapy for these histologies.

The approach to reduction in immunosuppression varies widely. Many groups initially hold CNIs and adjunctive antiproliferative agents, and maintain any corticosteroids that the patient might be receiving. Tacrolimus and cyclosporine levels may initially be high due to impaired hepatic metabolism. Subsequent practice varies widely. Among liver transplant programs, there is a tendency to withhold CNIs long term or even indefinitely [54]. This is unlikely to be possible in thoracic and intestinal transplantation. There has been considerable interest in replacing CNIs with inhibitors of the mammalian target of rapamycin (mTOR) once immunosuppression is reintroduced. Sirolimus has frequently been used in this setting. Interest in the use of this group of agents in PTLD, in part, reflects their antineoplastic properties, including inhibition of EBV-driven B-cell lymphomas. However, mTOR inhibitors are also potent T-cell inhibitors, so will impair T-cell immune surveillance. In the clinical setting, it is hard to know whether use of mTOR inhibitors is beneficial because they may suffice to prevent graft rejection (without the need for reintroduction of CNIs), or whether these agents are actually exhibiting anti-PTLD properties in vivo. Of note, patients managed with CNI-free immunosuppression, by using mTOR inhibitors, may still develop PTLD [58].

Surgery and radiation therapy

Excisional biopsy, generally performed for diagnostic purposes, may be curative for solitary PTLD lesions, but is usually combined with some reduction in immunosuppression. Thus, almost all patients receive a systemic approach to treatment and PTLD is probably best thought of as a systemic process. Surgery may also be indicated for management of local complications such as gastrointestinal hemorrhage, obstruction, or perforation.

Radiation therapy has a limited role in the management of PTLD, although many lesions will be responsive. It has been used when rapid local responses are required, e.g. when there is acute airway compression from tumor mass, or compression of other critical structures. It may also have a role in the treatment of some cases of CNS PTLD (see below).

Antiviral therapy

Initial interest in the role of antiviral chemotherapy for treatment of PTLD arose in 1982 when Hanto et al. described a patient whose EBV-associated PTLD lesion appeared to wax and wane in association with starting and stopping acyclovir [5]. As outlined above, both acyclovir and ganciclovir inhibit lytic EBV DNA replication in vitro and may be of value in treating the lytic phase of EBV infection. However, neither acts on latent infection or effectively treats proliferation of immortalized B cells; thus, the rationale for their use for the treatment of PTLD is far from clear. Nonetheless, use of these agents for the treatment of PTLD has become routine in many centers.

There has been progress in the development of other types of antiviral agents for the treatment of severe HHV infections, including those due to EBV [59,60]. Drugs that act independently of the viral enzyme target, thymidine kinase, may be particularly suitable candidates for investigation for the treatment of PTLD. Cidofovir has potential in this regard, but has important toxicities. Work suggests that lipid ester analogs of cidofovir and cyclic cidofovir may have much greater activity against EBV than the parent drug and may be suitable drugs for phase I clinical studies [60].

Another antiviral strategy is to induce EBV thymidine kinase in EBV-infected tumors, thus making the tumors sensitive to nucleoside-type antiviral agents such as ganciclovir. One agent that may achieve this goal is arginine butyrate. In a phase I/II clinical trial in 15 adults with refractory EBV-positive lymphoid tumors, continuous infusion of arginine butyrate along with standard doses of ganciclovir led to significant antitumor responses in two-thirds of patients [61]. More recently, it has been shown that short, discontinuous exposure to arginine butyrate (in contrast to continuous infusion) may suffice to initiate lytic phase EBV gene expression and thymidine kinase induction [62]. This therapy holds promise for patients with EBV-driven PTLD.

Interferon and other cytokines

The use of interferon (IFN) has been described in anecdotal reports as a therapeutic option in the management of PTLD [63,64]. IFN is both a proinflammatory cytokine and a natural antiviral agent, and appears capable of controlling proliferation of EBV-infected B cells. Since it is a non-specific immune stimulant, antidonor responses are often seen and severe rejection can develop during therapy. In a series of adult PTLD patients unresponsive to reduction in immunosuppression, only one of 13 (7%) achieved durable complete remission with IFN-α2b [57]. At the present time, most centers are not routinely using IFN in the management of PTLD.

IL-6 has been described as a growth factor for EBV-infected B cells. For this reason, an anti–IL-6 monoclonal antibody has been tested in a phase I/II clinical trial [65]. It was well tolerated and complete response was observed in approximately 40% of patients who had not responded to a brief period of reduction in immunosuppression. It is not currently used in routine clinical care.

As the biology of EBV-PTLD is further unraveled [12], more targets for biologic intervention are likely to be identified.

Intravenous immunoglobulin

A potential role for the use of IVIG for the treatment of PTLD has also been suggested. Several reports have documented an association between losses or absences of antibody against at least one of the EBNAs in EBV-infected organ recipients and the subsequent development of PTLD [66]. In addition, a correlation between increasing levels of anti-EBNA antibodies (including those introduced through transfusions) with a decrease in EBV viral load has been demonstrated. Taken together, these reports may provide a rationale for considering the use of antibodies in the prevention and/or treatment of EBV disease and PTLD, even though the primary mechanism for controlling EBV infection appears to be cytotoxic T-cell–mediated immunity. IVIG has been used alone and in combination with IFN-α as treatment for PTLD [64]. Both IVIG and CMV-IVIG have been used in the treatment of some patients with PTLD. As with the use of antiviral agents and IFN, there are no comparative trials evaluating the role of IVIG in general, or CMV-IVIG in particular, in the treatment of PTLD.

Anti–B-cell antibodies

Most PTLDs are of B-cell origin and most express CD20. The anti-CD20 human/mouse chimeric monoclonal antibody rituximab (Genentech Inc. and IDEC Pharmaceuticals) is currently commercially available for the treatment of certain $CD20^+$ B-cell non-Hodgkin's lymphomas in adult non-transplant recipients. In 2000,

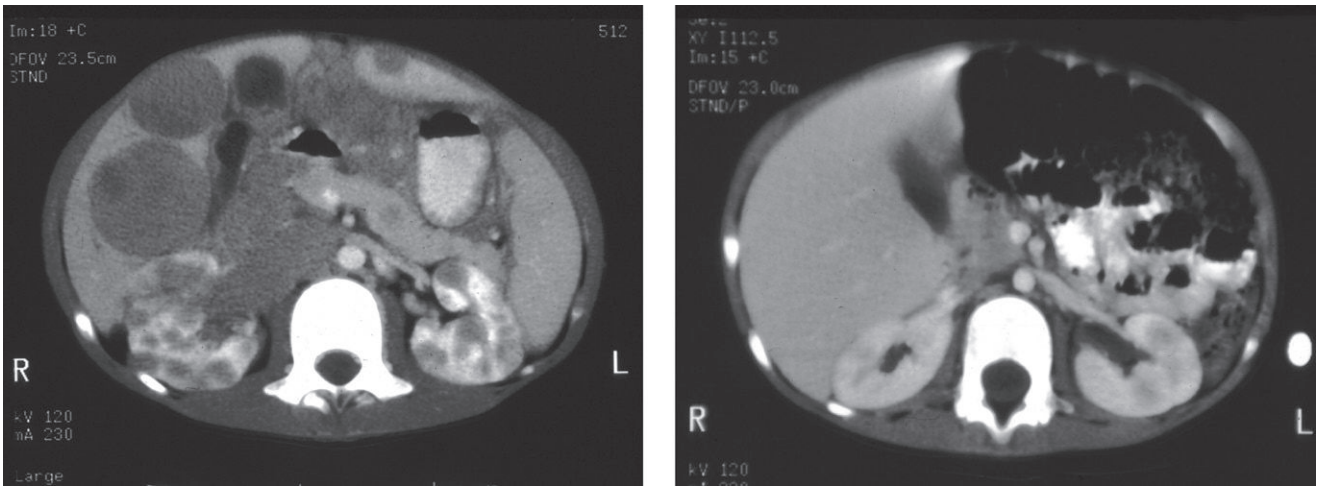

Figure 96.3. Burkitt's lymphoma in a pediatric heart transplant recipient. (A) There is diffuse disease throughout the abdomen, as well as disease in the jaw and orbit. (B) There was almost complete resolution of disease after two courses of chemotherapy. Sustained complete response was achieved. (Reproduced from Dharnidharka V, Green M, Webber SA (2010). Post-transplant lymphoproliferative disorders (figure 9.2), with kind permission from Springer Science+Business Media B.V. Copyright 2010, Springer–Verlag)

clinical investigators in France published a retrospective analysis of the use of rituximab in 32 patients with PTLD [67]. The overall response rate was 65% in SOT recipients, most of whom experienced long-term remission. However, relapse of PTLD developed in approximately 20% of responders a median of 7 months after completing their therapeutic course of rituximab. Several subsequent reports have emerged from single centers [53,68–70] and from multicenter phase II trials [71,72]. Overall response rates for adults vary significantly, with complete response rates ranging from 28% to 59% when rituximab is used as second-line therapy after failed reduction of immunosuppression [68,71,72]. However, late follow-up of a large series of 60 patients has revealed that 57% had progressive disease 1 year after completing treatment, with a median progression-free survival of only 6 months [73]. Despite the suboptimal results of rituximab in adults with PTLD, it has been pointed out that the drug is well tolerated, that a significant number will achieve durable complete responses without the need for chemotherapy, and those that do demonstrate progressive disease or relapse can still undergo chemotherapy [53]. Interestingly, rituximab may also be effective in some patients who have previously been treated with chemotherapy and have refractory or relapsed disease [74].

The results with rituximab may be superior in children, although less data are available to support this premise. In a small prospective multicenter phase II trial, 80% of children with refractory PTLD achieved complete response after four or eight doses, but with relapse in 25% [75].

There are several important questions regarding the use of rituximab in the transplant setting:

- Will the prolonged elimination of B cells result in additional opportunistic infections or other sequelae?
- Should rituximab be used in all patients at diagnosis, or only those who fail an initial period of reduced immunosuppression?
- If relapse occurs after the use of rituximab, how should management proceed?
- Is maintenance therapy every 6 months justified?
- Is the high relapse rate with rituximab due to use primarily in high-risk patients, i.e. those with refractory disease, or could therapy per se somehow be associated with subsequence risk of relapse?
- Are clinicians less aggressive in immunosuppression reduction when they use rituximab from the time of diagnosis?

It seems likely that durable remission cannot be achieved unless restitution of EBV-CTL responses is achieved [76]. Finally, it should be noted that there has been very little experience with newer radioconjugates of anti–B-cell antibodies (so-called radioimmunotherapy). Do these agents offer any advantage over rituximab and are they safe? Hopefully, ongoing and future studies will answer some of these important questions.

Chemotherapy

Chemotherapy is usually used as first-line treatment for aggressive post-transplant lymphomas such as Burkitt's disease (Figure 96.3) or T-cell lymphomas, and also for most cases of post-transplant classic Hodgkin's disease. As discussed earlier, it is unclear whether patients with monomorphic disease with histology of DLBCL should receive a trial of reduction in immunosuppression with or without rituximab, or whether they should receive chemotherapy as primary treatment. This is one of the most controversial areas in the current management of PTLD, with little evidence base to guide therapy.

Pediatric patients with refractory polymorphic PTLD have quite high initial response rates to "low-dose" chemotherapy with cyclophosphamide and prednisone, though 2-year event-free survival is suboptimal at around 58% [77]. The addition of rituximab to this regimen has also recently been studied [78]. It is unclear whether chemotherapy has a role before a trial of rituximab has been completed, since rituximab alone has given encouraging results in pediatric refractory disease [75]. Low-dose chemotherapy has not been compared directly to rituximab in children failing to respond to reduced immunosuppression. Chemotherapy should be given to children with progressive disease on rituximab, and is also useful for the rare cases of active PTLD with concomitant allograft rejection. Burkitt's disease in children and adults responds well to multidrug chemotherapy regimens (with or without rituximab; Figure 96.3), though the optimal regimen

remains to be defined [26,79,80]. We do not recommend rituximab alone for this condition.

The indications for chemotherapy in adults are broadly similar to those in children. Centers are divided on whether DLBCL (the most common pathology in adults) should be treated primarily with chemotherapy, or only after failure to respond to reduced immunosuppression and rituximab. Chemotherapy will generally protect the graft against rejection and impaired graft function [81], but is associated with higher infectious morbidity and mortality than comparable regimens used in the non-transplant setting [53]. Multiple different chemotherapeutic regimens have been used including cyclophosphamide-prednisone (with or without rituximab), "CHOP," "CHOP–rituximab," "ACVBP," and "ProMACE-CytaBOM" [53,57,77–86]. None has been directly compared to each other in controlled trials. Overall response rates are of the order of 65–80%, though late mortality appears to be significant [87].

Cellular immunotherapy

Inadequate EBV-specific T-cell responses are an important, if not critical, pathologic step in the development of EBV-driven PTLD, though other mechanisms may contribute. Infusion of EBV-specific CTLs has been employed both as treatment and prophylaxis against PTLD in bone marrow/stem cell transplantation [88]. In this setting, the PTLD are generally of donor origin, and the donor is usually available to provide a source of CTLs for the recipient. The success of this adoptive immunotherapy in stem cell recipients has stimulated investigation into applying this approach as a therapy for PTLD in SOT recipients [89]. This is a logical therapy as it is directed against the PTLD and should, in theory, cause little anti-donor response, i.e. rejection. However, the use of CTL infusions in solid organ recipients is made more difficult by the fact that the EBV-infected cells within PTLD lesions are typically of recipient origin. EBV-specific CTLs should ideally be HLA haplo-identical. For this reason, autologous CTLs are the obvious source for the development of EBV-specific CTL infusions for SOT recipients. However, the development of EBV-specific CTLs from organ recipients is challenging since most patients at risk for EBV-PTLD are EBV naïve at transplant, CTL generation is impaired by the presence of immunosuppression, and T-cell responses are further severely suppressed at the time of development of PTLD. Accordingly, techniques for adoptive immunotherapy of EBV-associated PTLD in SOT recipients have focused on developing strategies to "immunize" and stimulate the organ recipient's own T cells against EBV ex vivo and then subsequently infusing these EBV-specific CTLs back into the recipient at a time when the patient develops refractory EBV infection/PTLD [39,89,90]. Such an approach could also be used for prevention, with infusion performed when EBV viral loads start to climb after primary post-transplant EBV infection [39]. Ideally, the cells for culture should be obtained prior to transplantation and the initiation of immunosuppression in high-risk recipients (EBV seronegative at transplantation). Such an approach is expensive and labor intensive, as most candidates will never require CTL therapy. Furthermore, at this time, the success of adoptive immunotherapy using autologous CTLs after SOT has not paralleled that seen after bone marrow/stem cell transplantation. An alternative approach that has met with some success is the use of allogeneic T-cell infusions from EBV-positive blood donors who are as closely matched as possible to the recipient's HLA type. Success in about 50% of patients was observed in a phase II clinical trial using this approach [91]. While the use of adoptive immunotherapy represents an exciting advance in the management of PTLD after SOT, it remains a research tool at this time, limited to a very small number of SOT centers that offer cell-based therapies. Current research is focusing on the optimal strategy for developing ex-vivo cultures with high cytotoxicity against EBV-infected target cells. The nature of the stimulating cells, e.g. lymphoblastoid cell lines or dendritic cells loaded with EBV antigens, and the cytokine milieu of the culture, e.g. addition of IL-7 and IL-12, may be critical determinants in the success of development of ex-vivo generated CTL bulk cultures for clinical infusion [92,93].

Role of combination therapies

Since no therapies have been tested in large randomized controlled trials, it is evident that the use of combination therapies is also not evidence based. Nonetheless, as with other diseases, there may be logic in combining therapies that work by different mechanisms of action. The use of combination therapies also makes it very challenging to identify the efficacious components of a treatment regimen. For example, autologous EBV-specific CTLs immediately following polychemotherapy have been used successfully combined with rituximab [90]. Combination therapies are likely to remain largely empiric given the enormous challenges of performing randomized controlled trials in this disease.

Central nervous system disease

CNS disease may be primary [94,95] or may be an additional site of disease in patients with extracranial disease [94]. Disease is frequently multifocal, and may occur at any site, though is most commonly seen in the cerebral hemispheres [96]. Almost all lesions show contrast enhancement. The differential diagnosis includes other malignancies and infection, especially fungal. Biopsy is generally warranted to ensure the correct diagnosis. Prognosis has generally been considered to be very poor [94,95]. The CNS is generally considered a protected site in which it is harder for normal immune surveillance to gain control of disease. Nonetheless, normal immune surveillance may be sufficient to control disease in rare cases (Figure 96.4). Unfortunately, this occurs with insufficient frequency to justify reduction in immunosuppression as the only therapy. In renal transplant patients, complete cessation of immunosuppressive therapy seems justified until disease is eradicated. Adjunctive therapies are generally given, though their efficacy has been hard to establish. These have included systemic or intrathecal rituximab, chemotherapy (especially with use of high-dose methotrexate), and radiation.

Prognosis and outcome

Outcomes after PTLD are highly variable, and pooled estimates of graft and patient survival across age groups, e.g. pediatric versus adult, and across organ transplant types provide little useful information to guide the physician in individual patient management. When specific populations are studied, it is clear that PTLD does have a significant negative impact on long-term graft and patient outcomes [23]. Furthermore, death due to progressive disease is not necessarily the dominant force in determining outcome, with acute and chronic rejection being important determinants of outcome after diagnosis of PTLD [23]. Thus, therapeutic strategies must attempt to protect the graft during reduced immunosuppression as well as effectively treat the PTLD [81].

Factors predicting outcome have recently been reviewed [97] and are also the topic of several recent reports [98–100]. In many series,

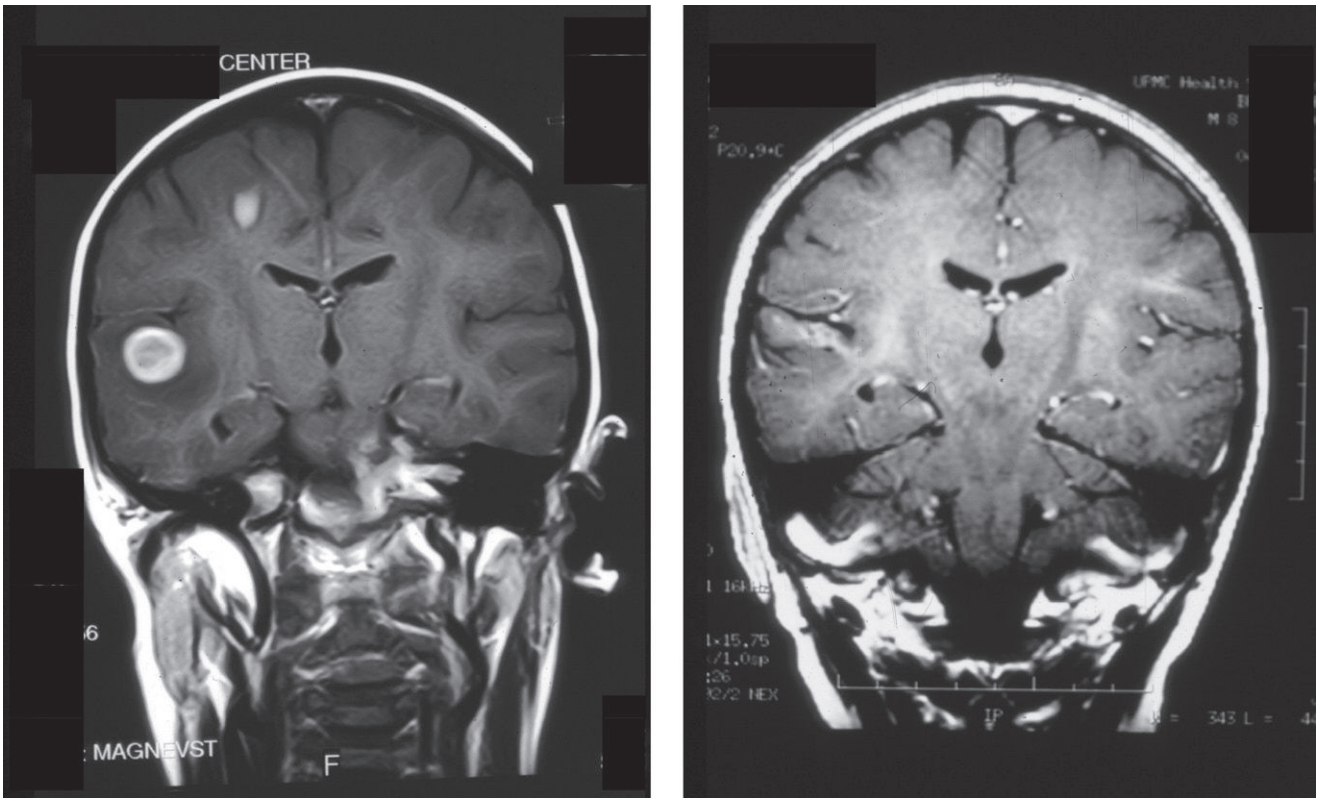

Figure 96.4. (A) Central nervous system monomorphic post-transplant lymphoproliferative disorder (PTLD) presenting with sixth nerve palsy and multiple contrast-enhancing space-occupying lesions (panel A). (B) There was complete resolution of disease after temporary withholding of immunosuppression. (Reproduced from Dharnidharka V, Green M, Webber SA (2010). Post-transplant lymphoproliferative disorders (figure 9.3), with kind permission from Springer Science+Business Media B.V. Copyright 2010, Springer–Verlag).

poor prognostic factors are similar to those for traditional non-Hodgkin's lymphomas in the non-transplant setting and include poor performance index, multifocal disease, bone marrow involvement, and increased age [97,100]. T-cell PTLDs and CNS PTLD are also associated with poor prognosis in most series, though recent data suggest that aggressive therapy can result in sustained remissions in some patients with CNS disease [96]. There is less consistency among series that monomorphic histology, absence of EBV, or late-onset disease are adverse prognostic factors. In the future, it seems likely that a more detailed understanding of the biology and molecular pathology of PTLD will improve our understanding of disease behavior and lead to individualized therapies [101]. Central to this goal is the understanding that PTLDs are a heterogeneous group of disorders clumped together under a common label and it is therefore unlikely that one optimal therapy will be appropriate for all PTLDs. Encouragingly, recent data suggest that new therapies may be modifying disease outcomes with evidence of improving survival in the current era [98].

Summary

PTLD continues to be an important potentially mortal condition in organ transplant recipients. Its association with EBV biology has provided important insights regarding the effects of immunosuppression on viral-driven malignancy, and has helped shape recognition of its clinical risk factors and the development of regimens for its treatment. Consensus has been reached on the classification of PTLD and this classification directs clinical therapy. Improvements in early diagnosis and treatment have been realized, but PTLD continues to contribute to post-transplant morbidity and mortality, especially in pediatric transplant recipients, and EBV-naïve recipients of organs from EBV-seropositive donors. Cognizance of the EBV status of all transplant patients is important and should be incorporated into the clinical routine of transplant clinicians.

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CHAPTER 97

Pregnancy and Contraception in Transplantation

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Introduction

Transplantation is the preferred therapeutic option for many patients with end-stage organ failure. The first successful human kidney transplant took place in December 1954 [1]. However, it was not until the 1960s that immunosuppression became available and not until the 1980s, with the introduction of cyclosporine, that consistently acceptable graft and patient survival was achieved (see Chapter 1). With the restoration of organ function, patients experience an overall improvement in their health, increased libido, and return of fertility. It is thus a natural consequence of successful transplantation that transplant recipients desire the same opportunities for parenthood as the general public, and indeed, one of the clearest measures of the success of transplantation in general, is the capacity for patients to experience this fundamental life goal. The first post-transplant pregnancy occurred in March 1958 and was reported in 1963 [2]. It occurred in a patient who had received a kidney from her identical twin. This pregnancy resulted in the cesarean delivery of a healthy boy.

As transplantation has improved, pregnancies have been reported in recipients of each organ type, though most outcomes reported are in kidney transplant recipients [3–10]. While successful pregnancy is possible, it is not without significant differences compared to a low-risk pregnancy in the general population. Issues that must be considered include maternal graft function and maternal health, the immediate and long-term effects of pregnancy on graft function, and the effect of the medications and graft function on the developing fetus. There is also the question of whether more subtle and long-term effects, although not apparent at birth, might affect the growth and development of the offspring of these recipients or future generations [11,12].

In this chapter we will review the current recommendations for contraception, the available guidelines for prepregnancy and prenatal management, and the available pregnancy outcome data in the solid organ recipient groups.

Contraception for the transplant recipient

Female transplant recipients should use safe and effective contraception and plan for pregnancy if so desired. In spite of the risks of pregnancy, many transplant recipients are not counseled about the need for contraception nor use any form of birth control [13,14]. In two recent questionnaire studies in renal transplant recipients, one from Iran and one from China, 29.1% and 88.2% of pregnancies in transplant recipients were unwanted or unplanned, respectively [15,16]. Of note, in the Chinese study, 94% of women who did not become pregnant used a method of contraception, whereas only 56% of those who became pregnant did so. Most women who did not use contraception in this study were unaware that fertility is often restored after transplantation [16]. Data regarding contraception failure in western countries are surprisingly sparse.

Currently there are myriad contraceptive options in the US: estrogen-containing patches, rings, and pills; progesterone-only pills; implants and intrauterine devices (IUDs); emergency contraception; as well as various methods of barrier contraception and sterilization. Many of these methods have non-contraceptive benefits as well as specific risks and side-effects.

In 2010 the Centers for Disease Control and Prevention (CDC) published the first US Medical Eligibility Criteria for Contraceptive Use and "solid organ transplantation" was included as a condition for which recommendations were given. This condition was further subdivided into "complicated: graft failure (acute or chronic), rejection, cardiac allograft vasculopathy" and "uncomplicated." Each method was categorized as: 1 = there are no restrictions for use of this method; 2 = the advantages of using this method generally outweigh the theoretical or proven risks; 3 = the theoretical or proven risks generally outweigh the advantages of using this method; and 4 = there is an unacceptable health risk if the method is used [17]. Risk assignments were based on a thorough review of the literature as well as expert opinion. Risks of other co-morbidities such as hypertension and coagulopathy were also taken into

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 97.1. Benefits and risk of different contraceptive methods in transplant recipients

Method	Benefits	Problems	Issues related to transplant	CDC category uncomplicated*	CDC category complicated*
Copper T IUD	Most effective Long acting	Heavy menses	Macrophage-mediated action not affected by most immunosuppressants	2	3 (initiation) 2 (continuation)
Progestin IUD	Most effective Long acting Decreased anemia	Irregular bleeding		2	3 (initiation) 2 (continuation)
DMPA	Highly effective Decreased anemia	Decrease in BMD Irregular bleeding	Possible cholestatic effect	2	2
Progestin implant	Most effective Long acting No decrease in BMD	Irregular bleeding		2	2
Combined oral contraceptive (COC)	Decreased anemia Menstrual regulation		First-pass liver metabolism- Metabolized in liver; levels may be affected by immunosuppressants Contraindicated in uncontrolled hypertension, active liver disease, and history of myocardial infarction, stroke, or deep venous thrombosis May be used if stable organ function and no contraindication Gastrointestinal disturbance by some immunosuppressants may decrease absorption Possible hyperkalemia caused by some immunosuppressant precludes use of drospirenone containing COCs	2 (do not use in women with a history of Budd–Chiari syndrome)	4
Contraceptive patch	First-pass liver metabolism avoided	Higher circulating levels of estrogen	Contraindicated in uncontrolled hypertension, active liver disease, and history of myocardial infarction, stroke, or deep venous thrombosis May be used if stable organ function and no contraindication	2 (do not use in women with a history of Budd–Chiari syndrome)	4
Vaginal ring	Lower circulating estrogen First-pass liver metabolism avoided		Contraindicated in uncontrolled hypertension, active liver disease, and history of myocardial infarction, stroke, or deep venous thrombosis May be used if stable organ function and no contraindication	2 (do not use in women with a history of Budd–Chiari syndrome)	4
Progestin-only pill		Less effective than COCs	*First-pass liver metabolism	2	2
Condoms	Protects against sexually transmitted infections No drug interactions	Less effective		1	1
Cervical cap/ diaphragm	No drug interactions	Less effective		1	1
Emergency contraception	Effective after sexual intercourse	Not effective as ongoing method		1	1
Withdrawal (coitus interruptus)	No drug interactions	Least effective		N/A	N/A

*CDC categories briefly defined: 1, no restriction for use; 2, advantages of using the method outweigh the risks; 3, risks outweigh the advantages of using the method; 4, unacceptable risk.
IUD, intrauterine device; DMPA, depot medroxyprogesterone acetate; BMD, bone mineral density.

account. A summary of the findings is given in Table 97.1 and discussed below.

Estrogen-containing contraception

Estrogen-containing contraceptives such as the combined oral contraceptives (OCs), contraceptive patch, and vaginal ring are often avoided in transplant recipients due to perceived thrombotic risk as well as possible liver toxicity. These formulations are thought to have many benefits: decreased menstrual bleeding, anemia, dysmenorrhea, acne, hirsutism, as well as ovarian and endometrial cancer [18]. The main risks of these compounds are related to thrombogenesis and increase in blood pressure.

Combined OCs currently available in the US vary in estrogen and progesterone formulations and doses, but are mostly considered "low dose." The contraceptive patch generally achieves higher blood estradiol levels and may have a slightly higher risk of venous thromboembolism (VTE) than OCs [19]. This risk, however, is lower than the risk of VTE during pregnancy. The contraceptive ring results in lower circulating estrogen levels, and avoids the first-pass effect in the liver; thus, it may be associated with lower VTE risk, but this remains unproven [19].

Studies regarding estrogen-containing contraceptives in transplant recipients are limited to small case series and several case reports. There are two prospective cohort studies of kidney transplant recipients treated with low-dose OCs (26 women) or the contraceptive patch (ten women) [20]. One of the recipients developed a thrombophlebitis after 18 months of combined OC use and a second developed rejection associated with deterioration of liver function approximately 10 years after transplant. In these studies there were no significant changes in liver enzymes, cholesterol, serum creatinine, and glucose after 18 months of use. There was, however, an increase in glucose and creatinine (from 1.31 mg/dL to 1.47 mg/dL; $P < 0.01$) at 6 months. Several women in these series required modification of their antihypertensive medications for

proper blood pressure control [20]. There was a significant increase in hematocrit in both OC and patch users. In a retrospective study of 15 liver transplant recipients, there were no changes noted in liver function, glucose metabolism, blood pressure, or body mass index (BMI) with combined OC use [21]. A case report identifies a recipient who was given a high-dose OC to control menorrhagia and she developed cholestasis which resolved upon withdrawal of the combined OC [22]. The recent questionnaire study from China included 34 recipients who used combined OCs, four of whom had unwanted pregnancies [16].

Based on the data from these small studies as well as expert opinion, the CDC guidelines assign a category 4 for use of combined hormonal contraception in "complicated" solid organ transplantation (SOT) (i.e. the health risks for these patients with use of combined hormonal contraception are unacceptable) [17]. For "uncomplicated" SOT, the risk is category 2 (i.e. the benefits generally outweigh the theoretical or proven risks) [17]. Therefore, in recipients with stable organ function and well-controlled hypertension who otherwise do not have other contraindications to estrogen-containing compounds, it is reasonable to offer estrogen-containing contraception.

As with any systemic medication, there is concern that the estrogen-containing contraceptives theoretically could affect immunosuppressant levels and vice versa. Estrogen is metabolized in the liver and immunosuppressants may activate the cytochrome P450 3A4 system. Thus, these medications may in theory decrease circulating levels and the efficacy of combined OCs. Existing data, however, suggest that interactions are minor and combined OCs remain effective in transplant recipients [19]. In addition, combined OCs may affect levels of immunosuppressants, though this has not been studied [23]. Therefore, women who have undergone SOT and are treated with estrogen-containing contraceptives must be closely monitored for hypertension, side-effects, and changes in immunosuppressant levels.

Progesterone-only hormonal contraception

Progesterone-containing systemic contraception such as depot medroxyprogesterone acetate (DMPA), etonorgestrel implant, and progestin-only pills has often been advocated in patients with contraindications to estrogen therapy. These products use different types and doses of progesterone. The progestin-only pill is taken daily, may have higher failure rates, and undergoes a first-pass effect in the liver. DMPA can be administered subcutaneously or intramuscularly every 12 weeks. It is metabolized in the liver and should be avoided in women with active liver disease, but its metabolism does not appear to be affected by other drugs [19]. Studies revealing reversible bone density decreases with DMPA use and the increase in osteoporosis in transplant recipients suggest that bone mineral density may need to be monitored (see Chapter 100). In addition, DMPA is associated with a delay in return of fertility and thus may not be the best method for a woman who wants a future pregnancy. The implant is one of the most effective methods of contraception and has a similar benefit profile to that of DMPA. Additionally, the implant does not decrease bone mineral density [19].

To date there are no studies evaluating these progesterone-only methods in transplant recipients. The CDC guidelines assign a category 2 for all three of the progesterone contraceptives in both "complicated" and "uncomplicated" cases of SOT [17]. Therefore, these forms of contraception are considered safe in transplant recipients.

Intrauterine devices

Currently in the US, two IUDs are available: the copper IUD and the levonorgestrel (progesterone)-containing IUD (LNG-IUD). The copper IUD can be associated with increased menstrual bleeding and should not be used in patients with Wilson's disease. The LNG-IUD has been shown to significantly decrease menstrual bleeding and increase hemoglobin [24]. A case report of a kidney recipient treated with a LNG-IUD for fibroids and menorrhagia noted significant improvement in dysmenorrhea and menorrhagia after 1 year of use [25].

IUDs are one of the most effective methods of birth control and yet in a study of renal transplant recipients, only 5.2% were recommended the IUD post transplant [14]. Two case reports from 1981 of pregnancy in renal transplant patients using the copper IUD for contraception sparked the speculation that immunosuppressive agents decrease the effectiveness of IUDs, but the evidence suggests that macrophages play the most important role in the destruction of ova. Macrophage function is minimally affected by current immunosuppressants [19,26]. In addition, in the recent Chinese questionnaire study, none of the 178 women who used the IUD became pregnant. The type of IUD used by these women was not reported [16]. Another study evaluating the ease of insertion of IUDs included 12 transplant recipients who received the LNG-IUD; no pregnancies were reported in this study after 1 year [27].

Another concern regarding IUD use is the risk of infection. Although there are no studies evaluating infection with IUD use in transplant recipients, studies in women with human immunodeficiency virus (HIV)/autoimmune immunodeficiency syndrome (AIDS) reveal a low incidence of pelvic inflammatory disease [28].

The CDC guidelines distinguish between *initiation* and *continuation* of the IUD. For women with "uncomplicated" SOT, both the copper and the progesterone-coated IUDs are assigned a category 2 (i.e. the benefits of the IUD generally outweigh the risks) [17]. The same category is assigned to "complicated" cases of SOT if the woman is *continuing* with this method (i.e. it is not necessary to remove an IUD if the recipient develops rejection, organ failure, or cardiac vasculopathy) [17]. For recipients with "complicated" SOT, *initiation* of either IUD is assigned a category 3 (i.e. the theoretical or proven risks of a condition usually outweigh the advantages of using the method) [17].

Emergency contraception

There are several methods used for emergency contraception: progesterone-only pills, antiprogesterone pills, combined OCs, as well as insertion of a copper IUD. It is important to stress that the risk of combined OC and progesterone-only pills when used as emergency contraception are classified as category 1 in the CDC guidelines. There should be no restrictions for use of this method in SOT recipients [17]. This recommendation is based on expert opinion only as there are no reports in the literature of emergency contraception in transplant recipients.

Barrier methods, withdrawal, and sterilization

Barrier methods and tubal ligation have been the most commonly used methods of contraception in renal and liver transplant recipients [14,16,29]. It is important to keep in mind that condoms have high failure rates but are the only method that prevents sexually transmitted infections. Patients often choose withdrawal to prevent pregnancy but the aforementioned study from Iran revealed that

92% of unwanted pregnancies resulted from coitus interruptus. In the Chinese study, use of the rhythm method, condoms, and withdrawal were the methods most often associated with unwanted pregnancy [16].

Sterilization in women can be performed through the laparoscope as well as the hysteroscope. The latter can be performed as an office procedure and may thus be a useful alternative for transplant recipients.

In summary, transplant recipients are at risk of unwanted pregnancy and should be counseled repeatedly about contraceptive options, beginning prior to transplant, specifically during the evaluation process. There are many options for these women. When considering a contraceptive method, it is important to consider the contraceptive and non-contraceptive benefits as well as risks and possible drug interactions (Table 97.1). Long-acting contraceptives such as IUDs and the progesterone implant are some of the most effective methods for preventing pregnancy [17]. These methods remain reversible, provide non-contraceptive benefits, and based on expert opinion, may be the best choice for women with SOT in whom unintended pregnancy could be associated with significant risks [17].

Pregnancy after transplantation

As already mentioned, this first pregnancy in a transplant recipient, Edith Helm, occurred in March 1958 [2]. The pregnancy was uneventful and worries about graft compression were allayed by a normal excretory urogram. A normal male infant weighing approximately 3300 g was delivered by cesarean section, undertaken to avoid the theoretical possibility of trauma to the transplant kidney as the fetus descended through the birth canal. There was no effect on transplant function. In 1960 the recipient had a second successful pregnancy with cesarean delivery of a healthy girl. The excretory urogram during pregnancy revealed prompt excretion but interestingly, there was less than the normal physiologic dilatation of the renal pelvis. It was concluded therefore that a solitary kidney transplanted into the pelvis could sustain a pregnancy and there were no compression problems. Indeed, Edith Helm lived to the age of 76 years, with her sister's kidney functioning 53 years post transplant.

In 1967 Hume et al. reported the first successful pregnancy in a kidney recipient with immunosuppression (azathioprine and prednisone) [30]. In 1976, one of the authors (JMD) and his colleagues in the UK published a report of the close follow-up of a kidney transplant recipient during a planned pregnancy [31]. This experience, with the authors' analyses of the available literature, led to the formulation of guidelines for counseling female kidney transplant recipients contemplating pregnancy. These guidelines have been only slightly modified through the years. Revisions have been incorporated into Consensus Guidelines through the American Society of Transplantation [32] (see below), with earlier European Best Practice Guidelines also available [33].

Given the need to provide better information for the transplant community, the National Transplantation Pregnancy Registry (NTPR) was established at Thomas Jefferson University in 1991, with the goal of maintaining an ongoing database to assess the safety of pregnancy in female transplant recipients as well as pregnancies fathered by male transplant recipients [3,9]. All reported pregnancy outcomes are analyzed, including livebirths, spontaneous abortions, therapeutic abortions, stillbirths, and ectopic pregnancies. The data include the follow-up of parents and offspring to determine long-term effects of post-transplant pregnancy for the recipient, graft, and offspring.

Table 97.2. NTPR: Pregnancies in female transplant recipients [3]

Organ	Recipients	Pregnancies	Outcomes*
Kidney	922	1490	1525
Liver	179	319	325
Liver–kidney	5	7	8
Intestine	2	2	2
Pancreas–kidney	50	90	95
Pancreas alone	2	5	6
Heart	60	105	109
Heart–lung	5	5	5
Lung	22	31	33
Totals	**1247**	**2054**	**2108**

*Includes twins, triplets, and quadruplets.
NTPR, National Transplantation Pregnancy Registry, December 2011.

Table 97.3. NTPR: Pregnancies fathered by male transplant recipients [3]

Organ	Recipients	Fathered pregnancies	Outcomes*
Kidney	605	925	941
Liver	69	109	115
Liver–kidney	3	7	7
Liver–heart–kidney	1	1	1
Liver–intestine–kidney	1	2	2
Intestine	1	1	1
Intestine–stomach–pancreas	2	2	2
Pancreas–kidney	34	43	45
Heart	108	158	162
Heart–lung	1	2	2
Heart–lung–kidney	1	3	3
Lung	4	4	4
Totals	**830**	**1257**	**1285**

*Includes twins and triplets.
NTPR, National Transplantation Pregnancy Registry, December 2011.

Table 97.4. NTPR: Pregnancies in female recipients transplanted at <21 years of age

Organ	Recipients	Pregnancies	Outcomes*
Kidney	299	499	511
Liver	76	150	153
Liver–kidney	2	4	5
Intestine	1	1	1
Pancreas–kidney	0	0	0
Pancreas alone	0	0	0
Heart	24	45	45
Heart–lung	0	0	0
Lung	1	1	1
Totals	**403**	**700**	**716**

*Includes twins, triplets, and quadruplets.
NTPR, National Transplantation Pregnancy Registry, January 2012.

Tables 97.2 and 97.3 show entries into the NTPR as of December 2011, totaling 2054 pregnancies in 1247 female recipients and 1257 pregnancies fathered by 830 male recipients. Numbers of pregnancies in recipients transplanted before the age of 21 years are listed in Table 97.4.

A National Transplant Pregnancy Register was established in the UK in 1997, but discontinued in 2002, the last report on pregnancy outcomes in the UK being published in 2007 [34,35]. Data from the UK report indicate outcomes similar to those of the NTPR [3,35] (Table 97.5).

Table 97.5. Pregnancy outcomes in kidney transplant recipients

	NTPR [3]	UK Transplant Pregnancy Registry [35]
Recipients	335	176
Pregnancies	515	193
Outcomes*	**527**	**188**
Therapeutic abortions (%)	8.4	6
Spontaneous abortions (%)	12	11
Ectopics (%)	0.6	1
Stillbirths (%)	3	2
Livebirths (%)	76	79
Livebirths	**401**	**121****
Mean gestational age (weeks)	35.8 ± 3.4	35.6 ± 0.3
Premature (<37 weeks)	53%	50%
Mean birthweight (g)	2490 ± 756	2316 ± 80
Low birthweight (<2500 g) (5)	46	54
Cesarean sections (%)	54%	72%
Newborn complications (%)	41	NR
Neonatal deaths (within 30 days of birth) [number (%)]	4 (1)	NR

*Includes twins and triplets.
**Number reported.
NR, not reported; NTPR, National Transplantation Pregnancy Registry.

Table 97.6. Food and Drug Administration (FDA) pregnancy categories for immunosuppressive drugs commonly used in transplantation [3]

Drug	FDA pregnancy category*
Corticosteroids (prednisone, prednisolone, methylprednisolone)	B or C
Azathioprine (Imuran®)	D
Cyclosporine (Sandimmune®)	C
Cyclosporine modified (Neoral®)	C
Tacrolimus (Prograf®)	C
Mycophenolate mofetil (Cellcept®)	D
Enteric-coated mycophenolate sodium (Myfortic®)	D
Sirolimus (Rapamune®)	C
Belatacept (Nulojix®)	C
Antithymocyte globulin (Atgam®, ATG)	C
Antithymocyte globulin (Thymoglobulin®)	C
Muromonab-CD3 (OKT3®)	C
Basiliximab (Simulect®)	B
Daclizumab (Zenapax®)	C

*FDA categories briefly defined: B, no fetal risk, no controlled studies; C, fetal risk cannot be ruled out; D, evidence of fetal risk.

Issues to be considered in pregnant transplant recipients

Although most of the outcomes have been positive, pregnancy after transplantation is a complex situation and the following issues must be considered:

1 The mother: risks to her long-term health and her ability and/or survival to be a parent.
2 The allograft: risks of allograft dysfunction and/or loss related to the pregnancy itself, the potential for changes in drug metabolism during pregnancy that could increase the susceptibility to rejection.
3 The fetus/neonate: potential teratogenic risks associated with immunosuppression and other medications, manifest not only as morphologic defects but as subtle developmental changes that might not become apparent until later during childhood, or could possibly have an effect on future generations.

Other long-term issues to be considered are the ability of a parent with a transplant to cope with unexpected illnesses and/or graft dysfunction while child rearing, and the impact on the child if the transplanted parent is ill or dies.

Immunosuppressive agents and teratology

The US Food and Drug Administration (FDA) pregnancy categories for immunosuppressive drugs are listed in Table 97.6. The mechanisms of action of these agents are covered in Chapters 98–102.

Corticosteroids

In animal studies, corticosteroids have caused cleft palate, although this has not been seen in humans [36,37]. Clinically, these agents are associated with an increased risk of preterm rupture of the membranes and adrenal insufficiency in newborns [38]. Prednisone has been used for >45 years for maintenance therapy in transplantation, as well as in other clinical areas, e.g. rheumatology.

Azathioprine (Imuran®)

Azathioprine at high doses along with prednisone was used for immunosuppression before the introduction of cyclosporine, and much clinical experience was accrued. Clinical data do not support the early concerns about teratogenicity, nor has a predominant structural malformation pattern been identified. Reviews show occasional attributable newborn problems, including thymic atrophy, leukopenia, anemia, thrombocytopenia, transient chromosomal aberrations, sepsis, and reduced immunoglobulin levels [39–41]. Although listed as pregnancy category D (evidence of fetal risk), azathioprine at adjunctive doses is considered a safe option for maintenance immunosuppressive therapy during pregnancy with respect to teratogenic risk.

Cyclosporine (Sandimmune®, Neoral®, Gengraf®, others)

The first calcineurin inhibitor (CNI), cyclosporine, became the mainstay of immunosuppression in the early 1980s. Cyclosporine is listed as pregnancy category C (fetal risk cannot be ruled out). Fetal toxicities and abnormalities have been reported in animal studies, but these occurred at dosages much higher than used clinically [42–44]. Although early reports suggested a greater risk of fetal growth restriction, this was not apparent in later studies [7]. The teratogenic risk appears minimal with no predominant pattern of newborn malformations reported. In the largest analysis of structural birth defects in the offspring of cyclosporine-treated kidney recipients, amongst 479 female kidney recipients maintained on cyclosporine reported to the NTPR, there were 362 (76%) livebirths [45], of whom 18 had birth defects, representing 5% of livebirths, which is reassuringly similar to the 3–5% rate reported in the general population [46].

Tacrolimus (Prograf®, others)

Since its introduction in 1995, tacrolimus has steadily replaced cyclosporine as maintenance CNI therapy in kidney transplantation in the US. In animal studies, fetal resorption occurred at dosages much higher than used clinically [47]. Transient neonatal hyperkalemia has been reported [48], as has a higher incidence of maternal diabetes. Similar to cyclosporine, no specific pattern or number of malformations has been identified with tacrolimus

exposure during pregnancy. The incidence of birth defects reported to the NTPR in kidney transplant recipients on CNIs, without adjunctive use of mycophenolic acid products [49], is similar to that in the general population.

Mycophenolic acid products (mycophenolate mofetil, CellCept® and others; and enteric-coated mycophenolate sodium, Myfortic®)

FDA-approved in 1994, mycophenolic mofetil (MMF) has supplanted azathioprine as adjunctive therapy with CNIs. In contrast to CNIs, there is greater concern about the risk of teratogenicity with mycophenolic acid products, based on reproductive toxicity studies in animals. Developmental toxicity in rats and rabbits included malformations and intrauterine growth restriction. Fetal death occurred at dosages that appeared to be within the recommended clinical dosages based on body surface area [50,51]. In the NTPR database, clinical data have demonstrated both an increased incidence of and a pattern of malformations, with additional reports of problems in newborns exposed to MMF [52–60]. Since 2007 the package inserts of CellCept® and Myfortic® have included a change in FDA category from C to D [29,30], and emphasized that females of childbearing potential taking mycophenolic acid products must use contraception, because during pregnancy there are increased rates of fetal loss and congenital malformations. This is also the recommendation of the European Best Practice Guidelines [33]. In combination with NTPR data, post-marketing data collected by Roche Laboratories, Inc. between 1995 and 2007 demonstrated that among 77 women exposed to systemic MMF during pregnancy, 25 had spontaneous abortions and 14 had a malformed infant or fetus. Six of these 14 had ear abnormalities.

Sirolimus (Rapamune®)

Sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, has been approved as adjunctive therapy with a CNI since 2000, and concerns have been expressed about its use during pregnancy. Teratogenicity has not been noted in animal studies, but decreased fetal weight and delayed ossification have been reported [61]. In conjunction with cyclosporine in pregnant animals, fetal resorption and loss rates were increased, suggesting increased toxicity. There is, however, a paucity of clinical pregnancy outcome data [52,62–66].

Males treated with sirolimus have a significantly lowered sperm count and spermatozoa motility, as well as a decrease in the fathered pregnancy rate [67]. Thus, male recipients who are taking sirolimus should be informed of this potential side-effect.

Other agents

Other agents used for induction or rejection have a minor role in pregnancy. In a small series of patients, muromonab-CD3 (OKT3®) [68], corticosteroids, and Thymoglobulin® were used to treat in pregnancy. Rituximab has been used to treat rejection, although there are no pregnancies reported with exposure.

The recently approved belatacept (Nulojix®) is a selective T-cell costimulation blocker used for maintenance immunosuppression. No pregnancy outcome data have yet been reported to the NTPR.

Organ groups

Kidney recipients

Literature surveys from the azathioprine era of the 1970s and 1980s attest to thousands of successful post-transplant pregnancies in kidney transplant recipients. The spontaneous abortion rate was approximately 14%, and the therapeutic abortion rate was approximately 20%. Of pregnancies that continued beyond the first trimester, >90% were successful. Renal impairment occurred in approximately 15% of women, and hypertension complicated approximately 30% of these pregnancies. Preterm delivery was common, affecting 45–60% of pregnancies, with fetal growth restriction occurring in approximately 20% [69,70].

Initial reports of cyclosporine exposure during pregnancy raised concerns because there was a higher incidence of fetal growth restriction and hypertension, which may have been related to the higher doses used in the early 1980s [43]. There were suggestions that cyclosporine might not be optimal for use in pregnancy and that recipients be switched back to azathioprine-based regimens due to the longer experience with this drug. As familiarity with the use of cyclosporine increased, case and individual transplant center reports and registry data reported increasing numbers of successful pregnancies, but some potential risks to mothers and newborns were apparent [71]. Outcomes of pregnancies in kidney recipients on CNIs as reported to the NTPR are detailed in Table 97.7 [3].

Maternal outcomes

Overall, compared with the general population, female transplant recipients are at greater risk for hypertension and pre-eclampsia during pregnancy. As reported to the NTPR, 53–64% of kidney recipients on CNIs have drug-treated hypertension during pregnancy. Kidney recipients are more likely to have pre-existing hypertension and this alone is a risk factor for worse pregnancy outcomes, including low birthweight [72]. Pre-eclampsia occurs in 7–10% of pregnancies in the general population, but is up to three times more common in transplant recipients. As many transplant recipients have pre-existing hypertension, pre-eclampsia can be more difficult to diagnose [70].

The use of insulin during pregnancy has been reported in up to 12% of kidney transplant recipients on CNIs reporting to the NTPR. Optimal glucose control is desired, especially in the first trimester given the association between gestational diabetes and birth defects [73]. Additionally, mild gestational diabetes not requiring insulin therapy has not been evaluated, so the actual frequency of gestational diabetes may be higher than reflected in the NTPR data.

The most common infectious complications reported to the NTPR during pregnancy are urinary tract infections. These typically can be successfully treated with the appropriate antibiotics.

Graft function

Clinical studies as well as those in animal models in the non-transplant population have shown that long-term renal function is unaffected by gestation if function prior to pregnancy is stable [74]. Similarly, in the transplant population, well-designed, case–control studies have demonstrated that pregnancy does not cause deterioration of graft function when prepregnancy graft function is stable [75–79]. A case–control study by Sturgiss and Davison [76], followed by an update [77], however, suggested that pregnancy may have a minor deleterious effect on kidney allograft function, but this was not statistically significant.

Rejection

The incidence of graft rejection during pregnancy is low, and does not appear to be any higher than reported in the non-pregnant transplant populations. Similarly, graft loss within 2 years of

Table 97.7. NTPR: Pregnancy outcomes in female kidney transplant recipients exposed to a calcineurin inhibitor during pregnancy [3]

	CyA (336 recipients, 517 pregnancies)	CyA modified (150 recipients, 241 pregnancies)	Tacrolimus (170 recipients, 278 pregnancies)
Maternal factors (number of pregnancies)	**517**	**241**	**278**
Mean transplant-to-conception interval (years)	3.6 ± 2.8	6.2 ± 4	4.4 ± 3
Hypertension during pregnancy (%)	62	64	53
Diabetes during pregnancy (%)	12	5	10
Infection during pregnancy (%)	23	18	21
Pre-eclampsia (%)	30	32	32
Rejection episode during pregnancy* (%)	1	2	2
Mean serum creatinine (mg/dL)			
Before pregnancy	1.4 ± 0.5	1.3 ± 0.4	1.2 ± 0.3
During pregnancy	1.4 ± 0.7	1.3 ± 0.5	1.3 ±0.8
After pregnancy	1.6 ± 0.97	1.4 ± 0.5	1.4 ± 0.7
Graft loss within 2 years of delivery (%)	9	6	6
Outcomes†	**529**	**254**	**283**
Therapeutic abortions (%)	8	1	1
Spontaneous abortions (%)	12	20	25
Ectopics (%)	0.6	1	0.4
Stillborns	3	2	2
Livebirths(%)	76	77	71
Livebirths	**402**	**196**	**200**
Mean gestational age (weeks)	35.9 ± 3.4	35.8 ± 3.4	35.3 ± 3.5
Premature (<37 weeks) (%)	52	52	53
Mean birthweight (g)	2490 ± 755	2501 ± 748	2494 ± 844
Low birthweight (<2500 g) (%)	46	43	43
Cesarean sections	54	43	57
Newborn complications	41%	44%	49%
Neonatal deaths (within 30 days of birth) [number (%)]	4 (1)	4 (2)‡	4 (2)

* Biopsy-proven acute rejection only.
† Includes twins, triplets, and quadruplets.
‡ Quadruplet 24-week pregnancy; all newborn died.
CyA, cyclosporine, , NTPR, National Transplantation Pregnancy Registry.

Table 97.8. Relationship between prepregnancy creatinine in kidney transplant recipients and estimates for pregnancy outcome (>24 weeks) and maternal kidney allograft function

					Loss of >25% kidney function		
Serum creatinine [mg/dL (μmol/L)]	Fetal growth restriction (%)	Preterm delivery (%)	Pre-eclampsia (%)	Perinatal deaths (%)	Pregnancy (%)	Persists postpartum (%)	ESRD in 1 year (%)
<1.4 (<125)	30	35	24	3	15	4	–
1.4–1.85 (125–160)	50	70	45	7	20	7	10
>1.85 (>160)	60	90	60	12	45	35	70

Estimates based on literature from 1991 to 2007, with all pregnancies reaching at least 24 weeks' gestation.
ESRD, end-stage renal disease.
Adapted from Armenti et al [88], with permission from Elsevier.

delivery does not typically appear to be precipitated by pregnancy. Irreversible and unpredictable graft events occur more often in recipients with impaired prepregnancy function.

From NTPR data, 2% of kidney recipients have reported biopsy-proven acute rejection during pregnancy. Treatment consists of pulsed steroids, lymphocyte depleting agents, and/or adjustment of baseline immunosuppression, and viable outcomes are possible. Rejection during pregnancy is associated with poorer outcomes for the newborn and graft [80]. Development of rejection during pregnancy or immediately postpartum represents a significant risk factor for kidney graft loss within 5 years after pregnancy [81].

Reports to the NTPR evaluating recipient and newborn variables do not reveal significant differences in the outcomes of pregnancies in living donor compared to deceased donor kidney transplant recipients [82].

Multiple and successive pregnancies

Many recipients have had successful successive pregnancies, and some have had successful outcomes with multiple gestations or in-vitro fertilization [82–87]. Of 478 recipients with a first pregnancy, 189 had one to four subsequent pregnancies. The proportion of livebirths was not statistically different between the groups. With successive pregnancies there was a trend toward increased gestational age, leading to a significant decrease in the number of preterm newborns of both low and very low birthweights. There were similar rejection rates among the groups [82]. Successive pregnancies were not associated with adverse fetal outcomes or increased maternal graft loss. Therefore, kidney transplant recipients with adequate allograft function who desire more than one pregnancy should not be discouraged in this.

Predictors of adverse events

Successful pregnancy outcomes for both recipients and newborns are the norm when kidney function is satisfactory and remains stable during gestation and after delivery. Pregnancy success, defined as livebirth, low risk of maternal and fetal complications, and preservation of kidney allograft function, is related to stable prepregnancy serum creatinine [88] (Table 97.8).

Women starting pregnancy with poorer allograft function [i.e. serum creatinine >1.5 mg/dL, (>133 μmol/l)], have a greater likelihood of allograft dysfunction during and after pregnancy [89]. Moreover, an increase in serum creatinine during pregnancy is more likely to be associated with adverse graft effects, as well as poorer newborn outcomes [88]. An upward trend in serum creatinine during pregnancy should not be attributed to pregnancy alone, and it warrants prompt investigation. As serum creatinine normally decreases during pregnancy due to increased glomerular filtration rate, allograft rejection during gestation may be signaled by only a minor increase in serum creatinine.

An NTPR analysis of 133 female kidney transplant recipients maintained on tacrolimus showed that a high level of serum creatinine before pregnancy and the development of rejection during pregnancy or immediately postpartum represent significant risk factors for kidney graft loss within 5 years post pregnancy. If serum creatinine before pregnancy was >1.3 mg/dL (>115 μmol/l), the relative risk of graft loss within 5 years post pregnancy was 3.3 ($P = 0.005$), while for a prepregnancy creatinine of >1.6 mg/dL (>140 μmol/L), the risk of graft loss increased to 7.4 ($P = 0.00001$) [81].

Deshpande et al. performed an international systematic review and a meta-analysis of 50 studies from 25 countries [90]. Overall, successful outcomes have been reported after kidney transplantation and the trends are consistent at an international level. As already described, factors associated with an adverse pregnancy outcome included prepregnancy hypertension, elevated serum creatinine, and proteinuria.

Newborn outcomes

Kidney transplant recipients, on average, deliver 1 month early, with a preterm (<37 weeks) delivery rate of 50% and mean birthweight of approximately 2500 g. Two large reports on the two primary CNIs, cyclosporine and tacrolimus, examined the prevalence of malformations in newborns. In the offspring of cyclosporine-treated recipients, the malformation rate was 4.1% (14 of 339 births) in a meta-analysis [91]. NTPR data on the offspring of cyclosporine-treated kidney recipients showed malformations in 5% of 362 liveborn infants [45]. The types of malformations varied among different systems, with no predominant type noted. In a report of recipients treated with tacrolimus during pregnancy (84 women, 100 pregnancies), four of 71 liveborn infants (5.6%) had evidence of structural malformations, but no specific pattern was evident [92].

A higher incidence of structural malformations was seen with MMF exposures during pregnancy compared to the overall kidney transplant offspring [52]. NTPR data and case reports have suggested a pattern of malformations. The phenotype includes craniofacial malformations affecting the oral cavity (cleft lip and cleft palate), the ears (microtia), and ocular anomalies (hypertelorism) [3,52–60]. Some fetal malformations may be detected on prenatal ultrasound [93].

Genetic considerations must be taken into account in the assessment of risk to the development of the newborn, especially if these contributed to organ failure in the mother. Of additional concern is the potential for more subtle effects that may not be apparent at birth, but may affect long-term growth and development in the next generation as well.

Long-term follow-up of offspring

Clinical reports note that children of solid organ recipients are developing well, although there is concern that alterations in T-cell subpopulations may affect vaccinations or long-term immunity [11,12,94,95]. A series of 175 newborns of cyclosporine-treated kidney recipients reported to the NTPR showed no evidence of an increased incidence of developmental delays over that expected [11]. Similarly, study of neurodevelopment in children aged 3–15 years who had been exposed to cyclosporine in utero did not show significant differences in full-scale IQ or behavioral outcomes compared with unexposed children [12]. Long-term neurocognitive development of the children of kidney transplant recipients is reassuring considering their high rate of preterm delivery and low birthweight.

Pancreas–kidney recipients

Reports regarding pregnancy after pancreas–kidney (PK) transplant have appeared in the literature [4,96–101]. When compared to kidney-only recipients, issues of concern in the PK population are assessment of pancreas graft dysfunction and glycemic control, higher incidences of hypertension and lower newborn birthweight, as well as whether graft survival is more adversely affected. Data are limited in this group.

The 94 outcomes (including twins and triplets) of 89 pregnancies in 50 PK transplant recipients reported to the NTPR are shown in Table 97.9. Immunosuppression was CNI based in 88 of the 89 pregnancies (eight with mycophenolic acid product exposure) and MMF in one [3]. Three recipients (all with adequate pancreas function prior to pregnancy) reported insulin use during pregnancy. In total, 11 recipients lost five pancreata and four kidneys, with two recipients losing both grafts within 2 years postpartum; rejection occurred in five during pregnancy. Mean gestational age of the 65 liveborns was 34.1 ± 3.2 weeks; 75% were premature (<37 weeks). The mean birthweight was 2075 ± 709 g; 63% were low birthweight (<2500 g). There was one neonatal death in a 26-week gestation newborn due to sepsis. At last follow-up of these children (mean age 8.5 ± 5.6 years), all 64 were reported healthy and developing well. There were some continuing issues reported: one child was medicated for attention deficit hyperactivity disorder (ADHD), one had insulin-dependent diabetes mellitus (IDDM), one had ADHD and IDDM, and one had ADHD and is being treated for epilepsy. At last maternal follow-up (mean 11.2 ± 5.3 years), both pancreas and kidney function were reported adequate in 30 (60%) recipients; seven recipients had died and 13 had varying degrees of graft dysfunction/losses.

Successful pregnancies after PK transplant in women with a low incidence of insulin use during pregnancy have also been reported to the NTPR. There is certainly a high incidence of preterm delivery and low birthweight in the offspring of this recipient group, so these pregnancies must be considered high risk and managed by an interdisciplinary team. Furthermore, the long-term impact of pregnancy on transplant survival in PK recipients requires continued surveillance.

It must be emphasized that many of the management approaches outlined for kidney recipients are applicable to all transplant groups. The following sections will review the issues that are specific to non-renal organ recipients.

Liver recipients

Amenorrhea or menstrual abnormalities with decreased fertility occur in up to 50% of women with chronic liver disease [102–113]. A successful transplant almost uniformly leads to a prompt return of normal menstrual cycles and to reproductive functions because of the recovery of the gonadotrophic function [104,109,110].

Table 97.9. NTPR: Pregnancy outcomes in pancreas–kidney (PK) recipients with exposed to a calcineurin inhibitor during pregnancy [3]

	CyA (17 recipients, 23 pregnancies)	CyA, modified (14 recipients, 23 pregnancies)	Tacrolimus (22 recipients, 42 pregnancies)
Maternal factors (number of pregnancies)	**23**	**23**	**42**
Mean transplant-to-conception interval (years)	3.4 ± 2.3	5.5 ± 3.5	3.9 ± 2.2
Hypertension during pregnancy (%)	95	78	41
Diabetes during pregnancy (%)	0	4	5
Infection during pregnancy (%)	62	55	21
Pre-eclampsia (%)	22	31	32
Rejection episode during pregnancy (%)	14	0	5
Graft loss within 2 years of delivery (%)	13	17	10
Outcomes*	**24**	**24**	**45**
Therapeutic abortions (%)	8	4	2
Spontaneous abortions (%)	8	25	31
Ectopics (%)	4	0	2
Stillborns (%)	0	0	0
Livebirths (%)	79	71	64
Livebirths	**19**	**17**	**29**
Mean gestational age (weeks)	34 ± 3	34.8 ± 2.5	33.7 ± 3.6
Premature (<37 weeks) (%)	84	65	76
Mean birthweight (g)	1936 ± 647	2195 ± 624	2096± 797
Low birthweight (< 2500 g) (%)	68	65	59
Cesarean sections (%)	61	69	65
Newborn complications (%)	44	53	52
Neonatal deaths (within 30 days of birth) [number (%)]	1 (5)	0	0

Some recipients may have had more than one pregnancy on a different calcineurin inhibitor. Not included in this table are those recipients on other regimens or for whom the regimen could not be determined.
*Includes twins.
CyA, cyclosporine; NTPR, National Transplantation Pregnancy Registry.

This is an important component of the social rehabilitation for recipients of child-bearing age, as evident from the increasing number of post-transplantation pregnancies reported worldwide [5,48,102–113].

Maternal and newborn outcomes

In contrast to kidney recipients, pregnancies in liver recipients are characterized by lower incidences of hypertension and pre-eclampsia during pregnancy, and a higher percentage of term deliveries. The current outcomes of pregnancies in liver recipients reported to the NTPR are shown in Table 97.10 [3].

Among liver recipients transplanted under the age of 21 years, 75% of pregnancies resulted in a livebirth. Pregnancy, potential risks for the mother and newborn, and long-term maternal survival should be discussed with the recipient and with the parents of the recipient, if the transplant recipient is a minor [108].

Graft function

Pruritus and cholestasis occur frequently during pregnancy. Elevated alkaline phosphatase levels are found in approximately 35% of "normal" pregnancies after liver transplantation. Graft rejection needs to be considered and differentiated from other conditions. Hemolysis, low platelets syndrome, and anemia have been reported [104].

Rejection

The incidence of biopsy-proven acute rejection ranges between 2% and 8% [3,9,105]. When acute rejection is suspected, percutaneous liver biopsy is not contraindicated in pregnant patients, although ultrasound visualization is recommended to reduce the risk of complications. Evaluation of transplant dysfunction includes liver Doppler ultrasound to exclude an anatomic source of graft dysfunction. Although rejection is a concern, it can usually be successfully managed with adjustment of immunosuppressive medications. For more serious rejections, the use of steroids as antirejection therapy is safe. There have been reports of lower birthweights and preterm births in mothers who had experienced rejection during pregnancy [105].

Particular attention should be reserved for patients with infrarenal aortic grafts for hepatic arterial flow. One death as a result of clotting of the graft by external compression from the gravid uterus has been reported [111].

Multiple and successive pregnancies

The NTPR analyzed pregnancy outcomes in liver recipients who had more than one pregnancy. Of the 125 liver recipients who had a first pregnancy, 61 had between one and four subsequent pregnancies. There were 217 outcomes of 213 pregnancies, including twins. There were no significant differences in newborn outcomes, rejection during pregnancy, and graft loss within 2 years of delivery of subsequent pregnancies. Therefore, female liver recipients with excellent graft function without significant recurrent disease or chronic rejection who wish to have more than one pregnancy should not be discouraged from conceiving [49].

Predictors of adverse effects

An NTPR analysis of 161 female liver recipients showed that the strongest risk factor for graft loss within 5 years of delivery is represented by rejection during pregnancy, followed by younger age at the time of conception [112]. Although viral hepatitis did not remain significant in the final model, it was the only liver disease etiology among those analyzed that was associated with graft loss within 5 years of delivery.

Coffin et al. performed a population-based survey using data from the 1993–2005 US Nationwide Inpatient Sample database [113]. They compared obstetric hospitalizations in liver transplant recipients (n = 206) with a control group (n = 4060), with the majority of pregnancies in liver recipients having favorable

Table 97.10. NTPR: Pregnancy outcomes in liver transplant recipients with exposed to a calcineurin inhibitor during pregnancy [3]

	CyA (62 recipients, 100 pregnancies)	CyA, modified (35 recipients, 64 pregnancies)	Tacrolimus (85 recipients, 140 pregnancies)
Maternal factors (number of pregnancies)	**100**	**64**	**140**
Mean transplant-to-conception interval (years)	3.6 ± 3.1	8.8 ± 5.5	5.9 ± 4.9
Hypertension during pregnancy (%)	40	39	17
Diabetes during pregnancy (%)	3	2	13
Infection during pregnancy (%)	31	31	19
Pre-eclampsia (%)	24	20	20
Rejection episode during pregnancy (%)	11	2	5
Graft loss within 2 years of delivery (%)	8	3	5
Outcomes*	**101**	**66**	**143**
Therapeutic abortions (%)	10	0	1
Spontaneous abortions (%)	15	17	20
Ectopics (%)	0	2	1
Stillbirths (%)	3	0	1
Livebirths (%)	72	82	76
Livebirths	**73**	**54**	**109**
Mean gestational age (weeks)	36.4 ± 3.9	37.3 ± 2.7	36 ± 3.7
Premature (<37 weeks) (%)	40%	30%	48%
Mean birthweight (g)	2619 ± 803	2757 ± 669	2747 ± 875
Low birthweight (<2500 g) (%)	36	31	30
Cesarean sections (%)	44	29	45
Newborn complications (%)	27	37	40
Neonatal deaths [number (%)] (within 30 days of birth)	1 (1)	0	1 (1)

Some recipients may have had more than one pregnancy on a different calcineurin inhibitor. Not included in this table are those recipients on other regimens or for whom the regimen could not be determined.
* Includes twins, triplets
CyA, cyclosporine; NTPR, National Transplantation Pregnancy Registry.

outcomes. Variables that were significantly higher in liver recipients included fetal mortality, antepartum admission, and maternal and fetal complications, prompting the conclusion that both mothers and their infants have an increased risk of complications.

Etiology of liver disease

Recurrent liver disease, especially viral hepatitis, appears to be a serious risk for both mother and child. Vertical transmission of hepatitis B virus (HBV) occurs in 10–20% of HBsAg-positive (HBeAg-negative) non-transplant mothers without immunoprophylaxis. For prevention of vertical transmission, all babies born to HBsAg-positive women should receive immunoprophylaxis [hepatitis B immunoglobulin (HBIg)] and HB vaccine within 12 h of birth. This combination prevents >90% of neonatal HBV infection [114]. The vertical transmission rate in pregnant hepatitis C (HCV) RNA-positive subjects is about 3–5% (in the absence of other viral co-infections) [115]. Special attention should be given to patients with high viral load post transplant, since this is a well-documented risk factor for vertical transmission of HCV.

Recipients transplanted for autoimmune hepatitis (AIH) typically require more immunosuppression than those transplanted for other causes. The NTPR analyzed pregnancy outcomes for women with the diagnosis of AIH [3]. The AIH liver transplant recipients were on average older at transplant and at conception, and had shorter transplant to conception intervals, yet there were no significant differences in maternal, pregnancy, and newborn outcomes between the AIH and non-AIH liver recipients.

Heart recipients

In recent years a greater proportion of young patients with complex congenital heart disease have been referred for heart transplant, and therefore there is a greater need to assess the potential for parenthood in this population [116].

In a heart transplant recipient contemplating pregnancy, there should be baseline tests including an electrocardiogram (ECG), echocardiogram, and potentially coronary angiography, with the option of right heart catheterization and endomyocardial biopsy (EMB) if clinically indicated [117]. Avoidance of pregnancy is recommended in heart transplant female recipients who have allograft dysfunction or cardiac allograft vasculopathy.

In contrast to the outcomes in kidney recipients, pregnancy outcomes in cardiac recipients are typically characterized by a greater percentage of term deliveries, lower pre-eclampsia rates, and lower incidence of graft loss within 2 years of delivery. However, maternal survival, independent of pregnancy-related events, should be considered as part of prepregnancy planning. Table 97.11 shows the outcomes of pregnancies in heart recipients on CNIs [3].

Graft function

Effects of pregnancy on the transplanted heart include increased cardiac workload, increased cardiac output, and elevated maternal oxygen consumption [118,119]. The transplanted heart appears to adapt well to these changes when graft function is stable [120,121].

Rejection

Monitoring for rejection in patients with a cardiac transplant may require EMB. Graft rejection during pregnancy was reported in 11% of 103 pregnancies in 57 heart recipients reported to the NTPR, with many of the episodes being low grade and without clinical consequence [3]. Treatment of rejection included corticosteroids, increased cyclosporine dose, or no change in several recipients with low-grade rejection [122]. One report that heart transplant recipients developed antibody-mediated rejection postpartum, so heart recipients who develop rejection during or after pregnancy should be assessed for anti-HLA antibodies [123].

Table 97.11. NTPR: Pregnancy outcomes in heart transplant recipients exposed to a calcineurin inhibitor during pregnancy [3]

	CyA (23 recipients, 43 pregnancies)	CyA, modified (16 recipients, 25 pregnancies)	Tacrolimus (20 recipients, 35 pregnancies)
Maternal factors (number of pregnancies)	**43**	**25**	**35**
Mean transplant-to-conception interval (years)	4.4 ± 3.6	5.9 ± 4.1	8.5 ± 5.4
Hypertension during pregnancy (%)	51	28	31
Diabetes during pregnancy (%)	2	4	0
Infection during pregnancy (%)	12	8	23
Pre-eclampsia (%)	10	10	25
Rejection episode during pregnancy (%)	21	4	3
Graft loss within 2 years of delivery (%)	0	4	3
Outcomes*	**43**	**25**	**39**
Therapeutic abortions (%)	9	4	0
Spontaneous abortions (%)	19	44	36
Ectopics (%)	2	0	3
Stillbirths (%)	0	4	0
Livebirths (%)	70	48	62
Livebirths	**30**	**12**	**24**
Mean gestational age (weeks)	36.9 ± 3.0	37.8± 1.4	36.1 ± 2.5
Premature (<37 weeks) (%)	37	8	54
Mean birthweight (g)	2658 ± 590	2690 ± 540	2483 ± 570
Low birthweight (< 2500 g) (%)	37	33	46
Cesarean section	30	42	57
Newborn complications	24	25	46
Neonatal deaths (within 30 days of birth) (%)	0	0	0

Some recipients may have had more than one pregnancy on a different calcineurin inhibitor. Not included in this table are those recipients on other regimens or for whom the regimen could not be determined.
*Includes twins.
CyA, cyclosporine; NTPR, National Transplantation Pregnancy Registry.

Statins

Statins are a common part of therapy in heart transplant recipients, but are not recommended during pregnancy due to their potential teratogenic effects. A study from the Motherisk program suggested that the actual risks for an exposed pregnancy appear to be small and exposure itself does not warrant termination of pregnancy [124]. It is advisable to avoid the use of these drugs in order to reduce fetal risks as much as possible. In the NTPR, ten pregnancies have been evaluated in heart recipients exposed to a statin drug, with six livebirths and no structural birth defects in these neonates [125].

Labor and delivery

Vaginal delivery is the recommended method of delivery in heart transplant recipients, similar to all other solid organ recipients [32]. Cesarean section should be performed only for obstetric indications. In cardiac recipient pregnancies reported to the NTPR, 40% were cesarean deliveries. During labor, ECG monitoring is mandatory because of the increased risk of arrhythmias, but more invasive cardiovascular monitoring is generally not necessary. Epidural anesthesia reduces pain-induced sympathetic responses and may reduce acute blood pressure fluctuations during labor [13]. Healthcare providers must be aware that cardiac recipients may have an unpredictable response to vasoactive medications and may not respond to vasolytics (atropine) as the heart is denervated.

Maternal outcomes

Most patients maintain adequate graft function following pregnancy. Among 57 heart recipients reported to the NTPR, 40 maintained adequate transplant function postpartum (70%). Two recipients had graft loss within 2 years of pregnancy, one died 17 years after transplant, and the second loss occurred 9 months after delivery necessitating retransplant; the latter recipient is currently healthy with adequate function [3]. Maternal death was reported in 16 of the 57 female heart recipients by the NTPR, all occurring >2 years after pregnancy. Causes of death included vasculopathy, atherosclerosis, acute rejection, sepsis, pneumonia, and sepsis. As emphasized previously, prepregnancy counseling must include the potentially limited lifespan of the heart transplant recipient.

Lung recipients

By comparison to heart recipients, lung recipients have a higher incidence of more significant rejection as well as graft loss in the peripartum period, with smaller newborns [8,126]. Given the overall higher risk for this recipient group, there has been less enthusiasm for advocating pregnancy. Management guidelines have not been published specific to this population.

Thirty-one pregnancies in 22 female lung recipients with 33 outcomes (including one triplet pregnancy) have been reported to the NTPR [3] (Table 97.12). Immunosuppressive regimens included: cyclosporine based (eight recipients), cyclosporine modified (two), and tacrolimus based (21). All recipients experiencing rejection were maintained on a cyclosporine-based regimen and received their transplant prior to 1996. Eleven of the 22 recipients were transplanted for cystic fibrosis and accounted for 13 of the pregnancy outcomes (eight livebirths, three spontaneous abortions, and two therapeutic abortions). Three cystic fibrosis recipients had rejection during pregnancy. At last recipient contact, 14 had adequate function, one had reduced function, five had died (two with cystic fibrosis), and one had a non-functioning transplant (single lung transplant removed; regain of native function). Mean gestational age of the 19 liveborns was 33.9 ± 5.0 weeks and mean birthweight was 2206 ± 936 g. There were two neonatal deaths in a triplet pregnancy born at 22 weeks (one spontaneous abortion at 14 weeks). At last follow-up, all 17 surviving children were reported healthy and developing well.

Table 97.12. NTPR: Pregnancy outcomes in 22 lung transplant recipients [3]

Organ	Lung
Maternal factors (number of pregnancies)	**31**
Mean transplant-to-conception interval (years)	3.6 ± 3.2
Hypertension during pregnancy (%)	52
Diabetes during pregnancy (%)	26
Infection during pregnancy (%)	21
Rejection episode during pregnancy (%)	16
Pre-eclampsia (%)	5
Graft loss within 2 years of delivery (%)	14
Outcomes*	**33**
Therapeutic abortions (%)	15
Spontaneous abortions (%)	27
Ectopics (%)	0
Stillbirths (%)	0
Livebirths (%)	58
Livebirths	**19**
Mean gestational age (weeks)	33.9 ± 5.0
Premature (<37 weeks) (%)	63
Mean birthweight (g)	2206 ± 936
Low birthweight (<2500 g) (%)	61
Cesarean sections	32
Newborn complications (%)	63%
Neonatal deaths [number (%)] (within 30 days of birth)	2 (11)†

* Includes triplets.;
† Triplet pregnancy: one spontaneous abortion at 14 weeks; two born at 22 weeks and died within 24 h of birth.
NTPR, National Transplantation Pregnancy Registry.

Successful pregnancy is possible after lung transplantation, including in those recipients with a diagnosis of cystic fibrosis. More rejection during pregnancy was evident in recipients transplanted before 1996. Analyses of a larger number of cases is needed to identify trends in pregnancy after lung transplantation. The impact of pregnancy on long-term maternal survival also requires further study.

Intestine recipients

Pregnancy after small bowel transplant has been reported in the literature and to the NTPR [3,6,127], but outcome data are limited in this group. Two cases of pregnancy post small bowel transplant have been reported to the NTPR. Both recipients delivered term infants with no malformations. Both recipients reported no transplant complications during pregnancy or in the postpartum period.

Other organ groups

Limited data are available, but successful pregnancy outcomes have been reported in female liver–kidney and heart–lung recipients [3].

Breastfeeding

Breastfeeding for female transplant recipient mothers remains an area of controversy [3,32,128–132]. The NTPR has received data from women who have chosen to breastfeed on various immunosuppressive regimens. Sixty-four kidney recipients have breastfed their 80 infants, 21 liver recipients their 28 infants, one liver–kidney recipient her infant for 3.5 months, and three PK recipients their four infants [3]. Two lung recipients have reported breastfeeding their infants; one for 10 weeks and another for 3 months. Six heart recipients have reported breastfeeding their 10 children, ranging from a few days up to 2 years. At last follow-up no specific problems related to breastfeeding in any of these children had been reported.

Table 97.13. Pregnancy after transplantation management options

Prepregnancy
- Patients should defer conception for at least 1 year after transplantation, with adequate contraception
- Assessment of graft function (organ specific):
  - Recent biopsy
  - Proteinuria
  - Hepatitis B and C status
  - CMV, toxoplasmosis, herpes simplex status
- Maintenance immunosuppression options:
  - Azathioprine
  - Cyclosporine
  - Tacrolimus
  - Corticosteroids
  - Mycophenolate mofetil
  - Enteric-coated mycophenolate sodium
  - Sirolimus
  - Everolimus
  - Belatacept
- The effect of co-morbid conditions, i.e. diabetes and hypertension, should be considered and their management optimized; non-renal recipients should have their baseline kidney function assessed
- Vaccinations should be given if needed, i.e. rubella, etc.
- Explore etiology of original disease; discuss genetic issues, if relevant
- Discuss the effect of pregnancy on renal allograft function
- Discuss the risks of intrauterine growth restriction, prematurity, low birthweight

Prenatal
- Accurate early diagnosis and dating of pregnancy
- Clinical and laboratory monitoring of functional status of transplanted organ and immunosuppressive drug levels:
  - Every 4 weeks until 32 weeks
  - Every 2 weeks until 36 weeks
  - Then weekly until delivery
- Monthly urine culture
- Surveillance for rejection with biopsy if it is suspected
- Surveillance for bacterial or viral presence, i.e. CMV, toxoplasmosis, hepatitis
- Fetal surveillance
- Monitor for hypertension and nephropathy
- Surveillance for pre-eclampsia
- Screening for gestational diabetes

Labor and delivery
- Vaginal delivery is optimal; cesarean delivery for obstetric reasons
- For heart/heart–lung/lung recipients:
  - Vigilance for poor or absent cough reflex, the need for airway protection
  - Unpredictable response to vasoactive medications
  - Judicious use of intravenous fluids

Postnatal
- Monitor immunosuppressive drug levels for at least 1 month postpartum, especially if dosages increased during pregnancy
- Surveillance for rejection with biopsy if it is suspected
- Breastfeeding discussion
- Contraception counseling

Adapted from Armenti et al [88], with permission from Elsevier.

Thus, while any immunosuppressive drug exposure to the infant could potentially exceed the threshold for safety, the relatively small amount of drug transferred and the lack of reported adverse effects together with the documented benefits of breastfeeding may outweigh the theoretical risks of this exposure. The breastfed infant should have a blood level checked for the mother's immunosuppressant; a detectable level is an indication to stop breastfeeding. Continued study in this area is warranted, especially the subtle effects of infant drug exposure on immunologic development.

Management options

Recommendations for the management of pregnancy after transplantation have been published in detail elsewhere [3,32,133–140]. A summary of management guidelines is given in Table 97.13.

Prepregnancy counseling

Transplantation restores fertility so recipients should be counseled about contraceptive options prior to transplant and early post transplant. An interval from transplant to conception is advisable in all transplant recipients to allow establishment of stable graft function and reduction of immunosuppression to maintenance levels. NTPR data have shown a higher incidence of pregnancy termination and peripartum rejection with transplant-to-conception intervals of <6 months compared with longer intervals in cyclosporine-treated kidney recipients. While the shortest safe interval from transplant to conception has not been established, 1 year is a reasonable milestone, given the prerequisites of stable, adequate graft function and maintenance level immunosuppression [32,141,142].

Immunosuppressive medications

The potential teratogenicity of mycophenolic acid products highlights the dilemma of protecting the transplanted organ from rejection versus fetal risk. There are no definitive pregnancy outcome data available on the risks of switching patients to alternative regimens that have a presumed lower risk of malformations to the newborn and no reliable data on rejection prophylaxis. Thus, for each recipient contemplating pregnancy, an individual assessment and decision must be made. One must judge the risk to the transplanted organ of switching immunosuppressants, particularly since organ function directly affects fertility and the ability to safely carry a pregnancy. This must be weighed against the risk of continuing a prepregnancy immunosuppressive regimen that could potentially harm the fetus. At this time, such decisions are best made preconception with the involvement of the patient, her partner, and the transplant team. Unplanned pregnancies do occur, however, such that decisions may have to be made after exposure has occurred.

Antihypertensive medications

Many transplant recipients have hypertension and may be receiving a combination of antihypertensive medications. Angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists are contraindicated during pregnancy, although some controversy exists with regard to the timing of greatest exposure risk [32,143]. If dosage adjustments or changes to hypertensive agents are needed, these could be made in anticipation of pregnancy.

Maternal co-morbid conditions

In addition to hypertension and pre-eclampsia, attention should focus on other co-morbidities that are likely to occur, including infections and gestational diabetes. Infectious complications during pregnancy are most often urinary tract infections. A monthly urine culture should be performed during pregnancy. Occasionally, yeast infections, pneumonia, sepsis, or unspecified viral infections complicate pregnancy. Cytomegalovirus (CMV) is usually asymptomatic but can be detected by serologic, antigen, or viral monitoring. CMV acute infection is the most common type of infection in the first year post transplant. It is particularly dangerous in early pregnancy. CMV infection is a causative agent of congenital malformation (microcephaly, cerebral palsy, sensorineural deafness) or congenital liver disease. Such abnormalities are seen in approximately 10–15% of infected pregnancies. The antiviral agents used for prophylaxis and treatment of CMV infection are ganciclovir and valganciclovir. The safety of their use during pregnancy has not been established [105].

The rate of HCV transmission from mother to child in the non-transplant population is 5% [144]. Recipients with HBV infection may also transmit this to their offspring [114]. As mentioned above, administration of HBIg and HBV vaccine to the newborn within a few hours of birth usually prevents transmission. Estimates of the incidence of acute infection with toxoplasmosis are 0.2–1%, with most cases undiagnosed and asymptomatic. Congenital toxoplasmosis can have severe consequences, and the diagnosis is dependent on culture, direct antigen detection, or serologic tests [145].

Scrutiny for hypertensive changes and pre-eclampsia is essential, although the diagnosis of pre-eclampsia may be difficult because serum uric acid levels and urinary protein excretion may be well above expected normal ranges without pre-eclampsia, as a result of drug nephrotoxicity. Other potential complications include the HELLP syndrome, ureteral obstruction, and complications of cesarean delivery. Peripartum ultrasound assessment to exclude urinary obstruction is warranted if serum creatinine rises.

Rejection

Unless immunosuppressive toxicity or rejection occurs, it is best to maintain prepregnancy immunosuppressive dosing and blood levels. Blood concentrations are likely to decrease during pregnancy, given the increased maternal volume of distribution as well as fetal metabolism of drugs. On occasion, a recipient may be non-compliant, choosing to stop medications during pregnancy for fear these might harm the fetus. Reports to the NTPR show that, in most pregnancies, immunosuppressive doses have been kept the same or increased during pregnancy. Regardless of dosing during pregnancy, changes that occur during the peripartum period mandate that postpartum immunosuppressive dosing be managed by determining blood concentrations when possible.

Rejection during pregnancy, although not common, must be considered in the differential diagnosis of graft dysfunction. The usual pattern for the serum creatinine level is a slight decrease in early pregnancy, with a return towards baseline postpartum. In kidney recipients, any increase in serum creatinine of ≥15% during pregnancy and postpartum must be evaluated. For all SOT recipients, any deterioration in graft function should be assessed by biopsy, and if the diagnosis of acute rejection is made, then appropriate antirejection treatment is necessary. Given the risks of rejection and of pre-eclampsia, more frequent monitoring is warranted from mid pregnancy onward, including blood pressure, assessment of graft function, and measurement of immunosuppressive drug levels.

Labor and delivery

A high incidence of cesarean sections has been reported for pregnant transplant recipients, ranging from 29% to 69% (Tables 97.7, 97.9, 97.10, 97.11, 97.12). However, it is recommended that cesarean delivery be performed for obstetric indications only. Immunosuppression must not be interrupted during labor and delivery. Prophylactic antibiotics are advisable.

Postnatal care

Most oral maintenance medications are easily absorbed and treatment can be resumed shortly after cesarean section. When oral treatment cannot be resumed, intravenous formulations are available for most, but not all agents. Immunosuppressive drug levels should be monitored and dosage adjusted appropriately, which may then affect blood pressure, renal function, and other toxicities. One must be aware of postpartum depression among transplant

Table 97.14. NTPR: Selected pregnancies fathered by male transplant recipients [3]

	Kidney	Liver	Pancreas–kidney	Heart
Number of recipients	594	69	34	106
Pregnancies	915	109	43	157
Outcomes*	930	115	45	160
Livebirths (%)	93	89	91	88
Mean gestational age (weeks)	39.1 ± 2.2	39.1 ± 1.9	38.7 ± 2.4	38.9 ± 2.3
Mean birthweight (g)	3329	3296	3321	3440
Newborn complications (%)	16	19	15	19

*Includes twins and triplets.
NTPR, National Transplantation Pregnancy Registry.

recipients, as medications may be missed or not taken. Therefore, close monitoring is required for several months postpartum.

As already mentioned, breastfeeding remains controversial. Although exposure of the newborn to any immunosuppressive drug may be detrimental, the amount present in the breast milk is thought to be minimal. The benefits of breastfeeding may possibly outweigh the minimal risk.

Pregnancy after living donation

Pregnancy after living kidney donation was first described by Murray et al. in 1963 [2]. Earlier studies concluded that donor nephrectomy is not detrimental to the prenatal course or the outcome of a future pregnancy [146–148]. Two recent studies, however, extend our current knowledge and do highlight some concerns [149,150].

Reisæter et al. examined the data from the Medical Birth Registry of Norway. They described 326 female kidney donors, with 620 pregnancies before donation and 106 pregnancies after donation, and used as control a random sample from the Norwegian birth registry [149]. There was no difference in maternal and newborn outcomes between kidney donors and the general population, except for a higher incidence of pre-eclampsia post donation compared to predonation (5.7% vs. 2.6%). As the number of pregnancies at risk and the number of events was extremely low, the results should be interpreted with caution. Most importantly, pregnancy outcome after donation was good for both mother and child, and similar to non-donor controls.

Ibrahim et al. surveyed 1589 female donors regarding pregnancy pre and post donation from a single large-volume transplant center in the US; 1085 donors reported 3213 pregnancies [150]. Fetal and maternal outcomes after kidney donation were similar to those for the general population. However, when post-donation pregnancies were compared to predonation pregnancies, it was apparent that post-donation pregnancies are associated with a higher likelihood of adverse outcomes such as a lower proportion of term infants, higher incidences of fetal loss, gestational diabetes, gestational hypertension, proteinuria, and pre-eclampsia. It is worth mentioning that the rates of adverse outcomes in predonation pregnancies were exceedingly rare, suggesting a selection bias; and it is conceivable that women with a history of gestational diabetes, hypertension, or pre-eclampsia are excluded from donation and are less likely to come forward to become kidney donors.

The existing data are reassuring that kidney donation is not associated with increased incidence of adverse maternal and fetal outcomes compared to the general population. Nevertheless, it is prudent to closely monitor pregnancies in kidney donors, including with blood pressure, urine protein excretion, and fetal ultrasound [151].

Table 97.15. Key points

- Recommendations to optimize pregnancy outcomes include consideration of the following:
  - Good general health ≥1 year since transplant
  - Stable graft function [ideally with serum creatinine <1.5 mg/dL (<130 μmol/L)] and no evidence of recent graft rejection
  - Well-controlled or no hypertension
  - Minimal or no proteinuria (<1 g/24 h)
  - Stable immunosuppressive regimen with suitable medication(s)
- Potential maternal and fetal complications include pre-eclampsia, preterm birth, fetal growth restriction, and low birthweight
- Pregnancy in and of itself does not affect previously stable allograft function
- The effect of co-morbid conditions, i.e. diabetes and hypertension, should be considered and their management optimized
- Maintenance of current immunosuppression in pregnancy is usually recommended, except for mycophenolic acid products, for which fetal risks should be discussed and alternatives sought if possible

The timing of pregnancy after kidney donation is not well established. A consensus conference recommended delaying pregnancy until at least 2 months after nephrectomy to assess renal compensation prior to conception [152]. Others have suggested 1 year post nephrectomy to allow a woman to recover from the emotional process of becoming a living donor and to adapt to a new level of kidney function [151]. Issues related to pregnancy post donation and potential concerns should be discussed with all potential donors of child-bearing age and their recipients [151,153,154].

Of interest, Soyama et al. have managed a woman who conceived within 6 months of a right liver lobe donation and she had a successful obstetric outcome [155].

Male recipients

Table 97.14 shows the percentages of livebirths and neonatal outcomes among selected pregnancies fathered by male transplant recipients reported to the NTPR, including pregnancies fathered by recipients after multivisceral transplantation. Overall, the outcomes of pregnancies fathered by male transplant recipients appear similar to those of the general population. The NTPR continues to collect pregnancy outcome data on male recipients who have fathered pregnancies after SOT. Issues related to fertility in male transplant recipients require additional studies.

Summary

The key points for pregnancy following transplantation are summarized in Table 97.15.

With the constant new developments and modifications in immunosuppressive regimens in transplant medicine, clinicians are responsible for providing pregnancy counseling in all pre- and post-transplant recipients of child-bearing age. As individual physicians and centers accrue experience with these major therapeutic decisions, it is critical that both positive and negative pregnancy outcomes be reported in appropriate settings. For the future, efforts also need to be directed toward developing better and more organ-specific management guidelines. Continued close collaboration among specialists will help to better identify potential pregnancy risks in these populations, particularly as new immunosuppressive agents are developed.

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CHAPTER 98

Drugs Specifically Approved for Transplant Indications

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Introduction

The Food and Drug Administration (FDA) regulates over a trillion dollars of products, ranging from 80% of the US food supply to all human healthcare products, electronic products that emit radiation, animal products, and cosmetics. This includes $466 billion in food sales, $275 billion in drugs, and $60 billion in cosmetics [1]. The FDA is expected to protect citizens of the United States (US) from harm through a vigorous and at times daunting approval and monitoring process. The FDA is expected to approve agents in an expeditious manner while balancing the risks of potential safety concerns or unpredicted adverse events. Given the nature of scientific advancement, it is no surprise that this agency and its statutory framework remain a work in progress attempting to evolve to meet emerging needs.

This chapter will provide a brief overview of the practical aspects of the FDA work model and the process of drug approval, as a prominent example of the complexities of immunosuppressive drug approval. While each country has specific rules that govern the approval process, the general themes covered here are similar to those used in most countries. It will also provide a summary description of those products that have received approval from the FDA for one or more transplant indications, and list the recommendations based on the approved label for each drug; this is often not the way the drugs evolve into common clinical use. In reality, drug regimens used in clinical practice are a hybrid of drugs being used as indicated, and drugs being used "off-label," that is used in a manner that was not part of their approval testing. This is necessary to allow for drug use in situations where efficacy can be reasonably assumed based on a drug's mechanism of action, but where the target population, or specific use is limited so as to preclude practical demonstration of efficacy in a fiscally practical clinical trial. This chapter should thus be considered in tandem with Chapter 101, which covers off-label drug use. The details of the mechanism of actions of the drugs listed in this chapter are covered in Chapter 17, and the practical clinical application of these agents are addressed in the many organ-specific chapters on post-operative management and treatment of rejection. Consideration should also be given to relating the content of this Chapter to that in Chapter 135, which deals with clinical trial design, as FDA approval is often a primary consideration in trial design.

Disclaimer: The opinions offered in this chapter are the personal opinions of the authors and should not be interpreted as the official policy of the U. S. Food and Drug Administration.

The evolution of the FDA

Prior to 1902, the US government had minimal role in the regulation of drugs. There were no laws, regulations, nor standards that existed for clinical efficacy. The US Pharmacopeia (USP) was established in 1820 as the first official registry of agents. The USP set standards for strength and purity to guide pharmacists and clinicians who would extract and compound the agents. The first American drug regulatory law was passed in 1848, the Drug Importation Act; it was enacted in response to US troops serving in Mexico who were adversely affected by contaminated quinine. This law required laboratory inspection and destruction of product that did not meet established standards.

The origins of the FDA are most often traced to the US Department of Agriculture's Division of Chemistry [2]. Under Dr. Harvey W. Wiley, appointed chief chemist in 1883, this division began to investigate the nation's food and drugs. Through controlled human clinical trials, he was able to demonstrate that many of America's foods and drugs were "adulterated" and products had inadequate labeling. Contemporaneously with Dr. Wiley's efforts, the nation was becoming more aware of the hazards of the food industry especially the publications of Upton Sinclair's *The Jungle* (which described the putrid conditions of meat industry). In 1902, the Virus, Serum, and Toxins Act (Biologics Control Act) was passed in response to tetanus-infected diphtheria antitoxin, which was produced by a small laboratory in St. Louis. Here, a horse named "Jim", a retired milk wagon horse was used to develop the diphtheria antitoxin. Unfortunately, he contracted tetanus and was humanely destroyed. Yet, his serum samples were not screened for tetanus and thus more than ten school-age children died as a result of the tainted serum.

In 1906, Congress passed and President Theodore Roosevelt signed the first food and drug law, the United States Pure Food and

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Drug Act (USPDA, also known as the Wiley Act). The Wiley Act prohibited the interstate sales of contaminated foods or misbranded drugs based on their labeling. Subsequently, in 1927, the Bureau of Chemistry was reorganized under a new USDA body called the Food, Drug and Insecticide Organization which evolved to the FDA for short [3]. Unfortunately, judicial decisions restricted the newly formed FDA to only enforce if the product's ingredients were incorrectly labeled even if intentionally false therapeutic claims were made. In 1937, a tragedy occurred that changed the future of modern medicine [4]. Sulfa was seen as a miracle drug that was used to treat life-threatening infections. Unfortunately it was unpleasant to consume and companies sought ways to create a palatable product. S.E. Massingill Co. of Bristol TN developed a raspberry liquid called Elixir of Sulfanilamide. However, it used diethylene glycol to dissolve the sulfa. Over 100 people died as a result of this single agent.

The result was the passage of one of the most comprehensive acts in American healthcare law, the federal Food Drug and Cosmetic Act of 1938 (FDCA). This law required all new drugs to be tested by their manufacturers and that those tests are submitted for review by the government for marketing approval via a new drug application (NDA). The FDCA still represents the broad basis for the statutory authority of the FDA today. In 1962, the Kefauver-Harris Act was implemented [5]. The critical component of this act was that all new drug applications would have to demonstrate convincing evidence of the drug's efficacy for the proposed indication in addition to previously required safety data. In addition, documented "informed consent" needed to be obtained prior to any human trials. Yet these new regulations led to emergence of a lengthy process for drug development. In 1984, Congress passed the Price Competition and Patent Restoration Act or better known as Waxman-Hatch Act [6]. This legislation was composed of two parts or "titles." The first one benefited generic pharmaceutical industry and allowed for easier market access to copies of pioneer drugs as long as they were not "significantly" different in absorption, action, and dosage. The second arm of this law was aimed at encouraging industry development of novel products by extending the patent life of the products for time lost during the FDA "review" process.

The length of the drug approval process fell under severe scrutiny during the emergence of the acquired immunodeficiency syndrome (AIDS) epidemic [7]. Pressures from AIDS activists in particular were critical in changing the rule. In 1992, the US Congress passed the Prescription Drug User Fee Act (PDUFA) which allowed the FDA to collect fees from drug sponsors to fund the new drug approval process [8]. In order to continue to collect such fees, the FDA is subjected to meet certain benchmarks; specifically the speed of certain activities within the review process. The law provided for exemptions and waivers for applications from small businesses, drugs aimed at orphan diseases or unmet public needs. In 1997, Congress expanded the scope and authority of the FDA with the FDA Modernization Act (FDAMA) in order to further develop with the evolving need of its citizens [8].

The FDA now has the authority to create an accelerated approval and a fast track process to speed lifesaving therapies to market. The fast track development program is a designation by the FDA that accelerates the approval of investigational new drugs which show promise in treating serious, life-threatening medical conditions where there are no other agents that either exist or work well. This status simply allows for more frequent meetings with the FDA to discuss drug development that should facilitate earlier drug approval and access by patients. This is distinct from the accelerated approval status. Here, the FDA allows a drug to be approved on the basis of an alternative or surrogate endpoint, which is reasonably likely to predict clinical benefit. A common approach is to identify a biological marker that "correlates" with the clinical efficacy endpoint and to document the effect of the novel therapy on this biomarker. For instance, in oncology, one approach is to report effects on tumor size or improved levels of biomarkers such as prostate-specific antigen (PSA). This would be distinct in that the traditional primary end-points, such as patient survival, would often require larger sample size or longer follow-up.

The structure of the FDA

As described above, the FDA originated in 1906 and is one of several divisions within the US Department of Human Health Services (HHS). Other agencies of the HHS include CDC, NIH, and the Centers for Medicare and Medicaid Services (CMS). The FDA is organized into a number of offices/centers. It is led by a commissioner who is appointed by the President and requires Senate confirmation. Figure 98.1 demonstrates the complexity of its responsibilities and operations. The FDA is headquartered in Silver Springs, Maryland where most of its operations are based. There are currently six centers: the Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation (CBER), Center for Devices and Radiological Health (CDRH), Center for Food Safety and Applied Nutrition (CFSAN), Center for Tobacco Products, and Center for Veterinary Medicine. There are additional offices which have unique responsibilities for drug development such as: Office of Combination Products and Special Programs, the Office of Ombudsman, and the Office of Orphan Products Development; the Office of Regulatory Affairs serves as the lead role in establishing and maintaining the standards and protocols for this organization. Each of these entities has their own defined role. However, with the rapidly advancing technologies, it is not surprising that there may be overlap in oversight. Drugs that are small molecular products are under the supervision of CDER, any of the other centers or offices may become involved as necessary. Biologics are substances that are derived from, or made by, the aid of living organisms such as antitoxins, serums, blood products, recombinant proteins, and cell therapies. Manufacturers of biologics must hold a license of the products that are issued by CDER. CBER is responsible for polyclonal antibody preparations and blood/tissue product derivatives such as IVIG or vaccines. If the drug is part of a combination product that has been deemed device, then it is under CDRH.

New drug development process

The FDA considers a "new" drug to be any substance that has a pharmacodynamic effect when administered with the intent for the diagnosis, treatment, or prevention of a disease state [9]. The critical piece is the intended use or the "indication" of the substance which reflects the foundation for its labeling and the necessary studies to demonstrate safety and efficacy. A "new" drug can also apply to an already FDA approved agent if there is a new intended use of the agent, new route of administration, or any other significant clinical difference [10]. In these situations, the substance must undergo the proper testing and FDA approval as if it was a novel agent. These are typically submitted as supplemental NDA (sNDA), which have the advantage of focusing on clinical studies, as the preclinical (e.g.

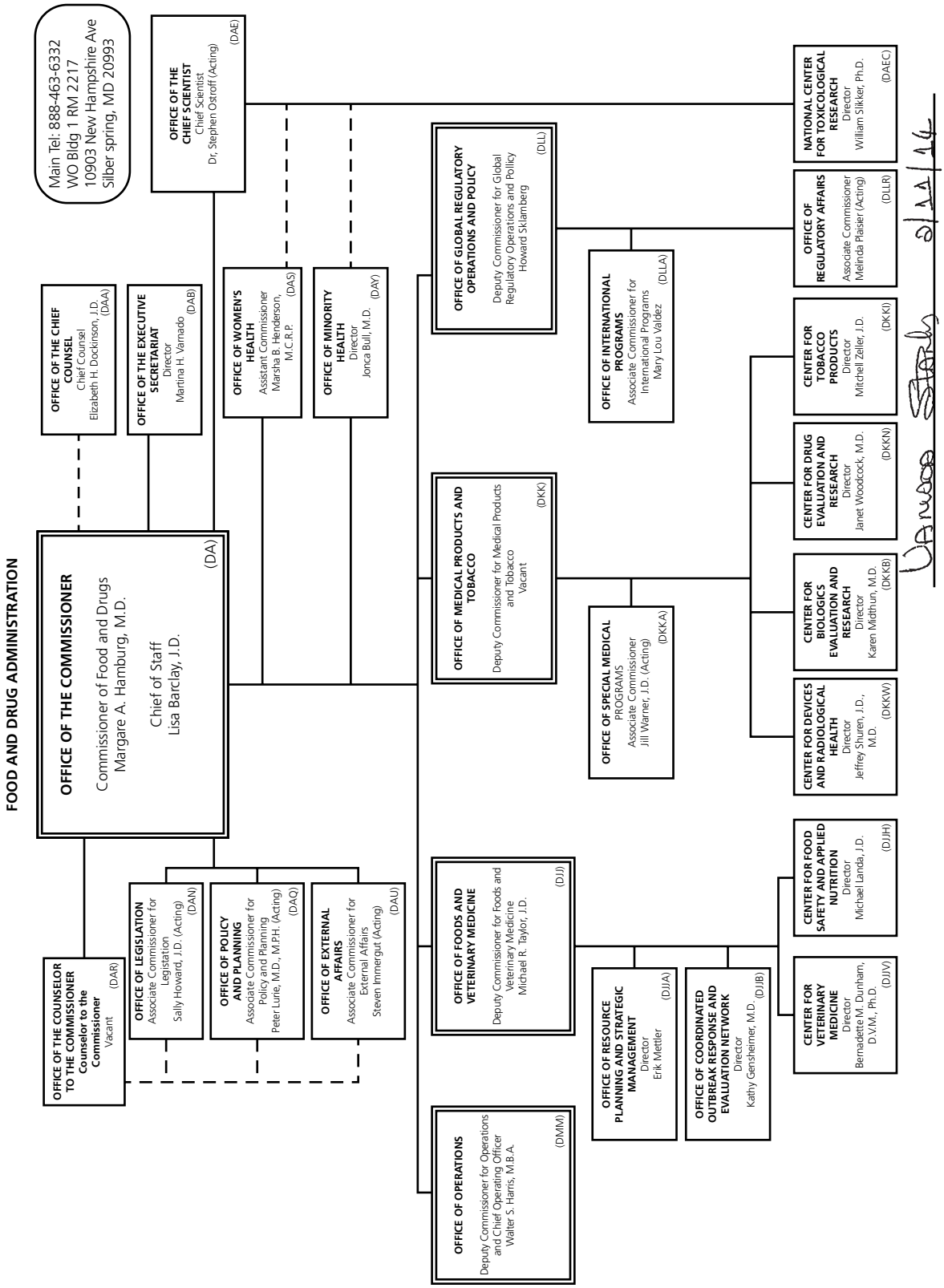

Figure 98.1. FDA Organization Chart. Reproduced from http://www.fda.gov/downloads/AboutFDA/CentersOffices/OrganizationCharts/UCM291886.pdf.

toxicology, animal studies, etc.) have already been reviewed in the original NDA. Briefly, the process begins with non-clinical studies, which involve in vitro studies and animal subject trials. This precedes the three phases of human clinical trials. Pertinent sections in the US Code of Federal Regulations (CFR) describe the elements of an adequate and well-controlled investigation, including details regarding the role of objectives, endpoints, controls, proper statistical analysis, and eligibility criteria. The regulations also include provisions regarding the importance of randomization and blinding. Importantly, the FDA through the CFR and FDA guidances makes clear that specific quantitative standards exist for proof of efficacy (whereas determinations of safety and overall risk-benefit generally rely on qualitative or semi-quantitative analyses). The FDA will review the results of these trials to determine final approval of the agent. Drugs are also subject to a fourth phase, known as post-market surveillance, which may include further education and patient monitoring.

Non-clinical investigations

A new molecular entity whose clinical safety has not been previously established will be required to undergo preclinical in vitro and in vivo animal testing. This represents the first major step in the drug development process. The principal goal of these investigations is to collect the necessary data to support the safety of early human trials [11]. The initial step is the drug discovery stage in which compounds are screened to determine if there is the desired pharmacologic activity. Subsequently, more complete in vitro studies are intended to elucidate the mechanisms of action, dose-response relations, and the pharmacodynamic effects of the novel agent. Pharmacokinetics analyses are performed in multiple species in order to provide information on the extent and duration of the systemic exposure to the new agent. These experiments are designed to describe the drug's absorption, distribution, metabolism, and excretion (ADME profile). Toxicity studies are critical to determine the short and long term physiological adverse effects. The "lethal" dose 50 (represents the dosage level that kills 50% of tested animals) has been replaced by the single dose with increasing dosage approach. The goal is to determine the no observed adverse (toxic) effect level (NOAEL) or the lowest dose necessary to see the desired pharmacological effect without adverse effects. The FDA does not regulate the conduct of non-clinical studies. However, to ensure the quality of the data produced, the laboratories must comply with Good Laboratory Practice (GLP) regulations. These establish basic standards for the organization, personnel, maintenance, and operating procedures of the facilities [12]. The FDA will perform on-site inspections to monitor compliance with GLP.

The investigational new drug application (IND)

The FDA carefully monitors clinical trials to protect the health of human subjects and to assure the highest level of integrity and value of the obtained data. Once the preclinical phase has accumulated sufficient evidence that the new agent exhibits the anticipated pharmacological effect to justify human experiments while demonstrating that it should be safe to proceed, then the sponsor will submit an investigational new drug application (IND). The IND is a process in which the FDA is notified of intent to initiate clinical trials with an investigational agent. Technically, an IND is a request for exemption from the Federal Food, Drug, and Cosmetic Act (FD&C) from prohibiting the interstate commerce of a new drug. Simply speaking, an IND allows one to legally ship an unapproved drug. The IND application provides the FDA with the necessary data to decide whether the proposed study is reasonably safe to proceed. An IND is required anytime one wants to perform clinical trials of an unapproved drug (such as novel indication, new dosage form, in combination with another drug) [13]. The regulations also exempt studies of approved drugs if the following criteria are met [14].

1. It is not for a new indication, or change in labeling or advertising for the product.
2. It will not involve a novel route of administration or dose level or patient population.
3. The studies will be in compliance with an IRB and informed consent.

The sponsor will submit the IND application to the FDA which primarily consists of: introductory statement, basic investigator plan, comprehensive protocols, the investigator brochure (the preclinical studies such as animal pharmacology and toxicology studies, pharmacological effects of the agent, pharmacokinetics studies), manufacturing information, and information on the investigators. After submission, the sponsor must wait 30 days prior to initiating any clinical studies. The FDA does not actually approve an IND, but rather would place a clinical hold if there were any objections to proceeding. Prior to any human testing, a clinical study protocol also must be reviewed and approved by an Institutional Review Board (IRB). The IRB is a committee of medical and ethical experts who oversee clinical research and are commissioned to uphold the most vigorous standards to ensure the safety of human subjects. Once the IRB has reviewed and approved the protocol and the necessary documents including the informed consent form, then human testing can begin. Clinical trials are classically conducted in three phases each with their specific purpose.

Phase I — "first in man" — studies, are designed to confirm the preclinical data in human subjects. Its purpose is to determine the pharmacokinetics actions, and confirm the safety of the agent's administration into healthy humans. These studies will often investigate if there was the desired pharmacological effect, although they often are not statistically designed to confirm this. Phase II studies are the clinical trials where subjects with the disease or condition are introduced to the new agent. This represents a fundamental shift on the focus of the process away from safety towards effectiveness. These studies will sometimes involve hundreds of human subjects. The purpose of this phase is to determine if the new agent has the desired efficacy against the disease. Typically, the investigational agent at several different treatment doses is compared to a placebo. Again, the results of this study are analyzed and used to design future protocols. A critical component of Phase II trials is the observed magnitude of treatment effect which will be used to determine the sample size for Phase III studies. At the end of the Phase II trial, the FDA and the sponsor will meet to discuss the results and plans for future studies.

Finally the "pivotal" Phase III study is conducted where the new agent is compared to FDA recognized and approved standard of care. These trials are designed to involve the patient population and clinical scenario that the sponsor seeks a label for. These studies are usually very large and involve hundreds to thousands of subjects. The larger patient pool allows the investigators to identify potential adverse effects, which are often relatively rare. At the conclusion of the trial, the investigators must be able to demonstrate "substantial evidence" or evidence from well-controlled investigations that could fairly be concluded by experts that the drug will have the effect under the conditions it is prescribed. With rare exception, at

least two adequate studies are necessary in order to obtain FDA approval to meet the scientific standards of reproducibility. However, the two studies do not have to be identical design; they could be performed in patient populations with different severities of disease or, in the case of transplantation, in different types of donors. Once all of the data has been collected and analyzed and the investigational agent has met the predetermined end-points of the Phase III study, then the sponsor is ready to ask the FDA for market approval for the intended indication.

Guidances relevant to FDA standards for establishing efficacy

Since a critical component of drug approval process involves efficacy, great care and foresight must be placed on this issue during trial design. Laws and regulations can outline general expectations, but the interpretations of their wording are often as important. As the agency responsible for their enforcement, FDA must regularly make determinations regarding the intent of the laws and regulations. In some instances, FDA may choose to issue guidance documents to elucidate its current thinking on a given topic. Although guidances do not have the force of law and do not bind the FDA to adhere to a given policy, they can provide important insight and may prove helpful to stakeholders interested in product development.

To date, no guidances specifically addressing transplantation trial design have been published. FDA, however, has produced several guidances regarding standards for clinical evidence. For instance, "Guidance for Industry: Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products" published in 1998 describes the quantity of evidence necessary to support efficacy — including the usual requirement for at least two adequate and well-controlled trials, but also contexts where reliance on a single trial may be appropriate [15]. Three other guidances, produced in conjunction with the regulatory bodies of the European Union and Japan as part of the International Conference on Harmonisation (ICH) Tripartite Guidelines, are also particularly worthy of notice: ICH E8 (General Considerations for Clinical Trials), ICH E9 (Statistical Principles for Clinical Trials), and ICH E10 (Choice of Control Group and Related Issues in Clinical Trials) – published in 1997, 1998, and 2000, respectively [15]. These three ICH documents provide considerable insight into the FDA view on a variety of design issues, including substantial discussions about the use of non-inferiority comparisons to active concurrent controls, for trials intended to support product approval.

Superiority and non-inferiority trials

Demonstrations of efficacy may be achieved through either superiority or non-inferiority trials. In superiority trials, the demonstration of efficacy for a given indication requires that the test product shows statistical superiority, typically using 95% confidence intervals, for a pre-determined primary clinical or surrogate endpoint. The interpretation of such a trial design is straightforward: if the endpoint is met, one would conclude, with the appropriate degree of confidence, that the product has at least some efficacy for the indication studied. Non-inferiority trials (previously known at FDA as equivalence trials), on the other hand, demonstrate the efficacy of a product by proving that the new treatment is not substantially inferior to an already marketed product. Non-inferiority trials seek to show that the differences between the two treatments are small enough to allow the investigators to conclude that the new drug has efficacy. A successful well conducted superiority trial has results that are fully interpretable, that speak for themselves. Non-inferiority trials are based on an assumption that the novel therapy has the expected effect which is used to determine assay sensitivity (the ability to determine an effective from non-effective agent) which relies on external or historical information.

New drug application

Since landmark legislation in 1938, the New Drug Application (NDA) has been the pathway for drug development in the United States. A sponsor would submit thousands of pages of documents covering the nonclinical and clinical data and analyses, the drug brochure, and information on many different areas. The information compiled here will serve as the basis to support the proposed labeling of the drug that the sponsor intends to market the drug. The NDA is reviewed by a panel of highly specialized experts who collectively decide whether to approve the application. A Biologics License Application (BLA) is used for biologic product rather than a NDA though the review process is similar. The required content of the NDA is outlined in the Food, Drug, and Cosmetic Act and Title 21 of the US Code of Federal Regulations (CFR). Applicants must provide significant evidence to allow the reviewers to decide that: the drug is safe and effective in its proposed use, the benefits of the drug outweigh the risks of using it or not having access to it, the drug's proposed labeling is appropriate, and the manufacturing of the agent is adequate. In addition, the FDA has established numerous guidance documents that may represent the current thinking process on the NDA review process [15]. As described earlier, the Prescription Drug User Fee Act (PDUFA) requires a speedier, more efficient review and establishes a time frame for a response to the sponsor.

The "clock" starts when the NDA arrives at the Controlled Document Room and the FDA was 60 days to decide whether to review the NDA. In order to file an NDA and begin the review process, the sponsor must pay the Prescription Drug User Fee, currently (for 2013) at $1,958,800 for an application requiring clinical data and $979,400 for an application not requiring clinical data [15]. The NDA is assigned a project manager who will perform a preliminary review to assure all the necessary components are present and there are no deficiencies. Next, the project manager will forward the appropriate sections of the NDA to different members of the review team, which may include clinicians, pharmacists, toxicologists, statisticians, and chemists to perform a second review to determine if the NDA should undergo a formal review (usually occurs with 45 days of receiving the NDA). Finally, the NDA is subjected to a formal review, which will include inspections of the drug manufacturing sites and potentially the clinical trial sites. Each member of the team will prepare a written evaluation of his or her section of the NDA. An "approval" means that the drug is ready to be marketed under its label. The review team could also recommend "approvable" which mean the sponsor needs to resolve additional issues before it can be marketed. Finally, the review team could recommend "nonapprovable" which would require justification for this decision.

Review of NDA and BLA applications received from sponsors represents a top priority of the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) at FDA. The system has the advantage of allowing an efficient allocation of resources within FDA, as the onus of producing and organizing the data supporting an NDA or BLA

application lies with its sponsor. The system relies on economic forces to provide incentive to sponsors to conduct the necessary trials and submit the relevant data to the FDA for review. An important consequence of the system is that applications determine the approvals (and many of the other labeling changes) considered by the FDA – a fundamental prerequisite of the FDA granting an approval for an indication related to solid organ transplantation is the submission of an application by a sponsor.

The public may infer that all FDA approved products have demonstrated their safety and efficacy in studies submitted to and reviewed by FDA. The absence of an FDA approval for a given indication implies that either an application has not been submitted or that the application has not yet been determined to be adequate to demonstrate the safety and efficacy of the product in the relevant context. In special circumstances, the FDA can require the sponsor to place a boxed warning, "black box warning", on the labeling of a prescription drug and in literature describing it. It is the strongest warning that the FDA requires, and signifies that medical studies indicate that the agent carries a significant risk of serious or even life-threatening adverse effects [16].

Post marketing surveillance

Over the past decade, a series of safety issues arose with a number of drugs after their approval, including rofecoxib, rosiglitazone, and natalizumab. Even after the NDA is approved, the applicant must conduct post-marketing monitoring for safety and newly reported adverse events. Since drugs are approved on the basis of well controlled clinical trials, post-marketing monitoring can further define the safety of the drug after it is introduced to the general population who has a wide variety of medical conditions. In 2007, Congress passed legislation called FDA Modernization Act of 2007 (FDAAA) which contained new strategies and safety programs specifically to evaluate and minimize the risks of new agents. Congress introduced the concept of risk evaluation and mitigation strategies (REMS) and post marketing requirements (PMR) that provide FDA greater authority to mandate risk aversion strategies.

Under Title IX, Subtitle A, Section 901 of the FDAAA created Section 505-1 of the Federal Food, Drug and Cosmetic Act, the FDA has the authority to require a company, at the time of NDA or any time after approval, to submit REMS if the agency determines it necessary to ensure that the benefits of the drug outweigh its risks [17]. REMS is defined as a strategy to manage known or potential serious risk associated with the use a drug. All REMS must have specific goals and the elements constitute the tools by which the goals are achieved. The elements of the REMS may include one or more of the following: medication guide and/or Patient Package Insert (PPI), Communication Plan for Healthcare Providers, and Elements to Assure Safe Use (ETASU). Medication guides are designed to provide potential patients with information about the safe use of the product. The purpose of the guide is to prevent serious adverse events by potentially affecting the patient's decision to use or continue the medication, or when specific instructions can aid in improving patient's adherence. PPI are designed to instruct patients on the safe use of the drug product. The medication guide, but not the PPI, must be provided to the patient every time the produce is dispensed [18]. Communication plans are designed to educate healthcare providers on the appropriate and safe use of the product through various communications means including letters or materials to the providers. Finally, ETASUs are utilized when safe access for patients to products with known risks are otherwise unavailable. ETASUs may include special certification of healthcare providers who prescribe the product or special certification of pharmacies that dispense the product, or restrictions of product distribution (limited to patient enrolled in a registry, or in specific treatment settings). As part of REMS, the sponsor must demonstrate a post-marketing plan to monitor for the potential risk and provide the FDA with an assessment no less frequent than 18 months, three years, and seven years post approval. This report should comment on the success of the REMS elements in achieving their goals. Between 2008 and 2011, roughly 35% of all NDAs have required REMS; though the majority has only required a medication guide. Of the 178 REMS, there have been 48 REMS which required a communication plan and only 21 have an ETASU [15]. In the setting of a generic drug requiring REMS, both innovator and generic medications will share the same REMS, including the systems required by an ETASU.

Orphan drugs

Orphan drugs are approved using most of the processes described above with a few significant differences. An orphan drug is one that is used to treat a "rare" disease defined as a disease that afflicts fewer than 200 000 patients in the United States. Importantly, organ transplantation is considered an orphan indication by these standards and, as such, this policy is relevant to every drug being developed for a transplant indication. In addition, the drug's development would ordinarily be of limited interest to commercial manufactures because of the inability to recover the financial costs. The Orphan Act of 1993 offers financial incentives to sponsors to promote the development of these necessary agents such as federal grants for clinical research, tax credits for development, and seven year market exclusivity for sponsor who obtains federal approval.

Abbreviated new drug applications

The FDA approves generic drugs through the abbreviated new drug application (ANDA) pathway. The manufacturer is able to submit this abbreviated application so that the approval will be effective immediately once the patent on the original product has expired. Unlike NDA applications, ANDA applications are not required to include clinical trial data to establish safety and efficacy. Instead, the FDA approves ANDA applications based on data establishing the bioequivalence and pharmaceutical equivalence of a generic drug to the approved innovator product. It is important to note that equivalence is defined as not being statistically significantly different. The FDA has set the range for pharmacokinetic data for the new product to be within 80–125% of the PK of the branded product. The field of transplantation has recently entered an era where generic versions of the mainstay agents such as mycophenolate mofetil (MMF) and tacrolimus have become widespread.

Challenges in drug development specific in solid organ transplantation

Efficacy endpoints considered in transplantation maintenance immunosuppression trials

Products approved as either initial or maintenance immunosuppressive agents since the advent of cyclosporine have generally

used a combined endpoint of acute rejection, graft loss, and death as a primary efficacy endpoint. In transplant literature, this combined endpoint has sometimes been referred to as the "traditional FDA combined endpoint". While such analyses have been used to establish the effectiveness of newer agents, it may be worth noting that FDA does not consider such analyses to represent either an "FDA" endpoint or a "combined" endpoint. Rather, during those reviews, the FDA agreed that acute rejection represented a significant clinical event constituting a treatment failure for therapies intended to modulate immune responses. From the FDA perspective, the endpoint of those trials has been acute rejection. Patients with missing data – whether due to death, graft loss, or loss to follow up – were simply imputed to have had episodes of acute rejection.

The use of an endpoint based on the incidence of acute rejection for the approval of immunosuppressive agents in transplantation has well-recognized limitations. The endpoint does not weigh, for instance, clinically important elements such as the severity of the rejection episodes. It has been suggested that use of alternative endpoints, such as those based on glomerular filtration rates (GFRs) and protocol biopsies, might augment the evaluation of patient outcomes in transplantation trials of immunosuppressive regimens. Indeed, for instance, GFR endpoints were considered during the reviews of both the belatacept and everolimus NDAs.

FDA is open to examining alternative endpoints, both for the prophylaxis of acute rejection as well as for more novel indications. However, the mandate to establish efficacy based on adequate and well-controlled investigations is non-identical with the desire of clinicians to identify optimal treatment regimens using randomized controlled trials. The most important function of a primary efficacy endpoint is to provide quantitative evidence of the effectiveness of an investigational agent. It should afford a clear demonstration of the contribution of the test product. Not all outcome measures, regardless of clinical importance, are well suited to this end in all contexts. Acute rejection endpoints have remained relevant because of the wealth of experience and data from past trials using such endpoints that are available to inform the design and interpretation of new trials.

Currently available immunosuppressive maintenance regimens offer reasonably potent efficacy as measured by endpoints based on acute rejection. But as in all drugs, they are associated with known toxicities (including reversible and non-reversible decreases to GFR as well as some particular nephrotoxicities evident on histopathology). Given the potency of the marketed products, the effectiveness of newer products is frequently established using trials designed to determine the non-inferiority rather than the superiority of the test product. Non-inferiority trials examine whether a newer product is not much less effective than the active control.

In order to perform a meaningful non-inferiority analysis, one must know the contribution to the effect of the element of the active control that the test product is replacing. To determine this contribution, one must have access to historical trials, which studied the desired endpoint by comparing outcomes with patients who received the active control and patients who received the putative placebo. Establishing non-inferiority margins for transplantation trials using endpoints based on acute rejection is challenging but sometimes possible, as many historical trials exist which captured reliable data for such endpoints. Establishing non-inferiority margins for trials using novel endpoints is often not possible as the requisite historical data do not exist or are not available.

Given the paucity of relevant historical trial data, more novel endpoints often must rely on trials designed to test the superiority of the investigational agent over the active control agent. As currently available therapies are potent, it can be challenging to prove that an experimental product is more effective than a marketed product. As marketed products have known toxicities, which limit their usefulness, it may be possible to demonstrate that use of a novel product results in a better outcome due to the absence of the relevant toxicity. Caution must be exercised, however, when designing such trials: absence of toxicity cannot itself constitute the efficacy of a test product. If an outcome is confounded by a known toxicity of the active comparator, the outcome may not be appropriate for use as a primary endpoint in a trial intended to support product approval.

Previous experience with GFR and CAN as efficacy endpoints for indications related to maintenance immunosuppression in kidney transplantation

GFR in kidney transplantation trials is illustrative of an important clinical outcome that is not always amenable to use as a primary efficacy endpoint. As a measure of kidney function, GFR would appear to represent a reasonable measure of the efficacy of an immunosuppressive agent in the setting of a kidney transplant: in the absence of effective immunosuppression, immune-mediated injury to the kidney will lead to a deterioration of kidney function and decreased GFR. Indeed, FDA might agree that such an endpoint would be interpretable in the setting of a placebo-controlled trial. Frequently, however, its use has been proposed in clinical trials where an investigational agent replaces a calcineurin inhibitor in the active comparator arm. In theory, one might pursue a non-inferiority trial design, but one would require reliable historical data regarding GFR outcomes associated with the putative placebo. Rather, sponsors typically propose to study GFR using a trial design to establish the superiority of the newer product. Calcineurin inhibitors (particularly cyclosporine), however, are known to cause vasoconstriction of the afferent renal artery, resulting in a physiologic decrease in GFR secondary to hemodynamic (not immunosuppressive) effects. The observation of statistically superior GFRs among patients randomized to the investigational arms, then, does not necessarily prove that the test products have greater (or even equivalent) efficacy. The deficiencies of previous trial designs do not preclude the use of GFR as a primary efficacy endpoint in the context of a maintenance immunosuppression trial; such trials, however, should rely on designs that ensure the endpoint is not confounded.

Protocol allograft biopsies have also been proposed as alternative efficacy endpoints in trials of newer products intended for maintenance immunosuppression but face similar challenges. Sponsors have proposed comparing the incidence of histopathologic diagnosis such as chronic allograft nephropathy (CAN) across study arms. CAN is a non-specific term used to describe various changes in renal histology thought to occur secondary to immunologic and non-immunologic injury. Insufficient historical trial data have been submitted and reviewed to allow the determination of non-inferiority margins based on a protocol biopsy endpoint. Furthermore, the use of CAN as a primary endpoint for a trial design intended to show the superiority of the test product is problematic for two reasons: 1) calcineurin inhibitor toxicity is one of the potential sources of non-immunologic injury to the kidney and 2) the clinical significance of a diagnosis of CAN is uncertain. If the incidence of CAN can be attributed, in at least some cases, to exposure

to the active control agent, the absence of CAN cannot prove the efficacy of the test agent. Proper use of efficacy endpoints based on protocol biopsies to show efficacy in the context of a maintenance immunosuppression trial would require trial designs that avoid confounding and that rely on a histopathologic finding with validated clinical significance.

FDA approved products for solid organ transplant

Immunosuppressants

The purpose of this section is to briefly review the immunosuppressive agents that have an FDA approved indication and are currently utilized in the US. Briefly, the critical features of the prescribing information will be reviewed in addition to the results of the clinical trials that led to each respective drug approval. The reader will quickly note that the approved indication of most drugs is substantially more limited than the common use of the drug in transplantation. Furthermore, it is apparent to any transplant practitioner that institutional dosing guidelines frequently stray from the approved dosing, and indeed, it is reasonable to say that there is no established regimen that clearly stands out as "best" for even the most common indications in transplantation. Furthermore, there are no guidelines included in approved labels recommending alterations in dosing over time, and with patients now on these drugs for decades, it is common for dosing to be generally reduced, without any clear regulatory approval of that practice. A comprehensive review of the mechanisms of action of these agents, and more detail on the "off-label" use of these and other medications will be discussed in Chapters 17 and 101, respectively. Practical uses are described in organ specific chapters on post transplant management. Several medications with FDA approved indications in organ transplant are not discussed due to the fact that they are no longer available for use in solid organ transplant recipients. These medications include: equine anti-thymocyte globulin, daclizumab, and muromonab-CD3.

Azathioprine (Imuran)

Azathioprine and steroids were considered the backbone of immunosuppression in organ transplantation from the early 1960's to early 1980's. Azathioprine received FDA approval in March of 1968 based on the NDA submitted by Prometheus Laboratories [19]. Currently, azathioprine is available as a generic through several companies who have submitted ANDAs. Azathioprine is indicated as an adjunct therapy option for the prevention of rejection in renal transplantation. According to the prescribing information, "experience with over 16 000 transplants shows a 5-year patient survival of 35% to 55%" [20]. This statement was made with the caveat that this was dependent on several factors including: the donor, match for HLA antigens, anti-donor or anti-B cell alloantigen antibody, and other variables not tested in controlled trials. Azathioprine is thought to suppress cell-mediated hypersensitivities and causes variable alterations in antibody production with little effect on established graft rejections or secondary responses. The safety and efficacy of azathioprine in pediatric patients has not been established. Dosing recommendations in the prescribing information include an initial dose of 3 to 5 mg/kg daily, beginning at the time of transplant. Therapeutic plasma level monitoring is of little predictive value due to the extensive metabolism of azathioprine, conversion to 6-mercaptopurine, and subsequent conversion to thiopurine nucleotides. Patients with reduced levels of thiopurine S-methyl transferase (TPMT) may be at an increased risk for severe, life threatening abnormalities, requiring reduced doses of azathioprine. TPMT genotyping or phenotyping can be used to identify patients at risk in conjunction with complete blood count monitoring for all patients. Finally, it appears that the data resulting in the FDA approved indication for renal transplantation does not necessarily reflect current transplant medication practices, as the prescribing information does not contain information regarding the use of azathioprine in conjunction with other maintenance immunosuppressants [20].

Cyclosporine (Sandimmune/Neoral/Gengraf)

Cyclosporine is a powerful immunosuppressive medication that revolutionized organ transplantation as the first of the calcineurin inhibitors. The impact of using cyclosporine occurred not only in kidney transplantation, but also heart and liver transplantation as well. Cyclosporine received its original FDA approval in November of 1983 for oral and injectable solutions; however, due to its poor bioavailability newer formulations were developed and approved in 1990 and 1995 respectively. The capsule formulation of cyclosporine, marketed under the brand name Sandimmune® was approved in 1990 with the original patent held by Novartis (Basel, Switzerland) [21]. Modified cyclosporine, also produced by Novartis and marketed under the brand name Neoral®, received FDA approval in 1995 [22].

Both cyclosporine and modified cyclosporine are indicated for the prophylaxis of organ rejection in kidney, liver, and heart allogeneic transplantations. However, there are slight variations between the indications for cyclosporine and cyclosporine modified. The prescribing information for cyclosporine states that it is always to be used with adrenal corticosteroids and may be used in the treatment of chronic rejection in patients previously treated with other immunosuppression agents [23]. The prescribing information for modified cyclosporine states that it has been used in combination with azathioprine and corticosteroids [24]. The differences in the prescribing information are also reflected in the clinical pharmacology for the two agents. The clinical pharmacology of cyclosporine is predicated by the statement that the exact mechanism of action of cyclosporine is not known, but "experimental evidence suggests the effectiveness of cyclosporine is due to specific and reversible inhibition of immunocompetent lymphocytes in the G0 or G1 phase of the cell cycle" [23].

Dosing recommendations for cyclosporine and modified cyclosporine are reflective of the differences in the pharmacokinetic properties of the formulations, and it is safe to say that the approved dosing is infrequently followed in modern clinical practice. An initial oral dose of cyclosporine of 15 mg/kg is recommended to be administered 4 to 12 hours prior to transplantation. This single daily dose should be continued for up to 2 weeks followed by a tapering strategy of a 5% daily dose reduction to doses as low as 3 mg/kg/day [23]. Modified cyclosporine can be given 4–13 hours prior to transplantation or postoperatively, with doses ranging from 7 to 9 mg/kg/day administered in two equally divided doses. Subsequent doses should be adjusted to obtain the target cyclosporine blood concentrations. The target range for trough levels is identical between cyclosporine and modified cyclosporine, although patients will receive greater exposure of modified cyclosporine at any given target range as compared to cyclosporine. Therefore, lower target troughs of modified cyclosporine may provide adequate immunosuppression. In addition, converting patients from modified cyclosporine to cyclosporine utilizing a 1 : 1 ratio may result in lower cyclosporine blood concentrations [24]. The prescribing information for both formulations also includes

dosing recommendations for adjunct therapy with adrenal corticosteroids with a 2-month tapering strategy. Though no adequate and well controlled pediatric studies have been conducted, patients as young as 6 months of age have received the drug with no "unusual" adverse effects. Pediatric dosing recommendations are weight based using adult dosing with similar pharmacokinetic differences between formulations observed [23,24].

Multiple ANDA applications for cyclosporine have been approved, utilizing both cyclosporine and modified cyclosporine as the reference product. In order to differentiate between all of the products available, the following are the full generic names utilized for each formulation. Cyclosporine capsules, USP and cyclosporine oral solution, USP refers to the oil-based product Sandimmune®, whereas cyclosporine capsules, USP Modified and cyclosporine oral solution, USP Modified refers to the microemulsion product, Neoral®. Branded generic modified cyclosporine is available under the trade name Gengraf® with the following generic drug name: cyclosporine capsules, USP Modified.

Tacrolimus (Prograf®)

Tacrolimus is an immunosuppressive agent that has significantly improved clinical outcomes in liver and kidney transplantation. Unlike other agents, tacrolimus first received FDA approval in 1994 for use in liver, as opposed to kidney, allograft transplantation recipients [25]. In 1997, tacrolimus received an indication for use in kidney transplantation allograft recipients. Finally, tacrolimus received an indication for use in heart allograft transplantation in 2006, representing the most recent indication to be added to tacrolimus. Since 2009, the FDA has approved several ANDA applications for generic versions of tacrolimus [26].

The full FDA approved indication for tacrolimus is for prophylaxis of organ rejection in kidney, liver, and heart transplant. Tacrolimus is recommended to be used concomitantly with azathioprine or MMF and adrenal corticosteroids for kidney and heart transplant. For liver transplant patients, tacrolimus is recommended to be used concomitantly with adrenal corticosteroids [27]. As described in the prescribing information, tacrolimus inhibits T-lymphocyte activation by binding the intracellular protein FKBP-12. This complex then binds with calcium, calmodulin, and calcineurin to prevent the formation of lymphokines, which prevents T-lymphocyte activation [27]. As with cyclosporine, the actual use of tacrolimus strays significantly from its indicated regimen.

Due to its significant intersubject variability and based on its pharmacokinetic properties, tacrolimus requires individualized dosing for optimal therapy. Tacrolimus is incompletely and variably absorbed, with decreased absorption associated with the presence of food. The trough blood level at 10 to 12 hour post-dose or Cmin level was found to correlate with the AUC in small pharmacokinetic studies with tacrolimus. Tacrolimus is 99% plasma protein bound and undergoes extensive hepatic metabolism by the cytochrome P-450 system, specifically CYP3A4. Tacrolimus is 92.6% fecally eliminated, as demonstrated by a small study in healthy volunteers using radiolabeled tacrolimus. Although pharmacokinetic studies for tacrolimus in the pediatric transplant population is limited, there was sufficient experience with pediatric liver transplant patients for the inclusion of pediatric liver transplantation dosing recommendations in the prescribing information.

The FDA approved dosing recommendations for tacrolimus are included for adult heart, liver, and kidney transplant patients as well as for pediatric liver transplant patients in Table 98.1. All doses are weight based in milligrams per kilogram of body weight, administered as two divided doses 12 hours apart. The prescribing information includes the following starting doses for tacrolimus with the target whole blood trough concentrations for individualized dose titration. In addition, the prescribing information suggest that both African American and pediatric liver transplant patients may require higher doses of tacrolimus per body weight in order to reach the target trough level [27].

Table 98.1. Summary of Initial Oral Dosage Recommendations and Observed Whole Blood Trough Concentrations

Patient Population	Recommended Initial Oral Dosage[a]	Obrerved Whole Blood Trough Concentrations
Adult kidney transplant patients		
In combination with azathioprine	0.2 mg/kg/day	mouth 1–3: 7–20 ng/mL mouth 4–12: 5–15 ng/mL
In combination with MMF/IL-2 receptor antagonist[b]	0.1 mg/kg/day	month 1–12: 4–11 ng/mL
Adult liver transplant patients	0.10–0.15 mg/kg/day	month 1–12: 5–20 ng/mL
Pediatric liver transplant patients	0.15–0.20 mg/kg/day	month 1–12: 5–20 ng/mL
Adult heart transplant patients	0.075 mg/kg/day	month 1–3: 10–20 ng/mL month ≥4: 5–15 ng/mL

[a] Note: two divided doses, ql 21 h.
[b] In a second smaller study, the initial dose of tacrolimus was 0.15–0.2 mg/kg/day and observed tacrolimus concentractions were 6–16 ng/mL during month 1–3 and 5–12 ng/mL during month 4–12 (see **CLINICAL STUDIES**).
Reproduced from [27] with permission.

As mentioned previously, the clinical trials that resulted in the original FDA approval of tacrolimus in 1994 were first conducted in liver transplantation. The safety and efficacy of a tacrolimus-based regimen was assessed in two prospective, randomized, non-blinded phase III multicenter trials. The studies evaluated 12-month patient and graft survival, comparing a regimen consisting of tacrolimus and adrenal corticosteroids to a regimen of cyclosporine, azathioprine, and adrenal corticosteroids. The first study was conducted in the US (n = 529) [28] while the other was conducted in Europe (n = 545) [29]. Both studies reported similar one-year patient and graft survival between treatment arms. Overall outcomes were also similar between the two studies in terms of patient and graft survival. The study reported trough blood concentrations up to one-year post transplant that ranged from 9.8 ng/ml to 19.4 ng/ml. As a result, of these studies, tacrolimus was found to be equivalent to cyclosporine-based regimens and received FDA approval for liver transplantation.

Subsequent trials with tacrolimus in renal transplantation resulted in the addition of kidney transplantation as an FDA approved indication for tacrolimus in 1997. Tacrolimus was initially evaluated in combination with azathioprine and corticosteroids in a Phase III randomized, multicenter, non-blinded prospective study [30]. All patients (n = 412) received induction immunosuppression therapy consisting of an antilymphocyte antibody preparation, corticosteroids, and azathioprine. Patients were randomized to either tacrolimus (n = 205) or cyclosporine (n = 207) with the first dose being initiated once the serum creatinine was less than 4 mg/dl. In terms of one-year outcomes, there were no significant differences in patient or graft survival in comparing tacrolimus and cyclosporine. Patient survival was 95.6% and 96.6% respectively, whereas graft survival was 91.2% and 87.9% respectively. However, there was a significant reduction in biopsy proven acute rejection in the tacrolimus treatment group (30.7% vs. 46.4%, P = 0.001). The authors

Table 98.2. Estimated creatinine clearance at 12 months in renal transplant patients

Group	eCLcr [mL/min] at Month 12[a]				
	N	MEAN	SD	MEDIAN	Treatment Difference with Group C (99.2%CI[b])
(A) CsA/MMF/CS	390	56.5	25.8	56.9	−8.6 (−13.7, −3.7)
(B) CsA/MMF/CS/ Daclizumab	399	58.9	25.6	60.9	−6.2 (−11.2, −1.2)
(C) Tac/MMF/CS/ Daclizumab	401	65.1	27.4	66.2	–
(D) SRL/MMF/CS/ Daclizumab	399	56.2	27.4	57.3	−8.9 (−14.1, −3.9)
Total	1589	59.2	26.8	60.5	

[a] All death/graft loss (n = 41, 27, 23 and 42 in Groups A, B, C and D) and patients whose last recorded creatinine values were prior to month 3 visit (n = 10, 9, 7 and 9 in Groups A, B, C and D, respectively) were inputed with Glomerular Filtration Rate (GFR) of 10 mL/min; a subject's last observed creatinine value from month 3 on was used for the remainder of subjects with missing creatinine at month 12 (n = 11, 12, 15 and 19 for Groups A, B, C and D, respectively). Weight was also imputed in the calculation of estimated GFR, if missing.
[b] Adjusted for multiple (6) pairwise comparisons using Bonferroni corrections.
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Table 98.3. Incidence of BPAR, graft loss, death or loss to follow-up at 12 months in renal transplant patients

	Tacrolimus/ MMF (n = 212)	Cyclosporine/MMF (n = 212)
Overall Failure	32 (15.1%)	36 (17.0%)
Components of efficacy failure		
BPAR	16 (7.5%)	29 (13.7%)
Graft Loss excluding death	6 (2.8%)	4 (1.9%)
Mortality	9 (4.2%)	5 (2.4%)
Lost to follow-up	4 (1.9%)	1 (0.5%)
Treatmert Difference of efficacy failure compared to Prograf/MMF group (95% CI[a])	–	1.9% (−5.2%, 9.0%)

[a] 95% confidence interval calculated using Fisher's Exact Test.

concluded that tacrolimus was more effective at reducing acute rejection episodes as compared to cyclosporine, while reducing the amount of antilymphocyte antibody preparation needed [30]. This study resulted in FDA approval of tacrolimus for use in conjunction with adrenal corticosteroids in liver or kidney transplant patients.

More recently, two phase III studies evaluated the combination of tacrolimus with MMF for use in kidney transplant patients. The ELITE-Symphony study involved 1589 kidney transplant patients, who were randomized into four treatment arms consisting of tacrolimus (Group C, n = 401), sirolimus (Group D, n = 399), or one of two cyclosporine regimens (Group A (standard dose), n = 390 and Group B (low dose), n = 399) [31]. In addition to MMF and corticosteroids, all groups with the exception of Group A received induction immunosuppression therapy with daclizumab. The primary outcome of the study was a comparison of renal function at 12 months using the estimated creatinine clearance (calculated by Cockcroft-Gault). Patients receiving tacrolimus had improved renal function at 12 months with significantly higher calculated creatinine clearance when compared to the other treatment groups (Table 98.2).

In terms of the secondary outcomes, treatment failure was defined as biopsy proven acute rejection, graft loss, death, and/or patient lost to follow up. Patients receiving tacrolimus (Group C) demonstrated fewer treatment failures, with the differences between Group C and each treatment group achieving statistical significance. Of note, patients receiving tacrolimus in combination with MMF were more likely to develop diarrhea and diabetes [31].

The second study was a phase III, randomized (1:1:1) open-label, multicenter, three arm noninferiority study conducted in the United States, Canada, and Brazil [32]. This study compared three regimens consisting of MMF, corticosteroids, and basiliximab induction for kidney transplant patients in combination with: extended release tacrolimus (n = 214), standard tacrolimus (n = 212), and cyclosporine (n = 212). The primary endpoint of this study was a combined efficacy failure at 1 year comprising of biopsy proven acute rejection, graft loss excluding death, mortality, and lost to follow up. Extended release tacrolimus is not currently FDA approved and, therefore, the prescribing information only reports the results of this study with regard to the standard tacrolimus treatment group (Table 98.3), although both tacrolimus groups achieved noninferiority to cyclosporine in terms of the primary outcome. Although the difference in mortality was not statistically significant, there was a higher mortality in the standard tacrolimus group (4.2% tacrolimus vs. 2.4% cyclosporine). No trends were identified, although three deaths attributed to sepsis were identified in the tacrolimus group as compared to none in the cyclosporine group. As a result of this study, as well as the ELITE-Symphony study, tacrolimus received an indication for kidney transplantation in combination with MMF [32].

Tacrolimus received an FDA approved indication for heart transplantation in 2006 as a result of two Phase III studies that compared the safety and efficacy of tacrolimus-based and cyclosporine-based regimens. The first study was conducted in Europe (n = 314) and stratified patients to treatment for 18 months with either tacrolimus or modified cyclosporine in combination with antibody induction, corticosteroids, and azathioprine [33]. The second study (n = 331) was conducted in the US and stratified patients to treatment for one year according to three groups consisting of: corticosteroids plus tacrolimus and sirolimus, tacrolimus plus MMF, or cyclosporine modified plus MMF for 1 year [34]. In each study, there was no significant difference in patient/graft survival at the treatment endpoint of either 18 months (91.7% tacrolimus vs. 89.2% cyclosporine) or 1 year (91.7% tacrolimus vs. 86.1% cyclosporine) respectively. The sirolimus arm of the study conducted in the US did find an increased risk of wound healing complications and insulin dependent post-transplant diabetes mellitus, and is therefore not recommended [34].

Mycophenolate mofetil (Cellcept)

Mycophenolate mofetil is a powerful anti-metabolite that was first approved for use in kidney transplantation in 1995 [35]. Subsequent studies resulted in additional indications added for heart and liver transplantation in 2000. Since 2009, the FDA has approved several ANDA applications for generic versions of MMF [36]. The current FDA approved indication of MMF is the prophylaxis of organ rejection in patients receiving allogeneic kidney, heart, and liver transplants [37]. The prescribing information also states that MMF is to be used in combination with cyclosporine and corticosteroids. Mycophenolate mofetil is a prodrug of mycophenolic acid, which is the active metabolite. Mycophenolic acid is a potent, selective, uncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase that inhibits de novo purine synthesis. Due to the fact that T- and B-lymphocytes lack a salvage pathway for

purine synthesis, mycophenolic acid is able to prevent lymphocyte proliferation [37].

The pharmacokinetic properties of MMF are well described. It is rapidly absorbed following oral administration and hydrolyzed to form mycophenolic acid. The mean absolute bioavailability of oral versus intravenous MMF is 94%. There is evidence to suggest that mycophenolic acid undergoes enterohepatic recirculation, which contributes to sustained plasma concentration levels. Due to its teratogenic effects, MMF is pregnancy category D. The use of MMF in pregnancy is associated with increased risk of first trimester pregnancy loss and congenital malformations. This is based on animal reproductive toxicology studies and data from the National Transplantation Pregnancy Registry (NTPR) [38]. This risk is currently being addressed by the FDA in the form of a Risk Evaluation and Mitigation Strategy program for all MMF containing compounds, implemented in November of 2012 [38].

Mycophenolate mofetil received approval for use in kidney transplantation based on three randomized double blind, multicenter prospective trials. All three studies compared two doses (1 gram and 1.5 gram) of MMF twice daily with either azathioprine (2 studies) or placebo (1 study). All of studies utilized concomitant cyclosporine and corticosteroid therapy, with one study utilizing equine antithymocyte globulin. One study was conducted in the United States (n = 499; Table 98.4), the second study was conducted in Europe (n = 503), and the last study was conducted in Europe, Canada, and Australia (n = 491) [39–41]. The primary efficacy end-point of all three studies was treatment failure within the first six months, defined as biopsy proven acute rejection, death, graft loss, and early termination from study. Patients receiving MMF had a statistically significant reduced incidence of treatment failure within the first 6 months following transplantation. More patients receiving MMF discontinued therapy without biopsy proven acute rejection, death, or graft loss than in the control arms, with the highest discontinuation rates in the MMF 3 grams per day groups. This was likely due to increased drug intolerance at the higher dose. There was no advantage to MMF compared to control with respect to one year patient or graft survival [37].

In 2000, MMF received an indication for heart transplantation based on the results of a double-blind, randomized, parallel-group, multicenter study, the results of which are summarized in Table 98.5 [42]. This multination study was conducted in primary cardiac transplant recipients, who received either MMF 1.5 g (n = 289) twice daily or azathioprine (n = 289) in combination with cyclosporine and corticosteroids. The two primary end-points were endomyocardial biopsy-proven rejection with hemodynamic compromise and graft failure or death in the first six months, and the proportion of patients who died or were retransplanted in the first 12 months. There were no differences between the treatment groups for the incidence of biopsy proven rejection or patient survival. Based on this study, MMF was shown to be at least as effective as azathioprine with respect to rejection and survival [42].

The final indication for liver transplantation was approved by the FDA in 2000 based on the results of a double blind, randomized, comparative, parallel group multinational, multicenter study in primary liver transplant patients [43]. Patients (n = 565) receive modified cyclosporine and corticosteroids in combination with either MMF 1 gram twice daily intravenously for 14 days followed by MMF 1.5 g twice daily or azathioprine intravenously followed by oral azathioprine. The two primary end-points were as follows: the proportion of patients with biopsy proven rejection, death, or retransplantation within the first six months; and the proportion of patients with graft loss due to death or retransplantation within the first 12 months. The study (summarized in Table 98.6) found that patients on MMF mofetil experienced significantly lower rates of

Table 98.4. Incidence of treatment failure in renal transplant patients within 6 months post-transplant

USA Study[a] (N = 499 patients)	**MMF 2 g/day (n = 167 patients)**	**MMF 3 g/day (n = 166 patients)**	**Azathioprine 1 to 2 mg/kg/day (n = 166 patients)**
All treatment failures	31.1%	31.3%	47.6%
Early termination without prior acute rejection[b]	9.6%	12.7%	6.0%
Biopsy-proven rejection episode on treatment	19.8%	17.5%	38.0%
Europe/Canada/Australia Study[c] (N = 505 patients)	**MMF 2 g/day (n = 173 patients)**	**MMF 3 g/day (n = 164 patients)**	**Azathioprine 100 to 150 mg/day (n = 166 patients)**
All treatment failures	38.2%	34.8%	50.0%
Early termination witout prior acute rejection[b]	13.9%	15.2%	10.2%
Biopsy-proven rejection episode on treatment	19.7%	15.9%	35.5%
Europe Study[d] (N = 491 patients)	**MMF 2 g/day (n = 165 patients)**	**MMF 3 g/day (n = 160 patients)**	**Placebo (n = 166 patients)**
All treatment failures	30.3%	38.8%	56.0%
Early termination-without prior acute rejection[b]	11.5%	22.5%	7.2%
Biopsy-proven rejection episode on treatment	17.0%	13.8%	46.4%

[a] Antithymocyte globulin induction/MMF or azathioprine/cyclosporine/corticosteroids.
[b] Does not include death and grafc loss as reason for early termination.
[c] MMF or azathioprine/cyclosporine/corticosteoids.
[d] MMF or placebo/cyclosporine/corticosteroids.

Table 98.5. Rejection at 6 months and death or retransplantation at 1 year in cardiac transplant patients

	All Patients		**Treated Patients**	
	AZA N = 323	**MMF N = 327**	**AZA N = 289**	**MMF N = 289**
Biopsy-proven rejection with hemodynamic compromise at 6 months[a]	121 (38%)	120 (37%)	100 (35%)	92 (32%)
Death or retransplantation at 1 year	49 (15.2%)	42 (12.8%)	33 (11.4%)	18 (6.2%)

[a] Hemodynamic compromise occurred if any of the following criteria were met: pulmonary capillary wedge pressure ≥20 mm or a 25% increase; cardiac index <2.0 L/min/m2 or a 25% decrease; ejection fraction ≤30%; pulmonary artery oxygen saturation ≤60% or a 24% decrease; presence of new S3 gallop; fractional shortening was ≤20% or a 25% decrease; inotropic support required to manage the clinical condition.

Table 98.6. Rejection at 6 months and death or retransplantation at 1 year in liver transplant patients

	AZA N = 287	**CellCept N = 278**
Biopsy-proven, treated rejection at 6 months (includes death or retransplantation)	137 (47.7%)	107 (38.5%)
Death or retransplantation at 1 year	42 (14.6%)	41 (14.7%)

acute rejection at six months when death or retransplantation were included as part of the primary endpoint.

When the primary endpoint of rejection was censored for graft loss due to death or retransplant, the difference between MMF and azathioprine did not retain statistical significance. With regards to patient survival at one year, there were no differences in graft loss between treatment groups [43].

Enteric-coated acid as mycophenolate sodium (Myfortic)

Mycophenolate sodium delayed release tablets are an enteric coated formulation that directly delivers the active moiety mycophenolic acid. Enteric-coated mycophenolate sodium (EC-MPS) was approved in 2004 for the prophylaxis of organ rejection in patients receiving allogeneic renal transplants in combination with cyclosporine and corticosteroids [44]. Mycophenolate sodium has an identical mechanism of action to MMF due to the fact that they share the same active metabolite. EC-MPS is an uncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase, which inhibits de novo purine synthesis and therefore, proliferation of B- and T-lymphocytes, which lack a salvage pathway for purines [45].

Due to its enteric-coated formulation, mycophenolate sodium has unique pharmacokinetic properties. There is an anticipated delay in the rise of mycophenolic acid concentration that ranges from 0.25 to 1.25 hours, with a median time to max concentration ranging from 1.5 to 2.75 hours. In comparison, the median time to max concentration of MMF ranged from 0.5 to 1.0 hours. The absolute bioavailability of EC-MPS was 72%. In terms of equimolar dosing, mean systemic mycophenolic acid exposure as determined by the AUC was similar in both single and multiple-dose cross-over trials with doses of mycophenolate sodium 720 mg and MMF 1000 mg, both dosed twice daily. Similarly to MMF, EC-MPS is pregnancy category D [45]. The use of EC-MPS in pregnancy is associated with an increased risk of first trimester pregnancy loss and congenital malformations. This is based on animal reproductive toxicology studies and data from the National Transplantation Pregnancy Registry (NTPR). This risk is currently being addressed by the FDA in the form of a Risk Evaluation and Mitigation Strategy program for all mycophenolate containing compounds, implemented in November of 2012 [38]. The safety and effectiveness of EC-MPS in pediatric de novo renal transplant patients has not been established. In stable pediatric renal transplant patients (ages 5 through 16), the safety and effectiveness is supported by adequately controlled adult studies; however, there is limited pharmacokinetic data [45].

The prescribing information for EC-MPS includes dosing recommendations for both adult and pediatric patients. The recommended adult dose of EC-MPS is 720 mg twice daily on an empty stomach, one hour before, or two hours after food intake. The delayed release tablet should not be chewed, crushed, or cut prior to ingestion. The recommended pediatric dose of EC-MPS is 400 mg/m^2 twice daily with a maximum dose of 720 mg twice daily, rounded to the nearest whole 180 mg tablet. Pediatric patients with a body surface area less than 1.19 m^2 [2] should not receive EC-MPS as the dose cannot be accurately administered with the currently available tablet formulations [45].

Mycophenolate sodium received FDA approval for use in kidney transplant patients based on two Phase III multicenter, randomized, trials in de novo and maintenance renal transplant patients. Both studies compared EC-MPS at a dose of 1.44 grams per day to MMF 2 grams per day. The de novo study was a multinational study with patients randomized either to MMF (n = 213) or EC-MPS (n = 210) within 48 hours post-transplant in combination with modified cyclosporine and corticosteroids [46]. Of note, the use of antibody induction was not controlled with only 41% of patients receiving antibody induction. The primary end point of the study was treatment failure, defined as first occurrence of biopsy proven acute rejection, graft loss, death, or lost to follow up at 6 months. The results of this study, shown in Table 98.7, suggest that there was no difference in the occurrence of treatment failure at 6 or 12 months between EC-MPS and MMF.

Table 98.7. Treatment failure in *de novo* renal transplant patients at 6 and 12 months therapy in combination with cyclosporine* and corticosteroids

	EC-MPS 1.44 g/day (n = 213)	mycophenolate mofetil 2 g/day (n = 210)
6 Months	**n (%)**	**n (%)**
Treatment failure#	55 (25.8)	55 (26.2)
Biopsy-proven acute rejection	46 (21.6)	48 (22.9)
Graft loss	7 (3.3)	9 (4.3)
Death	1 (0.5)	2 (1.0)
Lost to follow-up**	3 (1.4)	0
12 Months	**n (%)**	**n (%)**
Graft loss or death or lost to follow-up***	20 (9.4)	18 (8.6)
Treatment failure	61 (28.6)	59 (28.1)
Biopsy-proven acute rejection	48 (22.5)	51 (24.3)
Graft loss	9 (4.2)	9 (4.3)
Death	2 (0.9)	5 (2.4)
Lost to follow-up**	5 (2.3)	0

* USP (MODIFIED).
** Lost to follow-up indicates patients who were lost to follow-up without prior biopsy-proven acute rejection, graft loss or death.
*** Lost to follow-up indicates patients who were lost to follow-up without prior graft loss or death (9 Myfortic patients and 4 mycophenolate mofetil patients).
# 95% confidence interval of the difference in treatment failure at 6 months (Myfortic – mycophenolate mofetil) is (−8.7%, 8.0%).

The maintenance study was conducted in 322 patients who were at least six months post transplant and were on MMF with modified cyclosporine with or without corticosteroids [47]. Patients were randomized to either mycophenolate sodium or MMF with a primary end point of treatment failure, as defined by the first occurrence of biopsy proven acute rejection, graft loss, death, or loss to follow up at 6 or 12 months. Once again, there were no observed differences in the primary endpoint between treatment groups at either time point (Table 98.8).

Sirolimus (Rapamune)

Sirolimus is the original member of a relatively new class of immunosuppressive agents that inhibits the mammalian target of rapamycin (mTOR). Sirolimus was approved by the FDA in 1999 for use in kidney transplantation [48]. Sirolimus binds to FK506 binding protein-12 to form an immunosuppressive complex that inhibits the mTOR. By inhibiting mTOR, sirolimus prevents protein synthesis, cell cycle progression from G1 to S, and ultimately T-lymphocyte proliferation [49].

Sirolimus is indicated for the prophylaxis of organ rejection in patients aged 13 years or older receiving renal transplants. Combination therapy with cyclosporine and corticosteroids is also recommended initially post-transplant based on immunologic risk. It is recommended that patients at low or moderate immunological risk should withdraw cyclosporine therapy 2 to 4 months after transplantation. For patients at high immunologic risk, it is recom-

Table 98.8. Treatment failure in conversion transplant at 6 and 12 months therapy in combination with cyclosporine* and with or without corticosteroids

	EC-MPS 1.44 g/day (n = 159)	mycophenolate mofetil 2 g/day (n = 163)
6 Months	**n (%)**	**n (%)**
Treatmeant failure[#]	7 (4.4)	11 (6.7)
Biopsy-proven acute rejection	2 (1.3)	2 (1.2)
Graft loss	0	1 (0.6)
Death	0	1 (0.6)
Lost to follow-up**	5 (3.1)	7 (4.3)
12 Months	**n (%)**	**n (%)**
Graft loss or death or lost to follow-up***	10 (6.3)	17 (10.4)
Treatment failure	12 (7.5)	20 (12.3)
Biopsy-proven acute rejection	2 (1.3)	5 (3.1)
Graft loss	0	1 (0.6)
Death	2 (1.3)	4 (2.5)
Lost to follow-up**	8 (5.0)	10 (6.1)

* USP (MODIFIED).
** Lost to follow-up indicates patients who were lost to follow-up without prior biopsy-proven acute rejection, graft loss or death.
*** Lost to follow-up indicates patents who were lost to follow-up without prior graft loss or death (8 Myfortic patients and 12 mycophenolate mofetil patients).
[#]95% confidence interval of the difference in treatment failure at 6 months (Myfortic – mycophenclate mofetil) is (−7.4%, 2.7%).

mended that triple therapy with sirolimus, cyclosporine, and corticosteroids be continued for the first year after transplantation. High immunologic risk is defined as black recipients, patients with a high panel-reactive antibodies (peak PRA >80%), or those undergoing re-transplantation who have lost a previous kidney allograft for immunologic reasons [49]. It is important to note that the post approval experience has shown substantial synergistic exacerbation of cyclosporine-associated nephrotoxicity and as such, the use of sirolimus with cyclosporine as indicated on the label is relatively uncommon in modern practice.

The prescribing information includes the following dosing recommendations for sirolimus for patients greater than the age of 13. An initial loading dose of sirolimus should be administered equivalent to three times the maintenance dose (i.e. 6 mg loading dose, followed by 2 mg daily). An initial maintenance dose of 2 mg is recommended although 5 mg daily has been reported in clinical studies. Patients older than the age of 13, who are less than 40 kg, should be dosed based on body surface area (loading dose of 3 mg/m^2, maintenance dose of 1 mg/m^2). Sirolimus oral solution and tablets were found to be clinically equivalent in a randomized, multicenter controlled trial for the prevention of organ rejection following kidney transplantation. Due to the long elimination half-life of sirolimus of 62 hours, frequent dose adjustment with non-steady state levels can result in overdosing or underdosing. Therefore, a new maintenance dose should continue for at least 7 days prior to being adjusted based on a trough level. Several target blood trough concentrations are described in the prescribing information, ranging from 10–15 ng/ml for high immunologic risk patients on concomitant cyclosporine therapy to 16–24 ng/ml for low to moderate risk patients who have withdrawn cyclosporine therapy. In reality, these target levels are substantially higher than those used in modern practice. It is important to note that different assay methodologies are available to measure sirolimus levels [49]. As a result, levels from one assay are not interchangeable with levels from another.

Sirolimus is one of the few immunosuppressive agents with two distinct "black box" warnings. According to the prescribing information, the safety and efficacy of sirolimus in liver or lung transplant patients has not been established and therefore is not recommended. For liver transplantation, the warning is specific for excess mortality, graft loss and hepatic artery thrombosis in de novo liver transplant patients. For lung transplantation, sirolimus is not recommended for use in de novo lung transplant patients due to the risk of potentially fatal bronchial anastomotic dehiscence [49].

Two studies involving the use of sirolimus in de novo liver transplant patients (either with tacrolimus or in combination with cyclosporine) demonstrated excess mortality, graft loss, and increased incidence of hepatic artery thrombosis. In the first study, the use of sirolimus with tacrolimus was found to have increased mortality and graft loss at a rate of 22% versus 9% on tacrolimus alone [50]. The combined incidence of hepatic artery thrombosis from the two studies was 7% in patients receiving sirolimus compared to 2% in the control arm. Most cases occurred within 30 days post-transplant and resulted in graft loss or death [49]. In addition, in a 2 : 1 randomized, multicenter controlled clinical study of stable liver transplant patients converted from a calcineurin inhibitor based regimen to sirolimus, an increased number of deaths were observed in the sirolimus cohort compared to those remaining not converted (3.8% vs. 1.4%, P = NS) [51]. Sirolimus is not recommended for de novo lung transplant due to numerous cases of bronchial anastomotic dehiscence, most of which have been fatal [49].

The safety and efficacy of sirolimus oral solution for the prevention of renal allograft rejection was assessed in two Phase III randomized double-blind, multicenter, controlled trials. The first study (Table 98.9a) compared sirolimus 2 mg daily (n = 284), sirolimus 5 mg daily (n = 274), and azathioprine (n = 161), all in combination with cyclosporine and corticosteroids [52]. The second study (Table 98.9b) compared sirolimus 2 mg daily (n = 227), sirolimus 5 mg daily (n = 219), and azathioprine (n = 130), all in combination with cyclosporine and corticosteroids [53]. In both studies, antilymphocyte antibody induction was prohibited. The primary endpoint was efficacy failure, defined as biopsy proven acute rejection, graft loss, or patient death at six months. In both studies, sirolimus at either dose (2 mg or 5 mg) significantly reduced efficacy failure compared to either azathioprine or placebo. Patient and graft survival was similar between treatment groups at 1 and 2 years for the first study and 1 and 3 years for the second study. The mean glomerular filtration rate at later time points was found to be lower in patients receiving sirolimus for both studies. When the patients in the first study were stratified by race, sirolimus 5 mg daily had a reduced efficacy failure rate compared to azathioprine in black patients. However, the use of higher dose sirolimus in black patients must be weighed against the increased risk of dose-dependent adverse events observed in this cohort [52].

The use of a calcineurin inhibitor withdrawal strategy with sirolimus was evaluated in a Phase III, randomized multicenter controlled study (Table 98.10) [54,55]. All patients received sirolimus, cyclosporine, and corticosteroids for the first three months prior to randomization into either continuing cyclosporine (n = 215) or withdrawal of cyclosporine (n = 215). The primary end-point was graft survival at 12 months post-transplant. There was no difference in patient or graft survival at 1, 2, or 3 years post-transplant. There was a significant increase in the occurrence of biopsy proven acute rejection for patients receiving renal allografts with 4 or more HLA mismatches (15.3% cyclosporine withdrawal vs. 3.0% cyclosporine). For patients receiving allografts with 3 or less HLA mismatches, there was no difference in rejection rates

Table 98.9a. Incidence (%) of efficacy failure at 6 and 24 months for study 1[a,b]

Parameter	Sirolumus Oral Solution 2 mg/day (n = 284)	Sirolumus Oral Solution 5 mg/day (n = 274)	Azathioprine 2–3 mg/kg/day (n = 1.61)
Efficacy failure at 6 months[c]	18.7	16.8	32.3
Components of efficacy failure			
Biopsy-proven acute rejection	16.5	11.3	29.2
Graft loss	1.1	2.9	2.5
Death	0.7	1.8	0
Lost to follow-up	0.4	0.7	0.6
Efficacy failure at 24 months	32.8	25.9	36.0
Components of efficacy failure			
Biopsy-proven acute rejection	23.6	17.5	32.3
Graft loss	3.9	4.7	3.1
Death	4.2	3.3	0
Lost to follow-up	1.1	04	0.6

[a] Patients received cyclosporine and corticosteroids.
[b] Includes patients who prematurely discontinued treatment.
[c] Primary endpoint.

Table 98.9b. Incidence (%) of efficacy fallure at 6 and 36 months for study 2[a,b]

Parameter	Sirolumus Oral Solution 2 mg/day (n = 227)	Sirolumus Oral Solution 5 mg/day (n = 219)	Placebo (n = 130)
Efficacy failure at 6 months[c]	30.0	25.6	47.7
Components of efficacy failure			
Biopsy-proven acute rejection	24.7	19.2	41.5
Graft loss	3.1	3.7	3.9
Death	2.2	2.7	2.3
Lost to follow-up	0	0	0
Efficacy failure at 36 months	44.1	41.6	54.6
Components of efficacy failure			
Biopsy-proven acute rejection	32.2	27.4	43.9
Graft loss	6.2	7.3	4.6
Death	5.7	5.9	5.4
Lost to follow-up	0	0.9	0.8

[a] Patients received cyclosporine and corticosteroids.
[b] Includes patients who prematurely discontinued treatment.
[c] Primary endpoint.

between groups. The mean glomerular filtration rate was significantly higher at 1, 2, and 3 years post transplant for patients receiving sirolimus with cyclosporine withdrawal compared to those continuing cyclosporine [54,55].

The de novo use of sirolimus without calcineurin inhibitors has not been established in kidney transplantation. A Phase III study conducted in de novo renal transplant patients compared sirolimus versus cyclosporine in combination with IL-2 receptor antagonists, MMF, and steroids. The sirolimus cohort had significantly higher acute rejection rate and numerically higher death rates [49]. Finally, conversion from a calcineurin inhibitor (CNI) to sirolimus was

Table 98.10. Calculated glomerular filtration rates (ml/min) by Nankivell equation at 12, 24, and 36 months Post Transplant: study 3[a,b,c]

Parameter	Sirolumus with Cyclosporine Therapy	Sirolumus Following Cyclosporine Withdrawal
Month 12		
Mean ± SEM	53.2 ± 1.5 (n = 208)	59.3 ± 1.5 (n = 203)
Month 24		
Mean ± SEM	48.4 ± 1.7 (n = 203)	58.4 ± 1.6 (n = 201)
Month 36		
Mean ± SEM	47.0 ± 1.8 (n = 196)	58.5 ± 1.9 (n = 199)

[a] Includes patientes who prenaturely discontinued treatment.
[b] Patients who had a graft loss were included in the analysis and had their GFR set to 0.0.
[c] All patients received corticosteroids.

assessed in stable renal transplant patients at least 6 months post-transplant. This study was a randomized, multicenter, global study involving 111 centers and 830 patients [56]. The study was intended to demonstrate that renal function would improve through conversion from a calcineurin inhibitor to sirolimus. However, there was no benefit associated with conversion and a greater incidence of proteinuria in patients converted to sirolimus. In addition, the enrollment of patients with a glomerular filtration rate less than 49 was discontinued due to higher rates of serious adverse events.

Everolimus (Zortress)

Everolimus is the second member of the mTOR inhibitor class of immunosuppression agents. Everolimus received FDA approval in the US for an indication in transplantation in 2010 under the trade name Zortress® [57]. Everolimus is also available in the US under the trade name Afinitor® for use in oncology. Additionally, it is interesting to note that everolimus has been available since 2003 in more than 70 countries outside the US under the trade name Certican® for use in organ transplantation [58].

Everolimus is indicated for the prophylaxis of organ rejection in adult patients at low-moderate immunological risk receiving a kidney transplant [59]. Everolimus should be used with basiliximab and low doses of cyclosporine and corticosteroids. This lower dose recommendation reflects the post approval experience gleaned from sirolimus, in which standard dose cyclosporine was found to be prohibitively toxic. The prescribing information also includes several limitations to the use of everolimus. As mentioned previously, everolimus is an mTOR inhibitor that prevents T- and B-lymphocyte proliferation. Everolimus binds FK506 binding protein-12 to form an immunosuppressive complex that inhibits mTOR, thereby preventing protein synthesis and cell proliferation. The prescribing information for everolimus specifically warns of an increased risk of kidney arterial and venous thrombosis, resulting in graft loss, mostly within the first 30 days post-transplant. Additionally, the prescribing information includes warnings of increased mortality in a clinical trial of de novo heart transplant patients. Therefore, the use of everolimus is not recommended in heart transplantation. The safety and efficacy of everolimus in pediatric patients has been established at this time.

The FDA approved dosing recommendations for everolimus are for an initial dose of 0.75 mg orally twice daily for adult patients with reduced dose cyclosporine. Doses should be adjusted to target the recommended therapeutic range for whole blood trough

Table 98.11. Efficacy Failure by Treatment Group (ITT Population) at 12 Months

Efficacy Endpoints[3]	Everolimus 1.5 mg/day With reduced dose CsA N = 277 n (%)	Mycophenolic Acid 1.44 gm/day With standard dose CsA N = 277 n (%)
Primary Efficacy Failure Endpoint[1]	70 (25.3)	67 (24.2)
Treated Biopsy Proven Acute Rejection	45 (16.2)	47 (17.0)
Death	7 (2.5)	6 (2.2)
Graft Loss	12 (4.3)	9 (3.2)
Loss to Follow-up	12 (4.3)	9 (3.2)
Graft Loss or Death or Loss to Follow-up[2]	32 (11.6)	26 (9.4)
Graft Loss or Death	18 (6.5)	15 (5.4)
Loss to Follow-up[2]	14 (5.1)	11 (4.0)

[1] Includes treated BPAR, graft loss, death or loss to follow-up by month 12 where loss to follow-up represents patient who did not experience treated BPAR, graft loss or death and whose last contact date is prior to 12 month visit.
[2] Includes treated BPAR, graft loss, death or loss to follow-up by month 12 where loss to follow-up represents patient who did not experience treated BPAR, graft loss or death.
[3] Loss to follow-up (for graft loss, death or loss to follow-up) represents patients who did not experience death or graft loss and whose lost contact is prior to 12 month visit.

concentration levels of 3 to 8 ng/mL. Again, the reduced target levels are consistent with the practical migration of sirolimus dosing to lower target levels. Due to the long elimination half-life of 30 hours, it is recommended that dose adjustments be made at 4 to 5 day intervals to reach the therapeutic range. The prescribing information as includes recommendations for reduced whole blood trough concentrations for cyclosporine, with target ranges starting at 100–200 ng/ml in the first month post-transplant titrated down to 25–50 ng/ml between months 6 and 12 post-transplant [59].

The safety and efficacy of concentration controlled everolimus dosing was evaluated in a 24-month, multi-national, open-label, randomized trial in kidney transplant patients, the results of which are shown in Table 98.11 [60]. This was a three arm study comparing two different everolimus doses in combination with basiliximab, reduced dose cyclosporine, and corticosteroids versus the control arm (n = 277) receiving basiliximab, standard dose cyclosporine, mycophenolic acid, and corticosteroids. The two everolimus doses evaluated were 1.5 mg per day with target trough levels of 3 to 8 ng/mL (n = 277) and 3 mg per day with target trough levels of 6 to 12 ng/mL (n = 279). The study population consisted of patients undergoing their first renal transplant, who were at low to moderate immunologic risk (ABO compatible transplant, Class I PRA <20%, and negative T-cell cross-match). The primary end point of the study was efficacy failure at 12 months defined as biopsy proven acute rejection, death, graft loss, or patients lost to follow. Everolimus dosed at 1.5 mg per day was found to be comparable to the control arm with respect to efficacy failure at 12 months (25.3% everolimus 1.5 mg per day vs. 24.2% control). In addition, renal function at 12 months, as measured by the calculated glomerular filtration rate was found to be comparable between everolimus 1.5 mg per day (54.6 ml/min/1.73 m^2) and the control group (52.3 ml/min/1.73 m^2). Discontinuation of everolimus at a higher dose (3 mg/day) was 34% including 20% due to adverse events and therefore is not recommended.

Fixed dosing of everolimus without therapeutic drug monitoring was evaluated by two multicenter double blinded, randomized trials for de novo renal transplant patients [61,62]. Both studies compared two different fixed doses of everolimus (1.5 mg per day and 3 mg per day) in combination with standard cyclosporine and corticosteroids versus control patients treated with cyclosporine, MMF, and corticosteroids. The 12 month analysis of renal function utilizing the glomerular filtration rate demonstrated increased rates of renal impairment in both everolimus groups as compared to the control patients. As a result of these studies, it was concluded that therapeutic drug monitoring should be performed to maintain everolimus trough levels of 3 to 8 ng/ml. In addition, the doses of cyclosporine should be reduced when used with everolimus in order to avoid renal dysfunction [59].

Belatacept (Nulojix)

Belatacept is the first in a new class of immunosuppressive agents targeting specific costimulation molecules and pathways. The FDA approved belatacept in 2011 for use in kidney transplantation [63]. Belatacept is indicated for the prophylaxis of organ rejection in adult kidney transplant patients who are Epstein-Barr virus (EBV) seropositive [64]. It is to be utilized in combination with basiliximab, mycopheonolate mofetil, and corticosteroids. The use of belatacept to prevent rejection in transplanted organs other than the kidney has not been established.

Belatacept is a novel immunosuppressive agent that provides selective T-lymphocyte costimulation blockade. It is a fusion protein that binds to CD80 and CD86 on antigen presenting cells, thereby blocking the CD28 mediated costimulation of T-lymphocytes. This inhibition prevents signal 2 of T-lymphocyte activation, thereby inhibiting cell proliferation and production of the cytokines interleukin-2, interferon-γ, interleukin-4, and TNF-α. Belatacept has an extended elimination half-life of 9.8 days for the 10 mg/kg dose and 8.2 days for 5 mg/kg dose. As a result, the FDA approved dosing regimen for belatacept is 10 mg per kg intravenous infused once on day 1, 5, and the end of weeks 2 and 4; then every 4 weeks through week 12 post-transplantation. Starting on week 16, belatacept 5 mg per kg intravenous should be infused every 4 weeks thereafter. Doses should be calculated based on the actual weight of the patient at the time of transplant and should not be modified during the course of therapy, unless the patients has a change in body weight greater than 10%. All doses of basiliximab should be infused over 30 minutes [64].

The prescribing information for belatacept contains a black boxed warning for post-transplant lymphoproliferative disorder (PTLD), infection, and other malignancies. Patients receiving belatacept have an increased risk for the development of PTLD with predominantly central nervous system (CNS) involvement as compared to those on a cyclosporine-based regimen. In addition, CNS PTLD, PML, and other CNS infections occurred more frequently in patients receiving a higher cumulative and higher frequency dosing regimen of belatacept as compared to the FDA approved dosing regimen. Six of the eight cases of PTLD in this treatment regimen presented with CNS involvement. Therefore, the administration of this higher regimen is not recommended. Overall in patients treated with belatacept, the rate of PTLD was 9-fold higher in those who were EBV seronegative or with unknown serostatus (8/139) compared to those who were EBV seropositive (5/810). Therefore, belatacept should not be utilized in patients who are EBV seronegative or with unknown serostatus due to the increased risk of PTLD in patients without immunity to EBV. As a result of these safety concerns, the manufacturer was required to incorporate a Risk Evaluation and Mitigation Strategies program as part of the product approval [64].

Table 98.12. Efficacy outcomes by years 1 and 3 for BENEFIT: Recipients of living and standard criteria deceased donor kidneys

Parameter	Belatacept Recommended Regimen N = 226 n (%)	Cyclosporine (CSA) N = 221 n (%)	Belatacept-CSA (97.3% CI)
Death	4 (1.8)	7 (3.2)	
Lost to follow-up	0	1 (0.5)	
Efficacy Failure by Year 3	58 (25.7)	57 (25.8)	−0.1 (−9.3, 9)
Components of Efficacy Failure*			
Biopsy Proven Acute Rejection	50 (22.1)	31 (14)	
Graft Loss	9 (4)	10 (4.5)	
Death	10 (4.4)	15 (6.8)	
Lost to follow-up	2 (0.9)	5 (2.3)	
Patient and graft survival†			
Year 1	218 (96.5)	206 (93.2)	3.2 (−1.5, 8.4)
Year 3	206 (91.2)	192 (86.9)	4.3 (−2.2, 10.8)

* Patients may have experienced more than one event.
† Patients known to be alive with a functioning graft.

Table 98.13. Measured and calculated GFR for BENEFIT: Recipients of living and standard criteria deceased donor kidneys

Parameter	Belatacept Recommended Regimen N = 226	Cyclosporine (CSA) N = 221	Belatacept-CSA (97.3% CI)
Measured GFR* mL/min/1.73 m^2 mean (SD)			
Year 1	63.4 (27.7) (n = 206)	50.4 (18.7) (n = 199)	13.0 (7,3, 18.7)
Year 2†	67.9 (29.9) (n = 199)	50.5 (20.5) (n = 185)	17.4 (11.5, 23.4)
Calculated GFR‡ mL/min/1.73 m^2 mean (SD)			
Year 1	65.4 (22.9) (n = 200)	50.1 (21.1) (n = 199)	15.3 (10.3, 20.3)
Year 2	65.4 (25.2) (n = 201)	47.9 (23) (n = 182)	17.5 (12, 23.1)
Year 3	65.8 (27) (n = 190)	44.4 (23.6) (n = 171)	21.4 (15.4, 27.4)

* GFR was measured using the cold-iothalamate method.
† Measured GFR was not assessed at Year 3.
‡ GFR was calculated using the MDRD formula.

In addition, belatacept carries a black boxed warning recommending against use in liver transplantation due to increased risk of graft loss and death. This black box warning was based on the results of a Phase III trial [64]. Belatacept was utilized at dosing regimens with more frequent administration than those studied in kidney transplantation, in combination with MMF and corticosteroids. Belatacept therapy was associated with a higher rate of graft loss and death as compared to the tacrolimus treatment arms. In addition, there were two cases of PTLD involving the liver allograft (one fatal) and one case of fatal PML [64].

The safety and efficacy of belatacept in de novo kidney transplantation was assessed in two open-label, randomized, multicenter trials [65,66]. Two different dosing regimens of belatacept were compared to a cyclosporine based regimen with all patients receiving basiliximab, MMF, and corticosteroids. The two belatacept regimens were the currently recommended regimen, and a regimen with higher cumulative and more frequent dosing. All treatment groups received basiliximab induction with concurrent MMF and corticosteroids. Both studies excluded patients at high immunologic risk, defined as having a panel reactive antibody (PRA) >50%, re-transplants with PRA >30%, HIV, Hepatitis C, active TB, or active Hepatitis B. Efficacy failure at one year was defined as biopsy proven acute rejection, graft loss, death or patients lost to follow-up.

The first study (BENEFIT) only included kidney transplant recipients with living donor and standard criteria donors [65,67]. The rate of biopsy proven acute rejection at one and three years was higher in both belatacept groups compared to cyclosporine (Table 98.12). Among the patients who experienced acute rejection in the belatacept groups, 70% of patients experienced rejection by month three and 84% experienced rejection by month six post-transplant. In addition, patients receiving belatacept experienced biopsy proven acute rejection by one year, classified as Banff Grade IIb or higher more frequently than the control patients (6% belatacept vs. 2% control). However, there was no difference in patient and graft survival at one- and three-years post-transplant. Renal function was assessed at one and two years by glomerular filtration rate measurement and calculated using the Modification of Diet in Renal Disease equation at one-, two-, and three-years post-transplant. Both measured and calculated glomerular filtration rates were higher in patients receiving belatacept as compared to cyclosporine across all time points (Table 98.13). In interpreting the endpoint for renal function in this study, the FDA utilized the glomerular filtration data to assess the safety of belatacept with regards to renal function, rather than as an efficacy measure [68].

The second study (BENEFIT-EXT) included kidney transplant recipients with extended criteria deceased donors, and is the first trial ever to specifically seek indication in the ECD population [66,69]. This study found that there were no differences in the rate of biopsy proven acute rejection at one- and three-years post-transplant. The proportion of biopsy proven acute rejection classified as Banff Grade IIb or higher by one year was also comparable between treatment groups (5% in belatacept compared to 4% in cyclosporine-treated patients). There was no difference in patient and graft survival at one- and three-years post-transplant. Additionally, both measured and calculated glomerular filtration rate was higher in patients treated with belatacept compared to patients treated with cyclosporine (Figure 98.2). Similarly to the first study, the FDA interpreted these glomerular filtration rate results as a safety assessment of belatacept for renal function, rather than as an efficacy measure [68].

Rabbit anti-thymocyte globulin (Thymoglobulin)

Rabbit anti-thymocyte globulin (Thymoglobulin®, RATG) is a potent immunosuppressive agent that initially received FDA approval in December 1998 becoming the second anti-thymocyte globulin available on the market [70]. The first anti-thymocyte globulin preparation (ATGAM®) was horse derived and initially FDA approved in 1981. However, it has since been replaced by RATG as the anti-thymocyte agent of choice at the majority of transplant centers in the US [71].

It is important to note that despite its use for the prophylaxis of organ rejection in solid organ transplant patients, the only FDA approved indication for RATG is the treatment of adult renal

transplant acute rejection in conjunction with concomitant immunosuppression. RATG is a polyclonal antilymphocyte preparation that is obtained by immunizing rabbits with human thymocytes. It is a gamma immune globulin product that consists of polyclonal cytotoxic antibodies directed against T-lymphocyte antigens. The mechanism of action of RATG for inducing immunosuppression is not fully understood, but may involve T cell clearance, activation modulation, homing and cytotoxic activity. RATG is contraindicated in patients with allergy or anaphylaxis to rabbits or in those with acute or chronic infections for whom immunosuppression would be contraindicated [71].

The approved dosing and administration recommendations for RATG in the treatment of acute rejection is 1.5 mg per kg of body weight daily for 7 to 14 days administered intravenously via a high flow vein. It should be infused through a 0.22 micrometer filter over a minimum of 6 hours for the first infusion and 4 hours for subsequent infusions. Infusion associated reactions consistent with cytokine release syndrome occur with the first or second infusions due to the lysing of lymphocytes. Signs and symptoms of cytokine release syndrome include: fever, chills, rigors, dyspnea, hypotension, hypertension, nausea, vomiting and diarrhea, malaise, rash or headache. Both a reduction in the infusion rate and premedication with acetaminophen, corticosteroids, and antihistamines has been shown to decrease the incidence of infusion reactions. Serum sickness has been reported with signs and symptoms consisting of: fever, rash, arthralgias, and myalgia with symptom onset 5 to 15 days post therapy. Significant adverse events associated with RATG include leukopenia, thrombocytopenia, infection, and malignancy.

The prescribing information references one Phase III study conducted in the US that resulted in the approval of RATG [72]. This double blind, multicenter, parallel group randomized non-inferiority trial compared patients receiving RATG versus ATGAM for the treatment of acute graft rejection in kidney transplant patients. Patients were included in the study if they had biopsy proven Banff Grade II, III or steroid resistant Grade I acute cellular rejection in their first or second renal transplant. Rejection treatment was administered for 7 to 14 days with either 1.5 mg/kg/day of RATG (n = 82) or 15 mg/kg/day of ATGAM (n = 81). The primary endpoint of this study was the reversal of acute rejection episode, defined as a return to baseline serum creatinine levels within 14 days of rejection diagnosis. RATG was found to have a clinically significant higher rejection reversal rate than ATGAM (88% vs. 76%, $P = 0.027$) with rejection recurrence 90 days post therapy occurring less frequently in patients treated with RATG (17% vs. 36%, $P = 0.011$). Adverse events and one-year patient and graft survival were comparable between treatment groups. Although off protocol use of tacrolimus and mycophenolate occurred in both treatment groups during the second half of the study, there was no effect on the overall result. The overall weighted treatment difference was 11.1% (RATG-ATGAM success rate) with a lower confidence bound of 0.07% (Table 98.14). As a result, RATG was found to be at least as effective as ATGAM in the reversal of acute graft rejection in renal transplant patients [72].

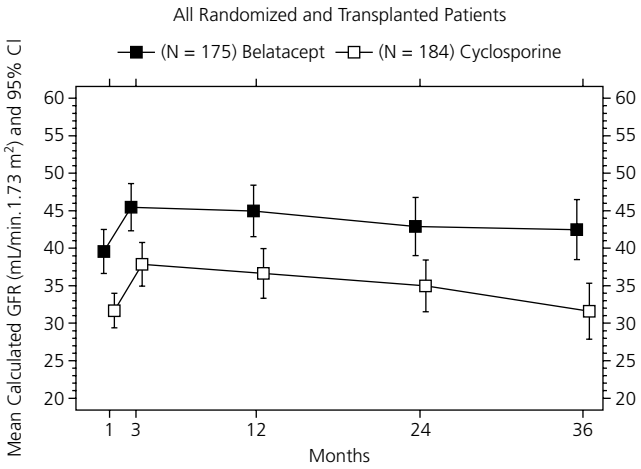

Figure 98.2. Calculated (MDRD) GFR through Month 36; Study 2: Recipients of extended criteria donor kidneys.

Basiliximab (Simulect)

Basiliximab is a non-lymphocyte depleting induction immunosuppression agent that received FDA approval in May 1998 [73]. Basiliximab is a member of the class of medications known as the interleukin 2 receptor antagonists [74]. The other member of this medication class was daclizumab (Zenapax®), which received FDA

Table 98.14. Response to Study Treatment by Rejection Severity and Study Half

Success/n	Total		First Half		Second Half	
	RATG	ATGAM	RATG	ATGAM	RATG	ATGAM
Risk Factor: Baseline Rejection Severty						
Mld	9/10 (90.0%)	5/8 (62.5%)	5/5 (100%)	1/3 (33.3%)	4/5 (80.0%)	4/5 (80.0%)
Moderate	44/58 (75.5%)	41/58 (70.7%)	22/28 (84.8%)	22/32 (68.8%)	22/32 (68.8%)	18/26 (73.1%)
Severe	11/14 (71.6%)	8/14 (57.1%)	6/8 (75.0%)	3/8 (37.5%)	5/8 (83.3%)	5/8 (83.3%)
Overal	64/82 (78.0%)	54/80 (87.5%)	33/39 (84.8%)	26/43 (60.5%)	31/43 (72.1%)	28/37 (75.7%)
Weighted estimate of difference (Thymoglobulin – Algam)		11.1%[a]		19.3%		−3.2%
Lower one-sided 95% confidence bound		0.07%		4.6%		−19.7%
p-Value[b]		0.061[c]		0.008[d]		0.625[d]

[a] across rejection severity and study half.
[b] under null hypothesis of equivalence (Codhran-Mantel-Haenszel test).
[c] one-sided straffied on rejection severity and study half.
[d] one-sided straffied on rejection severity.

approval in 1997 [75]. In 2010, the manufacturer discontinued production of daclizumab due diminishing market demand for the drug. As a result, basiliximab is the only interleukin 2 receptor antagonist available for use in the US.

Basiliximab is indicated for the prophylaxis of acute organ rejection in renal transplant patients as part of an immunosuppression regimen consisting of modified cyclosporine, and corticosteroids [74]. It is a chimeric (murine/human) monoclonal antibody designed to inhibit signal 3 of T cell activation. This occurs due to the high affinity of basiliximab for interleukin 2 receptor alpha (also known as the CD25 antigen) on the surface of activated T-lymphocytes. The duration of interleukin 2 receptor alpha saturation in adult and pediatric patients was 36 days when used in combination with modified cyclosporine and corticosteroids, whereas the duration was 50 and 59 days respectively when used in combination with modified cyclosporine, corticosteroids and either azathioprine or MMF. Basiliximab is generally well tolerated with the majority of reported adverse events consisting of gastrointestinal disorders (nausea, vomiting, constipation, abdominal pain, dyspepsia, and diarrhea).

The prescribing information contains dosing recommendations for both adult and pediatric patients. For adults, the recommended dosing regimen consists of two doses of basiliximab 20 mg intravenous. The first dose should be administered two hours prior to surgery and the second dose should be administered 4 days post-transplant. The pediatric dosing regimen is stratified based on weight according to the same schedule as adult dosing, with patients less than 35 kg receiving two doses of 10 mg and patients greater than 35 kg receiving two dose of 20 mg.

There were four adult, randomized, double blind, placebo controlled clinical trials and a pediatric pharmacokinetic safety study that resulted in FDA approval for basiliximab in kidney transplantation [74]. The initial studies utilized basiliximab in combination with modified cyclosporine and corticosteroids. The first study was conducted in Europe and Canada, whereas the second study was conducted in the US [76,77]. Although both studies were conducted in patients receiving deceased donor kidney transplants, the US based study also included living donor kidney transplantation. Patients were stratified to treatment with basiliximab or placebo. The primary efficacy endpoint for both studies was the incidence of death, graft loss, or acute rejection episodes during the first 6 months post-transplant. Secondary end points included biopsy proven rejection at 6 and 12 months, all the primary endpoints evaluated at 12 months, and 1 year patient and graft survival. In comparing patients from the European and Canadian study who received basiliximab (n = 190) versus placebo (n = 186), there was a statistically significant reduction in the incidence of the primary efficacy endpoint in patients receiving basiliximab (42% vs. 57%, $P = 0.003$) [76]. In comparing patients from the US based study who received basiliximab (n = 173) versus placebo (n = 173), there was a statistically significant reduction in the incidence of the primary efficacy endpoint in patients receiving basiliximab (38% vs. 55%, $P = 0.002$) [51,77]. In terms of secondary end points for both studies, the significant reduction in the primary efficacy endpoint was maintained at 12 months, as well as a significant reduction in the incidence of rejection episodes at 6 and 12 months. Patient and graft survival at 12 months were comparable between groups for both studies. The five-year graft and patient survival was evaluated in 71% of patients from the European/Canadian study and 58% of the patients from the US based study. There was no difference in graft survival in both studies. For the US based study, patient survival was comparable between groups; however, for the European/Canadian study, there was a numerically lower patient survival (87% basiliximab vs. 95% placebo). The prescribing information states that the cause of this difference is unknown with no increase in malignancy or infections noted [74].

The use of basiliximab in triple therapy based maintenance immunosuppression regimens was evaluated in two double blind, randomized, placebo-controlled studies. Both studies were conducted in deceased or living donor adult kidney transplant patients receiving their first or second transplant. The primary endpoint for both studies was acute rejection episodes 6 months post-transplant. The first study compared patients treated with (n = 168) or without (n = 172) basliximab in combination with cyclosporine, corticosteroids and azathioprine [78]. For the primary end point, there was a significant reduction in acute rejection episodes in patients treated with basiliximab (21% basiliximab vs. 35% placebo $P = 0.005$). The second study compared patients treated with (n = 59) or without (n = 64) basiliximab in combination with cyclosporine, corticosteroids and MMF [79]. For the primary end point in this study, there was no significant difference in biopsy proven acute rejection at 6 months (15% basiliximab vs. 27% placebo, $p > 0.05$). Patient and graft survival were comparable at 12 months for both studies.

A safety and pharmacokinetic study was conducted in 41 pediatric patients with a median age of 8.1 years (ages 1–11 years [n = 27] and ages 12–16 [n = 14]) [80]. These patients were treated with basiliximab in addition to standard immunosuppression with modified cyclosporine, azathioprine, MMF, and/or corticosteroids. Acute rejection rates at 6 months were found to be comparable with adult patients receiving triple therapy based immunosuppression with a comparable adverse event profile. The most frequent adverse events reported were fever, hypertrichoses, hypertension, rhinitis, and urinary tract infections [74].

Anti-viral agents

All transplant patients receive a cocktail of prophylactic agents to prevent the adverse effects of the immunosuppressants used. While peri- and post-transplant management of infection prophylaxis is covered in depth in Chapters 94, most agents used are not specifically approved for use in transplantation. The following agents are described as they have been formally approved for use in the solid organ transplant setting.

Ganciclovir sodium (Cytovene-IV)

Ganciclovir sodium is a synthetic guanine derivative active against cytomegalovirus (CMV), which was initially approved by the FDA in 1989 for the treatment of CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS) [81]. In 1992, ganciclovir received an expanded indication for the prevention of CMV disease in transplant recipients at risk for CMV disease [82]. Initially, ganciclovir was only available intravenously in the form of ganciclovir sodium; however in 1994, an oral ganciclovir formulation was introduced to the market [81]. Oral ganciclovir was indicated for the prevention of CMV disease in patients at risk for CMV disease, including solid organ transplant recipients and patients with AIDS. Recently, oral ganciclovir was discontinued from the market after a prolonged drug shortage.

The pharmacokinetic properties of intravenous and oral ganciclovir have been well described. In terms of absorption, oral ganciclovir is poorly bioavailable as compared to intravenous ganciclovir with a reported absolute bioavailability of 8.53% [83]. The major elimination pathway for ganciclovir is via renal excretion of

Table 98.15. Dosing for patients with renal impairment

Creatinine Clearance* (mL/min)	Ganciclovir-IV Induction Dose (mg/kg)	Dosing Interval (hours)	Ganciclovir-IV Maintenance Dose (mg/kg)	Dosing Interval (hours)
≥70	5.0	12	5.0	24
50–69	2.5	12	2.5	24
25–49	2.5	24	1.25	24
10–24	1.25	24	0.625	24
<10	1.25	3 times per week, following hemodialysis	0.625	3 times per week, following hemodialysis

*Creatinine clearance according to Cookcroft-gault-equation.

unchanged drug. As a result, careful adjustment for renal impairment is needed when dosing ganciclovir. With regards to pediatric patients, the pharmacokinetics properties are similar to those observed in adults. However, since ganciclovir is not indicated for use in pediatric patients, no dosing recommendations are included in the prescribing information. Finally, women of childbearing age are advised to use proper contraception while being treated with ganciclovir due to the mutagenic and teratogenic potential of ganciclovir [82].

The recommended FDA approved dosing of ganciclovir for patients with normal renal function for the prevention of CMV disease in transplant recipients is 5 mg/kg every 12 hours for 7 to 14 days, then 5 mg/kg once daily (seven days per week) or 6 mg/kg once daily (five days per week). The duration of treatment is dependent on the degree of immunosuppression with no clear recommendations from the prescribing information.

Ganciclovir dosing should be adjusted for renal impairment based on the creatinine clearance, as described in Table 98.15 [82].

Ganciclovir was evaluated in three randomized, controlled trials of prevention of CMV disease in organ transplant patients. The first Phase III study was conducted in 149 heart transplant patients who were at risk for CMV disease, defined as either CMV recipient seropositive or donor seropositive and recipient seronegative [84]. Patients were randomized to receive either ganciclovir (n = 76) for a total of 28 days or placebo (n = 73). There was a statistically significant reduction in the overall incidence of CMV disease in patients treated with ganciclovir (12/76 or 16%) as compared to placebo (31/73 or 43%) during the 120-day post-transplant observation period. The second study was a randomized, double-blinded, placebo-controlled trial of 72 bone marrow transplant patients with asymptomatic CMV infection (CMV positive culture of urine, throat, or blood) [85]. Patients were stratified to receive either placebo or ganciclovir for a total of 100 days. There was a statistically significant reduction in the incidence of CMV disease in patients receiving ganciclovir at day 100 and 180. In addition, the overall rate of survival was also significantly higher in ganciclovir treated patients at day 100 and day 180. The third randomized open label study evaluated 40 allogeneic bone marrow transplant recipients at risk for CMV disease [86]. Patients underwent bronchoscopy and bronchoalveolar lavage on post-transplant day 35. Those with histologic or virologic evidence of CMV infection in the lung were then randomized to observation or treatment with ganciclovir. The incidence of CMV disease was significantly lower in the ganciclovir-treated patients with 4 of the 20 (20%) patients treated with ganciclovir and 14 of the 20 (70%) control patients developing interstitial pneumonia. These results were found to be consistent with the previously mentioned study in bone marrow transplant recipients.

Valganciclovir (Valcyte)

Valganciclovir is a prodrug of ganciclovir that was initially approved by the FDA in 2001 for the treatment of CMV retinitis in patients with AIDS [87]. In 2003, the FDA expanded the indications to include the prevention of CMV disease in specific types of solid organ transplant for both adult and pediatric patients at high risk for CMV, defined as patients who are CMV IgG donor positive and recipient negative. The FDA approved indication for valganciclovir is the prevention of CMV disease in kidney, heart, or kidney-pancreas adult transplant patients at high risk for CMV and in kidney or heart pediatric (ages 4 months to 16 years) transplant patients at high risk for CMV [88]. It is important to note that valganciclovir is not indicated for use in adult or pediatric liver transplant patients.

Valganciclovir is an L-valyl ester of ganciclovir that is rapidly converted to ganciclovir by intestinal or hepatic esterases. The pharmacokinetics of valganciclovir has been evaluated in solid organ transplant patients, as compared to ganciclovir. The mean systemic exposure to ganciclovir was 1.7 × higher following 900 mg of valganciclovir daily compared to 1000 mg ganciclovir three times a day [88]. The systemic exposures attained were comparable for kidney, heart and liver transplantation. The pharmacokinetics of valganciclovir was similar in pediatric organ transplant recipients compared to adult patients. Due to its active metabolite of ganciclovir, valganciclovir should be considered a potential carcinogen.

The dosing recommendations for valganciclovir include both adult and pediatric dosing recommendations for patients at high risk for CMV. For adult heart or kidney pancreas transplant patients, the recommended dose is 900 mg orally once daily starting within 10 days post-transplant to be continued until postoperative day 100. For adult kidney transplant patients, the recommended dose is 900 mg orally once daily starting within 10 days post-transplant to be continued until postoperative day 200. Of note, all adult doses of valganciclovir should be adjusted for renal function based on the creatinine clearance as calculated by the Cockcroft-Gault equation. In heart or kidney pediatric transplant patients, the once daily dose of valganciclovir should be started with 10 days post-transplant and be dosed according to the following equation:

$$\text{Pediatric Dose (mg)} = 7 \times \text{body surface area} \times \text{CrCl (as calculated using a modified Schwartz formula, not to exceed 150 ml/min/1.73 m}^2\text{)}.$$

$$\textit{Mosteller BSA}\,(m^2) = \sqrt{\frac{\textit{Height}\,(cm) \times \textit{Weight}\,(kg)}{3600}}$$

$$\textit{Schwartz Creatinine Clearance}\,(mL/\text{min}/1.73\,m^2) = \frac{k \times \textit{Height}\,(cm)}{\textit{Serum Creatinine}\,(mg/dL)}$$

where k =

0.45 for patients aged 4 months to <1 year,

0.45 for patients aged 1 to <2 years (note k value is 0.45 instead of the typical value of 0.55),

0.55 for boys aged 2 to <13 years and girls aged 2 to 16 years, and

0.7 for boys aged 13 to 16 years.

Table 98.16. Percentage of Patients With CMV Disease, Tissue-Invasive CMV Disease or CMV syndrome by Organ Type: Endpoint Committee, 6 Month ITT Population

Organ	CMV Disease[1]		Tissue-Invasive CMV Disease		CMV Syndrome[2]	
	VGCV (N = 239)	GCV (N = 125)	VGCV (N = 239)	GCV (N = 125)	VGCV (N = 239)	GCV (N = 125)
Liver (n = 177)	19% (22/118)	12% (7/59)	14% (16/118)	3% (2/59)	5% (6/118)	9% (5/59)
Kidney (n = 120)	6% (5/81)	23% (9/39)	1% (1/81)	5% (2/39)	5% (4/81)	18% (7/39)
Heart (n = 56)	6% (2/35)	10% (2/21)	0% (0/35)	5% (1/21)	6% (2/35)	5% (1/21)
Kidney/Pancreas (n = 11)	0% (0/5)	17% (1/6)	0% (0/5)	17% (1/6)	0% (0/5)	0% (0/6)

GCV = oral ganciclovir: VGCV = valganciclovir.
[1] Number of patients with CMV disease = Number of patients with tissue-invasive CMV disease or CMV syndrome.
[2] CMV syndrome was defined as evidence of CMV viremia accompanied with fever ≥38°C on two or more occasions separated by at least 24 hours within a 7-day period and one or more of the following: malaise, leukopenia, atypical lymphocytosis, thrombocytopenia and elevation of hepatic transaminases.

Table 98.17. Percentage of Kidney Transplant Patients With CMV Disease, Tissue-Invasive CMV Disease or CMV Syndrome, 12 Month ITT Population

	CMV Disease[1]		Tissue-Invasive CMV Disease		CMV Syndrome[2]	
	100 Days VGCV (N = 163)	200 Days VGCV (N = 155)	100 Days VGCV (N = 163)	200 Days VGCV (N = 155)	100 Days VGCV (N = 163)	200 Days VGCV (N = 155)
Cases	36.8% (60/163)	16.8% (26/155)	1.8% (3/163)[3]	0.6% (1/155)	35.0% (57/163)	16.1% (25/155)

VGCV = valganciclovir.
[1] Number of patients with CMV disease = Number of patients with tissue-invasive CMV disease or CMV syndrome.
[2] CMV syndrome was defined as evidence of CMV viremia accompanied with at least one of the followings: fever ≥38°C), severe malaise, leukopenia, atypical lymphocytosis, thrombocytopenia, and elevation of hepatic transaminases.
[3] Two patients m the 100 day group had both tissue-invasive CMV disease and CMV syndrome; however, these patients are counted as having only tissue-invasive CMV disease.

With respect to the indication of organ transplantation, there are three major clinical trials that are referenced in the prescribing information. The first was a double blind, double-dummy active comparator study conducted in 372 liver, kidney, heart, and kidney pancreas transplant recipients who were considered high risk for CMV (donor CMV seropositive and recipient CMV seronegative) [89]. Patients were randomized 2:1 to valganciclovir 900 mg daily (n = 239) or oral ganciclovir 1000 mg three times daily (n = 125) starting within the first 10 days until 100 days post-transplant. The proportion of patients who developed CMV disease during the first six months post-transplant was comparable between valganciclovir (12.1%) and ganciclovir (15.2%). The authors concluded that once daily valganciclovir was as clinically effective and well tolerated as oral ganciclovir three times daily in high-risk solid organ transplant patients. However it is important to note that when stratified by organ type, liver transplant patients receiving valganciclovir were found to have a significantly higher incidence of tissue-invasive CMV disease compared to the ganciclovir group (see Table 98.16) [89].

The second study was designed to assess the safety and efficacy of extending CMV propylaxis from 100 to 200 days [90]. This was a double-blind, placebo controlled study in 326 CMV high risk kidney transplant patients, who were randomized to receive valganciclovir 900 mg daily for either 200 or 100 days followed by 100 days of placebo. The extended CMV prophylaxis regimen with valganciclovir was superior in preventing CMV disease within the first 12 months post-transplant (see Table 98.17). However, by 24 months post-transplant, the CMV disease rate was similar between treatment groups.

Finally, the third study was conducted in pediatric transplant patients, ages 4 months to 16 years old to assess the safety and efficacy of valganciclovir in preventing CMV [91]. A total of 63 pediatric solid organ transplant patients, who were at risk for CMV disease, were enrolled into an open-label safety, pharmacokinetic study of oral valganciclovir. The pediatric patients included 33 kidney, 17 liver, 12 heart, and 1 kidney liver transplant patients. The daily dose of valganciclovir was calculated based on body surface area and creatinine clearance. The pharmacokinetics of ganciclovir was similar across organ transplant types. The mean daily ganciclovir exposures were comparable to the adult recipients receiving 900 mg daily. It is important to note that because valganciclovir is not approved for use in adult liver transplantation; it is also not recommended in pediatric liver transplant patients.

Cytomegalovirus immune globulin intravenous (CytoGam)

Cytomegalovirus (CMV) Immune Globulin Intravenous (CMV-IGIV) is a fractionated plasma product consisting of immunoglobulin G (IgG) that contains a standardized amount of antibody specific to cytomegalovirus. CMV-IGIV was initially approved in 1991 for the attenuation of primary CMV disease in kidney transplant patients at high risk for CMV infection, defined as patients who are CMV IgG donor positive and recipient negative [92]. The indication was expanded in 1998 to include other transplanted organs. The current FDA approved indication of CMV-IGIV is for the prophylaxis of CMV disease associated with kidney, lung, liver, pancreas, and heart transplantation. For transplant patients at high risk for CMV infection, except for those undergoing kidney transplantation, CMV-IGIV should be considered in combination with ganciclovir [92].

CMV-IGIV is derived from a large pool of adult human plasma selected for high CMV antibody titers. As a result, the globulin contains a relatively high concentration of antibodies directed

Table 98.18. Dosing instructions for *Cytomegalovirus immune globulin intravenous (CytoGam)*

	Type of Transplant	
	Kidney	Liver, Pancreas, Lung, Heart
Within 72 hours of transplant:	150 mg/kg	150 mg/kg
2 weeks post transplant:	100 mg/kg	150 mg/kg
4 weeks post transplant:	100 mg/kg	150 mg/kg
6 weeks post transplant:	100 mg/kg	150 mg/kg
8 weeks post transplant:	100 mg/kg	150 mg/kg
12 weeks post transplant:	50 mg/kg	100 mg/kg
16 weeks post transplant:	50 mg/kg	100 mg/kg

against CMV. When administered, it can raise the CMV antibody to sufficient levels to attenuate or reduce the incidence of serious CMV disease. Since CMV-IGIV is derived from human plasma, it carries the possibility for blood-borne viral agent transmission as well as the theoretical possibility of Creutzfeldt-Jakob disease agent transmission. Additionally, since CMV-IGIV is an immune globulin based product, it carries risk for adverse events that have been associated with other immune globulin products [92]. Aseptic meningitis syndrome typically begins within hours to days following treatment and is associated with severe headache, nuchal rigidity, drowsiness, fever, and photophobia. Noncardiogenic pulmonary edema has been reported in patients 1 to 6 hours after receiving IGIV therapy and is associated with severe respiratory distress, pulmonary edema, normal left ventricular function, fever and hypoxemia. Renal dysfunction, thrombosis, and hemolysis have also been rarely reported in association with IGIV. The FDA approved recommended total dosage per infusion of CMV-IGIV is 150 mg/kg. Complete dosing recommendations based on time post-transplant are described in Table 98.18.

CMV-IGIV was initially shown to be effective for CMV prophylaxis in kidney transplant patients based on two clinical trials published in 1987 and 1991. The first randomized trial demonstrated that the incidence of CMV syndrome was significantly reduced from 60% in control patients (n = 35) to 21% in CMV-IGIV treated patients (n = 24) [93]. The incidence of serious CMV disease was reduced from 46% to 13% in patients receiving CMV-IGIV. In the second non-randomized trial 36 renal transplant patients were treated with CMV-IGIV and were found to have a 36% incidence of CMV associated syndrome [94]. In addition, the rate of serious CMV disease, concomitant fungal, and parasitic superinfection were also lower in both studies, as compared to the control group from the first study.

Additional studies utilizing CMV-IGIV in other transplanted organs allowed for the FDA approved indication of CMV-IGIV to be modified in 1998 to include liver, lung, heart, and pancreas transplantation. In a randomized, double-blind, placebo control study in liver transplant patients comparing patients receiving CMV-IGIV (n = 69) with control (n = 72), the incidence of serious CMV disease was significantly reduced from 27% to 12% in those treated with CMV-IGIV [95]. Of note, the reduction of serious CMV disease in liver transplant patients at high risk for CMV was less than in transplant with other donor and recipient serologic statuses. In a follow-up study in liver transplant patients comparing the control patients to those receiving CMV-IGIVI, the one year survival was 72% versus 86% respectively ($P = 0.03$) [96].

Additional studies have suggested that combined CMV prophylaxis with CMV-IGIV and ganciclovir may reduce the incidence of serious CMV associated disease in patients at high risk for CMV disease, below that expected from one drug alone. One such study conducted in solid organ transplant patients, including 14 high risk CMV kidney-pancreas transplant patients, reported reduced incidence of serious CMV disease with combined therapy, as compared to previous experience with single drug prophylaxis [92]. Another study in cardiothoracic transplantation compared prophylaxis with CMV-IGIV in combination with intravenous ganciclovir versus ganciclovir alone [97]. In CMV high risk heart transplant patients, the actuarial incidence of CMV disease was reduced from 55% in the ganciclovir group (n = 16) to 46% in the combined therapy group (n = 16). Additionally, there was a statistically significant increase in patient survival from 61% to 94% ($P \leq 0.001$). In heart-lung or lung alone transplant patients at high risk for CMV, the incidence of CMV disease was decreased from 85% in ganciclovir alone to 36% in combined therapy. Survival also improved from 60% to 80% respectively ($P \leq 0.01$). As a result of these studies, CMV-IGIV received an expanded indication to include kidney, pancreas, liver, lung, and heart transplant recipients.

Summary

Drug approval is a complex and highly regulated process in most countries, and knowledge of the approval process is necessary to not only to facilitate drug trial design, but also to have some practical cognizance of the manner in which dosing recommendations are established and associated with a particular drug. This drugs that have been formally approved for use in transplantation have clear evidence of relative safety and efficacy that refers back to the standard of care at the time of the drug's approval. While these drugs form the foundation on which modern immune management of transplant recipients is built, their use migrates over time based on emerging knowledge, introduction of additional adjuvant therapies, and clinician experience. It is reasonable to say that all transplant patients receive some combination of approved and off-label therapy during their post-transplant course. This chapter and Chapter 101 similarly will provide the reader with a good foundation on which to understand modern immune management, while understanding that clinical practice varies considerably.

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CHAPTER 99

Infectious Disease Prophylaxis after Organ Transplantation

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Introduction

"An ounce of prevention is worth a pound of cure." This saying is often attributed to Benjamin Franklin. However, the origins of the saying are much older than Franklin. The first written account dates to the thirteenth century in a manuscript by Henry de Bracton. Despite the sayings origins, it is very appropriate in the transplant setting. It is often much easier to prevent an infection than to try and cure the infection once established. For transplant patients and providers this prevention may take many forms — medications, immunizations, or disease avoidance through hygiene or avoiding sick contacts.

Preventive measures in the transplant recipient may not always avoid disease or infection altogether. They may mitigate disease making infectious episodes less severe. Or, they may delay the onset of disease until later in the post-transplant period. Delaying the onset of an infection may have benefits. In the current era, where more potent immune suppression induction is more often being used, delaying the onset of an infection until the immune system has recovered from induction may be desirable. Similarly, pretransplant vaccination may not prevent disease post-transplant altogether but may lessen the severity and duration of disease. This appears to be the case of an experimental cytomegalovirus (CMV) vaccine where vaccine recipients needed fewer days of antiviral therapy when compared to placebo recipients. When considering infections in solid organ transplantation, opportunistic infections most often present between 1 and 6 months (see Figure 94.1 in Chapter 94). However, in the presence of effective prophylactic medications, this time frame may be shifted to between 6 and 12 months.

In this chapter we will discuss effective prevention measures used in solid organ transplantation. The chapter complements specific chapters (Chapters 92, 93 and 94) on the diagnosis and management of donor-derived, perioperative and postoperative infections diseases respectively.

Prevention in kidney, pancreas, and kidney/pancreas transplant

Table 99.1 has a summary of recommended antimicrobial dosages and durations for kidney, pancreas and kidney/pancreas recipients.

Bacterial prevention

Surgical prophylaxis

Kidney transplantation is considered a clean surgical wound, although can be a clean contaminated wound if the bladder is colonized. In either case, there is low risk of developing a surgical-site infection (SSI), generally less than 2%. The bacteria that typically cause SSIs are *Staphylococcus* sp. Kidney transplantation is typical of clean surgery. As such, the antibiotics that can and should be used for surgical prophylaxis are those which have activity against Staphylococci. These include the antistaphylococcal penicillins and first generation cephalosporins. It is preferable to administer the prophylactic antibiotic within 30 minutes prior to skin incision [1]. Antibiotics may be continued for up to 24 hours postoperatively, but longer durations have not been shown to reduce rates of SSIs. For patients who are allergic to penicillins the primary alternative is vancomycin [1]. Because vancomycin is typically administered as a prolonged infusion, the dose of vancomycin should be started 60 minutes prior to skin incision. Vancomycin is also acceptable to use in patients who have documentation of colonization with methicillin-resistant *Staphylococcus aureus* (MRSA). Second generation cephalosporins are also acceptable for patients without a penicillin or cephalosporin allergy.

Pancreatic transplantation is considered a clean-contaminated wound. This is because the duodenum is opened during the surgical procedure (see earlier chapter for detail). Therefore, in addition to *Staphylocccoci*, the microorganisms typically involved in SSI related to pancreas transplant are those that colonize the duodenum. These include *Enterococcus, Klebsiella* sp., *Escherechia coli, Enterobacter*, anaerobic gram-negative rods, and *Candida* sp. Despite a wider variety of organisms causing surgical site infections in pancreas transplantation, cefazolin is still an effective prophylactic agent. This is because it has good activity against most aerobic gram-negative rods. Like in kidney transplant, if the patient has a penicillin or cephalosporin allergy, then vancomycin may be used [1]. However, it should be combined with either gentamicin or levofloxacin in order to protect against gram-negative organisms. Some centers choose to add fluconazole for prophylaxis against *Candida* sp. though there are no strong data to support this practice [2]. If used, fluconazole is often continued for 5–14 days after transplantation. Many centers culture the perfusate of abdominal organs and target therapy against any potential pathogens that may grow.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 99.1. General prevention measure recommendations for kidney or kidney/pancreas transplant recipients

	Preferred prevention measure	Alternative prevention measures
Surgical prophylaxis	Cefazolin 1 g iv 0–30 minutes before skin incision	Vancomycin 1 g iv 60 minutes before skin incision
CMV	Valganciclovir, acyclovir, and valacyclovir requiring dosage adjustment for renal dysfunction	
D+/R−*	Valganciclovir 450–900 mg daily for 3–6 months	Pre-emptive monitoring
Any R+†	Valganciclovir 450–900 mg daily for 3 months	Pre-emptive monitoring
D−R−‡		Acyclovir 400–800 mg TID§ or valacyclovir 500 mg BID for 3–6 months
Fungal		
Candida	Fluconazole 200 mg daily up to 14 days	
PCP**	Smx/tmp 1DS†† thrice weekly	Atovaquone 1500 mg daily Dapsone 100 mg daily Inhaled pentamidine Clindamycin + pyrimethamine

* Donor seropositive/recipient seronegative; † recipient seropositive; ‡ donor seronegative/recipient seronegative; § thrice daily (every 8 hours), twice daily (every 12 hours); ** *Pneumocystis* pneumonia; †† Double strength (sulfamethoxazole 800 mg — trimethoprim 160 mg).

Dental prophylaxis

There are few data to support the use of routine antibiotic prophylaxis for transplant recipients prior to undergoing dental procedures. However, most centers recommend that patients take antibiotics before dental procedures whether invasive or not [3]. If used, most transplant centers use regimens recommended by the American Heart Association endocarditis prevention guidelines [3,4].

Treatment of asymptomatic bacteriuria

The Infectious Diseases Society of America (IDSA) guidelines recommend against the treatment of asymptomatic bacteriuria [5]. However, these guidelines make exceptions for patients who have just had genitourinary surgery. Many centers leave ureteral stents in until about 90 days post-transplant. If asymptomatic bacteriuria is detected while the stents remain in place most centers will treat. This is because more severe infections, such as sepsis or pyelonephritis, are associated with asymptomatic bacteriuria in the early post-transplant period [6]. The choice of antimicrobial should be guided by susceptibility testing, and although not well defined, the duration should likely be 7 to 10 days.

Infection with *Mycobacterium tuberculosis* is relatively uncommon in the US. However, patients who attend a dialysis center are at a 30-times risk of being latently infected with *M. tuberculosis* as compared to the general population [7]. It is recommended that all potential transplant recipients be screened for latent tuberculosis (TB) prior to transplantation [8]. However, the timing of treatment for latent TB infection can be done either prior to or immediately following transplantation. A Korean study evaluated performing an interferon-γ release assay (IGRA) for latent TB infection at the time of transplant [9]. All four patients who eventually developed TB post-transplant were IGRA positive. Additionally, all patients who were skin test positive for latent TB infection were treated with 9 months of isoniazid (INH) post-transplant [9]. None of these patients (n = 24) developed active TB [9]. This study suggests that even if all treatment for latent TB is given post-transplantation it is still effective at reducing the risk of active TB. It is also positive to screen only patients who proceed to transplant with an IGRA as opposed to screening all potential recipients. However, the sensitivity of IGRA testing post-transplantation has not been assessed. Therefore it is important that some form of latent TB screening be performed prior to transplantation.

Fungal prevention

Except as mentioned above in regards to pancreas transplantation, specific antifungal prophylaxis is generally not warranted in kidney, pancreas, or kidney/pancreas transplantation. Kidney and pancreas transplant recipients are at risk for developing cryptococcosis post-transplant. Current IDSA guidelines recommend a minimum of 6 months of fluconazole maintenance, then after stopping fluconazole following the serum cryptococcal antigen (CrAg) [10]. If the serum CrAg rises after cessation of therapy and there are no signs of active disease, most transplant infectious diseases experts would restart fluconazole and continue indefinitely.

Pneumocystis jirovecii (formerly *P. carinii*) has been reclassified from a parasite to a fungus. All transplant recipients receive some sort of prophylaxis against developing *Pndeumocystis* pneumonia (PCP). Sulfamethoxazole/trimethoprim (smx/tmp) is the preferred agent for preventing PCP. Many patients have a stated allergy to smx/tmp. Because smx/tmp is markedly better at preventing PCP than second-line agents, it should be elicited what kind of adverse reaction the patient had. Many people have a photosensitivity reaction while taking smx/tmp. This can be avoided by limiting sun exposure while taking smx/tmp. The duration of PCP prophylaxis is typically 6 months after transplant. However, if patient receives antilymphocyte antibodies to treat an episode of rejection consideration should be given to reinstituting smx/tmp for three to 6 months. Also, some centers that use antithymocyte immunoglobulin for induction of immunosuppression extend the length of PCP prophylaxis to at least two years and some even keep patients on PCP prophylaxis for life.

Alternatives to smx/tmp include dapsone, atovaquone, inhaled pentamidine and clindamycin+pyrimethamine [11]. Dapsone has a sulfa moiety. Thus, patients with a true sulfa allergy should not be given dapsone. Dapsone also has been associated with hemolytic anemia in lung transplant patients [12]. While dapsone is cleared through the kidneys, there are no specific recommendations for adjustment for renal function. In the study of lung recipients on dapsone who developed hemolytic anemia, there was an association between peak creatinine and the eventual development of hemolytic anemia [12]. This suggests that patients with diminished renal function post-transplant (e.g. delayed graft function) should have either a reduced dose of dapsone or use atovaquone for PCP prophylaxis. Atovaquone is generally well tolerated from a medical standpoint. Atovaquone comes as a liquid and can be mixed with any drink. However, many patients do not like the taste of atovaquone

nor having to drink the large volume of liquid needed to disguise the taste.

Parasitic prevention

In the US, parasites are usually not a major problem for most transplant recipients. Additionally, smx/tmp, dapsone, and atovaquone, which are given for PCP prevention, also have activity against *Toxoplasma gondii*, the most common parasitic infection in transplant recipients. Even though toxoplasmosis is not a major issue across the transplant population, patients should still be counseled not to scoop cat-litter boxes or eat under-cooked game meat.

With an increasing proportion of immigrants from Latin America representing both donor and recipient populations, *Trypanosoma cruzi* is becoming more concerning. Recently conducted surveillance in the US shows a seroprevalence of 0.026% overall. However, in donors born in Mexico the prevalence was 0.3% and those born in Central American the rate was 0.5% [13]. Chagas' disease (American trypanosomiasis) is concerning because currently there are no FDA-approved medications available in the US to treat Chagas' disease. While heart transplantation has been performed successfully for Chagas' heart disease, the outcomes are less than favorable as early mortality is 18% [14]. Although Chagas' disease did not develop in any of the non-heart organs from two Chagas'-positive donors, using a kidney or pancreas from a *T. cruzi* seropositive donor should probably only be done in the setting of a life-saving transplant. If a potential transplant recipient tests positive for previous Chagas' disease then it is wise to treat prior to transplantation. Benznidazole or nifurtimox both have some activity against *T. cruzi*. However, cure is generally not possible. If an organ from a *T. cruzi* seropositive donor is being used then consideration should be given to treating the recipient. The duration of treatment should be 30–60 days using benznidazole [15].

Viral prevention

Apart from CMV, respiratory viruses (influenza, parainfluenza, respiratory syncytial virus, etc.) are the most common viral infections seen in transplant recipients. Currently, an annual influenza immunization is the best measure to reduce respiratory viral infection. Recommended vaccines will be discussed later in the chapter. Although there are concerns of influenza immunization eliciting alloantibodies [16–18], it appears that routine influenza immunization is safe. In a large study examining Medicare claims between 2000 and 2006, Hurst et al. found that influenza immunization in the first year was not associated with acute rejection [19]. However, influenza immunization was associated with a reduced risk of graft loss (adjusted hazard ratio (HR) = 0.77: 95% confidence interval (CI); 0.69–0.85, $P < 0.001$) and death (HR = 0.82: 95% CI; 0.76–0.89, $P < 0.001$) [19]. Because of limitations in the detail of the data, the authors were not able to associated timing of immunization with rejection episodes. Nor were they able to evaluate timing of influenza illness with rejection [19]. Multiple other studies have looked at surrogate end points of neutralizing antibodies against neuraminidase and hemagglutinin as well as adverse effects (fever, pain at injection site, change in creatinine). All of these studies have concluded that influenza immunization appears safe and effective at generating antibodies in renal transplant recipients [20–23].

CMV has been the viral infection most associated with transplantation. CMV is capable of infecting many organ systems including the gastrointestinal tract, the liver, the bone marrow, the central nervous system, and the eye. Untreated CMV disease can have disastrous consequences including permanent brain damage, blindness, and death. In 1989, Balfour and colleagues evaluated the use of acyclovir in preventing CMV disease after kidney transplant. Patients took acyclovir or placebo, based on randomization, for 12 weeks after transplant. The rate of symptomatic CMV disease was 7.5% in the acyclovir group compared to 29% in the placebo group, $P = 0.002$ [24]. Thus, many centers adopted using acyclovir for primary prophylaxis of CMV disease in high-risk patients, such as, those that are donor positive, recipient negative for CMV(D+/R−). However, in vitro acyclovir has poor activity against CMV. Ganciclovir was developed as an antiviral against CMV. Flechner et al. evaluated 3 months of either oral ganciclovir or oral acyclovir in a randomized fashion. In addition to CMV disease they performed routine blood cultures for CMV. By 6 months post-transplant CMV viremia occurred in 2.5% of ganciclovir-treated patients and 36% of acyclovir-treated patients, $P = 0.0001$. The highest benefit of ganciclovir over acyclovir was seen in the D+/R− patients, 54% vs. 0% ($P = 0.0008$). Thus, many centers converted to ganciclovir for routine prophylaxis against CMV in transplant recipients.

However, as diagnostic technology advanced and as ganciclovir-associated toxicities emerged, many transplant centers sought alternatives to universal prophylaxis with antiviral medications. The pre-emptive approach to treating CMV emerged. Improved diagnostic technology meant that CMV could be detected well before the development of symptoms. These assays included pp65 detection and CMV nucleic acid detection. The pp65 assay uses direct fluorescent antibodies against pp65, a surface protein on CMV. The test can be positive up to 1 week before the onset of symptoms. Nucleic acid amplification assays are usually more sensitive and can detect the presence of CMV in serum or plasma 7–14 days before symptoms. The pre-emptive principal relies on very sensitive assays and frequent monitoring of patients. The benefits of the pre-emptive approach are less exposure to antivirals, which may lead to less toxicity, potentially less ganciclovir resistance, and less cost to the patient. The drawbacks are higher costs to the transplant center, potential more severe disease especially if patients are non-adherent to the frequent testing regimen or if transplant center staff miss a positive result. When compared directly, universal prophylaxis and pre-emptive approaches appear to be equal at preventing CMV disease.

A prodrug of ganciclovir, valganciclovir, was developed and approved by the FDA in the early 2000s. Valganciclovir has improved bioavailability, which allows for comparable plasma levels to intravenous ganciclovir. A large, randomized clinical study evaluated oral valganciclovir at a dose of 900 mg daily versus oral ganciclovir 1000 mg three times per day for preventing CMV disease in D+/R− transplant recipients [25]. The study enrolled all organ transplant patients. Of the 364 evaluable patient enrolled, 120 (33%) were kidney transplants. Patient received study drug for 3 months post-transplant. At 6 and 12 months post-transplant the number of patients treated for CMV was comparable between the groups, 23% and 30.5% respectively for valgancicovir and 21.6% and 28.0% for oral ganciclovir [25]. The study met non-inferiority criteria and valganciclovir was rapidly adopted by most centers as the preferred agent for prevention against CMV. However, when the FDA approved valganciclovir there was not an indication for CMV prophylaxis in liver transplant recipients. This decision was based on a higher occurrence of tissue-invasive disease in liver transplant recipients who received valganciclovir (see the next section on prevention in liver transplant for more details).

Table 99.2. General prevention measures recommendations in liver transplantation

	Preferred prevention measure	Alternative prevention measures
Surgical prophylaxis	Ampicillin or vancomycin AND 3rd generation cephalosporin or carbapenem or fluoroquinolone or aminoglycoside AND Fluconazole (high-risk patients only)	Ampicillin/sulbactam or Piperacillin/tazobactam or carbapenem
CMV	Valganciclovir, acyclovir, and valacyclovir requiring dosage adjustment for renal dysfunction	
D+/R−*	Pre-emptive monitoring	Valganciclovir 900 mg daily for 3 months
Any R+†	Pre-emptive monitoring	Valganciclovir 900 mg daily for 3 months
D−R−‡		Acyclovir 400–800 mg TID§ or valacyclovir 500 mg BID for 3–6 months
Fungal		
Candida	Fluconazole 200 mg daily up to 70 days	
PCP**	Smx/tmp 1DS†† thrice weekly	Atovaquone 1500 mg daily Dapsone 100 mg daily Inhaled pentamidine Clindamycin+pyrimethamine
Hepatitis B	Lamivudine + hepatitis B immunolobulin	Lamivudine + entecavir or Emtricitabine + tenofovir or Lamivudine + tenofovir

* Donor seropositive/recipient seronegative; † recipient seropositive; ‡ donor seronegative/recipient seronegative; § thrice daily (every 8 hours), twice daily (every 12 hours); ** *Pneumocystis* pneumonia; †† Double strength (sulfamethoxazole 800 mg — trimethoprim 160 amg).

Subsequent studies on the use of valganciclovir prophylaxis in kidney transplant recipients have further refined its role. Initially, studies were published demonstrating that in kidney transplant patients 450 mg orally daily resulted in blood levels which were adequate to prevent CMV. Then, a study by Humar et al. showed that prolonging valganciclovir 900 mg/day prophylaxis from 100 to 200 days resulted in significantly less CMV disease by 2 years post-transplant, 38.7% versus 21.3%, $P < 0.001$ [26]. There was no difference between graft rejection or patient survival [26]. A consensus guideline [27] published prior to the study by Humar et al. recommends using 900 mg/day of valganciclovir for three to 6 months in D+/R− patients and for 3 months in R+ patients. Prophylaxis against herpes simplex and varicella zoster viruses should be considered for patients who are D−R− for CMV [27].

Primarily because there is a lack of effective antivirals, routine prophylaxis for other viruses cannot be general recommended except for some very specific scenarios. Most patients with end-stage renal disease (ESRD) should be vaccinated against hepatitis B virus (HBV). However, if the recipient did not mount an effective antibody response to the vaccination and they eventually receive a kidney from a HBV-infected donor (HBV cAb+, HBV sAg+, or HBV DNA+) then the recipient should probably receive HBV immunoglobulin and/or be started on antiviral prophylaxis. Antiviral agents that are active against HBV include lamivudine, tenofovir, emtricitabine, and entecavir. In addition to antivirals, patients should have surveillance for infection with HBV. This would include testing for HBV sAg and HBV DNA at 1, 3, 6, and 12 months post-transplant. If either of these becomes positive, then the patient should remain on antiviral treatment until the patient develops a detectable HBV sAb. If a patient receives an organ from a donor acutely infected with influenza, then consider giving the recipient oseltamivir for 5–10 days post-transplant. Although this is a soft recommendation as there have not been documented transmission of influenza through kidney transplantation.

Prevention in liver transplantation

See Table 99.2 for a summary of recommended prevention strategies in liver transplantation.

Bacterial prevention

Bacterial infections are common following surgery for liver transplantation. According to CDC surveillance, surgical site infections occur in up to 20% of liver transplant surgeries [28]. These infections range from superficial wound infections to deep, organ space infections. Superficial and deep wound infections are typically with skin organisms just as in any surgical procedure. However, deep, organ-space infections are usually related to flora of either the biliary tract or the bowel, depending on the surgical procedure performed. During the author's training, Robert H. Rubin, considered one of the early leaders in transplant infectious diseases, once said, "The risk for infection in transplantation is directly related to the skill of the surgeon." A corollary and likely more accurate way to state this principle is that the more complicated a surgery the more likely to have an infectious complication. This is illustrated by the experience at Mayo Jacksonville where the authors reviewed 370 liver transplants over a 2 year period [29]. After multivariate analysis there were only 2 factors which remained significantly associated with the risk of infection — a prolonged operative time (>6 hours) or a low liver graft weight to recipient BMI ratio (<0.01) [29]. In addition, in this study a surgical site infection was significantly associated with an increased risk of graft loss or death. Those with a surgical site infection had a 12 month cumulative graft loss or death percentage over 20% as compared to about 8% for those without a surgical site infection [29]. Thus, it is important to choose an antibiotic for surgical prophylaxis that has good activity against the flora of the duodenum and the biliary tree.

A Spanish transplant group, which covers all transplant centers in Spain, reviewed their cohort of liver transplants over a 2 year period to see if antibiotic choice impacted the rate of surgical

infections. There were 1222 liver transplants in the cohort. The cumulative 12 month surgical site infection rate was 8.8%. Most of these (42%) were incision infections with only 26% being deep abscesses [30]. In univariate analysis the use of cefazolin was associated with an increased risk of surgical site infection. However, this association dropped out on multivariate analysis because of high use of cefazolin at a single center. There were several risk factors for developing a surgical site infection following multivariate analysis. These included: choledochojejunostomy (OR = 4.2: 95% CI; 1.6–10.7, $P = 0.003$), previous liver or kidney transplant (OR = 2.6: 95% CI; 1.1–6.3, $P = 0.029$), or red cell transfusion >4 units (OR = 2.0: 95% CI; 1.1–3.4) [30]. Of note, high-volume transfusion has also been found to increased risk for *Candida* infection [31]. Together all of these studies reinforce the principle stated by Dr. Rubin, if a surgery is complicated for whatever reason then there is an increased risk for infection.

However, how do these studies inform decisions about choosing surgical antibiotic prophylaxis? The Spanish study found that *Enterococcus* species were responsible for all infections in their cohort. Thus, it is important to choose an antibiotic regimen that has activity against *Enterococcus*. The authors of the Spanish study suggest that a second or third generation cephalosporin with or without ampicillin may be adequate. Their data would suggest that a glycopeptide plus a fluoroqinolone would also be an acceptable choice (OR = 1.2: 95% CI; 0.01–13.4, univariate analysis) [30]. This would also be an acceptable regimen in either a patient with a stated penicillin/cephalosporin allergy or in a patient who is known to be colonized with MRSA. These regimens are supported by older guidelines currently undergoing revision [1]. Despite the antibiotic choices, it is important that antibiotics be re-dosed if it has been 6 hours since initial or last dose, or if there is large-volume blood loss. Continuing antibiotics for 48 hours postoperatively is generally recommended [1], antibiotics are often continued for longer following complicated surgeries.

Outside of surgical prophylaxis routine antibacterial prophylaxis is generally not needed. Clearly, if bacteria are isolated from fluid collections postoperatively, then using antibiotics in this setting would now be considered therapy rather than prophylaxis.

Fungal prevention

Candida infections are the most common fungal infections following liver transplantation [32]. Routine use of fluconazole for 70 days post-transplant was shown to significantly reduce the rate of *Candida* infections in liver transplant [33]. However, the rate of *Candida* infections in the fluconazole arm of this randomized, placebo-controlled study was higher than the rate encountered in most centers [32,33]. Therefore, many centers use fluconazole in patients at high-risk for developing a fungal infection. Criteria used to determine high-risk are: known *Candida* colonization (urine, wound, or respiratory), a choledochojejunostomy biliary anastomosis, retransplantation, intraoperative administration of >40 units of cellular blood products (red blood cells or platelets), preoperative creatinine ≥2.0 mg/dL, or return to the operating room repair of viscous leak or bleeding complication [34]. If a patient has two or more of these criteria then they are considered at high risk for a fungal infection and antifungal prophylaxis perioperatively is warranted [34,35]. However, patients who do not have two or more of the above criteria do not need routine antifungal prophylaxis [36].

Pneumocystis prophylaxis is recommended for all transplant recipients, including liver transplant, for the first 6–12 months post-transplant [11]. Antibiotic choices are the same as those for kidney recipients and include sulfamethsoxazole/trimethoprim, dapsone, atovaquone, inhaled pentamidine and clindamycin+pyramethamine [11]. For sulfa-allergic patients atovaquone or pentamidine are acceptable alternatives. Pentamidine has the convenience of being administered monthly. However, pentamidine aerosol administration requires specialized equipment and experienced personnel for effective administration [11].

While aspergillosis and cryptococcosis occur post liver transplantation, these are typically late infections with a low rate and routine antifungal prophylaxis is not warranted. Although, there may specific instances where disease is detected in the donor and the use of prophylaxis to prevent a disease transmission is acceptable.

Parasitic prevention

As for kidney transplant recipients, some agents used for PCP prophylaxis are effective against *T. gondi*. However, toxoplasmosis prevention usually is not needed in liver transplantation. Also similar when found in kidney transplantation, Chagas' disease in liver transplantation might be a consideration in a recipient born in rural areas of Latin America. If identified and time permits, patients should be treated prior to transplantation. Alternatively, recipients may be treated for recurrences post-transplant.

Viral prevention

CMV

Like with kidney transplantation CMV is the most common viral infection encountered after liver transplantation. However, unlike in kidney recipients, routine CMV chemoprophylaxis cannot be recommended. Valganciclovir was shown to be non-inferior to oral ganciclovir in preventing CMV disease in D+/R− recipients. However, when the FDA analyzed the data they found that tissue-invasive CMV disease was higher in liver transplant recipients receiving valganciclovir as compared to oral ganciclovir [37]. Therefore, valganciclovir did not receive an indication for prevention of CMV disease in liver transplant. The studied, and approved dose, of 900 mg po daily appears to be associated with an increased risk of toxicity when compared to 450 mg po daily. In a yet unpublished abstract, Kalil et al. found that the higher dose of valganciclovir was associated with a higher risk of leukopenia (OR = 5.24: 95% CI; 2.09–13.15, $P < 0.001$) [38].

If CMV prophylaxis is not used, then consideration should be given to preventing herpes simplex virus (HSV) and varicella zoster by using valacyclovir or acyclovir [39,40]. HSV typically occurs in the first month post-transplant.

Hepatitis B

Patients who are unimmunized against hepatitis B prior to transplantation should receive the immunization series. This may be given on accelerated schedule if needed. Patients with chronic hepatitis B who undergo liver transplant should be instituted on therapy prior to transplantation. Therapy usually consists of combination therapy because of high rates of failure with lamivudine monotherapy [41]. Lamivudine is well tolerated and has an excellent safety profile. It is usually combined with either tenofovir or adefovir. Lamivudine in combination with chronic hepatitis B

Table 99.3. General prevention measures recommendations lung transplantation

	Preferred prevention measure	Alternative prevention measures
Surgical prophylaxis	Ampicillin or vancomycin AND 3rd generation cephalosporin or carbapenem or fluoroquinolone	Piperacillin/tazobactam or carbapenem OR Clindamycin+ Ceftazidime or fluoroquinolone
CMV	Valganciclovir, acyclovir, and valacyclovir requiring dosage adjustment for renal dysfunction	
D+/R−*	Valganciclovir 900 mg daily for 12 months	Pre-emptive monitoring
Any R+†	Valganciclovir 900 mg daily for 12 months	Pre-emptive monitoring
D−R−†		Acyclovir 400–800 mg TID§ or valacyclovir 500 mg BID for 3–6 months
Fungal		
Mold	Voriconazole 200 mg twice daily for ≥4 months	Itraconazole 200–400 mg po daily — BID
PCP**	Smx/tmp 1DS†† thrice weekly	Atovaquone 1500 mg daily Dapsone 100 mg daily Inhaled pentamidine Clindamycin + pyrimethamine

* Donor seropositive/recipient seronegative; † recipient seropositive; † donor seronegative/recipient seronegative; § thrice daily (every 8 hours), twice daily (every 12 hours); ** *Pneumocystis* pneumonia; †† Double strength (sulfamethoxazole 800 mg — trimethoprim 160 mg).

immunoglobulin is also effective in preventing recurrences post-transplant [42].

Hepatitis C

As of yet there are no specific agents that are used to prevent a hepatitis C recurrence post-transplant. However, many centers use approaches to minimize the risk of post-transplant hepatitis C recurrence. This is accomplished by: rapid taper of steroids, minimizing rejection risk by slow steroid taper or avoiding CMV [43].

Respiratory viruses

Routine season influenza immunization is the most effective means of preventing influenza in liver transplantation [44,45]. Recipients who are exposed to influenza post-transplant can receive oseltamivir for 10 days [46,47].

Prevention in lung transplantation

See Table 99.3 for general prevention recommendations in lung transplantation.

Bacterial and fungal prevention

Lung transplantation is similar to other surgical procedures in that patients are at risk for developing superficial wound infections most likely from skin flora. However, unlike other surgical procedures, lung transplantation involves cutting into the respiratory tree. Therefore, the choice of surgical prophylaxis must also account for bacteria, which may be present in this body site. This includes both the donor and recipient airways. Many centers and organ procurement organizations routinely obtain cultures of the airways. Then antibiotics are chosen which target whatever organisms grow. This practice is primarily based on observational studies showing bacterial pneumonias were the most common infections following lung transplantation [48]. Guidelines from the American Society of Health-Systems Pharmacist suggest cefazolin, cefuroxime or vancomycin for penicillin allergic patients. However, these guidelines are based on no data and therefore default to prophylactic regimens used for non-transplant cardiothoracic surgery [1]. These guidelines do state that therapy should be adjusted based on the results of donor cultures. For empiric perioperative prophylaxis, many centers choose antibiotics which are likely to cover ventilator-associated or aspiration pneumonia which may be present or developing in the donor at the time of procurement. At our center the empiric regimen is vancomycin and ceftazidime. This covers oral flora, possible MRSA and *Pseudomonas*. However, a carbapenem, anti-*Pseudomonal* penicillin, or an anti-*Pseudomonal* cephalosporin +/− ampicillin or vancomycin would also be reasonable coverage. For patients with septic lung disease, e.g. cystic fibrosis or bronchiectasis, the perioperative regimen is often designed based on the most recent cultures in the recipient. This regimen should include antimicrobials active against gram-positive oral flora. Or, if MRSA is a problem locally, vancomycin should also be included in the antibiotic regimen. Of course, the timing of antibiotic administration should follow existing guidelines from the Healthcare Infection Control Practices Advisory Committee [49], or more recent data demonstrating that except for vancomycin and fluoroquinolones, which should be dosed within 1 hour of skin incision, antibiotics should be administered between 0 and 30 minutes before skin incision [50].

While pneumonias are an important post-transplant complication, the bronchial anastomosis is a critical site for infection. Because the bronchial anastomosis takes 6–12 weeks to heal, it is susceptible to infection with both pathogenic and saprophytic organisms. This is especially true for fungi where *Candida* and *Penicillium* have been shown to cause anastamotic infections possibly leading to breakdown of the anastomosis [51]. Additionally, apart from small bowel transplants, lung transplant recipients are at highest risk for invasive fungal infections, especially aspergillosis. By 12 months post-transplant 8.6% of lung transplant patients will have developed an invasive fungal infection, and 64% of these cases will be aspergillosis or an unspecified mold [32]. Therefore, preventing fungal infections potentially has high favorable impact in lung transplantation. However, the most effective antifungal agent is not well defined. Most centers use universal prophylaxis with voriconazole with or without inhaled amphotericin B [52]. However, some centers use only an inhaled amphotericin B product, and lipid complex amphotericin B has been shown to be tolerable with only intermittent dosing — once per week after first 2 weeks [53]. While this study by Drew et al. was not powered for efficacy, there were 14.3% prophylaxis failures in the inhaled amphotericin B arm and 11.8% failures in the inhaled lipid complex amphotericin B arm [53]. These rates compare favorably to a retrospective analysis from the University of Pittsburgh where previously universal prophylaxis with fluconazole for 3 months

was used. However, if patients were found to be colonized with an *Aspergillus* species, then targeted intervention with systemic itraconazole combined with inhaled amphotericin B was used [54]. Employing this approach they experienced 23% invasive aspergillosis at one year. However, after switching to a universal prophylaxis approach using voriconazole 200 mg twice daily for 4 months minimum post-transplant, they reduced their invasive aspergillosis rate to 1.5% [54]. Thus, it is clear that universal inhaled amphotericin B — deoxycholate or lipid complex — is better than fluconazole, but universal prophylaxis with voriconazole appears to be better than inhaled amphotericin B, 1.5% versus 11–15% respectively. Although, these have never been compared head-to-head. Unfortunately, voriconazole is not without issues. Studies have linked voriconazole use in lung transplant recipients with increased rates of skin cancers [55–57]. While skin cancers are clearly problematic, this risk must be weighed against the high risk of developing invasive aspergillosis and the high associated mortality. Fortunately, there may be other options in posaconazole, itraconazole, or a currently investigational agent, isavuconazole. Of these, only itraconazole has been shown to be effective when employed in a universal prophylaxis manner [58]. However, itraconazole has poor bioavailability and many centers follow blood levels to ensure levels are adequate. Posaconazole is effective in preventing fungal infections in patients with acute myelogenous leukemia or myelodysplastic syndrome [59,60]. So it may be reasonable to presume posaconazole would be effective in lung transplant prophylaxis, but this has not been demonstrated.

Lung transplant recipients are at increased risk for PCP. However, unlike other transplant recipients, this risk is probably elevated for life. Therefore, current guidelines recommend continuing on PCP prophylaxis for life [11]. As with other organ transplant recipients, smx/tmp is the preferred agent. Alternative agents include dapsone, atovaquone, inhaled pentamidine, and clindamycin plus pyrimethamine. However, dapsone should be used with caution in lung transplant recipients as there may be an increased risk of hemolytic anemia without glucose-6 phosphatase deficiency (G6PD), especially if there is accompanying renal failure [12].

Parasitic prevention

As for kidney transplant recipients, some agents used for PCP prophylaxis are effective against *T. gondi*. However, toxoplasmosis prevention usually is not needed in liver transplantation. Also similar when found in kidney transplantation, Chagas' disease in liver transplantation might be a consideration in a recipient born in rural areas of Latin America. If identified and time permits, patients should be treated prior to transplantation. Alternatively, recipients may be treated for recurrences post-transplant.

Viral prevention

Immunization against hepatitis B virus prior to transplantation may help diminish the chance of hepatitis B being transmitted with the organ. However, if lungs known to be procured from an actively infected donor or concerns for recent infection (e.g. HBV cAb IgM positive), then consideration should be given to using lamivudine with HBIG to prevent infection. If a lung transplant recipient does develop chronic, active HBV infection, then treatment with combination therapy with antivirals is warranted.

Vaccination against seasonal influenza is effective at inducing an immune response in lung transplant recipients [61–63]. And, in the general population neutralizing antibodies are correlated with protection against seasonal influenza. Therefore, it is recommended that lung transplant recipients be immunized annually, preferably as soon as vaccine becomes available.

CMV is a major cause of morbidity post-transplant in lung recipients. CMV has been associated with the development of bronchiolitis obliterans syndrome (BOS) [64,65] In the pivotal study by Paya et al. [25] there were an insufficient number of lung transplant recipients enrolled for the FDA to give valganciclovir an indication for CMV prevention in these patients. However, a follow-up study examined whether 12 months versus 3 months of valganciclovir 900 mg daily (adjusted for renal dysfunction) was better following lung transplantation [66]. Patients were randomized to an additional 9 months of therapy or placebo following in initial, standard 3-month prophylaxis period. At one year post transplant, the patients in the extended prophylaxis period had, both statistically and clinically, significantly less CMV disease, 3.6% versus 35% [66]. This benefit was gained without an increase in toxicity as manifest by study withdrawal or the development of ganciclovir resistance. Therefore, it is recommended that lung transplant recipients who are positive for CMV either donor or recipient seropositive, should receive valganciclovir for 12 months post-transplant. It is advisable that some form of monitoring for CMV viremia be undertaken while on prophylaxis to insure there are no breakthroughs.

If CMV prophylaxis is not used, then consideration should be given to preventing herpes simplex and varicella (HSV) zoster by using valacyclovir or acyclovir [39,40]. HSV typically occurs in the first month post-transplant.

Prevention in heart transplantation

See Table 99.4 for general prevention recommendations in heart transplantation.

Bacterial prevention

Surgical site infection rates following heart transplantation are not any higher than coronary artery bypass grafting surgery [28]. Therefore, it is acceptable to use similar perioperative prophylactic antibiotics. Current guidelines [1] recommend either cefazolin or cefuroxime for prophylaxis. For patients with a penicillin or cephalosporin allergy, then vancomycin is an acceptable alternative. The cephalosporin should ideally be given in the 30 minutes prior to skin incision and vancomycin infusion should start 30–60 minutes before skin infusion [49].

Fungal prevention

Like other transplant recipients, all heart transplant recipients should receive PCP prophylaxis. Smx/tmp is the preferred agent for preventing PCP [11]. Also, if a patient receives antilymphocyte antibodies to treat an episode of rejection, consideration should be given to reinstituting smx/tmp for 3 to 6 months. Many patients have a stated allergy to smx/tmp. Alternatives to smx/tmp include dapsone, atovaquone, inhaled pentamidine and clindamycin plus pyrimethamine [11]. Dapsone has a sulfa moiety. Thus, patients with a true sulfa allergy should not be given dapsone. As previously discussed, dapsone has also been associated with hemolytic anemia in lung transplant patients [12]. Therefore, hemolytic anemia should be considered in a heart transplant recipient with anemia on dapsone. This occurs even without G6PD.

Heart transplant recipients have a relatively low risk of developing a fungal infection post-transplant [32]. As such, routine antifungal prophylaxis (other than PCP) is not indicated.

Table 99.4. General prevention measures recommendations in heart transplantation

	Preferred prevention measure	Alternative Prevention Measures
Surgical prophylaxis	Cefazolin 1 g iv 0–30 minutes before skin incision	Vancomycin 1 g iv 60 minutes before skin incision
CMV	Valganciclovir, acyclovir, and valacyclovir requiring dosage adjustment for renal dysfunction	
D+/R−*	Valganciclovir 900 mg daily for 3 months	Pre-emptive monitoring
Any R+[†]	Valganciclovir 900 mg daily for 3 months	Pre-emptive monitoring
D−R−[‡]		Acyclovir 400–800 mg TID[§] or valacyclovir 500 mg BID for 3–6 months
Fungal		
PCP**	Smx/tmp 1DS[††] thrice weekly	Atovaquone 1500 mg daily Dapsone 100 mg daily Inhaled pentamidine Clindamycin + pyrimethamine

* Donor seropositive/recipient seronegative; [†] recipient seropositive; [‡] donor seronegative/recipient seronegative; [§] thice daily (every 8 hours), twice daily (every 12 hours); ** *Pneumocystis* pneumonia; [††] Double strength (sulfamethoxazole 800 mg — trimethoprim 160 amg).

Parasitic prevention

Heart transplant recipients have the highest risk of significant morbidity from *T. gondi*. Heart transplant recipients also have the highest risk of acquiring *Toxoplasma* from the donor. The risk of toxoplasmosis is highest in seronegative recipients who receive a heart from a seropositive donor. Fortunately, all heart transplant recipients receive PCP prophylaxis, and smx/tmp, atovaquone, and dapsone are all effective prophylaxis against *Toxoplasma*. High risk patients (D+/R−) should remain on prophylaxis for life since relapses do occur after stopping prophylaxis [67]. In addition to chemoprophylaxis, heart transplant recipients should avoid acquiring *Toxoplasma* post-transplant. This means avoiding eating undercooked meat and animal feces, especially cats or other carnivores. Patients should be reminded that animal feces can contaminate soil. So, care should be taken to insure cats do not use gardens and flower beds for defecation.

Chagas' cardiomyopathy is a leading reason for heart transplantation in Brazil [14]. However, the early mortality rate is high, 18%. Treatment with benznidazole is recommended for Chagas' positive patients prior to transplantation. The duration of treatment should be 30–60 days using benznidazole or nifurtimox [15]. While benznidazole and nifurtimox are not FDA-approved in the US, either may be obtained through the CDC's Division of Parasitic Diseases and Malaria (404-718-4745; e-mail chagas@cdc.gov) or outside of regular business hours, call the CDC Emergency Operations Center (770-488-7100) and ask for the person on call for Parasitic Diseases. If treatment cannot be feasibly administered prior to transplant then patients should be closely monitored for the development of symptoms post-transplant and treated if they become symptomatic.

Viral prevention

CMV infection has been associated with post-transplant vasculopathy in heart transplant recipients [68,69]. Based on the study by Paya et al. there is a benefit of heart transplant recipients who are CMV D+/R− receive valganciclovir prophylaxis for 3 months after transplant [25]. There were 56 heart recipients enrolled in this study. By 12 months, patients in the valganciclovir arm had investigator-treated CMV disease 30.5% of the time, which compared favorably to oral ganciclovir (28.0%) [25]. Since oral ganciclovir is no longer available in the US, oral valganciclovir or high-dose valacyclovir are the only feasible prophylactic agents available. Many centers choose to use a pre-emptive approach for CMV in heart transplant recipients. This may result in a higher number of patients being treated for CMV. However, there may be less CMV disease or toxicity from valganciclovir. Although, studies have demonstrated that CMV prophylaxis results in less intimal thickening and presumably less post-transplant vasculopathy [70].

If CMV prophylaxis is not used, then consideration should be given to preventing HSV by using valacyclovir or acyclovir [39,40]. HSV typically occurs in the first month post-transplant.

As with other organs, heart transplant recipients should receive annual influenza immunization as it reduces symptomatic, influenza-like illness [71]. Influenza immunization is also safe post heart transplant. Patients who are on the waiting list for a heart transplant should also receive influenza vaccination. Flu shots have been demonstrated to reduce the occurrence of cardiovascular events and potentially overall mortality [72,73]. This is especially true for patients with ischemic cardiomyopathy. If a post-transplant patient is definitely exposed to someone with influenza, then secondary prophylaxis with oseltamivir can be considered. The usual dose and duration are 75 mg once daily for 10 days.

If the deceased donor of the heart has evidence of recent or active HBV (e.g. HBV cAb IgM positive or positive HBV sAg), then consideration should be given to using lamivudine with HBIG to prevent infection. If the heart transplant recipient does develop chronic, active HBV infection, then treatment with combination therapy with antivirals is warranted.

Prevention in small-bowel and composite vascular allografts (CVA)

Very little quality data has been accumulated to guide prevention strategies in small bowel and CVA transplantation. Even so, the principles outlined above can be empirically applied to these two transplant scenarios. Like liver transplant, small-bowel transplant involves cutting into the intestines. Therefore, surgical antimicrobial prophylaxis should include agents active against small bowel flora. These would include a combination of ampicillin with a third-generation cephalosporin, or a beta-lactam combined with a beta-lactamase inhibitor (ampicillin plus sulbactam or piperacillin plus tazobactam). Antibiotics should be redosed for large-volume blood loss or prolonged operative time. Like other non-viscous surgical procedures, cefazolin or vancomycin should be adequate prophylaxis for CVA limb surgery. However, if the CVA surgical procedure involves the face, then additional Gram-negative coverage should be added to vancomycin, such as, fluoroquinolone, aminoglycoside, or aztreonam.

Based on observational reports, CMV-disease rates are higher in both small-bowel and CVA transplantation. Most experts recommend antiviral prophylaxis with valganciclovir in both of these

Table 99.5. Recommended vaccines for transplant recipients (adults)

Vaccine	Comments
Influenza — trivalent, inactivated	Give annually as soon as available
Tetanus + diphtheria + acellular pertussis (Tdap)	Give once if never received
Tetanus + diphtheria (Td)	Give every 10 years
Human papilloma virus (HPV)	Consider giving pretransplant if potential recipient is <26 years old and unvaccinated. Vaccines are different based on gender.
Hepatitis A	Give series if unvaccinated and non-immune, pre or post-transplant is acceptable.
Hepatitis B	Give series if unvaccinated and non-immune, pre or post-transplant is acceptable though pretransplant is preferable.
Pneumococcal	Give every 5 years
Meningococcal	Give every 5 years if felt at risk
Live virus vaccines — should not be given post-transplant	
Varicella (chicken pox)	Give pretransplant if non-immune
Varicella-zoster (shingles)	Consider pretransplant if potential recipient is >50years
Measles + mumps + rubella	Consider checking rubella titers and immunizing pretransplant if the vaccination history is questionable.
Live attenuated influenza vaccine (intranasal)	Currently (2012) not recommended for transplant recipients or household contacts, or healthcare workers who care for transplant recipients.

patient populations. Three months post-transplant should be a minimum. However, longer courses may be desired.

Immunizations in solid organ transplantation

See Table 99.5 for vaccination recommendations in transplantation.

In general, transplant patients should be immunized similarly to adult or pediatric patients of the same age. The CDC's Advisory Committee on Immunization Practices issues periodic updates for both children and adults [74]. However, there is one important caveat with solid organ transplant recipients — **no live vaccines should be administered to a post-transplant recipient receiving immunosuppression**. Recipients with failed allografts who are on minimal immunosuppression may receive live vaccines. Also, if the benefit of receiving a live vaccine is felt to outweigh the risks of developing a chronic infection with the vaccine strain, then administration of a live vaccination is warranted. Transplant recipients should not change the diaper of an infant who has received either rotavirus vaccine or oral polio vaccine. Also, household contacts should thoroughly wash their hands after changing diapers in order to prevent transmission of the vaccine strain. Of note, oral polio vaccination is no longer routinely done in the US. However, a foreign-born infant may have received oral polio vaccination. Other than these two instances transplant recipients do not routinely need to avoid contact with live-virus vaccine recipients. One caveat to this, if a varicella-vaccine recipient develops a rash, then the transplant recipient should avoid contact until the rash resolves [74].

Summary

The field of transplantation has evolved toward the use of effective strategies to prevent infections when possible, and numerous validated approaches for chemoprophylaxis now exist for use in transplant procedures. A thorough knowledge of the organisms to be anticipated, combined with robust institutional protocols for disease avoidance, combine to lessen the risk of infectious disease, and provide improved outcomes. However, regardless of the prophylactic measures taken, good clinical surveillance and a high index of suspicion should be combined with a collaborative relationship between the clinical transplant team and the infectious disease service to anticipate and if necessary respond to infectious illness.

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CHAPTER 100

Metabolic and Endocrine Management of the Organ Transplant Recipient

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Introduction

Solid organ transplant recipients may develop a number of metabolic and endocrine disorders. These disorders, which arise on a background of pre-existing co-morbid conditions, result from complex interactions among physiological changes related to the transplanted organ, post-transplant lifestyle changes, immunosuppressive medications and genetic susceptibility.

In the immediate post-transplant period, patients require close monitoring for numerous electrolyte abnormalities. Acutely these are related to the operative procedure but quickly these give way to disorders brought on by changes in organ function and the requirement for electrolyte-altering immunosuppressive agents. Specific management of hyperkalemia, hypercalcemia, hypomagnesemia, and hypophosphatemia is frequently required. Acute hyperglycemia is not uncommon, having been observed in as many as 87% of kidney transplant recipients with no history of diabetes mellitus [1]. In the long term, the prevalence of clustered metabolic risk factors, called the metabolic syndrome consisting of diabetes mellitus, hypertension, and dyslipidemia, may reach at least 40–50% in liver and kidney transplants [2,3]. The metabolic syndrome is associated with the development of allograft vasculopathy in heart transplant recipients and an increased risk for cardiovascular disease in kidney transplant recipients [3,4]. New onset diabetes after transplant (NODAT) is associated with graft failure and all-cause mortality after kidney [5,6] and liver transplantation [7].

The successful management of endocrine and metabolic disorders after solid organ transplantation requires careful consideration of co-morbidities, impaired kidney function, and medication interactions. There is some evidence of a genetic predisposition to the toxicity of transplant-related therapies. Single nucleotide polymorphisms (SNPs) have been linked to particular side effects of immunosuppressive agents [8–10]. Identification of genetic factors may shed light on our understanding of etiology behind metabolic and other complications of anti-rejection agents, and create an opportunity for future personalization of immunosuppression. Close collaboration among transplant nephrologists, endocrinologists, and primary care physicians well-versed in the management of post-transplant complications is necessary to optimize co-morbid conditions and minimize the risk of drug-related toxicities. The reader is also referred to Chapter 88, for additional information on long-term management of metabolic issues as they relate to cardiovascular morbidity post-transplantation.

Hyperglycemia and diabetes mellitus after solid organ transplantation

Post-transplant management of hyperglycemia differs depending on the timing and expected duration of elevated blood sugar levels (Figure 100.1). Immediately after transplant, hyperglycemia can occur from the stress of surgery and exposure to immunosuppression. Notably, acute hyperglycemia after transplant is associated with a four-fold increase in the incidence of NODAT [11]. Insulin is a preferred therapy in the perioperative period, as acute hyperglycemia is usually resistant to oral medications, and changes in the daily doses of immunosuppressants (especially corticosteroids) lead to unpredictable blood glucose levels.

In the long term, the incidence of NODAT may be as high as 30% across all transplanted organs, especially in adult solid organ transplant recipients (Table 100.1). While traditional risk factors (older age, obesity, ethnicity) are well-recognized contributors, hepatits C infection and exposure to immunosuppression also increase the risk (Figure 100.2) [3,6,12–18]. The pathophysiology of NODAT is complex and revolves around two important pathways: direct toxicity to the pancreatic β cells and increased insulin resistance (Figure 100.2). Moreover, the kidney play a substantial role in the clearance and degradation of circulating insulin, and therefore restoration of kidney function may "unmask" pre-existing diabetes [19]. An understanding of the etiology behind the diabetogenic action of individual agents may guide clinicians in choosing targeted therapies.

All non-diabetic solid organ transplant recipients should be screened for NODAT. The Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group recommends measurement of fasting blood glucose, an oral glucose tolerance test and/or assessment of HbA1C weekly for 4 weeks, every 3 months for 1 year, and annually thereafter [20].Given that many patients may experience postprandial hyperglycemia related to insulin resistance, the use of fasting blood glucose is limited due to a lack of sensitivity, and use as a screen may under-diagnose up to 25% of patients with NODAT [21]. HbA1C is useful for screening but values may be confounded by uremia, blood transfusions,

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

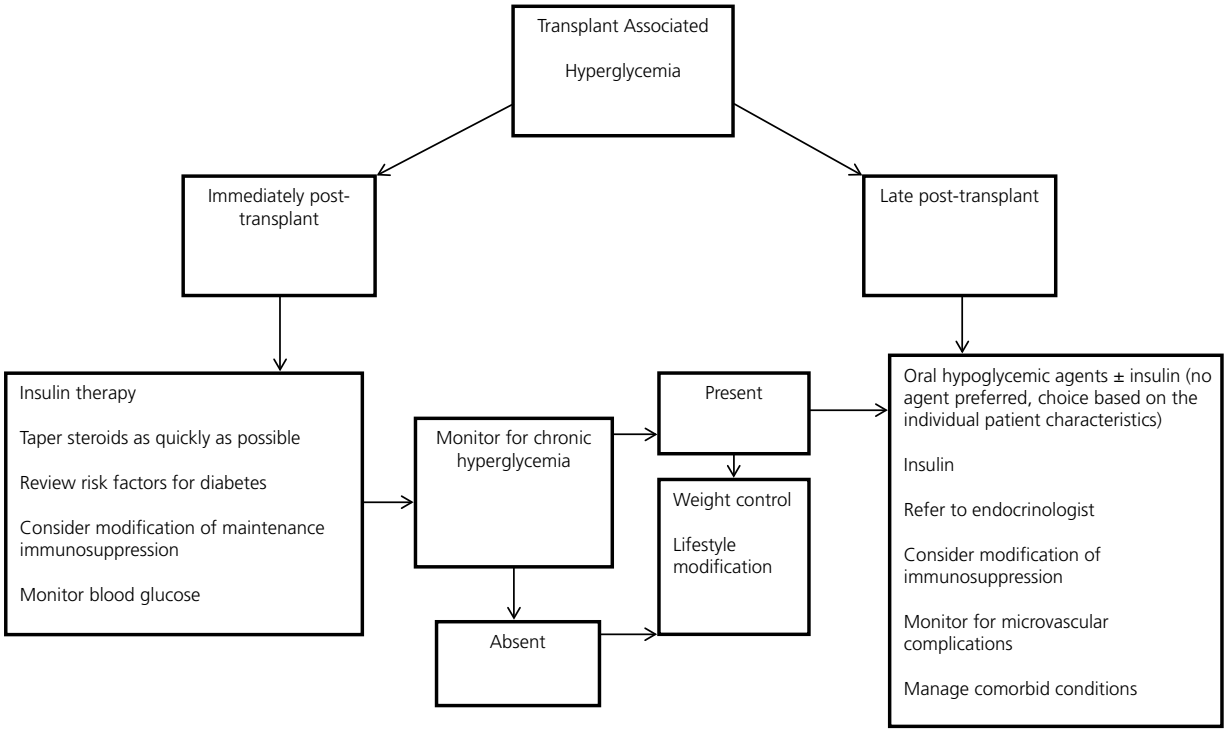

Figure 100.1 Transplant associated hyperglycemia.

erythropoietin administration, and hemolysis (not uncommon early post-transplant). Moreover, when measured in the early post-transplant period, HbA1C may reflect transient hyperglycemia.

The management of glucose disorders following pancreas transplantation is substantially different from that of other organ transplants and is covered in Chapter 77.

Role of immunosuppressive agents

Glucocorticosteroids

Glucocorticosteroids induce NODAT mainly by increasing insulin resistance, which results from increased hepatic gluconeogenesis and weight gain, as well as decreased glycogen synthesis and glucose uptake in muscle. Glucocorticosteroids also exert a direct negative effect on β cell function. Cumulative dose and duration of corticosteroids seem to influence the risk of NODAT [22]. Steroid-sparing protocols, developed to avoid unfavorable metabolic complications, are increasingly common [23]. These protocols have been associated with a decreased incidence of NODAT and may possibly decrease other cardiovascular risk factors including hypertriglyceridemia and weight gain [24–27]. Existing literature on the benefits of steroid-sparing protocols in other organ transplant recipients is limited.

Calcineurin inhibitors

Two calcineurin inhibitors (CNIs), cyclosporine and tacrolimus, form the backbone of maintenance immunosuppression after organ transplantation. Use of these medications, especially tacrolimus, contributes to the development of NODAT [28–30]. CNIs inhibit insulin transcription and synthesis through the disruption of calcineurin/transcription factors of the nuclear factor of activated T cells (NFAT) pathway, an essential regulator of β cell function [31].

Mammalian target of rapamycin inhibitors

Mammalian target of rapamycin inhibitors (mTOR inhibitors) were once considered alternative medications for patients on CNIs who developed NODAT. However, studies failed to demonstrate an improvement in glucose metabolism in kidney transplant recipients who underwent conversion from CNIs to sirolimus. Rather, the conversion was associated with a 30% increase in the incidence of impaired glucose tolerance [32]. The diabetogenic action of sirolimus may be related to impaired insulin-mediated suppression of hepatic glucose production, insulin resistance, and direct β cells toxicity [33–35].

Costimulation blockers

Belatacept is a first-in-class biological agent that selectively inhibits T cell activation by acting on a costimulatory pathway. Belatacept was designed as an alternative drug to CNIs in hopes of prolonging kidney allograft survival by avoiding CNI-related toxicity and minimizing metabolic complications. Two prospective trials compared the rates of metabolic complications in kidney transplant recipients randomized to cyclosporine or belatacept. In a prespecified pooled analysis, use of belatacept was associated with lower incidence of NODAT at 12 months [36]. Longer follow up is necessary to delineate true metabolic benefits of this new agent.

Other immunosuppressive agents

Azathioprine and mycophenolate mofetil have not been shown to have an independent diabetogenic effect, and in fact, were shown to have "protective" effects in one analysis [6]. This relationship needs to be interpreted with caution, as no good explanation exists.

Adjustment of immunosuppression

Given the important role of immunosuppressive agents in the development of NODAT, careful risk assessment in individual

Table 100.1. Incidence of new onset diabetes in solid organ transplant recipients

Study	Organ	Database	n	Time post transplant (months)	Incidence (cumulative) (%)	Increased risk	Risk not increased	Risk reduced
Israni et al [3]	kidney	PORT	1840*	12–60	13	MS 6–12 m post-transplant older age (per 10 years) AA race GFR (per 10) BMI >30 kg/m^2	—	—
Shah et al [17]	kidney	OPTN/UNOS	15309	Mean follow-up: 306 days	10	Older age Hypertension prior transplant AA race BMI >30 kg/m^2 HCV Tac	Extended criteria donor Living donor No of HLA –A,-B mismatches	Alemtuzumab
Kasiske et al [6]	kidney	USRDS	11659	3, 12, 36	9.1 16 24	Age >45 vs. 18–44 AA race Hispanic ethnicity Male donor HCV infection Tac at discharge	—	Age <17 years vs. 18–44 MMF AZA ESRD secondary to GN
Kuo et al [18]	pediatric kidney	OPTN/UNOS	2726	Median follow-up 693 days	4.6	Age >10 BMI <5% and >85% Steroid use at discharge	Gender hypertension Non-white race induction mTOR inhibitors Tac MMF	Living donor
Ye et al [15]	lung	OPTN/UNOS	3540	Median follow-up: 670 days	33.4	Gender (male) Age (>50) AA race BMI ≥25 kg/m^2 Cystic fibrosis (as an etiology for lung disease) Tac at discharge	HCV Immunosuppression (MMF, mTOR inhibitors , steroids)	Private insurance CMV negative (donor/recipient) BMI <18 kg/m^2
Kuo et al [14]	liver	OPTN/UNOS	15463	Median follow-up: 685 days	26.4	Age (>50) AA race BMI ≥25 kg/m^2 HCV Cirrhosis Donor age >60 Tac steroids	Recipient male gender Status 1A Cold ischemia time	Living donor Induction
Kuo et al [13]	pediatric liver	OPTN/UNOS	1161	Median follow-up: 770 days	10.1	Age >13 AA race ESLD secondary to AHN, cirrhosis, PSC or cystic fibrosis Serum bilirubin >3 mg/dL AR with steroid treatment	Gender Donor gender Donor age Donor type (living vs deceased) HCV Obesity Transplant factors(cold ischemia time, immunosuppression (CNI, MMF,mTOR inhibitors, steroids) Maintenance steroids at 1 year	—
Ye et al. [16]	adult heart	OPTN/UNOS	3763	Median follow-up 713 days	28.6	Increased age (>50 years) Non-white race BMI ≥25 kg/m^2 Ischemic heart disease CMV positivity (recipient) Tobacco use Tac use at discharge Steroids use at discharge	Recipient gender HCV MMF at discharge mTOR at discharge	—

*Patients with NODAT in first year were excluded; Tac, tacrolimus; MMF, mycophenolate mofetil; AZA, Azatioprine; mTOR, mammalian target of rapamacin; GFR, glomerular filtration rate; MS, metabolic syndrome, AA, African American; ESLD, end stage liver disease; AHN, acute hepatic necrosis; PSC, primary sclerosis cholangitis; HCV, hepatitis C; AR, acute rejection; CNI, calcineurin inhibitors

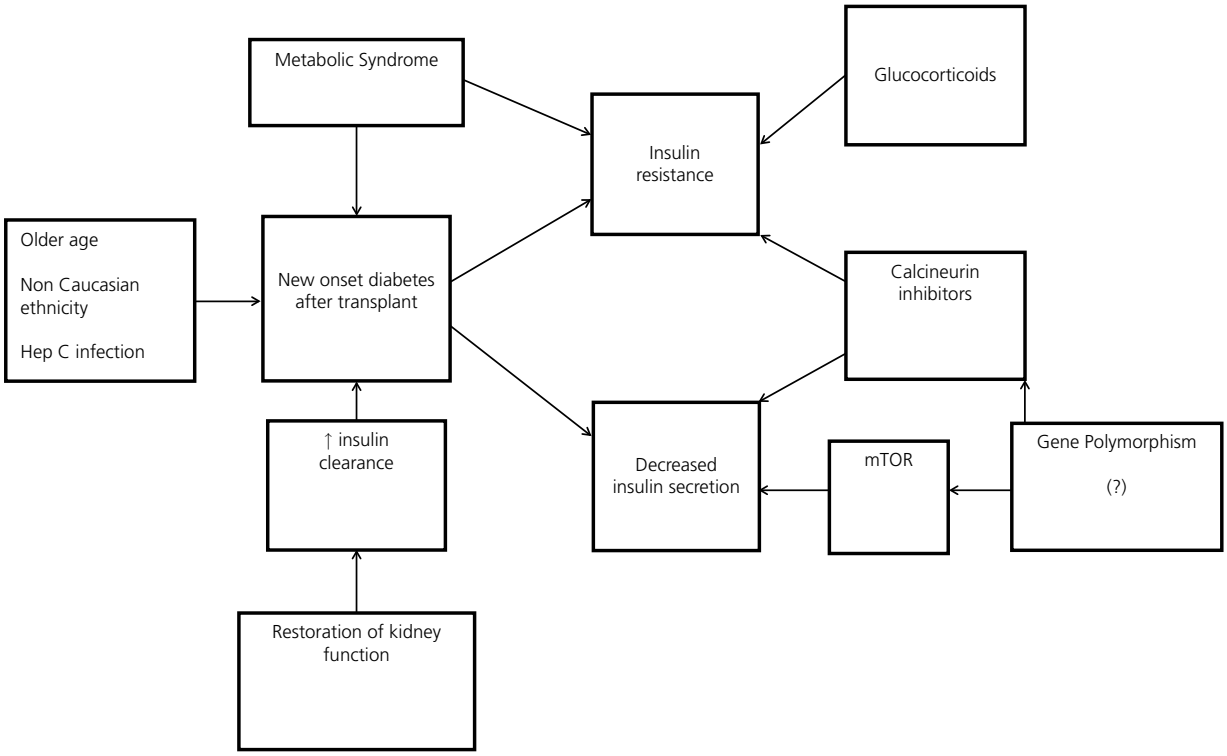

Figure 100.2 Risks factors for new onset diabetes after transplant.

patients may guide the clinician in the choice of maintenance immunosuppression. Certain risk factors are concordant across all solid transplant organs (Table 100.1). Tacrolimus has been repeatedly shown to be more diabetogenic than cyclosporine [37]. Older age, African American ethnicity, hepatitis C infection, and obesity predispose transplant recipients to NODAT and those patients may be best served with cyclosporine, rather than tacrolimus. Similarly, it has been previously shown that recipients with NODAT who are on maintenance tacrolimus may benefit from conversion to cyclosporine [38–40]. Belatacept may be a good option in those with high risk of metabolic complications, however longer follow-up is needed to adequately assess its long-term safety. Corticosteroids-minimization protocols, if deemed safe, should be considered in patients at risk for NODAT. For recipients on maintenance steroids, reduction of the dose to 5 mg/day may result in improvement of glucose metabolism [41].

Treatment of post-transplant diabetes mellitus with oral hypoglycemic Medications: safety profile and side effects (Table 100.2)

Insulin sensitizers

Metformin is recommended as a first-line therapy for non-transplant patients with type 2 diabetes mellitus. It has a favorable effect on weight stabilization, or even weight loss, and a limited side-effect profile (mainly gastrointestinal intolerance). Metformin monotherapy reduces HbA1C levels by 1.5%, and does not typically induce hypoglycemia [42]. The use of metformin has been limited in patients with renal disease because of the perceived risk of lactic acidosis; however, it was shown to be safe in patients with chronic kidney disease (CKD) stage 1–3, without severe co-morbidities [43,44]. Incidence of lactic acidosis in patients on metformin is very rare (4.3 cases per 100 000 patient years) and comparable to that in patients on non-metformin therapy (5.4 cases per 100 000 patient years) [45]. Risk for lactic acidosis increases with severe co-morbidities like hepatic dysfunction, kidney failure, heart failure, and hypoxia. Although data are limited, metformin has been shown to be a safe option in kidney transplant recipients with preserved kidney function [46]. In the non-transplant population, metformin use reduces the risk of metabolic syndrome and improves cardiovascular outcomes [44,47]. More research is needed to confirm these benefits in solid organ transplant recipients.

Insulin secretagogues

Sulfonylureas and glinides directly stimulate insulin secretion and may be especially beneficial in patients with CNI-induced insulin hyposecretion. Sulfonylureas and repaglinide lower hemoglobin A1C by 1.5%, while nateglinide is less effective when used as monotherapy [42]. The main side effect of these agents is prolonged hypoglycemia, which is more common in elderly patients.

Glyburide and its active metabolite are renally cleared and thus accumulate in patients with renal insufficiency, leading to frequent and prolonged hypoglycemic episodes [48]. In animal studies the hypoglycemic action of glyburide was inhibited by cyclosporine [49]. In contrast, glipizide is extensively metabolized in the liver to inactive metabolites. Glipizide does not interact with cyclosporine and causes hypoglycemia less frequently [48,50]. Clearance of glipizide is prolonged in patients with liver disease and therefore should be avoided in patients with significant liver impairment. Repaglinide and nateglinide are also hepatically metabolized and should be avoided in patients with liver failure, but do not need dose adjustment for kidney dysfunction. While repaglinide was shown to be a safe and effective option in kidney transplant recipients [51], it is metabolized through hepatic cytochrome p450 isoenzyme CYP 3A4. This isoenzyme is inhibited by CNIs,

Table 100.2. Oral hypoglycemic medications

Agent	Action	Metabolism/elimination	Benefits	Risks, precautions and contraindications
Insulin sensitizers				
metformin, buformin, phenformin	Decrease glucose production, increase glucose uptake by skeletal muscle	Major: renal tubular secretion	Low risk of hypoglycemia Weight neutral of weight reduction	GI intolerance Contraindicated in patients with renal disease and hepatic disease Increased risk of lactic acidosis with renal and liver disease
Insulin secretagogues				
the sulfonylureas glipizide, glyburide, glimepiride;	Increase pancreatic insulin secretion	Major: hepatic Glyburide is renally excreted	Rapid onset of glycemic effect	Hypoglycemia (highest risk with glyburide) Weight gain Adjust dose or avoid in patients with renal disease or liver failure
the meglitinides repaglinide, nateglinide		Major: CYP2C8 And 3A4	Rapid onset of glycemic effect	Hypoglycemia Weight gain Adjust dose or avoid in patients with liver failure Watch for immunosuppressive drug interactions
Others with different actions				
the thiazolidinedione derivatives pioglitazone, rosiglitazone;	Bind to peroxisome proliferator-activated receptors (PPARs) and stimulate insulin sensitive genes	Major: CYP2C8 Minor: CYP3A4	Low risk of hypoglycemia Improved lipid profile No adjustment needed for kidney disease	Weight gain Peripheral edema Heart failure Bone fractures Contraindicated in those with established NYHA class III and IV heart failure Avoid in patients with liver disease
the glucagon-like peptide-1 analogs exenatide, liraglutide;	Increase pancreatic insulin secretion	Major: renal	Low risk of hypoglycemia Weight loss ? β cells preservations	GI intolerance Not recommended in patients with history of pancreatitis Do not use (exenatide) or use with caution (liraglutide) in patients with advanced kidney disease
the dipeptidyl peptidase-4 inhibitors sitagliptin, saxagliptin	Increase endogenous incretins	Major: renal Minor: CYP3A4 2C8	Low risk of hypoglycemia Weight neutral May improve lipid profile	Reduce dose in moderate or severe kidney disease Watch for immunosuppressive drug interactions

therefore patients on calcineurin inhibitors may be at risk for hypoglycemia [52].

Thiazolidinediones

Thiazolidinediones (TZDs) increase the sensitivity of muscle, adipose tissue, and liver to endogenous and exogenous insulin. Monotherapy with TZDs lowers HbA1C by 0.5–1.4%, but their effect is more durable compared to sulphonylureas [53]. They typically require 3–6 weeks of therapy to show an effect. Main side effects are weight gain and peripheral edema; their use is also associated with a two-fold increased risk of heart failure [54]. While TZDs exert a positive effect on the lipid profile, their effect on the incidence of cardiovascular events remains controversial [55].There is also evidence of accelerated bone loss and higher risk of fracture in patients on TZDs, likely resulting from activation of peroxisome proliferator-activated receptor gamma (PPARγ), inhibiting bone formation and enhancing bone resorption [56]. TZD use in kidney transplant recipients was shown to be well tolerated without significant interactions with immunosuppressive medications in the short term [57]. Nonetheless, they should be used with caution in transplant recipients, many of whom are already predisposed to bone fractures. Additionally, the lack of positive effects on cardiovascular outcomes makes this class of drug less desirable in solid organ transplant recipients.

Glucagon-like peptide-1 analogs

The incretins, natural hormones produced by the small intestines, are secreted in response to carbohydrate ingestion, which potentiates glucose-stimulated insulin secretion. Incretin hormone-based treatments, including glucagon-like peptide 1 (GLP-1) agonists and dipeptide peptidase IV (DDP-4) inhibitors, are promising new therapeutic options for patients with type 2 diabetes mellitus. GLP-1 agonists bind to GLP-1 receptors and increase GLP-1 activity, leading to glucose-dependent insulin secretion, decreased glucagon secretion, and hepatic glucose production. Exenatide lowers HbA1C by 0.5–1% mainly by lowering postprandial glucose levels. Main side effects include gastrointestinal disturbances such as nausea, vomiting, and diarrhea. Exanetide promotes weight loss by inducing satiety and slowing gastric motility, and therefore is not recommended in patients with severe gastroparesis. Exanatide should not be used in patients with GFR <30 mL/min. Similarly, liraglutide should be avoided in patients with severe kidney dysfunction [42,58].

The DPP-4 inhibitors prevent endogenous GLP-1 and gastric inhibitory polypeptide enzymatic inactivation, leading to prolonged availability of physiological levels of endogenous GLP-1. They reduce HbA1C by 0.5–0.8%. Similar to GLP-1 agonists, the DPP-4 inhibitors exert a positive effect on weight loss and do not cause hypoglycemia. Dose adjustment is necessary for kidney

dysfunction. Overall, the use of this class of drugs in transplant recipients should be done with caution due to lack of data on safety and efficacy, as well as potential drug interactions [42,58].

Management of post-transplant diabetes mellitus

The target HbA1C has not been established in solid organ transplant recipients, but a goal of <7% is often extrapolated from guidelines for treatment of type 2 diabetes mellitus in the general population [42]. Analogous to the non-transplant population, lifestyle and dietary modifications play an important role in NODAT management, but patients with persistent hyperglycemia require pharmacological therapy.

The literature is lacking adequate studies addressing the safety and efficacy of oral hypoglycemic agents versus insulin in long-term management of diabetes after transplant. Despite increasing understanding of the pathophysiology of NODAT, a thorough assessment of insulin resistance versus decreased insulin secretion is not done routinely in clinical practice.

While no single oral hypoglycemic agent is preferred, the risks and benefits of each drug should be carefully examined for potential interactions with immunosuppressive agents in solid organ transplant recipients. Metformin appears to be a good option for patients with insulin resistance and stable kidney function. Metformin should be discontinued if a patient experiences acute changes in renal function, acute illnesses such as gastrointestinal disturbances, severe infections, or conditions that can cause hypoxia including either cardiac or respiratory failure. Metformin should be used cautiously in kidney transplant recipients, especially during the first six months post-transplant when the incidence of rejection, and therefore graft dysfunction, is greatest.

Insulin secretagogues can be used in the transplant population, especially if the predominant mechanism is suspected to be decreased insulin secretion. However, the particular hypoglycemic agent should be chosen based on pharmacokinetics in the context of an individual patient's co-morbidities. TZDs should be used with caution because they have a propensity to cause edema, heart failure, and bone fractures. Incretin-based therapies are a new, promising class of drugs, but their safety and efficacy in transplant patients has not been adequately studied. Combination therapy of oral hypoglycemic agents from different classes may be necessary based on the severity of hyperglycemia and initial response to therapy.

Hyperlipidemia after solid organ transplantation

Hyperlipidemia is common in solid organ transplantation and may affect over half of the liver, kidney and heart transplant recipients [59–61]. Contributing factors include genetic predisposition, obesity, nephrotic syndrome, hypothyroidism, diabetes mellitus, and medication use — including immunosuppressive agents.

Role of immunosuppressive agents

Corticosteroids

Corticosteroids have many deleterious effects on cholesterol metabolism. A correlation between high cholesterol levels and the cumulative dose of corticosteroids has been described. Their prolonged use induces insulin resistance and hyperinsulinism, thereby causing an increase in triglycerides (TG), total cholesterol (TC), and very low-density lipoprotein (VLDL) levels, and a decrease in high-density lipoprotein (HDL) levels. Other lipogenic mechanisms include up-regulation of the activity of acetyl-coenzyme A carboxylase and free fatty acid synthetase, an increase in the activity of HMG-CoA reductase, inhibition of lipoprotein lipase, and down-regulation of LDL receptor activity [62–65].

CNIs

The mechanisms of cyclosporine-induced hyperlipidemia include decreased synthesis of bile acids from cholesterol via inhibition of the enzyme 26-hydroxylase and the down-regulation of LDL receptors [66]. This effect is independent of concurrent corticosteroid use; withdrawing corticosteroids has a limited and modest effect on dyslipidemia [67]. A trial of late cyclosporine reduction in kidney transplant recipients, who were treated with both mycophenolate mofetil and corticosteroids, showed a dose-responsiveness reduction of lipid levels [68]. Kidney and liver transplant recipients treated with tacrolimus had a lower incidence of dyslipidemia than those treated with cyclosporine [69,70]. Some studies showed both de novo use and conversion from cyclosporine to tacrolimus had favorable effects on dyslipidemia [71,72].

mTOR inhibitors

Use of mTOR inhibitors significantly increases cholesterol and TG levels in a dose-dependent pattern. The mechanisms of adverse lipidemic effects of sirolimus are not known and may be multifactorial, including blockage of insulin-stimulated lipoprotein lipase, increased hepatic production of TG and lipoproteins, decreased plasma clearance and cellular transport, and decreased catabolism of apoB100-containing lipoproteins [73–78]. Conversion from CNIs to sirolimus is associated with a higher prevalence of hypertriglyceridemia and hypercholesterolemia [79]. Combination therapy of sirolimus with CNIs is associated with a significant increase of total cholesterol and TG levels [80–82].

Other immunosuppressive agents

There are no data suggesting that either azathioprine or mycophenolic mofetil causes dyslipidemia. Use of the costimulation blocker belatacept was associated with a 25–50% reduced increase in non-HDL and total cholesterol compared with cyclosporine, and a reduction in post-transplantation concentrations of TG [36,83].

Treatment with anti-dyslipidemic agents: safety profile and side effects (Table 100.3)

Hypolipidemic agents for clinical use include statins, ezetimibe, nicotinic acid, fibrates and fish oil. Statins are the most commonly prescribed agents, with potent LDL-lowering properties, excellent tolerability, and a proven ability to reduce cardiovascular events [84]. The major difference in the treatment of dyslipidemia of solid organ transplant recipients versus the general population stems from drug interactions between immunosuppressive agents and lipid-lowering medications.

Statins

Statins are HMG-CoA reductase inhibitors (the rate-limiting step in cholesterol biosynthesis) and the most powerful drugs for lowering LDL-cholesterol; reductions of 20–60% have been documented [85–87]. They also exhibit pleiotropic effects including inhibition of inflammatory activity and cytokine activation, attenuation of endothelial dysfunction, and attenuation of vascular hypercoagulability, adding to their cardiovascular benefits [88–91]. Randomized clinical trials have shown that pravastatin and simvastatin improve survival and reduce the incidence of acute rejection and transplant

Table 100.3. Pharmacotherapy of lipid-lowering agents

	Usual daily dosing in general population	Dosing Instructions	Metabolism	Selected Drug-Interaction	Side Effects
HMG CoA Reductase Inhibitors (Statins)					
Pravastatin	10–80 mg/day	Should be taken at bedtime	50% renally cleared as unchanged drug. Smaller amounts are cleared by biliary excretion or undergo non-CYP and CYP 3A4 hepatic transformation.	Cyclosporine: increased risk of myopathy due to additive toxicity and elevation of statin serum level by competitive inhibition of CYP 3A4.Pravastatin and fluvastatin are less likely than other statins to interact significantly with cyclosporine. mTOR inhibitors (sirolimus, everolimus): increased serum levels of sirolimus and everolimus in combination with atorvastatin The FDA recommends avoiding doses higher than 20 mg of simvastatin in patients receiving amlodipine	Transaminitis, GI intolerance, CPK elevations, muscle toxicity, rhabdomyolysis (rare), Close monitoring of transaminases may provide early evidence of elevated statin levels and toxicity.
Fluvastatin	20–80 mg/day	Regular release should be taken at bedtime	75% undergoes hepatic transformation by CYP 2C9 and 20% by CYP 3A4.		FDA warning of increased myopathy risk about the use of high dose of simvastatin (80 mg/day)
Atorvastain	10–80 mg/day	May be taken at any time of the day	Undergoes extensive CYP 3A4 transformation to active metabolites.		
Resuvastatin	5–40 mg/day	May be taken any time of the day	Cleared as unchanged drug. 10% undergoes CYP 2C9 transformation to a partially active metabolite.		
Simvastatin	10–80 mg/day (FDA warning for doses >40 mg/day)	Should be taken at bedtime	Undergoes extensive CYP 3A4 transformation to active and inactive metabolites.		
Cholesterol Absorption Inhibitors					
Ezetimibe	10 mg once daily	May be taken any time of the day	Undergoes glucuronide conjugation in liver and in small intestines to form active metabolite. Cleared by enterohepatic recycling and fecal excretion.	Statins: added LDL lowering in combination with a statin or as single agent if statin not tolerated. Fibrate: increased serum level of ezetimibe. Cyclosporine: increased serum level of ezetimibe and possible alteration of cyclosporine level. 50% dose reduction to 5 mg of ezetimibe daily in combination with cyclosporine.	Increased transaminases in combination with statins
Fibrates					
Gemfibrozil	600 mg twice daily	30 to 60 minutes before meals	Undergoes non CYP transformation by glucuronide conjugation with small amount of CYP 3A4 transformation. Gemfibrozil inhibits other drugs metabolized by CYP 2C8, 2C9 and 2C19	Cyclosporine: potentially nephrotoxic in cyclosporine treated patients. Statins: increased risks of hepatotoxicity, myopathy, and rhabdomyolysis Avoid combining gemfibrozil with statins. Statins: increased risk of myopathy and rhabdomyolysis.	Skin rash, gastrointestinal intolerance (bloating, cramping, nausea), cholelithiasis, myalgia, muscle toxicity, rhabdomyolysis
Fenofibrate	Dose varies, depending on preparation	Not recommended with renal dysfunction (especially in patients with GFR < 30 mL/min).	Metabolized to active intermediary by plasma and tissue esterases. Undergoes non CYP metabolism by glucuronide conjugation.	Cyclosporine: additive risk of nephrotoxicity	

(*Continued*)

Table 100.3. (*Continued*)

	Usual daily dosing in general population	Dosing Instructions	Metabolism	Selected Drug-Interaction	Side Effects
Bile Acid Sequestrants					
Cholestyramine	4–24 g/day	Should be taken within 30 minutes of a meal.	Not well-absorbed from the gut into the bloodstream. Thus, bile acid sequestrants, along with any bile acids bound to the drug, are excreted via the feces after passage through the GI tract	Affects other medications' absorption, bind and decrease absorption of fat-soluble vitamins, such as vitamin A, vitamin D, vitamin E, and vitamin K, digoxin, warfarin, thyroxine and phenobarbital.	Nausea, bloating, cramping and constipation, transaminitis
Colesevalem	3.75 g/day	Should be taken with meals daily			
Colespitol	5–30 g/day	Should be taken within 30 minutes of a meal.			
Miscellaneous					
Nicotinic acid	1–2 g/day (avoid higher doses)	Should be taken with meals daily TID. Start with low dose and titrate up.	Not absorbed from the gut into the bloodstream.	Bile Acid Sequestrants: May decrease the absorption of Niacin. Statin: niacin may enhance their toxic effect especially with niacin doses of 1 g or greater daily.	Skin flushing, headache, warm sensation and pruritus, nausea, vomiting, diarrhea, angina pectoris, and myositis
Omega-3-acid ethyl esters (Fish oil)	1–2 grams twice per day	May be taken at any time of the day	Not defined	No CYP mediated interactions with immunosuppressive agents, statins, fibrates or ezetimibe based on in vitro data	Dyspepsia, eructation, fishy breath and taste, modest increase in LDL, modest increase in bleeding time

vasculopathy in heart transplant recipients [92–94]. The effects of fluvastatin on cardiovascular outcomes in kidney transplant recipients were examined in the ALERT trial [95]. Indeed, this trial serves as an exemplary specimen of clinical trial design in Chapter 134. Although the initial results did not show a significant difference, extension of the trial demonstrated a 21% reduction of major cardiovascular events with fluvastatin use [96].

Statins vary in their potency, metabolism, and drug interactions (Table 100.3). Atorvastatin and rosuvastatin are the most potent, reducing LDL-cholesterol levels by approximately 60% at doses of 80 and 40 mg/day, respectively [97]. An additional benefit of atorvastatin and rosuvastatin is an improvement in triglyceride levels and modest elevation in HDL-cholesterol levels [98,99].

Most statins, including lovastatin, simvastatin, and atorvastatin, are metabolized by the hepatic isoenzyme CYP3A4. Systemic exposure to statins may be increased 3- to 20-fold when co-administered with CNIs due to its inhibitory effect on the CYP3A4 or CYP2C9 isoenzymes. This is especially seen in patients taking cyclosporine [100–103]. Cyclosporine also inhibits other transport proteins, like P-glycoprotein organic anion transport polypeptide (OATP1B1), which may explain elevated levels of non-CYP3A4-dependent statins such as pravastatin, fluvastatin, and rosuvastatin [104].

Greater exposure to statins increases the risk of statin-induced myopathy, ranging from muscle pain and weakness to the potentially fatal complication, rhabdomyolysis. In a retrospective analysis of 20 366 kidney transplant recipients in the US Renal Data System (USRDS) database, the incidence of rhabdomyolysis was 1.4 (95% CI, 1.1–1.8) per 1000 person years and was significantly more likely to occur with cyclosporine than with tacrolimus or sirolimus [105]. Statin-induced myopathy was found to be dose dependent in non-transplant population [106], leading to a Food and Drug Administration (FDA) warning regarding the use of high dose of simvastatin (80 mg/day).

Table 100.4. Inhibitors of cytochrome P450 isoenzymes that potentiate HMG-CoA reductase inhibitors levels*

Agent	P450 Isoenzyme**
Cyclosporine	3A4
Macrolides (erythromycin, clarithromycin)	3A4
Azole antifungals (ketoconazole, fluconazole, itraconazole, voriconazole)	mainly 3A4 and 2C9
Non-dihydropyridine calcium blockers (diltiazem, verapamil)	3A4
Amlodipine	3A4 (mainly with high dose simvastatin)
Gemfibrozil	3A4
Amiodarone	3A4 and 2C9
Omeprazole	2C9
Trimethoprim/sulfamethoxazole	2C9
Protease inhibitors	3A4

*Typically induces between less than twofold increase to more than twofold increase in the area under the curve plasma concentration of the statin; **P450 indicates the subfamily of P450 hepatic oxygenase enzyme.

In transplant recipients on CNIs, the maximum dose of statins metabolized by cytochrome P450 should be reduced to 50% of the maximal dose for the general population. Co-administration of medications inhibiting the hepatic cytochrome P450 enzymes should be avoided or managed with extreme caution [107]. An example of this interaction involves the use of amlodipine, which is commonly administered for hypertension, and requires further statin dose reduction because amlodipine is a weak inhibitor of the CYP3A4 isoenzyme [108]. Similar concerns exist regarding other agents sharing the same metabolic pathway (Table 100.4).

Statin use is associated with elevations in liver transaminases in up to 3% of treated patients. This occurs in a dose-dependent fashion, with spontaneous resolution occurring in the majority of cases. Statins rarely lead to serious drug-induced liver injury,

chronic liver disease, or acute liver failure. Liver function should be monitored shortly after initiating statins and with any dose increase. If liver transaminases are higher than three times the upper limit of normal, statin use should be discontinued or reduced in dose.

Ezetimibe

Ezetimibe is a selective cholesterol absorption inhibitor that effectively blocks intestinal absorption of dietary and biliary cholesterol. In kidney transplant recipients, ezetimibe can effectively decrease total cholesterol and TG levels with a good safety profile [109,110]. A review of available randomized trials in the non-transplant population indicates that addition of ezetimibe to statin therapy does not increase the risk of myalgias, creatine kinase level increases, rhabdomyolysis, transaminase increases, gastrointestinal adverse events, or discontinuations because of an adverse event [111]; therefore, ezetimibe may be considered in solid organ transplant recipients to avoid statin dose escalation. However, there is no convincing evidence that its addition improves clinical outcomes beyond treatment with statin alone.

Nicotinic acid

Nicotinic acid can reduce the level of triglycerides, small, dense LDL particles and raise the level of HDL. Nicotinic acid, in combination with statins, has been shown to be effective in reducing the progression of atherosclerosis in patients with hypertriglyceridemia who are at risk for premature coronary artery disease [112]. Possible side effects include flushing, dizziness and nausea, which often limit the use of this agent in clinical practice.

Fibrates

Fibrates are peroxisome proliferator-activated receptor-α (PPARα) agonists and are effective in decreasing TG levels. The risk of fibrate-induced myositis and rhabdomyolysis, especially seen with gemfibrozil, is higher in patients with renal dysfunction and when used concomitantly with statins. Fenofibrates have been associated with kidney function decline in kidney transplant recipients, and dose adjustments are necessary for renal dysfunction [113–115]. In the action to control cardiovascular risk in diabetes (ACCORD) trial, the rate of fatal cardiovascular events, non-fatal myocardial infarction, or non-fatal stroke did not significantly differ in patients with type 2 diabetes who were treated with a combination of fenofibrate and simvastatin as compared to patients receiving simvastatin alone [116]. Given their unproven benefits and potential for drug interactions, fibrates should be avoided in solid organ transplant recipients.

Fish oil

The advantages of fish oil include good tolerance with minor adverse effects and no drug interactions.

A meta-analysis of data from randomized controlled trials in kidney transplant recipients showed a decrease in triglyceride levels and increase in HDL levels in patients on fish oil therapy, but no effect on patient survival, graft survival, acute rejection, and CNI toxicity [117]. It may be used as an adjunct therapy to statins.

Management of dyslipidemia

The KDIGO Transplant Work Group recommends measuring fasting serum, total cholesterol, LDL, HDL and TG 2–3 months post-transplant, 2–3 months after a change in treatment of other conditions known to cause dyslipidemia, and annually thereafter [20].

Lifestyle modifications such as weight loss, a low-fat diet, increased physical activity, and treatment of diabetes mellitus are always recommended, however, pharmacological therapy is usually necessary to achieve the target LDL goal (Table 100.5). Current target lipid levels in transplant recipients have been extrapolated from the general population. In kidney transplant recipients, LDL levels greater than 100 mg/dL (>2.6 mmol/L) should be treated to a goal of less than 100 mg/dL (<2.6 mmol/L). Some experts now recommend a lower goal of less than 70 mg/dL (1.8 mmol/L) for

Table 100.5. Management of dyslipidemia in transplant recipients (Adapted from KDIGO 2009 guidelines [11])

Risk Category	Goal	First line therapy	Preferred agent(s)	Additional therapy	Alternative*	Special considerations
Fasting TG ≥500 mg/dL	TG <500 mg/dL	lifestyle modifications +/− niacin	Nicotinic acid	Statin	Fibrates (preferably gemfibrosil)	TG ≥500 mg/dL increases risks of acute pancreatitis Fibrates are generally not recommended in patients with renal failure especially with GFR <15 mL/min/1.73 m^2; Dose reduction by 50% in patients with GFR <60 mL/min/1.73 m^2
LDL 100–129 mg/dL	LDL <100 mg/dL	lifestyle modifications +/− statin	Atorvastatin (more potent) fluvastatin or pravastatin	Statin	Nicotinic acid alone	Statin dose need to be reduced by approximately 50% in patients treated with cyclosporine and also probably with tacrolimus
LDL ≥130 mg/dL	LDL <100 mg/dL	lifestyle modifications + statin	Atorvastatin (more potent) fluvastatin or pravastatin	Ezetimibe	Nicotinic acid alone	Cases refractory to pharmacological therapy may require decreasing the dose or discontinuation of the immunosuppressive agent and switching to another immunosuppressant agent
LDL <100 mg/dL Fasting TG ≥200 mg/dL non-HDL-C ≥130 mg/dL	Non-HDL-C <130 mg/dL	lifestyle modifications + statin	Atorvastatin (more potent) fluvastatin or pravastatin	Ezetimibe	Nicotinic acid alone	Relatively small number of patients who have normal or low LDL, increased TG and high non-HDL-C considered to have high levels of atherogenic lipoprotein remnants Treatment for these patients is similar to treatment for patients with high LDL

TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins; non-HDL-C, non-high-density lipoproteins cholesterol; GFR, glomerular filtration rate.* Alternative therapy: in the case when first line therapy is not tolerate.

secondary prevention of coronary heart disease among the general population. These more aggressive guidelines may also be applicable for solid organ transplant recipients, given that such patients are considered a high-risk equivalent. However, the risks of more aggressive therapy may outweigh the benefits, and these recommendations have not been adopted by the 2009 KDIGO guidelines. Treatment of hyperlipidemia in liver, heart, and lung transplant recipients is similar, with no formal guidelines.

Triglyceride levels >500 mg/dL

Among patients with TG levels >500 mg/dL lifestyle modifications are recommended as first line, unless severe hypertriglyceridemia is present. If levels are not adequate after 2–3 months, therapy with nicotinic acid could be initiated [112]. Fish oil has few side effects and should be considered as an alternative agent.

LDL ≥100 mg/dL

Among patients with LDL ≥100 mg/dL, lifestyle modifications are recommended along with statin therapy. In heart transplant recipients, the benefits of statins extend beyond the cholesterol treatment and should be used regardless of cholesterol level [93]. The safest statins are fluvastatin and pravastatin because they are not metabolized by cytochrome P450 3A4 and have little or no muscle toxicity. These statins have lower potency than others, however, and should therefore be selected for patients with mild LDL elevations. In most solid organ transplant recipients, a more potent statin such as atorvastatin may be a more appropriate choice, given its well documented effectiveness in decreasing cardiovascular events in the general population and excellent tolerability in combination with tacrolimus [104].

If a statin alone fails to adequately lower LDL-cholesterol levels, ezetimibe can be an additional agent. If adverse effects develop with statins, reducing the dose or switching to a different statin can be considered. Ezetimibe or nicotinic acid alone can be used for patients who are not able to tolerate statins. In severe cases of dyslipidemia refractory to pharmacological therapy, decreasing the dose of or discontinuing the patient's current immunosuppressive agent and switching to another may be necessary. Such adjustments should always be done under the guidance of a transplant specialist.

Bone and mineral disorders after solid organ transplantation

Bone and mineral disorders are common in solid organ transplant recipients. Contributing factors include advanced age, pre-existing osteodystrophy, underlying disease leading to end stage organ failure, vitamin D deficiency, smoking, diabetes and transplantation-specific therapies, in particular the immunosuppressive agents [118].

Bone loss is especially rapid within the first 6 to 12 months post solid organ transplantation and range from 0–24% at the spine and 2–11% at the hip [119]. In the later post-transplant period, rates of bone loss decrease, likely due to lower doses of immunosuppressive agents and improvement in metabolic/endocrine abnormalities, and there may be some recovery of bone mineral density (BMD), particularly seen at the spine [120]. Increase in glucocorticoid doses for treatment of rejection or other reasons may lead to accelerated bone loss. Accordingly, the frequency of bone fractures ranges from 6–45% for kidney transplant recipients to 22–42% for heart, lung, and liver transplant recipients [119–125].

Kidney transplant recipients are unique in having pre-existing chronic kidney disease (CKD), which affects many aspects of mineral metabolism including phosphate retention, hypocalcemia, and decreased vitamin D activation. These effects stimulate the parathyroid glands, leading to secondary hyperparathyroidism. If not appropriately treated, these metabolic disturbances can lead to parathyroid cell hyperplasia and tertiary hyperparathyroidism. Successful kidney transplantation reverses many of these abnormalities in mineral and bone metabolism, however, the degree of improvement is frequently incomplete [126–131]. Persistent hypercalcemia can have adverse effects on renal allografts including graft microcalcifications and dysfunction, renal failure from afferent arteriole vasoconstriction, bone demineralization and loss, soft tissue and vascular calcifications, and increased fracture rate [132–136].

Persistent hyperparathyroidism and hypercalcemia

The incidence of persistent hyperparathyroidism after kidney transplantation may be as high as 50% [132,137–144]. The magnitude of pretransplant hyperparathyroidism and renal function determine long-term post-transplant parathyroid function [133]. Although the parathyroid glands involute after transplantation, this process takes a few months to several years to complete [129,130,137,138].

Within the first 3 months after transplant, up to 41% of recipients will develop transient hypercalcemia [145]. After the first year, up to 20% of patients will have persistent hypercalcemia with elevated parathyroid hormone (PTH) levels [146,147]. The development of hypercalcemia correlates with duration of pretransplant dialysis, degree of parathyroid hyperplasia, and gland size; thus, spontaneous resolution of hypercalcemia after 1 year is uncommon [139]. While persistent hyperparathyroidism is the main cause of post kidney transplant hypercalcemia, other factors may contribute such as enhanced tubular calcium reabsorption in the new renal allograft and normalization of calcitriol production, which enhances the PTH effect on bone and also increases intestinal calcium absorption [132,148].

Therapeutic considerations for tertiary hyperparathyroidism

There are no universal recommendations for the indications, timing, and modality of therapy of tertiary hyperparathyroidism. Vitamin D analogs typically have a negative biofeedback effect on the parathyroid glands, but their use may be limited in patients with hypercalcemia. Calcimimetics may be promising therapy in those with persistent hyperparathyroidism and hypercalcemia, as their use effectively lowers calcium levels, but decrease in PTH levels has not been demonstrated consistently [149–154]. An interaction between cinacalcet and tacrolimus leading to a moderate, but significant decrease in systemic exposure of tacrolimus has been described in renal transplant recipients, while cyclosporine and mycophenolate mofetil pharmacokinetics are not affected [155]. Although an improvement in BMD was shown in a small number of kidney transplant recipients treated with cinacalcet for 12 months, further studies and longer follow-up is needed to better elucidate the efficacy/safety profile of calcimimetics, particularly with regard to long-term bone histology and renal outcomes [156].

Parathyroidectomy is thought to be a definitive therapy for tertiary hyperparathyroidism after kidney transplantation. Some studies have demonstrated resolution of hypercalcemia and an increase in bone mineral density, along with improved blood pressure and lipid control, following parathyroidectomy [157–161]. There are two major indications for parathyroidectomy in renal transplant

patients: severe symptomatic hypercalcemia, usually occurring in the early post-transplant period, and persistent, marked hypercalcemia despite medical therapy. There is no consensus regarding the optimal timing for parathyroidectomy. Given the lack of evidence-based guidelines, a conservative approach of waiting for at least 12 months before considering parathyroidectomy in patients with tertiary hyperparathyroidism has been advocated. The use of cinacalcet along with careful monitoring of tacrolimus trough levels and calcium levels may be an alternative to parathyroidectomy, although it is not FDA approved for use in renal transplant recipients. Further studies are needed to determine the relative risk and benefits to surgical therapy of tertiary hyperparathyroidism after renal transplantation.

Vitamin D deficiency is an important contributor to elevated PTH in chronic kidney disease patients and often persists even after kidney transplantation [162,163]. No guidelines exist for vitamin D replacement in transplant recipients; therefore, in the absence of hypercalcemia, it is advised to maintain a 25-OH-vitamin D concentration greater than 30 ng/mL based on the recommendations of the National Kidney Foundation in the non-transplant population. Treatment with vitamin D requires a minimum 1000–2000 IU of vitamin D3 daily [164].

Table 100.6. Immunosuppression medications and bone disease

Immunosuppressive agent	Mechanism(s) of bone mineral density loss
Corticosteroids	Impair osteoblast bone formation by direct toxicity Decrease calcium gastrointestinal absorption Increase calcium urinary excretion Reduce gonadal hormone production (estrogen, testosterone, adrenal androgen) Reduce insulin-like growth factor–1 production Decrease sensitivity to PTH Increase receptor activator of NF-κ β ligand Increase osteoclastogenesis
Cyclosporine	Conflicting reports ? Increases osteoclast number and bone turnover ? Suppresses osteoblasts ? Decreases mineral apposition and bone formation rates
Tacrolimus	? similar effects as cyclosporine vs. less toxic
Mycophenolate	No evidence of adverse bone effects
Azathioprine	No evidence of adverse bone effects
Sirolimus	No human data to date; animal data: interferes with proliferation and differentiation of osteoblasts
Everolimus	No human data to date; animal data: decreases osteoclast-mediated bone resorption

Hypophosphatemia

Hypophosphatemia can develop in up to 93% of patients during the first few months after kidney transplantation [165], and very rarely after transplantation of other solid organs. The condition typically resolves within 1 to 3 months post-transplant but may persist for up to 10 years [165]. In general, encouragement to resume food rich in phosphorous is a sufficient therapy that is usually welcomed by the patient.

Persistent hypophosphatemia is primarily induced by renal phosphate wasting due to hyperparathyroidism, relative calcitriol deficiency, and the use of glucocorticoids and other immunosuppressants (mTor inhibitors and cyclosporine more than tacrolimus) [165–169]. In the early post-transplant period phosphaturic fibroblast growth factor-23 (FGF-23), which accumulates in patients with CKD, is a major contributor [165,170–173]. Yet, persistent hyperparathyroidism, but not increased FGF-23, appears to underlie the phosphate wasting that persists beyond one year [143].

Most patients with low phosphorus levels are asymptomatic. Plasma phosphate levels below 1.5 mg/dL can cause generalized muscle weakness. Severe hypophosphatemia, although generally rare post-transplantation, can be associated with acute respiratory failure, muscle weakness, hemolytic anemia, rhabdomyolysis, and altered mental status. The net effect of hypophosphatemia on bone density and risk for fracture is not well known; however, prolonged and severe hypophosphatemia could lead to osteomalacia and ricketts.

Most post-transplant hypophosphatemia will resolve spontaneously. Without knowledge about factors predictive of persistent or symptomatic hypophosphatemia, it is unclear which patients might benefit from pharmacological therapy. Phosphate supplementation (intravenous and/or oral) has been supported by some studies [174], but there is concern that phosphate replacement might create bursts of high phosphorus levels that would trigger more PTH production and thereby delay the resolution of secondary hyperparathyroidism. Others advocate that phosphorus replacement is unlikely to have any more significant an impact than watchful waiting and resumption of normal diet. A more rational approach may be to supplement with calcitriol, which increases tubular Na+/phosphate co-transporter activity and thereby enhances phosphate reabsorption [175]. Lowering PTH levels by treating tertiary hyperparathyroidism (whether medically or surgically), as discussed earlier, would also help treat hypophosphatemia by inhibiting PTH-induced phosphate renal wasting.

Role of immunosuppressive agents (Table 100.6)

The largest influence on early post-transplant bone loss appears to be the use of corticosteroids. Steroids impair bone formation by direct osteoblast toxicity and increased osteoclast activity and promote a negative calcium balance (by decreasing gastrointestinal absorption and increasing urinary excretion). They also reduce the production of gonadal hormone and insulin-like growth factor-1, decrease sensitivity to PTH, increase receptor activator of NF-κ β ligand, and increase osteoclastogenesis [176,177]. Steroid dose positively correlates with the amount of bone loss. Even small doses of steroids can result in significant bone loss [178].

The data on cyclosporine's influence on bone loss are conflicting. There are reports of cyclosporine being utilized in a corticosteroid-free regimen with no significant bone loss [179,180]. However, other data suggest that cyclosporine may contribute to bone loss by increasing osteoclast number and bone turnover, as evidenced by increased osteocalcin [181,182]. Cyclosporine appears to have more negative effect on bone than does tacrolimus [180,183,184].

Epidemiologic studies could not establish an association between CNIs and fracture risk [125,185]. There are no available data regarding the effects of mycophenolate and azathioprine on bone. A recent in-vitro study suggests that sirolimus might interfere with the proliferation and differentiation of osteoblasts in mice [186]; while a study of ovariectomized rats found that everolimus might reduce trabecular bone loss by decreasing osteoclast-mediated bone resorption [187].

Treatment with anti-resorptive agents: safety profile and side effects (Table 100.7)

Calcium and vitamin D analogs

Calcium supplementation of at least 1000 mg daily should be considered in non-hypercalcemic patients, since glucocorticoid therapy

Table 100.7. Pharmacological agents used for the management of mineral and bone disorders in solid organ transplant recipients

Drug family (brand names)	Effects on serum calcium and phosphorus	Effects on Serum PTH	Notes/evidence
Bisphosphonates			
Alendronate (Fosamax)	Decrease	Decrease	Increase bone mineral density, particularly at the lumbar spine.
Pamidronate (APD, Aredia)	Decrease	Decrease	May cause low turnover bone disease.
Zoledronate (Zometa, Aclasta)	Decrease	Decrease	Unclear whether they really reduces the risk of fractures.
Risedronate (Actonel)	Decrease	Decrease	Uniform use is not recommended.
Ibandronate (Boniva)	Decrease	Decrease	Contra-indicated when GFR <30 mL/min.
Etidronate (Didronel)	Decrease	Decrease	
Clodronate (Bonefos, Loron)	Decrease	Decrease	
Vitamin D			
Ergocalciferol (Drisdol)	Increase	Decrease	Improve bone mineral density.
Cholecalciferol (Calciol, vitamin D3)	Increase	Decrease	May cause hypercalcemia.
25(OH)D (calcidiol, calcifediol)	Increase	Decrease	
Vitamin D receptor activators			
1-Alfa-calcidiol (One-alpha)	Increase	Decrease	Improve bone mineral density.
Doxercalciferol (Hectoral)	Increase	Decrease	May cause hypercalcemia.
Calcitriol (Calcijex, Rocaltrol)	Increase	Decrease	
Vitamin D mimetics			
Paricalcitol (Zemplar)	No change or Increase	Decrease	Improve bone mineral density.
Calcimimetics			
Cinacalcet (Sensipar)	Decrease	Decrease	Not FDA approved for use in renal transplant recipients for secondary/tertiary hyperparathyroidism. May improve bone mineral density May interact with tacrolimus (tacrolimus levels should be carefully monitored)
Miscellaneous Agents			
Teriparatide (Forteo)	Calcium: Increase Phosphorus: Decrease	Increase	No proven benefit in transplant patients. Not recommended post-transplantation.
Calcitonin (Miacalcin, Fortical, Calcimar)	Decrease	Decrease	No proven benefit in transplant patients.

reduces intestinal calcium absorption. There is a high incidence of vitamin D deficiency in the transplant population and treatment with both 25-hydroxy- and 1,25-hydroxy-vitamin D is associated with improved bone mineral density as compared with no treatment [188–190]. Careful monitoring for hypercalcemia is necessary in recipients on vitamin D therapy.

Bisphosphonates

In the non-transplant population, bisphosphonates are used to prevent bone mass loss and to treat osteoporosis. Several clinical interventional trials confirmed the efficacy of bisphosphonates in the prevention and treatment of bone loss after transplantation in liver, heart, lung and kidney transplant recipients [191–196]. Notably, a majority of the studies have had inadequate statistical power to detect differences in fracture among treated and untreated patients. Results of meta-analysis of trials involving the treatment with bisphosphonates or active vitamin D analogs in patients with kidney, liver, heart, or lung transplants in the first 12 months post-transplant suggest a reduction in the number of subjects with fractures, vertebral fractures and improvement in bone mineral density in patients on bisphosphonates or vitamin D therapy [197]. When bisphosphonate trials were analyzed separately, there was a reduction in number of subjects with fractures, but no improvement in vertebral fractures.

Bisphosphonates should be used with caution in certain populations. Kidney transplant recipients treated with biphosphonates may be at risk for low turn over bone disease due to pre-exsting renal osteodystrophy, although bone biopsy studies showed conflicting results [193,198].

Similarly, biphosphonates should be avoided in patients with advanced CKD (stage 4–5), since renal excretion is the only route of elimination. Bisphosphonates accumulate in bone, with a half-life of more than 10 years, and gradually are released back into the circulation and taken up again or excreted [199]. Long-term use of bisphosphonates may lead to over suppression of bone formation, therefore bone biopsy should be considered in patients with CKD to exclude low-bone-turnover disease before starting therapy.

Teriparatide

Teriparatide is an amino-terminal parathyroid hormone with full physiological activity. It increases bone mineral density and decreases fracture risk in post menopausal women and in patients on steroid therapy [200,201]. In transplant recipients, no benefit of teriparatide use has been demonstrated to date [202]. Until further studies are conducted, its use is not recommended post-transplantation.

Calcitonin and other therapies

Another potential therapeutic agent is calcitonin, but it has no effect on mortality, graft loss, and risk of fracture in patients after kidney transplantation [203,204]. Regular exercise and hormone replacement therapy were shown to reduce bone loss and risk of fracture in lung and heart transplant recipients, but they have not yet been examined in kidney transplant recipients [205,206]. Early stages of osteonecrosis are generally managed conservatively or with decompression accompanied by bone grafting.

Management of bone disease

Prevention and management of bone disease in solid organ transplant recipients should focus on correcting any metabolic derangements which can affect bone metabolism and life style modifications to increase physical activity level. Formal guidelines for bone disease prevention and therapy are only available for kidney transplant recipients. The National Kidney Foundation Disease Outcomes Quality Initiative (KDOQI) guidelines recommend obtaining a DEXA (dual-energy X-ray absorptiometry) scan at the time of transplant, then at 1-year and 2-years post-transplant. If the T-score

is equal to or less than −2 at the time of the transplant or at subsequent evaluations, consideration should be given to therapy with bisphosphonates [207]. The KDIGO guidelines suggest that, in patients with low bone mineral density and GFRs greater than approximately 30 mL/min per 1.73 mm^2, treatment with vitamin D, calcitriol, or bisphosphonates should be considered [208]. The choice of these agents is determined by abnormal calcium, phosphate, PTH, alkaline phosphate, and 25-hydroxy Vitamin D levels. In non-renal solid organ transplant recipients, data from clinical trials suggest that bisphosphonates and vitamin D analogs are safe and effective agents for the prevention and treatment of post-transplantation osteoporosis and fractures [119,197].

Management of solid organ transplant recipients with low bone mineral density and a GFR less than 30 mL/min per 1.72 mm^2 should include dietary phosphate restriction and replacement of vitamin D stores. Biphosphonates should be used with caution in patients with advanced CKD and bone biopsy should be ideally done prior to the initiation of therapy [209].

Steroid withdrawal or avoidance immunosuppression protocols have been developed and found to retain a higher bone mineral density than that observed with standard steroid immunosuppression protocols. The greatest differences were seen in the lumbar spine. However, randomized controlled trials showed that late steroid withdrawal (when done several weeks to months post-transplant) is associated with an increased risk of acute rejection in kidney transplantation [210,211]. Therefore, current KDIGO guidelines do not recommend steroid withdrawal and avoidance for the sole purpose of treating bone disease.

Hyperuricemia and gout after solid organ transplantation

Hyperuricemia is common after solid organ transplantation. The etiology is complex and related to decreased uric acid excretion with reduced GFR, older age at transplant, obesity, metabolic syndrome, the presence of pretransplant hyperuricemia, and the use of diuretics and immunosuppressive agents. The prevalence of hyperuricemia in kidney transplant recipients may be as high as 80%, especially in recipients taking cyclosporine and close to 50% in liver transplant recipients [212,213]. The incidence of gout is much lower (6–7%) [213,214].

Elevations in serum levels of uric acid are associated with an increased risk for both cardiovascular and all-cause mortality in men in the general population [215]. Hyperuricemia has also been associated with insulin resistance and the development of metabolic syndrome [216]. The question of whether uric acid plays a causative role in the development of disease versus merely serving as a surrogate for co-morbid disease remains unclear.

The impact of asymptomatic hyperuricemia on morbidity and mortality in solid organ transplant recipients is unknown. Similarly, data are conflicting regarding the impact of hyperuricemia on allograft function in kidney transplant patients. One study demonstrated an association of hyperuricemia with decreased allograft function in 405 kidney transplant recipients over 3 years [217], whereas the results from a much larger prospective trial did not find an association [212].

Role of immunosuppressive agents

Calcineurin inhibitors

Cyclosporine induces a decrease in GFR and a net increase in tubular resorption of the filtered urate, leading to hyperuricemia [218,219]. Tacrolimus shares many of the same hemodynamic and toxic effects as cyclosporine, but the incidence of hyperuricemia appears to be lower in tacrolimus-based regimens [220]. Dose reduction strategies of CNIs have been employed as part of immunosuppression management to address hyperuricemia and gout. One report documented a decrease in uric acid after conversion from cyclosporine to tacrolimus, but the data are limited [221].

Other immunosuppressive agents

Long-term use of glucocorticoids is associated with the development of insulin resistance and the metabolic syndrome. Hyperuricemia has also been associated with the metabolic syndrome, although there are no definitive studies linking glucocorticoid use and hyperuricemia [216,222].

Azathioprine, mycophenolate mofetil and sirolimus have not been shown to significantly influence uric acid.

Role of diuretics

Treatment with loop and thiazide diuretics leads to an increased risk of gout and hyperuricemia. Diuretic therapy has a dose-dependent association with increases in serum uric acid. This is due to an induced relative hypovolemia, leading to an increase in tubular urate reabsorption and a decrease in urate excretion [223].

Therapeutic considerations for hyperuricemia and gout

Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) act through the non-selective inhibition of cyclooxygenase enzymes, resulting in decreased synthesis of prostaglandin and various mediators of inflammation. While effective for the treatment of acute gouty attacks in the non-transplant population, the high prevalence of decreased kidney function in the transplant population and use of CNIs limit their use. Short-term therapy with NSAIDS may be used in transplant patients with normal kidney function.

Glucocorticoids

The anti-inflammatory effects of glucocorticoids are mediated through decreased peripheral migration of leukocytes into the area of inflammation. In a randomized control trial, therapy with systemic oral corticosteroids (35 mg of oral prednisolone for 5 days) was compared to naproxen in 120 non-transplant patients during an acute gout flare. The safety and efficacy of prednisolone was comparable to naproxen [224]. While similar studies in transplant recipients have not been conducted, a short course of corticosteroids is considered to be effective. Intra-articular preparations may be reasonable options for therapy in the setting of monoarticular disease.

Colchicine

Colchicine is commonly used in the non-transplant population for both acute gout flare and prophylaxis. It provides rapid pain relief (within 48 hours), but its significant side effect profile may limit its use in transplant recipients. Colchicine-induced myopathy is especially seen in the context of renal function impairment; accordingly, alternative treatment should be considered in patients with chronic kidney disease stage 3–5 [225]. Myotoxicity related to colchicine has been reported in both kidney and heart transplant recipients [226,227]. Cyclosporine co-administration increases colchicine toxicity by a dual mechanism: cyclosporine inhibits P-glycoprotein, which leads to increased intracellular colchicine concentrations and

decreased hepatic and renal excretion of the drug and cyclosporine interacts with CYP3A4, which decreases the hepatic elimination of colchicine [228,229]. Concomitant statin use increases the risk of muscle injury. Myelosuppression, not uncommon in kidney transplant recipients, may also be caused by colchicine.

Allopurinol

Allopurinol is a purine analog and inhibitor of the enzyme xanthine oxidase, which is necessary for production of uric acid [228]. Allopurinol is often the first choice of therapy for long-term treatment of hyperuricemia and gout. Co-administration with azathioprine may lead to severe myelosuppression due to inhibition of metabolism of its active metabolite, mercaptopurine; thus, alternative therapy should be considered. If that is not possible, the azathioprine dose should be reduced by 50–75% and a reduced dose of allopurinol should be used [230]. Despite dose adjustment, pancytopenia can be seen after months or even years of therapy [231]. Dose adjustment is necessary for CKD.

Febuxostat

Febuxostat is a non-purine inhibitor of xanthine oxidase, shown to be more effective in reducing uric acid than allopurinol in clinical trials [232]. While no dose reduction is required for patients with CKD stage 3, data are insufficient for patients with more advanced kidney disease [225]. The risk for myelosuppression is increased with co-administration of azathioprine.

Uricosuric agents

Probenecid lowers uric acid by increasing renal excretion. It is ineffective in patients with decreased renal function. Moreover, it may lead to deposition of uric acid crystals in the kidney, resulting in urate nephropathy and/or uric acid stones. A more potent uricosuric agent, benzbramorone, is effective in patients with kidney disease and has been shown to be effective in kidney transplant recipients, but hepatoxicity is a significant concern [230,233].

Losartan has been shown to have modest uricosuric effects along with its antihypertensive activity, an effect not demonstrated with other angiotensin receptor blockers [234].

Pegloticase

Peglioticase, mammalian recombinant uricase, allows for metabolism of uric acid to its solube metabolite, allantoin, and has effectively lowered uric acid in patients with gout who are intolerant to allopurinol or refractory to treatment [235]. It has not been thoroughly studied in patients with chronic kidney disease and solid organ transplant recipients.

Management of gout

The evidence outlining treatment goals for patients with asymptomatic hyperuricemia is conflicting and generally not recommended in transplant recipients because of insufficient data and the high potential of side effects. Treatment is necessary, however, for symptomatic hyperuricemia such as gout and uric acid stones. Therapeutic strategies target both the acute inflammatory response associated with gouty flares and hyperuricemia. Dietary modifications, such as decreased consumption of alcohol, seafood, and red meat, are important lifestyle components. Foods containing fructose are of concern because their consumption prompts a rapid increase in serum uric acid [222].

The risks and benefits of anti-gout therapeutic agents need to be carefully examined in the context of other co-morbidities and their treatment. Hypertension and hyperlipidemia are common in the transplant population, and agents for their treatment may affect uric acid levels or potentiate toxicity. Diuretics should be avoided, if possible, and alternative agents may need to be used for treatment of hypertension.

For the acute gout flare, short-term corticosteroids can be administered. Short therapy with NSAIDS may also be used in patients with normal kidney function. Prolonged use of colchicine should be avoided in patients with eGFR $<60\,mL/min/1.73\,m^2$. If absolutely necessary, colchicine can be used at reduced doses for <1 week in patients with eGFR $>10\,mL/min/1.73\,m^2$ and not requiring dialysis [20].

Long term, agent-lowering uric acid may need to be started. Allopurinol is commonly used, but adjustment of immunosuppression may be necessary and dose reduction is needed in kidney disease. Newer hypouricemic agents, like febuxostat or pegloticase, may prove to be useful in transplant recipients, but more studies are needed.

Electrolyte disturbances after solid organ transplantation

Hyperkalemia

Hyperkalemia is the most frequent electrolyte disturbance encountered after solid organ transplantation, especially in the first few weeks. Its cause is almost always multifactorial, including increased dietary intake, redistribution and decreased renal excretion. Medications commonly used in transplant recipients, such as trimethoprim and CNIs, increase the risk of hyperkalemia. Trimethoprim induces hyperkalemia by inhibiting potassium elimination in the distal nephron, and this effect is especially seen in patients with impaired kidney function [236]. CNIs impair aldosterone production, renal response to aldosterone, and inhibit potassium secretion in the collecting duct [237,238].

Acute treatment of hyperkalemia in solid organ transplant recipients follows general therapy in the non-transplant population. Options include intravenous calcium gluconate, glucose followed by insulin injection, sodium bicarbonate, or even hemodialysis (when other therapies fail). Treatment is guided by the degree of renal function, presence or absence of EKG changes, and the absolute level of potassium. Replacement of certain drugs such as trimethoprim may be necessary; in most cases it is sufficient to decrease serum potassium levels. Chronic management of hyperkalemia includes optimization of renal function, use of potassium-wasting agents such as resin binders and mineralocorticoids (e.g. fludrocortisone) and, in severe cases, minimization or withdrawal of calcineurin inhibitors.

Hypomagnesemia

Hypomagnesemia is very common in solid organ transplant recipients treated with CNIs. It typically manifests in the first few weeks and may persist with the chronic use of CNIs. Approximately 80% of serum magnesium is freely filtered in the glomerulus and more than 95% is reabsorbed throughout the tubules. CNIs have direct magnesiuric tubular effects; they decrease magnesium reabsorption and increase urinary magnesium wasting, thereby causing hypomagnesemia [239].

Serum magnesium levels are associated with a faster rate of decline in kidney allograft function and increased rates of graft loss in renal transplant recipients treated with cyclosporine [239]. Severe hypomagnesemia may be associated with arrhythmias and

seizures. Hypomagnesemia is also an independent predictor of new onset post-transplant diabetes mellitus in kidney transplant recipients [240]. Oral magnesium-oxide supplementation may be chronically needed when dietary supplementation fails to maintain the magnesium level, especially in patients treated with CNIs.

Metabolic acidosis

Metabolic acidosis after solid organ transplant can be caused by decreased kidney function and CNIs. In animal studies, CNIs were shown to induce metabolic acidosis through dysregulation of major acid-base transporters in the proximal tubule and the distal nephron [241]. Post-transplant recipients on CNIs may present with hyperkalemic metabolic acidosis (type IV renal tubular acidosis), resulting from inhibition of potassium secretion in the collecting duct [237]. Treatment to correct the acidosis and hyperkalemia should be promptly initiated. Pancreas transplant recipients with bladder-drained pancreata may present with metabolic acidosis due to bicarbonate loss in the urine from pancreas secretions. Due to this major side effect of pancreas bladder drainage, along with chronic volume depletion, enteric-drained pancreas transplantation has gained popularity. Moreover, enteric drainage conversion leads to correction of metabolic acidosis in most of the cases [242]. Oral sodium bicarbonate supplementation has been shown to preserve renal function in non-transplant chronic kidney disease patients, but data on post-transplant kidney outcomes are lacking [243].

Summary

Disorders of metabolic pathways and mineral homeostasis are common in solid organ transplant recipients. In the early postoperative period, electrolyte abnormalities like hyperkalemia, hypercalcemia, hypomagnesemia, and hypophosphatemia are frequently seen. In the long term, recipients require close monitoring and management of co-morbidities like diabetes, hyperlipidemia, bone disease and gout. Treatment is especially challenging due to potential interactions with the transplant medications and variations in renal and hepatic function. In this chapter we review the therapeutic options available for treating metabolic and electrolyte disorders post-transplant with a particular focus on the potential side effects and drug interactions of commonly used medications.

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CHAPTER 101

Off-Label Use of Immunosuppressive Agents in Solid Organ Transplantation

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Introduction

The Food and Drug Administration (FDA) is charged by statute with maintaining public safety for food and drugs, which is assured through a stringent regulatory drug approval process. In order for a drug to be approved by the FDA, the sponsor must provide satisfactory evidence regarding the safety and efficacy of a drug in a specific patient population and a defined clinical condition. Inherent to the drug approval process is the creation and definition of the product label, which includes dosing, administration, intended patient population and clinical condition. Use of the drug outside of these aspects of the label is generally considered off-label use. In theory, the FDA has regulations regarding off-label drug use and promotion by manufacturers but in practice this regulation is limited [1].

In the US it is legal, and common practice, for physicians to choose to use FDA approved drugs outside the defined label [2,3]. Off-label use may differ from labeled uses in one or more of the following aspects, including: clinical indications, patient populations, dosing, or route of administration. The term "unlabeled" is preferred to "unapproved" as the FDA has recognized that these unlabeled uses may be appropriate and accepted and supported by evidence in medical journals [4]. While the term "off-label" derives from the US FDA, the concept of drug use expanding beyond the specific drug indication is commonplace worldwide, and as such, this chapter's applicability extends well beyond national borders. Indeed, within solid organ transplantation, off-label use has become so common it is regarded as "standard of care" for most immunosuppressive regimens, and is supported by a substantial evidence base provided by numerous randomized trials (Table 101.1). This chapter will cover the concept and practice of off-label drug use, and in doing so, serve as a complement to Chapter 98, which deals with drugs formally indicated for transplant use. Detailed mechanistic information regarding many of the agents listed in this chapter can be found in Chapters 17 and 18.

Off-label use in transplantation

The extent of off-label drug use in modern medicine is significant, and especially common in the practice of transplantation. It has been estimated that off-label drug use represents 20% of the 750 million prescriptions per year in the US [2]. Transplantation and oncology are two medical specialties where off-label drug use has become common practice. It is estimated that 50% or more of the drugs use in oncology are off-label [5,6]. A primary reason for this has been the large number of individual cancers and that anticancer agents are generally approved initially for a specific cancer. The recent advent of molecular profiling in oncology has led to the recognition of shared gene mutations among a large number of cancers; therefore a drug that targets a specific gene mutation may enjoy substantial off-label use in other cancer types with similar gene mutations.

In transplantation, the extent of off-label use varies considerably between individual drugs and across various organ types. A highly visible example of off-label use in transplantation is the combination of tacrolimus and mycophenolate for the prevention of rejection in kidney transplant recipients. Tacrolimus was approved in 1994, shortly prior to FDA approval of mycophenolate. Studies for both tacrolimus and mycophenolate were ongoing simultaneously, but independently and therefore were not conducted with these agents in combination. When first approved, mycophenolate mofetil (MMF) was labeled for use with cyclosporine, as no data existed regarding its safety and efficacy with tacrolimus. Almost immediately after MMF approval, studies were initiated evaluating the tacrolimus/MMF combination, as many centers considered tacrolimus the preferred calcineurin inhibitor (CNI) over cyclosporine. Tacrolimus is widely considered a more potent immunosuppressive agent and is associated with better renal function [7]. Large multicenter randomized trials were conducted with the tacrolimus/MMF combination that confirmed the efficacy and safety of the regimen [8,9]. Today, tacrolimus is the most commonly utilized CNI in kidney transplant with approximately 80% of kidney transplant recipients discharged on tacrolimus. Similarly, the tacrolimus/MMF combination is the most commonly prescribed regimen, with over 70% of kidney transplant recipients receiving the combination at the time of hospital discharge from kidney transplantation operation [7,10].

Although the tacrolimus/MMF combination was the most frequently used immunosuppressive regimen in kidney transplant recipients for several years, it was regarded as off-label until 2009 [11]. In 2006, a supplemental New Drug Application (sNDA) was submitted by Astellas, the manufacturer of brand Prograf™ (tacrolimus), to seek FDA approval for the tacrolimus/MMF regimen for rejection prophylaxis in kidney transplantation. In a novel regulatory action for the solid organ transplant field, the FDA granted

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approval for the tacrolimus/MMF combination based on submission of a large number of multicenter, randomized trials that demonstrated efficacy and safety in lieu of prospectively conducted phase 3 registration trials. This action by the FDA set a precedent in the transplant field by accepting a large evidence base from non-registration randomized trials as a basis for approval.

The FDA has traditionally required the use of FDA approved immunosuppressive drug combination as the control arm in registration trials. Prior to the approval of the tacrolimus/MMF combination, this policy required control arms consisting of an interleukin-2 receptor antagonist (IL-2RA) for induction therapy with cyclosporine, MMF, and steroids as maintenance therapy [7]. The perception of therapeutic inferiority of this cyclosporine-based regimen as a control group substantially limited patient enrollment in registration trials, thereby creating a substantial burden for sponsors. This FDA policy, therefore resulted in sponsors avoiding drug development in the transplant field, and led to a shift in immunosuppressive drugs being developed in autoimmune diseases rather than transplantation. This trend has continued to the present day, to the disadvantage of transplant patients.

At the same time, endpoints historically considered acceptable to the FDA and practical in terms of drug development by sponsors were undergoing substantial change. As patient and graft survival rates continued to improve with advances in immunosuppression, the number of patients required to achieve statistical power exceeded budgetary limits for sponsors. Similarly, biopsy-proven acute rejection (BPAR), which is associated with significant morbidity and is considered a traditional endpoint for registration trials, had fallen to such a low rate that it also became an unfeasible endpoint due to the large numbers of patients required to achieve statistical power [12,13]. As a result, composite or surrogate endpoints (e.g. change in renal function) allow for more reasonable sample sizes, and have been more recently accepted by the FDA for registration trials. Clinical investigators largely consider the FDA approval of the tacrolimus/MMF regimen paramount in creating the ability to use the regimen as the control arm in future registration trials.

The problem of clinically inferior FDA labeled regimens being required as control group therapies has also complicated registration trials for rabbit anti-thymocyte globulin (rATG, Thymoglobulin™, Sanofi) for induction in kidney transplantation. Currently, rATG is only FDA approved for the treatment of rejection in kidney transplantation, and is not FDA approved for induction therapy following the transplant procedure, which is a greater volume of use. Compared to other available agents for induction therapy in kidney transplantation, rATG is the most commonly used agent, and is considered by most transplant programs the induction agent of choice for patients at high immunologic risk, delayed graft function, and corticosteroid avoidance or minimization protocols. These considerations made induction trials in kidney transplantation unfeasible because of anticipated negative effects on enrollment without rATG as an induction agent in the control group. An approach similar to that used for the tacrolimus/MMF combination has been suggested for the concerns related to rATG as the comparator for registration trials of induction agents in kidney transplantation. Investigators with substantial experience in drug development within the transplant field have vocalized this observation:

> I think it is important for transplant professionals (through their societies) to engage the FDA on regulatory matters for drugs used off label where it's clear either because of patent expiration or unwillingness of the transplant community to have a placebo arm such as depleting antibodies in high immunologic risk patients to approve them when there is enough data from registry and multicenter trials. As in the case of TAC-MMF combo, it's time to proceed with the approval of thymoglobulin for induction in high-risk patients. We have demonstrated that a respectful yet determined advocacy can yield results. [Personal Communication, Flavio Vincenti, MD] [14]

FDA regulatory process for new drug approval

In order to obtain FDA approval, a drug company must file a New Drug Application (NDA) or Biologic License Application (BLA), which provides data from registration trials that support a proposed indication for drugs proving efficacy and safety [15]. A sNDA is filed whenever a company wants to expand the labeled indication of a drug (e.g. the process through which the tacrolimus/mycophenolate combination was approved). An Investigational New Drug (IND) Application is filed when a company would like to gain approval for a new or novel clinical indication [15].

Orphan drug designation provides an alternative approach for FDA approval under specific conditions. Orphan drug designation can be sought for drugs used for treatment, prevention or diagnosis of rare diseases and conditions that affect fewer than 200 000 people in the US [15]. Orphan drug designation can also be given to drugs used in rare disease affecting more than 200 000 but which the market will never help to recover the cost of development. With approximately 16 000 kidney transplants performed in the US in 2009, and even fewer among other solid organs, all transplants would qualify as rare conditions [10].

Medicare reimbursement approval process for off-label drug use

Over the past several years, payor reimbursement has become the greatest barrier to off-label use of immunosuppressive agents (ISA). Private health insurance carriers and managed care providers have become increasingly resistant to reimburse drugs (particularly expensive drugs) when used outside the FDA approved indication. This issue often precludes off-label use of drugs as immunosuppressive agents.

The Office of Inspector General (OIG) of the Department of Health and Human Services (HHS) issues a yearly policy statement regarding prescription drug coverage that provides the basis for drug coverage by the Centers for Medicare and Medicaid Services (CMS), health insurance coverage for ISA use in transplant recipients. This provides the basis by which other insurance payors develop coverage policies. The fiscal year 2012 OIG policy stated that:

> . . . for prescription drugs to be covered, Federal law generally requires that they are prescribed according to the medically accepted indications, such as those approved by the FDA or supported in one or more of the authoritative drug compendia identified by the Secretary of HHS [16].

This policy thereby dictates that off-label use of drugs is covered if drug compendia report sufficient evidence that the drug is safe and effective for that clinical disease or condition.

Historically, the Social Security Act (Section 1861(t)(2)(B)(ii)(I)) recognized three compendia as acceptable sources for medically accepted drug indications for off label use. These three compendia included the Medical Association Drug Evaluations (AMA-DE),

United States Pharmacopoeia-Drug Information (USP-DI) or its successor publication [amended in Section 6001 (f)(1) of the DRA] and American Hospital Formulary Service-Drug Information (AFHS-DI). Current Medicare-recognized compendia have included American Hospital Formulary Service Drug Information (AHFS-DI), Clinical Pharmacology and DrugDex [16]. For anticancer drugs, the National Comprehensive Cancer Network (NCCN) Drugs and Biologics Compendium is utilized. Other insurance carriers may utilize the same or different compendia.

Thomson Reuters, maintains the DrugDex compendia (a database within the larger Micromedex resource) that has long been dedicated to including information regarding off-label drug use [17]. Off-label indications are monitored through ongoing evaluation of primary literature and influential sources of drug information, such as the FDA, National Institute of Health (NIH), and the Center for Disease Control and Prevention (CDC). Additionally, the database accepts requests for additions to off-label indications. For every drug, all FDA approved indications are included, as well as various off-label indications. Each indication is evaluated similarly with a rating for strength of efficacy, strength of evidence, and strength of recommendation (Table 101.2) [17]. Efficacy is given a Class I, IIa, IIb, or III designation based on whether or not available evidence favored efficacy. Class I is considered the highest level of effectiveness. The strength of evidence is given a category A, B, C or "no evidence." Category A evidence is based on meta-analysis and/or randomized, controlled trials. The overall strength of recommendation takes in to account the efficacy and evidence rating. Class I, IIa, or IIb are considered acceptable for use, Class III rating is not recommended [17].

American Hospital Formulary Service – Drug Information (AHFS-DI), another HHS-recognized drug compendium, is a resource that aims to provide information on the safe and effective use of drugs based on available evidence. A vital part of the drug resource's mission is to evaluate evidence for the off-label use of drugs. Off-label drug use recommendations are provided by AHFS-DI by the presence of supportive narrative. AHFS-DI data is also available through the Lexicomp drug resource [18].

The Medicare benefit policy states that an off-label drug use may be covered if supportive text is provided in AHFS-DI or Clinical Pharmacology; or is given Class I, IIa or IIb recommendation in DrugDex [19]. However, coverage decision processes are complicated by inconsistencies by the various drug compendia resources in their support and evidence for off-label ISA use. The support of off-label drug use by AHFS-DI is based on whether the use is discussed and whether the narrative present is supportive or not supportive (Table 101.2). However, the extent of this narrative is extremely limited. The majority of the ISA listed in Table 101.1 have no narrative within AHFS-DI regarding the off-label use and therefore no support for its use. In contrast, DrugDex provides a rating for whether the off-label use is acceptable or not. The compendium goes on to provide ratings for strength of evidence and efficacy.

Discrepancies exist between AHFS-DI and DrugDex compendia complicate coverage decisions regarding off-label ISA use. For example, the use of sirolimus to prevent rejection in heart transplant is rated III (unacceptable for off-label use) yet available evidence was found to favor efficacy. Given these facts, the basis for a rating of unacceptable is unclear. Interestingly, AHFS-DI did not provide a narrative for the same off-label use.

Significant discrepancies exist between AHFS-DI and DrugDex in supporting rATG use outside the labeled indication of treatment of renal allograft rejection (current FDA approved indication). Within AHFS-DI, off-label use listed for transplantation consists only for prevention of renal allograft rejection. Specifically, AHFS-DI does not mention rATG for treatment or prevention of rejection in heart or pancreas transplantation, and does not include narrative text supporting this use. In contrast, DrugDex supports the use of rATG for the treatment and prevention of rejection in heart transplant recipients, and is assigned a Class IIb recommendation, which is often considered satisfactory for OIG approval of off-label use.

The shortcomings of compendia for determination of coverage for ISA may be best represented by the DrugDex assignment for rATG use as induction therapy in kidney transplantation. DrugDex assigned rATG a Class III (i.e. not recommended) for prevention of rejection in renal allografts despite providing a supporting rating for efficacy and strength of evidence.

The lack of support for this off-label use of rATG for induction in renal transplantation is difficult to justify given the state of clinical use and the existing evidence base. According to the Scientific Registry of Transplant Recipients (SRTR), rATG was the most commonly used induction agent in 2009, nearly 48% of kidney transplant recipients received rATG, and the agent is largely considered standard of care across US transplant centers [10]. Additionally, this off-label use of rATG for induction therapy is extensively supported by scientific literature [20–24]. This discrepancy between the drug compendia, clinical practice, and available scientific evidence provides a cogent reason for a rational re-evaluation of the current state of compendia. The issue presents considerable challenges for transplant providers in providing optimal care for their patients. One approach for dealing with the problems presented by compendia would be for the major professional transplant societies (American Society of Transplant Surgeons and the American Society of Transplantation) to become engaged in developing and providing the scientific evidence base that is used in developing compendia-based classifications and narratives.

An additional reimbursement option of off-label ISA is providing source documentation of therapeutic efficacy in peer-reviewed journal articles. The use of off-label drugs may be considered acceptable if validated by authoritative medical literature or standard of care practices. For the off-label use of anticancer drugs and biologics, OIG and Medicare have provided specific details for how medical literature will be evaluated and accepted [19]. This policy provides a list of acceptable literature sources, specific to investigation of anticancer drugs. In terms of biologics in transplantation, the major solid organ transplantation academic journals are not included in the list of approved medical literature, thereby seriously complicating the issue of Medicare coverage for off-label ISA use. This situation offers an opportunity for major professional societies in the transplant field to engage and influence established approaches.

Off-label drug use within the oncology patient population has been well recognized and addressed through policies by OIG. However, OIG has not applied a similar degree of effort and rigor for off-label ISA use in transplantation. The lack of OIG policies regarding off-label drug use in transplantation is further complicated by the presence of more significant time constraints within transplantation — transplants are often performed on an urgent basis and acute rejection therapy must be instituted on an emergency basis. These observations provide a cogent rationale for a greater concentration of effort with respect to federal policy development and regulatory application of policy.

Table 101.1. FDA-Approved and off-label clinical uses of immunosuppression. Adapted from: Micromedex [Internet database]

Drug	FDA approved indication	Off-label clinical uses in transplant and drug compendia recommendation				
			AHFS-DI	DrugDex©		
				Overall	Evidence	Efficacy
Tacrolimus	• Heart: Prevention of rejection	Heart: Treatment of rejection	NN	None	None	None
	• Liver: Prevention of rejection	Liver: Treatment of rejection	NN	IIb	B	Favors, IIa
	• Kidney: Prevention of rejection	Kidney: Treatment of rejection	Supportive	IIa	B	Favors, IIa
		Lung Transplant	NN	IIb	C	Favors, IIa
		Kidney/pancreas: Prevention of rejection	NN	IIa	C	Favors, IIa
		Pancreas: Treatment of rejection	NN	IIa	B	Favors, IIa
Cyclosporine	• Heart: Prevention of rejection	Lung: Prevention of rejection	NN	IIa	B	Favors, IIa
	• Heart: Treatment of rejection	Lung: Treatment of rejection	NN	IIa	B	Favors, IIa
	• Liver: Prevention of rejection	Pancreas: Prevention of rejection	NN	None	None	None
	• Liver: Treatment of rejection	Pancreas: Treatment of rejection	NN	None	None	None
	• Kidney: Prevention of rejection					
	• Kidney: Treatment of rejection					
Sirolimus	• Kidney: Prevention of rejection (low, moderate, high immunologic risk)	Heart: Prevention of rejection	NN	III	B	Favors, IIa
		Kidney: Treatment of rejection	NN	IIb	B	Favors, IIa
		Liver: Prevention of rejection	Not Supportive	III	B	Inconclusive
Everolimus	• Kidney: Prevention of rejection	Heart: Prevention of rejection	NN	III	B	Favors, IIa
		Kidney: Treatment of rejection	NN	None	None	None
Belatacept	• Kidney: Prevention of rejection in when used in combination with basiliximab induction in EBV seropositive recipients	Kidney: Prevention of rejection when used in combination with anti-thymocyte globulin induction	NN	None	None	None
Mycophenolate mofetil	• Heart: Prevention of rejection	Kidney: Treatment of rejection	NN	IIb	B	Favors, IIa
	• Liver: Prevention of rejection	Kidney: Alternative regimen	NN	IIb	B	Favors, IIa
	• Kidney: Prevention of rejection					
Mycophenolic acid	• Kidney: Prevention of rejection	Kidney: Treatment of rejection	NN	None	None	None
Azathioprine	• Kidney: Adjunct therapy for prevention of rejection	Liver: Prevention of rejection	NN	IIb	B	Favors, IIa
		Pancreas: Prevention of rejection	NN	IIb	B	Favors, IIa
Basiliximab	• Kidney: Prevention of rejection in combination with cyclosporine and corticosteroids	Liver: Prevention of rejection	NN	IIb	B	Favors, IIa
Rabbit anti-thymocyte Globulin	• Kidney: Treatment of rejection, in conjunction with concomitant immunosuppression	Heart: Prevention of rejection	NN	IIb	B	Favors, IIa
		Heart: Treatment of rejection	NN	IIb	B	Favors, IIa
		Liver: Prevention of rejection	NN	IIb	C	Favors, IIa
		Kidney: Prevention of rejection	Supportive	III	B	Favors, IIa
		Lung: Prevention of rejection	NN	IIb	C	Favors, IIa
		Pancreas: Prevention of rejection	NN	IIb	B	Favors, IIa
		Pancreas: Treatment of rejection	NN	IIb	B	Favors, IIa
Horse anti-thymocyte globulin	• Kidney: Prevention of rejection	Alternative to rabbit anti-thymocyte globulin in non-renal organs	Supportive	None	None	None
	• Kidney: Treatment of rejection					
Alemtuzumab	• B-cell chronic lymphoid leukemia	Kidney: Prevention of rejection	NN	IIb	B	Favors, IIa
Rituximab	• Rheumatoid arthritis (moderate to severe)	Treatment of PTLD	NN	IIb	B	Favors, IIa
	• CD20-positive chronic lymphoid leukemia	Desensitization regimens	NN	None	None	None
	• CD20-postiive non-Hodgkin's lymphoma	Treatment of antibody-mediated rejection	NN	None	None	None
Bortezomib	• Multiple myeloma	Desensitization regimen in combination with plasmapheresis	NN	None	None	None
	• Mantle cell lymphoma					
		Treatment of antibody-mediated rejection	NN	None	None	None

Off-label use of immunosuppressive drugs in transplantation

Over the past several years, three drugs with FDA indications for oncology have seen used off-label as immunosuppressants in transplantation: alemtuzumab, rituximab, and bortezomib. The following sections detail the published clinical use of these oncologic agents in transplantation, and how they have become standard practice at a number of transplant centers.

Alemtuzumab

Alemtuzumab (Campath-1H™) is a monoclonal antibody specific for the CD52 antigen expressed on T and B lymphoctyes, monocytes, macrophages and eosinophils [25,26]. Alemtuzumab was originally FDA approved for treatment of B-cell chronic lymphocyte leukemia (CLL). This cytoloytic agent, which causes rapid and sustained lymphocyte depletion, has been extensively studied in solid organ transplant as induction therapy. Alemtuzumab was approved for use in the US in 2001 for CLL, but was used prior to this outside the US. In 1998, a study in the UK investigated the use of alemtuzumab as induction therapy in 13 kidney transplant recipients [26]. A 5-year follow-up study of a larger group of patients was published in 2005 [27]. Alemtuzumab, in combination with low-dose cyclosporine, was found to be safe and effective when compared to standard therapy with respect to patient and graft survival [27]. Another study conducted in three centers in Asia, suggested alemtuzumab was an effective induction agent in steroid-free regimens [28].

Randomized studies comparing alemtuzumab to rATG and IL-2RAs (daclizumab or basiliximab) have provided a substantial evidence base supporting the off-label use of alemtuzumab as induction therapy in kidney transplantation. Cianco et al. published findings at 15 months of patients randomized to alemtuzumab,

Table 101.2. Definition of recommendations and ratings in drug compendia. Adapted from: Micromedex [Internet database]

Drug Compendia			
AHFS-DI	Narrative text is ***supportive*** — considered acceptable off-label drug use Narrative text is ***non-supportive*** — considered unacceptable off-label drug use ***No narrative (NN)*** in supportive text		
DrugDex©	Acceptable off-label drug use	Class I	Recommended. Treatment has been proven to be useful and should administered
		Class IIa	Recommended, in most cases. Treatment is generally considered to be useful, and indicated in most cases
		Class IIb	Recommended, in some cases. Treatment may be useful, and is indicated in some, but not most, cases
	Unacceptable off-label drug use	Class III	Not recommended. Treatment is not useful, and should be avoided
	Strength of Evidence	Category A	Evidence is based on data derived from: meta-analysis of randomized controlled trials with homogeneity with regard to the directions and degrees of results between individual studies. Multiple, well-done randomized clinical trials involving large numbers of patients
		Category B	Evidence is derived from: meta-analysis of randomized controlled trials with conflicting conclusions with regard to directions and degrees of results between individual studies. Randomized controlled trials that involved small numbers of patients or had significant methodological flaws (e.g. bias, drop-out-rate, flawed analysis, etc.). Nonrandomized studies (e.g., cohort studies, case-control studies, observational studies)
		Category C	Evidence is based on data derived from: expert opinion or consensus, case reports or case series
		No Evidence	
	Efficacy	Class I	Effective. Evidence and/or expert opinion suggests that a given drug treatment for a specific indication is effective
		Class IIa	Evidence favors efficacy. Evidence and/or expert opinion is conflicting as to whether a given drug treatment for a specific indication is effective, but the weight of evidence and/or expert opinion favors efficacy
		Class IIb	Evidence and/or expert opinion is conflicting as to whether a given drug treatment for a specific indication is effective, but the weight of evidence and/or expert opinion argues against efficacy
		Class III	Evidence and/or expert opinion suggest that a given drug treatment for a specific indication is ineffective

rATG or daclizumab, and found no difference in acute rejection rates, renal function, or patient or graft survival [29]. The best evidence supporting off-label use of alemtuzumab in transplantation derives from the multicenter randomized INTAC trial in which alemtuzumab was compared to either basiliximab (in low immunologic risk patients) or rATG (in high immunologic risk patients). In this study, alemtuzumab was found to have similar efficacy to rATG and potential superiority to basiliximab in low-risk patients [24]. Additionally, there have been several other non-randomized published trials of alemtuzumab for induction. Despite this use of alemtuzumab as induction therapy, Genzyme-Sanofi, has not sought FDA approval for alemtuzumab in kidney transplantation.

In 2009, approximately 13% of transplant centers utilize alemtuzumab in kidney transplant recipients [10]. Although the use of alemtuzumab has been mostly in kidney transplant, alemtuzumab has been used in other solid organ transplant recipients. According to the SRTR alemtuzumab was used in 12% of intestinal transplants, 8% of lung transplants, and 11–12.6% of pancreas transplant (simultaneous pancreas-kidney, pancreas after kidney, and pancreas alone) [10]. Alemtuzumab has also been used in a more limited respect as induction therapy in liver transplantation and in pediatric transplant recipients.

Unfortunately, it appears that alemtuzumab is unlikely to receive FDA approval for use in transplantation. In September 2012, it was announced that alemtuzumab (under the proprietary name Campath) was no longer going to be made available through usual drug distribution mechanisms, and would only be available through the US Campath Distribution Program [30]. It is widely believed that this move was preparatory for Sanofi's efforts to gain FDA approval for alemtuzumab use in multiple sclerosis. This decision by Sanofi was likely based on market potential considerations, as inflammatory and autoimmune disorders have substantially greater market potential and therefore profitability margins. Despite the provision of free alemtuzumab via the US Campath Distribution Program for indications outside of multiple sclerosis, the long-term sustainability of such a program is subject to question. Alemtuzumab is an exemplary case of the issues faced by the transplant field in terms of drug development. The relatively smaller market size represented by transplantation provides a smaller potential financial profitability in comparison to autoimmune diseases and oncology indications. These considerations have resulted in a shift in drug development for new immunosuppressive agents away from transplantation and toward oncology or autoimmune diseases.

Rituximab

Rituximab is a monoclonal antibody specific for the CD20 antigen on B-lymphocytes. The exact mechanism of rituximab is complex. However, B cell depletion, the most prominent effect, is mediated via several potential pathways, including complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and apoptosis [31]. Rituximab is FDA approved for treatment of: CD20-positive non-Hodgkin's lymphomas, CD20-positive CLL, moderate to severe active rheumatoid arthritis (in combination with methotrexate) in adult patients with inadequate response to tumor necrosis factor (TNF) antagonists, Wegner's granulomatosis, and microscopic polyangiitis. Within solid organ transplant, rituximab has had several off-label uses investigated, including post-transplant lymphoproliferative disease (PTLD), prevention of rejection, ABO incompatible transplantation, treatment of rejection, and desensitization of HLA-sensitized patients [31]. The area with some of the greatest success has been the use of rituximab for PTLD, however this section will focus on rituximab for use in treatment of rejection and desensitization.

Rituximab use for treatment of refractory rejection has been limited to small, non-randomized studies. This use has been based in part on the observation that patients with resistant rejection demonstrate significant numbers of CD20+ staining B cells on renal allograft biopsies [31]. Becker and colleagues described a case series of 27 patients who received rituximab for steroid resistant or

antibody-mediated rejections (AMR). Nearly all of these patients also received steroids (24 of 27) and plasmapheresis and rATG (22 of 27). Only 3 patients experienced graft loss not associated with patient death. Although this case series was too small to evaluate outcomes, it did suggest that rituximab was an effective therapy for patients with resistant rejection episodes [32]. Another case series of 4 patients with chronic AMR described the use of rituximab in combination with intravenous immune globulin (IVIg). These patients showed improved kidney allograft function at 3 and 6 months after therapy, and 2 patients had reduction in donor specific antibodies (DSA) [33]. Other studies suggest benefit of rituximab for AMR when used in combination with plasmapheresis [34].

Tydén and colleagues performed a prospective, randomized, double blind study of rituximab for induction, in combination with tacrolimus, mycophenolate, and steroids. One hundred and forty patients were randomized to receive one dose of rituximab (375 mg/m^2 body surface area) or one dose of placebo within 24 hours of revascularization [35]. Among the 68 patients who received rituximab significant depletion of CD19/CD20 cells was observed. A trend toward fewer rejection episodes was observed at 6 months in the rituximab arm; however this was not statistically significant. In comparison, an open-label randomized study of comparing rituximab and daclizumab for induction was terminated after enrolling only 13 patients due to an 83% rate of acute cellular rejection within 3 months of transplantation in the rituximab group [36].

Rituximab has also been used in desensitization regimens for highly HLA-sensitized patients. In one study of 20 patients, 16 went on to transplantation following administration of two doses of 1 g rituximab (day 7 and 22) and 2 doses of 2 g/kg IVIg (day 0 and 30) [37].

In summary, the evidence base for rituximab is generally, not as robust as that for alemtuzumab. However, data from uncontrolled clinic trials suggest that rituximab may possess efficacy desensitization regimens and antibody-mediated rejection.

Bortezomib

Bortezomib is a small molecule, first in class proteasome inhibitor that is FDA approved for treatment of multiple myeloma and mantle cell lymphoma [38]. The mechanism of action of bortezomib is complex and involves a myriad of effects on cellular signaling pathways. However, few of these effects are thought to contribute to its efficacy in multiple myeloma. Current thinking holds that the predominant mechanism in multiple myeloma is derived from induction of endoplasmic reticulum (ER) stress, and a terminal unfolded protein response that culminates in apoptosis [39]. Bortezomib is also known to be a potent cell cycle inhibitor and inhibitor of nuclear factor κ B (NFκB)-mediated resistance, resulting in apoptosis.

The first report of bortezomib use in transplantation was for the treatment of refractory antibody mediated rejection (AMR) [40]. In this study, 6 patients with documented AMR refractory to standard therapy were treated with bortezomib. This off-label use of bortezomib resulted in AMR reversal in each patient, who had failed standard AMR therapies. Payor coverage for this initial off-label indication was variable, and ongoing inconsistency with payor coverage complicated the expansion of bortezomib therapy investigation. Following this experience, documentation of treatment success and provision of scientific publications, has been a substantial help in gaining payor coverage. In our experience, bortezomib use was detected and monitored by the hospital pharmacy, and due to its considerable cost (about $5000 for 4 doses during an 11 day cycle) the off-label use of bortezomib drew considerable attention. However, the potential allograft-saving effects helped defray resistance from hospital pharmacy and the formulary committee.

Publication of experiences in peer-reviewed scientific literature became the most effective strategy in obtaining coverage for off-label use of bortezomib for treatment of refractory AMR in kidney transplant recipients. However, had bortezomib therapy not been as effective as it was in the original series of patients, such an approach may very well have failed.

The use of off-label bortezomib illustrates important considerations for the future of off-label drug use in transplantation. In general, decisions regarding registration trials are based on several factors, including known mechanism of action, and also the size of the potential market. Although a drug may have several potential disease states that are amenable to further drug development, the exorbitant costs of clinical registration trials limits investigations to a single indication. The restriction to a single indication, results in off-label drug development the only viable option for other most other potential therapeutic indications. An active phase IV development program may provide substantial support for off-label evaluation in new therapeutic areas. However, when these funds are not available, off-label use becomes challenging. In the case of bortezomib, at the University of Cincinnati, the authors were fortunate secure funding for three clinical trials, including two small, randomized trials — one in induction therapy and another for acute mixed rejection in renal transplantation. The third trial was conducted as a desensitization trial in highly sensitized kidney transplant recipients.

A number of reports followed the initial report of bortezomib for refractory AMR. Unfortunately, results from these published reports had varying success rates. However, bortezomib demonstrated potential efficacy in reversal of AMR in heart and lung recipients, as well as pediatric transplant recipients [41,42]. A large collaborative effort has confirmed the original observations in kidney transplant recipients with refractory AMR [43]. More recently, a large desensitization study confirmed the effects of bortezomib in highly sensitized renal transplant candidates, thereby offering a potential alternative to IVIG-based regimens.

To summarize, off-label development of bortezomib was hindered by its high cost, resistance to coverage by payors, cost containment concerns from hospital pharmacy and formulary committees, and limited funding for phase IV studies by the sponsor. Scientific publication of clinical results, efficacy in preventing graft loss in patients with treatment refractory AMR, and lack of alternative FDA approved AMR therapies helped to improve coverage for off-label use of bortezomib.

French statutory framework for redefining off-label drug use

A recently passed French law and an associated "decree" have created a process for regulating off-label use of approved drugs. This novel regulatory framework intends to provide a means for supporting and assessing the use of newly approved drugs for off-label indications, while assuring public safety [44]. The major effect of the law is to require the sponsor (i.e. drug manufacturing company) to monitor off-label use, and determine if the following actions must be taken:

1 Inform the national regulatory agency (Agence Nationale de Securite du Medicament et des Produits de Sante (ANSM) of unconventional prescribing.

2 To take measures to educate health care professionals and prevent off-label use.

The decree ("Temporary Recommendations for Use" [TRU]) provides for authorization of a one-time TRU designation for a drug that requires reimbursement by payors if no other medications are available for the designated use. The regulatory agency (ANSM) may be notified of the need for a TRU by any of several groups with vested interests such as the national Ministry of Health, the national cancer institute or patient advocacy groups. ANSM may then decide to take the proposal for a TRU under consideration, whereby it will consider data submitted by the sponsor or from the published literature [44].

For a TRU to be granted, there must be no other approved drugs for the indication. Factors to be considered in approving a TRU include:

- the quality of the evidence base supporting the proposed TRU;
- the safety profile of the drug;
- the prognosis of the disease; and,
- the frequency of the disease/condition.

Although the French regulatory framework may enhance safety some factors create considerable concern. Most concerning is the fact that a TRU may be granted only once for each drug. Therefore, if a drug has several potential off-label uses (e.g. several different cancer types) the sponsor is given the decisions of which indication deserves a TRU application. What if a drug has two (or more) potential off-label uses, both of which are for potentially lethal conditions? What is the ethical framework by which such decisions will be made? Clearly, additional consideration is required before such a system would be acceptable to the medical community in the US and the US general public.

Off-label use, standard of care, and investigational use of drugs

Off-label use of drugs has been defined earlier in this chapter. However, off-label use may be further defined in terms of standard of care, or alternatively, may constitute investigational use. As discussed previously, off-label use may become such common practice that it becomes the standard of care (e.g. tacrolimus/MMF regimens for prophylaxis of rejection in renal transplantation), but determining the transition point of off-label use to standard of care is more difficult to define. Similarly, off-label use may or may not be investigational, and this definition raises important concerns regarding human subjects' protection and regulatory issues. Therefore, consideration of providing definitions for standard of care and investigational use are important in the context of off-label use of drugs.

Standard of care has been reasonably well defined from a legal perspective. Legal dictionaries define "standard of care" as "the watchfulness, attention, caution and prudence that a reasonable person in the circumstances would exercise [45]." The definition of standard of care is critical in legal malpractice actions, as the plaintiff is required to establish the standard of care for a physician practicing in a defined specialty or general practice of medicine.

In comparison, standard of care does not have a clearly established medical definition. Moreover, standard of care for medical practitioners may vary significantly based on location of a physician's practice. Even within the US, significant regional or even local differences may exist with respect to the standard of care. As an example, within the state of Ohio, marked differences exist amongst the six adult kidney transplant programs. Even between individual programs within the same city have significant differences in standard of care immunosuppressive regimens despite the physical proximity. There are a number of larger cities in the US that have multiple transplant programs, where the immunosuppressive regimens differ substantially from other programs within that same city.

Given these differences, it is important to understand how a program develops its standards of care. CMS and the Organ Procurement and Transplantation Network (OPTN) regulate the transplant field through program certification and establishing standards for certification. These standards require a team approach toward operating protocols, and also require that standards be developed for immunosuppression and prophylaxis of infection. CMS and OPTN do not dictate which drugs are used for immunosuppression or infection prophylaxis, only that policies for such are developed by a multidisciplinary group, led by certified transplant physicians and surgeons. In this local, program-specific, consensus approach physician leadership must consider the evidence base, prior experience, ongoing experience, financial cost, payor coverage, and individual patient populations in deciding on immunosuppressive protocols.

Electronically derived prescribing data is readily available for the most commonly prescribed immunosuppressive agents and immunosuppressive regimens, which may represent an alternative approach toward defining the standard of care. If a given regimen represents the most commonly prescribed treatment, such data can present a strong argument for the particular combination representing a standard of care. However, for smaller patient populations, such as a high-risk population, prescribing volume would be a more difficult approach to apply toward defining standard of care, unless such high-risk populations can be reliably defined by the prescribing database.

As another consideration, the most commonly prescribed immunosuppressive regimens are tracked in the US on a national, regional, and local basis from the electronic prescribing databases currently in existence. There are cases where local standards of care may differ substantially from those defined on a national basis by prescribing frequency data. In these cases, the local programs should be able to justify the basis used for their selection of a given regimen as their local standard of care.

Evidence based medicine approaches, and patient care guidelines represent another approach for defining a standard of care. However the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, established by the National Kidney Foundation KDIGO group, present an example of the difficulties inherent in establishing practice guidelines. The immunosuppression recommendations in KDIGO in particular, have been controversial; as some practitioners believed that they did not achieve an appropriate degree of objectivity and were unduly biased by the responsible authors (Dr. E. Steve Woodle, unpublished observation). Therefore, in cases where there are no clear superior approaches established by multicenter double blinded trials, issues of standard of care definitions are inherently and necessarily subjective in nature.

The issue of when off-label use constitutes investigational use is easier to define when an Institutional Review Board (IRB) sponsored protocol is in effect. However, an argument could be made that off-label use may be investigational in the absence of an IRB-approved protocol. As an example, a practitioner or group of practitioners may choose to treat patients with off-label drug use and collect data for purposes of evaluation on the effects of therapy. When such data is published, it begins to approximate

investigational use. An example of this can be provided by the issues and experiences that we encountered in our initial experience with the use of bortezomib to treat refractory antibody-mediated rejection (AMR) [40]. Prior to treating patients with AMR refractory to known therapeutic agents, including IVIg and rituximab (interestingly, also used off-label to treat AMR) internal programmatic discussions were held by faculty nephrologists, transplant surgeons, and transplant pharmacists as to whether patients with ongoing AMR that had failed IVIG and rituximab should be allowed to progress and lose their renal allograft (and return to dialysis) without additional attempts at rejection reversal. Because the return to dialysis and loss of the renal allograft is associated with major decreases in life expectancy for patients, we elected to consider use of bortezomib for this condition. Prior to employing bortezomib, its effects and toxicities and safety profile were carefully researched from scientific literature; we also engaged oncologists and oncology-specialty trained pharmacists. The safety and toxicity profile was judged reasonable to assume in the patient population being considered for treatment. The transplant team discussed these observations and a proactive decision was made to treat the next appropriate patient with refractory AMR, who was judged to be at high risk for renal allograft loss, with off-label bortezomib as rescue therapy. When patients were identified, the prognosis without further therapy was discussed with the patient, the lack of alternative options was discussed, and the potential off-label use of bortezomib was discussed, including potential costs, safety and toxicity. The first six patients treated all demonstrated substantial responses to bortezomib treatment, and experienced AMR reversal [40]. After two patients had been treated, we engaged the head of our local IRB in a discussion of this off label use, specifically addressing whether such treatment represented research that should be conducted under an IRB-approved protocol. It was agreed that at some point, the preferred approach would be to conduct an IRB sponsored trial; however, it was clear that such off-label use was clearly a prerogative of the treating physicians. To date, we have submitted research proposals to study bortezomib AMR therapy, but have been unsuccessful in obtaining funding for such a trial. In the history of transplantation, only a few trials have been funded for the study of AMR. To date, the collective experience with bortezomib therapy for AMR provides results similar to those provided historically achieved with IVIG [43].

In a perfect world, funding would be available for the study of all conditions, no matter how rare, to provide a proper evidence base for off-label drug therapy. However, the reality of modern day medicine is such that many new medications are quite expensive, and the cost of conducting these clinical trials is increasingly substantial. These considerations have largely precluded the potential of industry funding for such trials, leaving the NIH and other entities as the only potential alternatives for such studies.

Summary

Solid organ transplantation is well known for off-label drug use, in part because the field does not experience the same level and extent of new drug development as other fields such as autoimmune disease and oncology. Off-label ISA use and drug development are complicated by refusal of insurance carriers to provide reimbursement, which could result in costs of several thousands of dollars to patients and time-consuming prior authorizations to be completed by providers. Despite these considerable barriers, off-label drug use and development has been possible up to the present. However, the future does not appear to be robust, and with increasing cost of new drugs, it is likely that local and federal scrutiny of off-label drug use will increase, and greater restrictions will result. Historically, off-label drug use has progressively become standard of care for transplant recipients. However, this practice may change in the future. Statutory limitations, such as those created in France, are redefining this area of drug development and it is likely that other countries may soon follow.

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CHAPTER 102

Drug Interactions in Organ Transplantation

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Introduction

A drug–drug interaction occurs when one drug alters or interferes with the action of another drug resulting in unintended or unexpected effects. Drug interactions may have potentially life-threatening consequences in transplant recipients, who have several risk factors for drug-drug interactions including multiple concomitant diseases and the use of narrow therapeutic index drugs. The potential for adverse interactions also increases with increasing age. The frequency of drug interactions is not well-known in transplant recipients, although in the general population the incidence of emergency department visits, hospital admissions and re-hospitalization due to drug-drug interactions is 0–7.6% [1]. The incidence of actual drug–drug interactions during hospital stays is 5.3–14.3% [1]. The incidence in transplant patients could be easily envisioned as being higher given the high number of drugs introduced simultaneously at the time of transplantation, the common deployment of drugs in off-label indications, and the rapidly changing function of organs critical to drug metabolism, particularly in liver and kidney transplantation. Drug interactions can be divided into two general categories: pharmacokinetic and pharmacodynamic.

Pharmacokinetics

Pharmacokinetics is the study of drug absorption, distribution, metabolism, and excretion within and from the body. Pharmacokinetic drug interactions occur when one drug affects the absorption, distribution, metabolism, or excretion of another. See Table 102.1 for examples of selected drug-drug interactions.

Pharmacodynamics

Pharmacodynamics is the study of the relationship between drug exposure and its target tissue effects or clinical outcome. Pharmacodynamic drug-drug interactions may result in additive, synergistic or antagonistic pharmacologic effects. Either type of drug interaction can result in undesirable outcomes in patients. Evidence is now accumulating that genetic variation or pharmacogenetics may affect drug interactions.

The purpose of this chapter is to describe pharmacokinetic and pharmacodynamic drug-drug interactions with a focus on the immune suppressants, to explore the influence of pharmacogenomics in transplant recipients, and to present approaches for managing drug interactions. An overview of the common mechanisms for transplant immunosuppressants can be found in Chapter 17, and detailed descriptions of medications commonly used in transplantation can be found in Chapters 98–101.

Pharmacokinetic drug-drug interactions

Pharmacokinetic interactions may result in changes to the drug's pharmacokinetic parameters such as area under the time versus concentration curve (AUC), half-life ($t_{1/2}$), time to peak blood concentration (T_{max}), maximum blood concentration (C_{max}) or minimum blood concentration (C_{min}).

Absorption

Absorption is the process by which a drug reaches the systemic circulation. After a drug is ingested, the dosage form dissolves or disintegrates and then is absorbed. Absorption is dependent on several factors including drug formulation, the presence of food or bile at site of absorption, concomitant drugs, the gastrointestinal physical environment, and transporters.

The presence, composition and timing of food may affect the absorption of many of the immunosuppressive drugs [2–9] (Table 102.2). Because avoidance of food at time of medication administration is not always clinically practical, many clinicians recommend that patients be consistent and that they always take their medication in the same manner whether it is with or without food.

Another factor that may influence the absorption of transplant medications is concomitant administration of commonly used divalent cations. In a single-dose, crossover study in healthy volunteers, co-administration of tacrolimus and magnesium/aluminum hydroxide resulted in a 21% increase in the mean tacrolimus AUC and a 10% decrease in the mean tacrolimus C_{max} relative to tacrolimus administration alone [2]. In a clinical study, patients did not require tacrolimus dose adjustment when magnesium/aluminum was prescribed with tacrolimus therapy [10]. When tested with mycophenolate mofetil (MMF), aluminum/magnesium hydroxide decreased the AUC of mycophenolic acid (MPA) by 17% and the C_{max} by 33% [4, 9]. When given with enteric-coated mycophenolate sodium (EC-MPS), aluminum/magnesium hydroxide decreased the MPA AUC by 25% and the C_{max} by 37% [4] (Package insert). The effect of calcium on MPA absorption has also been studied. In a study of six healthy male volunteers, who received

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Table 102.1. Selected drug interactions with immunosuppressants

Immunosuppressant	Interacting agent	Study conditions (if reported)	Results	Reference
Azathioprine	Allopurinol	Not reported	Requires azathioprine dose reduction by approximately 1/3 to 1/4 the usual dose	[6]
Cyclosporine	Acetazolamide	3 men studied before and 72 hours after the co-administration of acetazolamide	Increased mean trough cyclosporine levels, 170 ng/mL (range 54–270 ng/mL) to 1130 ng/mL (range 517–1827 ng/mL)	[76]
Cyclosporine	Aliskerin	14 healthy subjects in an open-label, single-sequence, parallel-group, single-dose study received aliskiren 75 mg orally (period 1), followed by aliskiren 75 mg + cyclosporine 200 mg (period 2) after a 7-day washout period, and aliskiren 75 mg + cyclosporine 600 mg (period 3) after a 14-day washout period.)	Increased aliskerin AUC 4 to 5 fold, C_{max} 2.5 fold, increased mean $t_{1/2}$ from 25 to 45 hours. Did not alter the pharmacokinetics of cyclosporine	[77]
Cyclosporine	Ambrisentan	28 healthy subjects received ambrisentan 5 mg daily either alone or with cyclosporine 100–150 mg 2 times/day, and 24 other subjects received cyclosporine alone or with ambrisentan	Increased ambrisentan Cmax 1.5 fold, AUC 2 fold; CsA Cmax and AUC only increased slightly (906 vs. 1,014 ng/mL) and (3.05 vs. 3.37 μg h/mL) respectively. The recommended maximum dose of ambrisentan is 5 mg if co-administered with CsA.	[42]
Cyclosporine	Fluconazole	16 stable renal transplant recipients in a randomized, double-blind, placebo-controlled study received a constant cyclosporine dose and fluconazole 200 mg daily for 14 days or placebo	Increased cyclosporine concentrations slowly over two weeks of therapy, approximately doubling the cyclosporine trough concentrations	[78]
Cyclosporine	Fluconazole	Pharmacokinetics compared with those 2, 4 and 7 days after starting fluconazole orally in a dose of 200 mg/day	Increased CsA AUC from 2887 +/− 1729 ng h/mL on day 0 to 3842 +/− 1975 ng h/mL on day 2 ($P < 0.05$), 4750 +/− 1718 ng h/mL on day 4 ($P < 0.01$) and then decreased to 4052 +/− 1687 ng h/mL on day 7 ($P < 0.01$)	[79]
Cyclosporine	Indinavir and Nelfinavir	Kidney transplant patients concomitantly administered CsA , indinavir and nelfinavir	Required 85% dose reduction of CsA	[80]
Cyclosporine	Ketoconazole	Not reported	Increased AUC of CsA by almost three-fold, requiring up to 80% CsA dose reduction	[81]
Cyclosporine	Lopinavir/Ritonavir	Liver transplant patients receiving lopinavir/ritonativir and CsA	Required 5%–20% CsA dose reduction	[82]
Cyclosporine	Methotrexate	20 rheumatoid arthritis patients	Increased methotrexate concentrations AUCs approximately 30%, CsA concentrations did not appear to have been altered	[7]
Cyclosporine	Metoclopramide	14 kidney transplant patients with stable renal function, total dose of metoclopramide was 20 mg	Increased CsA AUC by 29%	[22]
Cyclosporine	Nefazodone	Case report of a cardiac transplant recipient maintained on CsA and started nefazodone	Increased CsA trough levels 10 fold after initiation of nefazodone	[83]
Cyclosporine	Posaconazole	3 heart transplant recipients on cyclosporine dosed 3 times/day for 6 weeks or longer were given posaconazole 200 mg/day for 10 days	Increased cyclosporine exposure and necessitated dosage reductions of 14–29%	[84]
Cyclosporine	Quinupristin/Dalfopristin	Concurrent administration of quinupristin/dalfopristin with CsA	Increase CsA trough concentrations by more than 2.5 fold, CsA AUC by 1.6 fold and CsA C_{max} by 1.3 fold within 2 to 3 days	[85]
Cyclosporine	Repaglinide	12 healthy males received two doses of 100 mg CsA capsule orally 12 hours apart with a single dose of repaglinide 0.25 mg orally 13 hours after the CsA initial dose	Increased repaglinide mean C_{max} 1.8 fold (range: 0.6–3.7 fold) and AUC 2.4 fold (range 1.2–5.3 fold)	[7]
Cyclosporine	Rifampin	Single dose of rifampin	Decreased CsA trough concentration by 73%	[86]
Cyclosporine	Rifampin	Kidney transplant recipients on CsA before and after the administration of rifampin	Required a CsA dose increase of 2.5 to 3 times to maintain optimal trough concentrations.	[87]
Cyclosporine	Telaprevir	10 healthy volunteers were administered CsA alone as a single 100 mg oral dose, followed by a minimum 8-day washout period, and subsequent co-administration of a single 10 mg oral dose of cyclosporine with either a single dose of telaprevir 750 mg or with steady-state telaprevir 750 mg every 8 hours	Increased CsA dose normalized AUC approximately 4.6 fold and increased the terminal elimination $t_{1/2}$ of cyclosporine from a mean (standard deviation [SD]) of 12 (1.67) hours to 42.1 (11.3) hours	[88]
Cyclosporine	Voriconazole	7 kidney transplant recipients in a randomized, double-blind, crossover study with the addition of voriconazole to CsA	Increased mean CsA AUC 1.7 fold	[89]
Everolimus	Atorvastatin	24 healthy subjects given a single oral dose administration of atorvastatin 20 mg and everolimus 2 mg	Decreased everolimus AUC 5%, and C_{max} 9%. No change in atorvastatin AUC, therefore no dosage adjustment needed	[90]
Everolimus	Cyclosporine	Single-dose study in healthy subjects which administered neoral 175 mg with everolimus 2 mg compared with administration of everolimus alone	Increased everolimus AUC by 2.7 fold (168% range, 46–365%) and C_{max} by 82% (range, 25–158%)	[91]
Everolimus	Erythromycin	16 healthy subjects given erythromycin 500 mg 3 times/day for a total of 9 days and a single 2 mg dose of everolimus coadministered on the fifth day	Increased everolimus AUC 4.4 fold (90% CI, 3.5–5.4) from 116+/− 37 ng h/mL to 524+/− 225 ng h/mL. Everolimus $t_{1/2}$ was prolonged by 39% from 32+/− 6 h to 44+/− 6 h	[92]

(*Continued*)

Table 102.1. (*Continued*)

Immunosuppressant	Interacting agent	Study conditions (if reported)	Results	Reference
Everolimus	Itraconazole	Renal transplant recipient case report	Decreased itraconazole clearance by 74%	[93]
Everolimus	Ketoconazole	12 healthy volunteers given ketoconazole 200 mg 2 times/day for a total of 8 days and a single 2 mg dose of everolimus coadministered on the fourth day of ketoconazole therapy	Increased everolimus AUC 15.0 fold (90% CI, 13.6–16.6), Increased C_{max} 3.9 fold (90% CI, 3.4–4.6), prolonged $t_{1/2}$ by 1.9 fold; everolimus did not appear to alter ketoconazole concentrations	[94]
Everolimus	Posaconazole	Renal transplant recipient case report who received posaconazole treatment in combination with everolimus	Increased everolimus blood trough concentrations.3.8 fold	[95]
Everolimus	Pravastatin	24 healthy subjects given a single oral dose of pravastatin 20 mg and everolimus 2 mg	Decreased everolimus C_{max} 10% and AUC 6%, decreased pravastatin AUC 5%, therefore no dosage adjustments are needed	[90]
Everolimus	Rifampin	12 healthy subjects given rifampin 600 mg once daily for 8 days followed by a single dose of 4 mg everolimus	Increased everolimus clearance 172%, decreased AUC by 63% and reduced the $t_{1/2}$ from 32 to 24 hours	[96]
Everolimus	Verapamil	16 healthy volunteers given verapamil 80 mg 3 times/day for 5 days with a single dose 2 mg everolimus on second day	Increased everolimus C_{max} 2.3 fold and AUC 3.5 fold	[97]
Everolimus	Voriconazole	Renal transplant patient case report who received voriconazole treatment in combination with everolimus.	Increased everolimus blood trough concentrations 7.5 fold	[95]
Mycophenolate mofetil	Ciprofloxacin or amoxicillin plus clavulanic acid	Kidney transplant recipients on MMF received either oral ciprofloxacin 500 mg bid or amoxicillin plus clavulanic acid 375 mg 3 times/day for 7–14 days	Reduced median trough MPA concentrations approximately 50% in 3 days	[3]
Mycophenolate mofetil	Levonorgestrel	18 women with psoriasis followed over 3 consecutive menstrual cycles were co-administered MMF (1 g bid) and combined oral contraceptives containing ethinylestradiol (0.02 mg to 0.04 mg) and levonorgestrel (0.05 mg to 0.20 mg), desogestrel (0.15 mg) or gestodene (0.05 mg to 0.10 mg)	Decreased mean levonorgestrel AUC(0–24h) by about 15%, mean AUC(0–24h) was similar for ethinylestradiol and 3-keto desogestrel	[3]
Mycophenolate mofetil	Magnesium Aluminum Suspension	10 rheumatoid arthritis patients given a single dose of MMF(2 g) and also taking Maalox® TC 10 mL four times/day	Reduced MPA C_{max} by 37% and AUC (0–24h) by 15%	[3]
Mycophenolate mofetil	Norfloxacin and Metronidazole	11 healthy volunteers administered a single-dose of MMF(1 g) on day 4 of a 5 day course of a combination of norfloxacin and metronidazole,	Reduced mean MPA AUC0-48 by 33% compared to the administration of MMF alone	[9]
Mycophenolate mofetil	Pantoprazole	36 patients with autoimmune diseases under stable MMF maintenance therapy; 23 patients received co-medication with pantoprazole and 13 patients received no treatment with PPIs or antacids	Decreased MPA AUC by 37% in pantoprazole treated patients ($P < 0.01$); MPA exposure correlated with the inhibition of IMPDH activity and the area of enzyme activity curve was 42% higher in the pantoprazole treated patients ($P < 0.01$)	[28]
Mycophenolate mofetil	Probenecid	Monkeys	Increased plasma MPAG AUC 3 fold in and plasma MPA AUC 2 fold.	[3]
Mycophenolate mofetil	Rifampin	Heart-lung transplant recipient case report	Decreased MPA exposure AUC (0–12) 67%	[3]
Mycophenolate mofetil	Rifampin	Heart–lung transplant recipient case report	Decreased MMF exposure by more than 2 fold	[73]
Mycophenolate mofetil	Sevelamer	Adult and pediatric patients	Decreased mean MPA C_{max} 36% and AUC(0–12h) by 26%	[3]
Mycophenolate mofetil	Sevelamer	Kidney transplant recipients with single and repeated concomitant dosing with sevelamer and MMF	Decreased MPA by an average of 25%	[20]
Mycophenolate mofetil/ Mycophenolate sodium	Omeprazole	12 healthy volunteers (6 male/6 female) received a single oral dose of MMF 1000 mg or an equimolar dose of EC-MPS 720 mg while fasting with and without co-administered omeprazole 20 mg 2 times/day	Decreased MPA AUC by 20% and the peak concentrations were halved with MMF but no change with EC MPS	[29]
Mycophenolate mofetil/ Mycophenolate sodium	Pantoprazole	12 healthy volunteers (6 male/6 female) received a single oral dose of MMF 1000 mg or an equimolar dose of EC-MPS 720 mg while fasting with and without coadministered pantoprazole 40 mg 2 times/day	Decreased MPA AUC (0-12) by 27% and C_{max} by 57% with MMF but pantoprazole does not change the pharmacokinetics of enteric-coated mycophenolate sodium	[25]
Mycophenolate sodium	Magnesium Aluminum Suspension	12 stable renal transplant patients taking magnesium-aluminum-containing antacids 30 mL and MMF	Decreased MPA mean C_{max} 25% and AUC 37%	[4]
Mycophenolate sodium	Pantoprazole	21 heart or lung transplant recipients treated with pantoprazole 40 mg once daily and EC-MPS 2 times/day at a mean dose of 960 mg	No significant difference in MPA AUC, C_{max}, T_{max} or IMPDH activity AUC	[98]

Table 102.1. (*Continued*)

Immunosuppressant	Interacting agent	Study conditions (if reported)	Results	Reference
Sirolimus	Cyclosporine	24 healthy volunteers in a single dose study were administered sirolimus tablets 10 mg either simultaneously or 4 hours after a dose of Neoral 300 mg	Simultaneous administration: Increased sirolimus mean C_{max} by 512% and AUC by148%, compared to sirolimus alone. Dosed 4 hours after CsA administration: increased sirolimus C_{max} and AUC by 33% compared to sirolimus alone	[5]
Sirolimus	Cyclosporine	24 healthy volunteers single dose study were administered sirolimus oral solution 10 mg either simultaneously or 4 hours after a dose of Neoral 300 mg	Simultaneous administration: Increased the sirolimus mean C_{max} by 116% and AUC of by 230%, compared to sirolimus alone. Dosed 4 hours after CsA: increased sirolimus C_{max} by 37% and AUC by 80%, compared to sirolimus alone	[5]
Sirolimus	Cyclosporine	33 healthy volunteers in a single dose cross-over study received sirolimus oral solution 5 mg alone, 2 hours before, and 2 hours after a dose of Neoral 300 mg	When dosed 2 hours before CsA: sirolimus C_{max} and AUC were comparable to those with administration of sirolimus alone. When given 2 hours after CsA: Increased sirolimus mean C_{max} by 126% and AUC by 141%, compared to administration of sirolimus alone.	[5]
Sirolimus	Diltiazem	18 healthy volunteers with simultaneous oral administration of sirolimus oral solution 10 mg and diltiazem 120 mg	Significantly increased sirolimus C_{max} 1.4 fold, T_{max} 1.3 fold, and AUC 1.6 fold ; sirolimus did not affect the pharmacokinetics of diltiazem	[5]
Sirolimus	Erythromycin	24 healthy volunteers administered sirolimus oral solution 2 mg daily and erythromycin ethylsuccinate tablets 800 mg every 8 hours at steady state	Increased sirolimus C_{max} 4.4 fold, AUC 4.2 fold , increased T_{max} by 0.4 hours; Increased erythromycin C_{max} 1.6 and AUC 1.7 fold, and increased by T_{max} 0.3 hours.	[5]
Sirolimus	Ketoconazole	Multiple dose ketoconazole administration with administration of Rapamune oral solution	Increased in sirolimus C_{max} 4.3 fold, T_{max} 38%, AUC 10.9 fold and the terminal t½ of sirolimus was not changed; single dose sirolimus did not affect steady state 12-hour plasma ketoconazole concentration	[5]
Siroilmus	Rifampin	14 healthy volunteers administered rifampin 600 mg daily for 14 days, followed by a single dose of sirolimus oral solution 20 mg	Decreased sirolimus AUC by 82% and C_{max} by 71%	[5]
Sirolimus	Verapamil	26 healthy volunteers given simultaneous sirolimus oral solution 2 mg daily and verapamil 180 mg every 12 hours at steady state	Increased sirolimus C_{max} 2.3 fold and AUC 2.2 fold without substantial change in T_{max}.	[5]
Sirolimus	Voriconazole	Healthy males given repeat doses of oral voriconazole 400 mg every 12 hours for 1 day then 200 mg every 12 hours for 8 days and a single dose sirolimus 2 mg	Increased sirolimus C_{max} 7 fold and AUC 11 fold	[99]
Tacrolimus	Darunavir	Kidney transplant recipient case report	Increased tacrolimus trough concentrations requiring a dose reduction corresponding to 3.5% of the usual dose	[100]
Tacrolimus	Lopinavir/Ritonavir	3 liver transplant recipients case reports in co-administered lopinavir/ritonavir in combination with tacrolimus	Increased tacrolimus exposure and $t_{1/2}$ approxiamately 10 fold	[101]
Tacrolimus	Nefazodone	2 renal transplant recipients case reports on stable tacrolimus and added nefazodone	Increased tacrolimus trough levels >3–5 fold	[102,103]
Tacrolimus	Nelfinavir	5 liver transplant recipients compared to historical controls and a case report in a liver transplant patient after addition of nelfinavir to a tacrolimus-based regimen	Required a 38–70 fold decrease in tacrolimus dose in order to maintain similar trough concentrations	[104,105]
Tacrolimus	Omeprazole and esomeprazole but not lansoprazole	Renal transplant recipient with stable blood concentrations maintained between 5–8 ng/mL while on lansoprazole (30 mg/day) was switched to esomeprazole (40 mg/day)	Presented one month later with tacrolimus blood concentrations elevated to 27.4 ng/mL. patient switched back to lansoprazole and tacrolimus conc normalized to target range of 5–8 ng/mL	[106]
Tacrolimus	Posaconazole	36 healthy volunteers received tacrolimus 0.05 mg/kg/day on days 1 and 14 and posaconazole 400 mg 2 times/day on days 7–14.	Increased tacrolimus C_{max} by 121% and AUC by 358%, did not affect posaconazole pharmacokinetics	[84]
Tacrolimus	Telaprevir	Single tacrolimus 2 mg oral dose, followed by a minimum 14-day washout period, and subsequent coadministration of a single 0.5 mg dose of tacrolimus with steady-state telaprevir 750 mg every 8 hours	Increased tacrolimus AUC 70-fold and increased $t_{1/2}$ of tacrolimus from a mean (SD) of 40.7 (5.85) hours to 196 (159) hours.	[88]
Tacrolimus	Voriconazole	A primary renal allograft recipient case report	Increased tacrolimus trough concentrations ≥5 fold after 3 days and 10 fold after 5 to 7 days	[107]
Tacrolimus	Voriconazole	Healthy volunteers with repeat oral dose administration of voriconazole 400 mg every 12 hours × 1 day, then 200 mg every 12 hours × 6 days and tacrolimus 0.1 mg/kg single dose	Increased AUC of tacrolimus 3 fold (90% CI, 2.7–3.8) and increased C_{max} by an average of 2 fold (90% CI, 1.9–2.5)	[99]

MMF, mycophenolate mofetil; CsA — cyclosporine; AUC, area under the time versus concentration curve; C_{max}, maximum blood concentration; $C_{min,}$ minimum blood concentration or trough; EC MPS, enteric coated mycophenolate acid sodium; $t_{1/2}$, half-life; $T_{max,}$ time to peak blood concentration

Table 102.2. Common immunosuppressant and food interactions, and their common metabolic and transport pathways [3–8]

Drug Class	Drug	Effect of food on absorption	Metabolism and transport	
			Effect on CYP P450 3A4/5	Effect on P-glycoprotein
Corticosteroids	Prednisone	No effect	Substrate	Substrate
Calcineurin inhibitors	Cyclosporine	Variable	Substrate, inhibitor	Substrate, inhibitor
	Tacrolimus	High fat meal — $\downarrow C_{max}$ by 77% and $\downarrow$AUC by 37% High carbohydrate meal — $\downarrow C_{max}$ by 65% and $\downarrow$AUC by 28%	Substrate, inhibitor	None
mTOR inhibitors	Sirolimus	High fat meal — $\downarrow$AUC by 23–35%	Substrate	Substrate
	Everolimus	High fat meal — $\downarrow C_{max}$ by 60% and $\downarrow$AUC by 16%	Substrate	Substrate
Anti-proliferative	Azathioprine	No effect	None	None
	Mycophenolate mofetil	$\downarrow C_{max}$ by 40%, no change in AUC	None	None
	Enteric coated mycophenolic acid	High fat meal — $\downarrow C_{max}$ by 33%, no change in AUC	None	None
Anti-T cell therapy	Antithymocyte globulin — horse	No effect	None	None
	Antithymocyte globulin — rabbit	No effect	None	None
IL-2 receptor blockers	Basiliximab	No effect	None	None
Costimulation blockade	Belatacept	No effect	None	None

AUC, area under the time versus concentration curve; C_{max}, maximum blood concentration or peak.

MMF and calcium polycarbophil there was a 50% decrease in MPA exposure [11]. This was thought to be due to chelation between MMF and calcium ions in the gastrointestinal tract [11]. A similar interaction was reported between MMF and iron ions. Sustained-release ferrous sulfate tablets given with MMF resulted in a 90% decrease in MPA exposure in seven healthy volunteers [12]. However, other studies found no reduction in MPA absorption in renal transplant recipients [13–16], therefore the importance of this interaction remains controversial. Monitoring of MPA and calcineurin inhibitor (CNI) concentrations and appropriate dosage adjustments are recommended when these drugs are used with divalent cations. To minimize these potential interactions, magnesium and aluminum containing products should not be administered within 2 hours of tacrolimus or the mycophenolate products.

Cholestyramine also reduces the absorption of many medications [17]. Although there are little data in transplant recipients, cholestyramine is typically reserved as the last choice to treat dyslipidemias [18,19]. Other medications including lanthanum and sevelamer may also inhibit the absorption of immunosuppressants. Sevelamer decreases the C_{max} and AUC of MPA and, if needed, the oral doses should be separated by at least 2 hours [3,20]. Lanthanum has the potential to interact with compounds that bind to cationic antacids [21] and thus has the potential to bind MPA.

Changes to the gastrointestinal tract may influence absorption of immunosuppressants. Gastrointestinal effects such as slowing of gastrointestinal motility (common following intra-abdominal operations) and delayed emptying (common in patients with type 1 diabetes-associated gastroparesis), changes in pH, low blood flow, and reduced surface area adversely affect absorption. Laxatives can reduce absorption of other drugs by accelerating their passage through the intestine, and drugs that decrease peristalsis (e.g. narcotics, atropine) prolong transit time in the intestine, increasing the time for absorption. In contrast, fluid intake such as an 250 mL glass of water can increase absorption of medications by improving dissolution. Likewise, prokinetic agents such as metoclopramide [22] and erythromycin may increase drug concentrations by increasing gastrointestinal motility, gastric emptying and absorption in the small bowel.

Interactions may be mediated through transporters and enzymes present in the gastrointestinal tract. It is thought that the potent interaction between sirolimus and cyclosporine is mediated through these mechanisms. Cyclosporine and sirolimus are both substrates for CYP3A4 and p-glycoprotein. In addition, cyclosporine is a potent inhibitor of CYP3A4 and p-glycoprotein Table 102.3 [23,24] When cyclosporine and sirolimus are simultaneously administered, cyclosporine is thought to inhibit sirolimus metabolism in the gut thereby enhancing sirolimus bioavailability. Cyclosporine inhibition of p-glycoprotein also likely reduces efflux of sirolimus back into the gut therefore also enhancing sirolimus bioavailability. The combination of both of these mechanisms leads to a large increase in sirolimus C_{max} and AUC when administered with cyclosporine. Spacing the administration of cyclosporine and sirolimus by 4 hours reduces the potency of this interaction but the interaction still remains of high significance (Table 102.4). Interestingly, this interaction has little effect on cyclosporine blood levels. Patients should be aware of the interactions and take their medications consistently. Clinicians should closely monitor sirolimus because cyclosporine dose requirements and blood concentrations may decrease with time.

Drug interactions also may occur with concurrent use of proton pump inhibitors (PPI) and MPA. Proton pump inhibitors suppress gastric acid secretion and increase the gastric pH. Studies suggest that the combination of MMF with pantoprazole, lansoprazole, rabeprazole and omeprazole results in reduced dissolution of MMF in the stomach at the higher pH, leading to reduced bioavailability and lower systemic MPA exposure [25–31]. Proton pump inhibitor co-medication reduces MPA exposure in heart transplant recipients receiving MMF [26,27]. However, a retrospective study did not demonstrate a reduction in MPA AUC when MMF was administered with PPIs [32].Therefore, the effect of PPI treatment on MMF absorption remains controversial. Patients receiving this combination should have MPA plasma concentrations obtained, although this is distressingly atypical in clinical practiced. The enteric-coated formulation of MPA, EC-MPS, passes the acidic environment of the stomach and dissolves after reaching the neutral pH environment

Table 102.3. Drugs that induce, inhibit, or act as a substrate for CYP3A4/5 by drug class [3–8,43,74,75]

Drug Class	Substrate for CYP450 3A4/5
Anti-convulsants	Carbamazepine, ethosuximide
Anti-infectives	Clarithromycin, dapsone, erythromycin, ketoconazole
Anti-retrovirals	Etravirine, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, telaprevir
Anti-neoplastic agents	Crizotinib, docetaxel, etoposide, flutamide, imatinib, irinotecan, sorafenib, sunitinib, tamoxifen, taxol, vincristine
Cardiovascular agents	Amiodarone, amlodipine, atorvastatin, diltiazem, drodarone, eplerenone, felodipine, isradipine, lovastatin, nicardipine, nifedipine, nimodipine, nisoldipine, quinidine, simvastatin, verapamil
Immunosuppressants	Cyclosporine, everolimus, dexamethasone, prednisone, sirolimus, tacrolimus
Pain medicines	Fentanyl, methadone
Psychiatric drugs	Sertraline
Miscellaneous	Aprepitant, armodafinil, budesonide, bromocriptine, cannabinoids, cisapride, conivaptan, dasatinib, dexamethasone, ethinyl estradiol, fexofenadine, hydrocortisone, imatinib, lansoprazole, modafinil, ondansetron, progesterone, rifabutin, zolpidem
Drug Class	**Inducers of CYP450 3A4/5**
Anti-infectives	Isoniazid, naficillin, rifampin, rifapentine, rifabutin
Anti-convulsants	Carbamazepine, fosphenytoin, oxcarbazepine, phenobarbital, phenytoin, primidone
Anti-retrovirals	Efavirenz*, etravirine, nevirapine
Immunosuppressants	Dexamethasone, prednisone , prednisolone
Others	Armodafinil, bosentan, bromocriptine, deferasirox, griseofulvin, modafinil, octreotide#, pioglitazone, St. John's wort
Drug Class	**Inhibitors of CYP450 3A4/5**
Antibiotics	Chloramphenicol, ciprofloxacin, norfloxacin (reported with cyclosporine only), quinopristin-dalfopristin
Anti-emetics	Aprepitant
Anti-neoplastics	Crizotinib, dasatinib, etoposide, imatinib, nilotinib, sunitinib, tamoxifen, vemurafenib
Anti-retrovirals	Amprenavir, atazanavir, boceprevir, darunavir, delavirdine, efavirenz*, fosamprenavir, indinavir (strong), nelfinavir (strong), ritonavir (strong), saquinavir (strong), telaprevir, tipranavir
Azole anti-fungals	Clotrimazole, fluconazole , itraconazole (strong), ketoconazole (strong), miconazole, posaconazole, voriconazole
Calcium channel blockers	Diltiazem, felodipine, nicardipine, verapamil
Cardiovascular agents	Amiodarone, dronedarone
Immunosuppressants	Cyclosporine (3A4 inhibitor only), everolimus, tacrolimus
Macrolides	Clarithromycin (strong), erythromycin, telithromycin (strong)
Psychiatric drugs	Fluoxetine, fluvoxamine, nefazodone (strong), sertraline
Others	Bromocriptine, cimetidine, cisapride, conivaptan, danazol, ethinyl estradiol, grapefruit juice, glyburide, methylprednisolone, modafinil, pimozide, propofol , quinine, star fruit, zafirlukast

*May induce or inhibit; #octreotide suppresses growth hormone secretion, which may decrease the metabolic clearance of drugs metabolized by CYP3A4.

Table 102.4. Calcineurin inhibitors, mTOR inhibitors and mycophenolic acid interactions [2,3,5,8,23,24]

	Sirolimus		Everolimus	Mycophenolic acid
Cyclosporine	**Concurrent administration** *Sirolimus solution:* ↑sirolimus C_{max} by 116%, ↑AUC by 230%, ↑trough, ↓T_{max} *Sirolmus tablet:* ↑sirolimus C_{max} by 512%, AUC by 148% No significant effect on CSA concentrations	**Separated by 4 hours** *Sirolimus solution:* ↑sirolimus C_{max} by 37%, ↑AUC by 80%, ↑trough, ↓T_{max} *Sirolimus tablet:* ↑sirolimus C_{max} and AUC by 33%	↑Everolimus C_{max} by 82% and AUC by 168% Everolimus may slightly increase CsA concentration over time	↓MPA concentrations 30–50% due to inhibition of enterohepatic recirculation No significant effect on CsA concentrations
Tacrolimus	↓Tacrolimus levels No apparent effect on sirolimus concentrations		No significant effect on tacrolimus or everolimus concentrations	No significant effect on tacrolimus or MPA concentrations

C_{max}, Maximum blood concentration; CsA, cyclosporine, MPA, mycophenolic acid.

of the small intestine [33]. Therefore, PPIs when combined with EC–MPS have limited effect on MPA bioavailability and systemic exposure. This has been demonstrated for pantoprazole and omeprazole [25,29].

Distribution

Distribution is the process by which a drug is transferred from the intravascular to extravascular space, both compartments that are in dynamic flux after a transplant. Many processes may affect distribution of drugs including ability of drug to bind to plasma proteins, drug transporters, lipid solubility, tissue permeability and blood flow. However, many drug interactions occurring by distribution mechanisms occur by protein or drug-induced changes in transport.

Many drugs are *protein bound*; however the extent of protein binding varies widely. In general, drugs that are highly protein are at risk for potential drug interactions that are mediated through changes in binding. Proteins such as α-1-acid glycoprotein, lipoprotein, or albumin can bind to drugs in the blood or tissue and decrease the rate and/or amount of drug that reaches the target site. Only unbound drug is pharmacologically active; therefore changes in protein binding are of importance to drugs that are highly bound to one of more of these proteins. Many drugs are bound to proteins; however, only a few drug-drug interactions that occur as a result of

protein binding are clinically relevant. Concomitant use of highly protein bound drugs can displace each from their common proteins thus increasing the unbound drug fraction. Weak acids primarily bind to albumin and basic drugs bind to α-acid glycoprotein. The most significant protein involved in the binding of drugs is albumin. Thus, certain acidic drugs that bind avidly to albumin (e.g. MPA, furosemide, non-steroidal anti-inflammatory drugs, penicillin, phenytoin, salicylates, and sulfonamides) may lead to an increase in unbound drug concentrations when combined with another drug bound to albumin. Likewise, basic drugs bound to α-1-acid glycoprotein (e.g. lidocaine, phenothiazines, propranolol, quinidine, and tricyclic antidepressants) may display an increased amount of available unbound drug when combined with another drug bound to α-1-acid glycoprotein.

Mycophenolic acid and its primary metabolite, mycophenolic acid glucuronide (MPAG), are highly protein bound (>98% and 82%, respectively) to albumin [3]. As MPAG accumulates (as it does with individuals with poor kidney function) it may displace MPA from albumin binding sites, causing an increase in MPA free fraction and potentially a greater pharmacologic effect [34]. Concomitant mycophenolate and phenytoin or theophylline therapy may result in a decrease in the protein binding of theophylline from 53% to 45% and phenytoin from 90% to 87% [3]. Therefore, in patients who require tightly controlled phenytoin or theophylline concentrations additional monitoring (unbound phenytoin, if available) may be required when given with mycophenolate. These types of interactions are difficult to detect and may be noted only through measurement of unbound drug concentrations. Patients at risk for these interactions should be monitored closely for adverse effects and excessive immunosuppression.

Active drug transporter proteins

Active drug transporter proteins control drug movement in and out of cells and can affect the bioavailability, concentration at target tissue and elimination of many agents. Common active drug transport systems include P-glycoprotein and human organic anion-transporting polypeptides (OATPs). These are regulated by genetic factors but can be influenced by food and drugs. Drugs can up or down regulate these proteins thereby altering intestinal absorption, proximal renal-tubular excretion, or biliary excretion. Drug-drug interactions can be mediated through transporter proteins although they are generally clinically difficult to detect. Drug interactions of this type are more likely if one drug is an inhibitor and the other is a substrate of the same transporter. Many of the drugs and foods that affect CYP450 also affect active drug transport systems, greatly confounding the underlying mechanisms by which these interactions occur [35].

P-glycoproteins

P-glycoproteins are part of a large superfamily of efflux transporters distributed to or expressed in many cells including intestinal epithelium, hepatocytes, renal proximal tubular cells, adrenal glands and the blood-brain barrier. P-glycoproteins also are known as multidrug resistance protein 1 (MDR1) or ATP binding cassette sub-family B member 1 (ABCB1) protein. P-glycoprotein is an efflux transporter involved in the movement of substrates across the cell membrane and plays a large role in the distribution and elimination of many clinically important therapeutic substances. Drugs, foods and substances made by the body may be inhibitors and/or inducers of these transporters. Cyclosporine, everolimus, sirolimus and prednisone are substrates of p-glycoprotein (Table 102.2). In vitro data suggest that tacrolimus is neither a substrate nor an inhibitor of P-glycoprotein [36]. Cyclosporine is an inhibitor of P-glycoprotein [37,38]. In an example of this type of interaction, cyclosporine can enhance the efficacy of daunorubicin, docetaxel, doxorubicin, epirubicin, etopisode, mitoxantrone, paclitaxel, vinca alkaloids by inhibiting P-glycoprotein and reducing the efflux of drug out of the tumor cell. Carvedilol inhibits P-glycoprotein and therefore may increase blood concentrations of cyclosporine [39].

Organic anion transporting polypeptides

Organic anion transporting polypeptides also play a role in drug-drug interactions. For example, cyclosporine inhibits the metabolism of repaglinide [40,41]. Cyclosporine appears to reduce the uptake of repaglinide into the liver by inhibiting OATP1B1, which is an active hepatic uptake transporter. In a randomized, crossover study of healthy volunteers, cyclosporine raised plasma concentrations of repaglinide by 175% and AUC by 244% compared to placebo [40]. Complex drug interactions involving CYP450, P-glycoproteins and OATP can occur. For example, cyclosporine is an inhibitor of CYP3A4, P-glycoprotein, and OATP. Ambrisentan is an ET(A)-selective endothelin receptor antagonist, a potential substrate for CYP3A4, OATPs and P-glycoprotein. Twenty-eight healthy subjects received ambrisentan 5 mg daily alone or with steady-state cyclosporine 100–150 mg twice daily, and 24 other subjects received cyclosporine alone at steady-state or with steady-state ambrisentan [42]. With the combination of cyclosporine and ambrisentan, ambrisentan C_{max} increased 1.5-fold, and AUC increased 2-fold. Cyclosporine C_{max} and AUC increased only slightly (906 vs. 1014 ng/mL and 3.05 vs. 3.37 μg h/mL, respectively). Therefore, the recommended maximum dose of ambrisentan is 5 mg if co-administered with cyclosporine.

Metabolism

Even as drug absorption and distribution are taking place, drug elimination begins. Before reaching the systemic circulation, orally administered medications pass through the liver via the portal circulation. The gastrointestinal tract and liver contain enzymes that can metabolize certain drugs before they reach the systemic circulation. Most drugs are metabolized to inactive or less active metabolites by these enzymes. In transplant recipients the most common type of pharmacokinetic drug interaction involves the cytochrome P 450 enzyme system. A summary of the immune suppressants metabolized through CYP3A enzymes is shown in Table 102.2.

Although there are many sites of metabolism, the liver is the most important location of oxidation, reduction, hydrolysis, and conjugation of drugs. Many lipophilic drugs undergo biotransformation to hydrophilic compounds to be excreted. Drug biotransformation reactions consist of Phase I (e.g. oxidation, reduction) or Phase 2 (e.g. conjugation) reactions that occur primarily in the liver. The CYP450 enzyme system is the key pathway of oxidative biotransformation reactions involved in drug metabolism. CYP450 is a superfamily of hemoproteins which can be divided into families, subfamilies and/or single enzymes. The CYP450 enzymes are designated by the letters CYP (representing CYP), followed by an Arabic numeral denoting the family, a letter representing the subfamily (when 2 or more exist) and another Arabic numeral designating the individual gene within the subfamily (e.g., CYP2D6). Of the different isoforms of CYP450, CYP3A4 and 5 are the prevalent

in humans and very important towards drug metabolism of drugs used in transplantation.

Drugs may be substrates for one or more of the CYP450 enzymes and/or also be an inhibitor or inducer of the same or different enzymes (Table 102.3) [43]. In humans, these commonly include CYP450 3A4, 3A5, 2C9, 2C19, 2D6, 2A6, 2E1, and 1A2. By definition, a substrate is a drug acted upon or affected by an enzyme. An inhibitor decreases the metabolic capacity of an enzyme and may therefore decrease the metabolism of specific substrates, generally leading to an increased drug effect. In contrast, inducers can increase the metabolism of specific substrates, generally leading to a decreased drug effect. Drug interactions of this type are more likely if one drug is an inhibitor or inducer and the other is a substrate of the same enzyme. Although many drugs are CYP inhibitors and inducers, their potency varies widely. The potency of the inhibitor or inducer greatly influences the clinical relevance of these interactions. Everolimus, sirolimus, cyclosporine, and tacrolimus are substrates for CYP3A4/5. The active metabolite of prednisone, prednisolone, is also a substrate of CYP3A4, although clinically significant interactions rarely occur. Cyclosporine is a CYP3A4/5 inhibitor and substrate; everolimus is a competitive inhibitor of CYP3A4/5 and a mixed inhibitor of CYP2D6.

Inhibitors and inducers of CYP3A4/5 are listed in Table 102.3. Patients should avoid these medications if receiving a CYP3A4/5 substrate. If a patient cannot avoid a strong inhibitor or inducer of CYP3A4/5, then drug concentrations and toxicities must be monitored closely. Limited data are available regarding precise dose adjustments when inhibitors are co-administered with cyclosporine and tacrolimus. When initiating therapy with voriconazole or posaconazole in patients receiving tacrolimus, the tacrolimus dose should be initially reduced to one-third of the current dose and the subsequent doses should be adjusted based on the tacrolimus whole blood concentrations [2].

Although most metabolism interactions occur via CYP450, especially in the case of CNIs and mammalian target of rapamycin (mTOR) inhibitors, other types of interactions occur with immunosuppressants that are not metabolized by the CYP450 enzyme system. Allopurinol inhibits xanthine oxidase resulting in reduced metabolism of azathioprine and severe bone marrow suppression and pancytopenia [44]. This combination is a potentially serious interaction and should be avoided. If not possible, this combination can be used with significant reductions in the azathioprine dose by 67–75% [6] along with close monitoring.

Mycophenolic acid is extensively metabolized by uridine-diphosphate glucuronosyl transferase (UGT) enzymes. However, no clinically relevant interactions have yet been identified with MPA through this pathway.

Excretion

Excretion is the last process involved in pharmacokinetics and involves drug elimination from the body. The kidneys are the principal site of excretion for water-soluble drugs. The urinary pH may affect drug reabsorption and excretion. Acidification of urine increases reabsorption and decreases excretion of weak acids and decreases reabsorption of weak bases. Alkalinization of urine has the opposite effect.

For some drugs, active secretion into the renal tubules is an important route of elimination. Drugs may also be excreted by the kidney through glomerular filtration, the passive process by which the kidney filters molecules. When drug concentration is high, secretory transport can become saturated. Mycophenolic acid and antivirals including acyclovir, ganciclovir, valacyclovir, valganciclovir undergo tubular secretion. Concomitant use of MPA and these antivirals may result in competition for tubular secretion and an increase in MPA and/or antiviral exposure. Mycophenolate mofetil (1 g) and acyclovir (800 mg) administered to 12 healthy volunteers resulted in no significant change in MPA AUC and C_{max}. However, MPAG and acyclovir plasma AUCs were increased 10.6% and 21.9%, respectively [3]. Following single-dose administration to 12 stable renal transplant patients, no pharmacokinetic interaction was observed between MMF (1.5 g) and intravenous ganciclovir (5 mg/kg). Although in the presence of renal impairment, MPAG and ganciclovir compete for tubular secretion and plasma concentrations of both are increased. In patients with renal impairment in which MPA and ganciclovir, valganciclovir, acyclovir or valacyclovir are coadministered, careful monitoring for hematologic toxicity and signs of over immunosuppression is warranted [3].

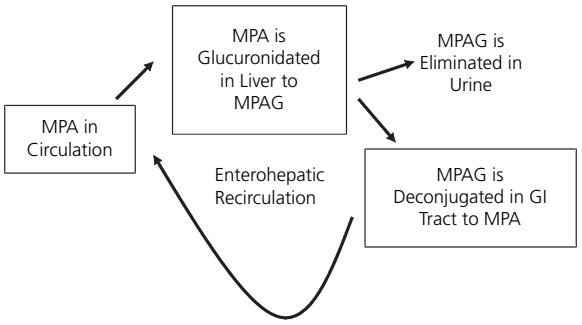

Figure 102.1. Mycophenolate enterohepatic recirulation. Mycophenolate undergoes enterohepatic recirculation and some MPA drug interactions are mediated through this mechanism. In enterohepatic recirculation, a drug secreted in the bile is reabsorbed into the circulation from the intestine.
MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide.

Other minor sites of excretion are the biliary system, saliva, sweat, and lung. Polar, lipophilic drugs with high molecular weights are excreted in the bile, via active secretory transport. Mycophenolic acid and MPAG biliary excretion, distal absorption and reabsorption may utilize several transport mechanisms including OATPs, and multidrug resistance-associated protein (MRP-2) [45].

Mycophenolate undergoes enterohepatic recirculation and some MPA drug interactions are mediated by interfering with this process. In enterohepatic recirculation, drug secreted in the bile is reabsorbed into the circulation from the intestine (Figure 102.1). In the case of mycophenolate, MPA is glucuronidated by UGT enzymes in the intestine and liver to form the primary glucuronide metabolite, MPAG, which is excreted into the bile. There, MPAG undergoes deconjugation back to MPA and is reabsorbed in the gastrointestinal tract into the systemic circulation, thus increasing plasma concentrations of MPA. Bile acid sequestrants, such as cholestyramine, reduce MPA AUC by inhibiting enterohepatic recirculation resulting in a decrease in MPA AUC by approximately 40% [3]. Cyclosporine also interrupts enterohepatic recirculation of MPA by inhibiting the MRP-2 transporter, thus reducing the amount of MPAG excreted into the bile and less available for conversion to MPA and reabsorption. Mycophenolic acid plasma concentrations are 30%–40% lower when given concomitantly with

cyclosporine than when the drug is given alone or co-administered with tacrolimus or sirolimus [13]. In kidney transplant patients, mean MPA $AUC_{0\text{-}12}$ was increased 30–50% when MMF was administered without cyclosporine compared with when MMF was co-administered with cyclosporine [3,33].

Antibiotics also may interact with mycophenolate. Mycophenolic acid glucuronide is converted to MPA in the gastrointestinal tract by glucuronidases. Glucuronidases are produced by bacteria, and in the presence of antibiotics less glucuronidase is available. In patients and healthy volunteers the use of antibiotics which diminish the gastrointestinal flora, reduce MPAG deconjugation and enterohepatic recirculation of MPA [46,47].

Pharmacodynamic interactions

Pharmacodynamics is the study of the relationship between drug exposure and the drug's pharmacologic effect or clinical response (efficacy or toxicity).

Factors that may contribute to a drug's pharmacodynamic effects include the relationship between a drug and its receptor, age, tolerance, and physiologic factors. Several terms are used to describe the interaction between a drug and its receptor. A synergistic drug effect is when the combination of two or more agents results in an effect that is greater than the sum of their individual effects. For instance, if furosemide and metolazone are given together they have a greater diuretic response than if given alone. Additive drug effects occur when the effects of two drugs combined form a sum effect. In transplant recipients, pharmacodynamics drug interactions generally result in enhanced toxicity or changes in efficacy.

Drug-drug interactions — CNIs

Common side effects of CNIs include neurotoxicity, nephrotoxicity, hypertension, diabetes mellitus, dyslipidemia, electrolytes disturbances. Drugs that also cause the same side effects should be avoided, if possible, in transplant recipients. Hyperkalemia is common after transplantation and therefore drugs that also are associated with hyperkalemia (e.g. potassium-sparing diuretics, ACE inhibitors, angiotension II receptor blockers, amiloride, spironolactone, triamterene) should be closely monitored when given concurrently with CNIs. Transplant recipients treated with cyclosporine and sirolimus had higher serum creatinine levels and lower glomerular filtration rates compared with cyclosporine and placebo or azathioprine controls [5]. Everolimus given with standard dose cyclosporine increases the risk of nephrotoxicity resulting in a lower glomerular filtration rate. Reduced doses of cyclosporine are required for use in combination with everolimus in order to reduce renal dysfunction [8].

Drug-drug interactions — antimetabolites

Bone marrow suppression (including anemia, leukopenia, and thrombocytopenia) is a common adverse event associated with mycophenolate and azathioprine therapy. Given that azathioprine and mycophenolate both inhibit purine metabolism, the combination is not recommended [3,4].

Drugs that increase the risk of bleeding (e.g. anticoagulants, platelet inhibitors and thrombotic agents) should be closely monitored when given with antimetabolites. Additive bone marrow suppression may occur when antimetabolites are given with antivirals such as acyclovir, ganciclovir, valacyclovir, and valganciclovir [3,4,48–50]. Therefore, complete blood counts should be obtained more frequently when these drugs are co-administered. Mycophenolate dose reductions or granulocyte colony stimulating factors are options for treating bone marrow suppression in patients receiving mycophenolate and antiviral agents.

Azathioprine and leflunomide are both associated with hepatoxicity. Regular liver function testing is necessary when these medications are given together. Administration of azathioprine and angiotensin converting enzyme (ACE) inhibitors may result in an increased risk of anemia.

Drug-organ interactions

Table 102.5 provides a list of potential pharmacodynamic interactions relevant to transplantation. The combination of two or more nephrotoxic agents is an example of a pharmacodynamic interaction that results in additive nephrotoxicity. For example, the use of a combination of an aminoglycoside and diuretic should be avoided especially in kidney transplant recipients who have underlying diminished kidney function and are receiving CNIs. Depending on the transplanted organ, certain pharmacodynamic drug-organ interactions may be quite significant. Table 102.6 gives drug-organ interactions that should be avoided or minimized due to the high risk of toxicity to the transplant graft. Herbal or natural products, although sometimes not considered drugs, can have significant toxic effects on the liver, particularly when combined with other hepatotoxins. Table 102.7 is a list of hepatotoxins that should be avoided in liver transplant recipients. Likewise pancreas, heart or lung transplant recipients should avoid drugs that may increase the risk of pancreatitis, hyperglycemia, or compromise cardiac or pulmonary function.

Drug–disease interaction

CNIs, which are known to cause hyperglycemia, have been associated with a worsening of hyperglycemia in those with pre-existing diabetes, or frank development of new onset diabetes, particularly in recipients of African descent [51–53]. To minimize this pharmacodynamic interaction, post-transplant patients should be monitored regularly for hyperglycemia and weight gain.

In general, transplant recipients should not receive live viral vaccines due to their impaired ability to mount an immune response and, therefore, high risk of viral replication. Transplant recipients may require higher doses or more frequent boosters of inactivated vaccines. Likewise, the risks and benefits of co-administration of any medication that may compromise the immune system, for example, antineoplastics, autoimmune disease therapies, natural products with immunosuppressive properties (i.e. Echinacea) should be carefully considered in transplant recipients [54].

Many other drug-disease state interactions might occur. A rare adverse event associated with CNIs and mTOR inhibitors is thrombotic microangiopathy. Concurrent administration of these immunosuppressant's has been associated with an increased risk of thrombotic microangiopathy [55]. Concurrent administration of tacrolimus and quinidine, dronedarone or amiodarone has been associated with prolongation of the QT interval and torsades de pointes [56–58]. Close monitoring with an EKG should occur if the medications are given concomitantly. The administration of sirolimus and everolimus with ACE inhibitors, nonsteroidal anti-inflammatory drugs, salicylates, cephalosporins or penicillins may increase the risk of angioedema [5,8,59]. Prednisone and bupropion combination may increase the risk of seizures [60].

Table 102.5. Pharmacodynamic interactions

Immunosuppressant	Interacting medication	Effect
All immunosuppression	Vaccines	Decreased effect of vaccine
All immunosuppression	Antineoplastics, autoimmune disease therapies	Additive immunosuppression and bone marrow suppression
Calcineurin inhibitors	Potassium sparing diuretics, amiloride, spironolactone, traimterene, TMP/SMZ	Additive hyperkalemia
Calcineurin inhibitors	Sirolimus/everolimus	Additive risk of thrombotic microangiopathy/hemolytic uremic syndrome/thrombotic thrombocytopenic purpura,
Calcineurin inhibitors	Hypoglycemics/antidiabetics	Direct beta cell toxicity – hyperglycemia
Calcineurin inhibitors	See Table 102.6 for a list of nephrotoxins	Additive risk of nephrotoxicity
Tacrolimus	Quinidine, dronedarone, amiodarone, alfuzosin, artemether/lumefantrine, chloroquine, ciprofloxacin, crizotinib, nilotinib, quetiapine, quinine, tetrabenazine, thioridazine, vandetanib, toremifene, ziprasidone, ranolazine, cisapride, dasatinib	Additive prolongation of the QT interval/Torsades
Cyclosporine	Minoxidil	Additive hypertrichosis
Cyclosporine	Androgens	Additive hepatotoxicity
Mycophenolate	Azathioprine	Additive bone marrow suppression
Mycophenolate	Anticoagulants, platelet inhibitors, thrombotic agent	Additive risk of bleeding
Mycophenolate	Antibiotics	May alter GI flora and change enterohepatic recirculation
Mycophenolate	Acylovir, ganciclovir, valacyclovir, valganciclovir, TMP-SMZ	Increased risk of neutropenia
Azathioprine	Leflunomide	Additive risk of hepatotoxicity
Azathioprine	ACEi	Additive risk of anemia
Sirolimus/everolimus	ARB/ACEi, cephalosporins, NSAID/salicylates, penicillin	Additive risk of angioedema
Prednisone	Bupropion	Additive risk of seizures

ARB,angiotension receptor blocker; ACEi, angiotensin-converting enzyme inhibitors; GI, gastrointestinal intestinal; TMP-SMZ, trimethoprim/sulfamethazole.

Table 102.6. Selected medications that may harm or counter the beneficial effects of the transplanted organ

Kidney (nephrotoxicity)	Liver (hepatotoxicity)	Lung (pulmonary toxicity)	Pancreas (pancreatitis)	Heart (cardiotoxicity)
Acyclovir	Acetaminophen	Amiodarone	Azathioprine	Doxorubicin
Adefovir	Amoxicillin-clavulante	Beta blockers	Estrogens	Trastuzumab
Aminoglycosides	Azathioprine	Bleomycin	Furosemide	
Amphotericin B	Captopril	Busulfan	Metronidazole	
ARB/ACEi	Carbamazepine	Cyclophosphamide	Pentamidine	
Carboplatin	Erythromycin	Methotrexate	Salicylates	
Cidofovir	HMG-CoA reductase inhibitors	Minoxidil	Sulfasalazine	
Cisplatin	Isoniazid	Mitomycin-C	Tetracycline	
Cyclosporine and tacrolimus	Nafcillin	Nitrosoureas	Thiazide diuretics	
Foscarnet	Nitrofurantoin	Procainamide		
Ganciclovir	Phenytoin	Quinidine		
Immunoglobulin IV	Rosiglitazone			
Pentamidine IV	Tetracycline			
Methotrexate	Trimethoprim-sulfamethoxazole			
NSAIDS				
Pamidronate				
Polymixin				
Trimethoprim-sulfamethoxazole				
Vancomycin				
Zoledronic Acid				

ARB, angiotension receptor blocker; ACEi, angiotensin-converting enzyme inhibitors; IV, intravascular.

Cholesterol medications and drug interactions

Dyslipidemia is common in transplant recipients, and frequently, it requires treatment; therefore, the potential for interactions between cholesterol-lowering drugs and immunosuppressants is high. Many of the HMG-CoA reductase inhibitors (statins) are substrates of CYP3A4 and are associated with rhabdomyolysis and myositis. Drugs that inhibit CYP3A4, such as cyclosporine, may significantly interact with statins, increase statin concentrations and increase the risk of muscle toxicity. To avoid or minimize these interactions, lower doses of statins are recommended when combined with cyclosporine (Table 102.8) [61,62]. Pitavastatin, an HMG-CoA reducatase inhibitor, is contraindicated with cyclosporine. Cyclosporine is also a known inhibitor of OATP transporter and pitavastatin is influxed into human hepatocytes mainly by OATP1B1. This interaction may result in increased pitavastatin concentrations. Rosuvastatin, pravastatin, fluvastatin are less likely than other HMG-CoA reductase inhibitors to interact significantly with cyclosporine since they are poor substrates for CYP3A4.

Other treatment options for dyslipidemias include fibric acid derivatives, ezetimibe and cholestryramine. Often the treatment of choice for hypertriglyceridemia is fibric acid derivatives. However, when gemfibrozil and cyclosporine are combined there is an additive risk of renal dysfunction. Cyclosporine also may increase ezetimibe serum concentrations and ezetimibe may increase the concentrations of cyclosporine. Patients who receive this drug combination should be monitored for the adverse effects of ezetimibe. Cholestyramine impairs the absorption of many medications by

Table 102.7. Herbal products that are potential hepatotoxins [54]

• African remedy	• Germander	• Pennyroyal
• Black colash	• Gordolobo tea	• Saw palmetto
• Bajiaolian	• Jin Bu huan	• Sassafras
• Chaparral	• Life root	• Skullcap
• Carparral leaf	• Kava	• Sho-saiko-to
• CHM	• Kombucha mushroom	• Usnic acid
• Chinese herbal tea	• Mate tea	• Valerian
• Comfrey	• Mediterranean remedy	• Venencapsan
• Chaparral leaf	• Margosa oil	• Zulu remedy
• European remedy	• Mistletoe	

Table 102.8. HMG-CoA reductase inhibitor doses when given with cyclosporine [7,61,62]

Drug	Metabolic pathway	Usual starting dose*	Dose with cyclosporine
Rosuvastatin	CYP2C9	10 mg once per day	5 mg once per day
Lovastatin	CYP3A4	10–20 mg once per day	10 mg once per day
Pitavastatin	Minor amounts by CYP2C9	2 mg once per day	contraindicated in patients taking cyclosporine
Pravastatin	No known CYP metabolism	40 mg once per day	cyclosporine 10 mg once per day
Fluvastatin	CYP2C9	20–40 mg once per day	No change
Atorvastatin	CYP3A4	10–20 mg once per day	10 mg per day
Simvastatin*	CYP3A4	10–20 mg once per day	contraindicated in patients taking cyclosporine^

HMG, 3-hydroxy-3-methyl-glutaryl; CoA, coenzyme A; *No more than 20 mg for patients taking amlodipine and ranolazine; and no more than 10 mg for patients on amiodarone, verapamil, and diltiazem; do not start patients on doses greater than 80 mg due to the risk of rhabdomyolitis.

binding to the drug, including immunosuppressive medications when administered simultaneously. Therefore, in transplant recipients cholestyramine is typically reserved as the last line to treat dyslipidemias.

Herbal products and grapefruit and drug interactions

Herbal products may have significant drug pharmacodynamic interactions with immunosuppressive agents leading to adverse immunologic outcomes and unwanted adverse effects such a bleeding. Unfortunately the effect of herbal products in transplant patients is poorly studied. St. John's wort is an example of an herbal product that interacts with immunosuppressive agents. St John's wort induces CYP3A4 and P-glycoprotein. As a result rejection has been reported in kidney, pancreas, liver and heart transplant recipients who took St. John's wort with immunosuppressive agents [63]. Echinacea is an herbal product that also may have adverse effects on the immune system. In vitro studies have shown that it activates phagocytes, T-lymphocyte proliferation and cytokine production [64]. Herbal medications that may harm the native liver or the transplanted liver are listed in Table 102.7 [54]. Several herbal products may also increase the risk of bleeding. Recommendations suggest that herbal products be discontinued at least 2–3 weeks prior to transplant surgery. Clinicians should carefully consider the influence of natural products on the immune system, the potential for interactions with transplant medications and the risk of harm to the transplanted organ. If there are equally efficacious and safe prescription medications available then herbal medications generally should be avoided.

Grapefruit is an excellent source of vitamins and phytochemicals, yet grapefruit has been linked to many significant drug interactions. A group of active chemical compounds in grapefruit, known as the furanocoumarins, are potent inhibitors of CYP3A4 enzyme. Mechanism-based inhibition of CYP3A4 enzymes in the intestine, by furanocoumarins, results in a complete inactivation of the enzyme [63]. This leads to prolonged CYP3A4 inhibitory effects on the intestinal clearance of CYP3A4 substrates, requiring de novo restoration of the isoenzyme for a return to normal metabolic function. With many oral agents undergoing first-pass metabolism by CYP3A4, the effects of enzyme inhibition can be significant and result in an increase in systemic exposure to drugs that are substrates for CYP3A4 (especially cyclosporine). It is important to educate patients about the interaction between grapefruit juice and their immunosuppressive agents. More frequent monitoring of blood CNI blood concentrations may be necessary in patients who regularly consume grapefruit. Star fruit also has similar potential for interaction.

Pharmacogenomics and drug interactions

Genetic variation has an important influence on the pharmacokinetics and pharmacodynamics of drugs. However, pharmacogenomic data are limited in transplantation relative to other therapeutic areas with the exception of tacrolimus where CYP3A5 enzyme variation has a well-established effect on its pharmacokinetics [65–68]. Increasing data suggest that drug interactions arising through inhibition of CYP3A, 2D6, 2C9 and 2C8 enzymes, which generally have limited clinical relevance, may become highly significant in individuals with underlying genetic variation [69–71].

Sixty-one kidney transplant recipients receiving MMF and tacrolimus with or without a PPI were genotyped for CYP2C19*1, *2 and *3 and multidrug resistance (MDR)1 C3435T alleles [31]. Individuals receiving lansoprazole 30 mg who also had the CYP2C19 *1/*2 or *1/*3, or the MDR1 CC genotypes had MPA AUCs and troughs 25–30% lower relative to those without lansoprazole and the same genotypes. These data suggest that individuals with these genotypes who require an acid blocker may be better managed with an alternative acid blocker or higher doses of MMF.

The addition of an azole antifungal to tacrolimus therapy generally requires a substantial tacrolimus dose reduction to maintain similar blood exposure. Kidney recipients (n = 79) receiving tacrolimus and ketoconazole 100 mg/day were genotyped for CYP3A5. Individuals with the CYP3A5*3/*1 or *3/*3 genotype had a 79–112% increase in tacrolimus troughs after starting ketoconazole whereas those with the *1/*1 genotype had only a 30% increase in dose normalized troughs [72]. A genotype effect also has been suggested for the interaction between tacrolimus and fluconazole in 29 kidney recipients [73]. Dose-normalized tacrolimus troughs occurring immediately following the addition of fluconazole increased 73% in those with the CYP3A5*3/*3 genotype and 45% in those with the *1/*3 genotype, relative to prior to fluconazole treatment. More subjects with the *3/*3 genotype than the *1/*3 genotype (73.9% vs. 16.6%) had trough tacrolimus concentrations >25 ng/mL after starting the fluconazole. These data demonstrate that the potency of tacrolimus drug interactions that are mediated through

CYP3A are in part a function of underlying genotype. Preemptive dose reductions may be determined more accurately with knowledge of the CYP3A5 genotype.

Summary

Careful consideration of pharmacokinetics, pharmacodynamics and pharmacogenomics must be taken into account when prescribing medications for transplant recipients, and drug interactions should be anticipated in essentially all patients. Approaches to prevent or minimize drug interactions should be considered. First, it is a general best practice to minimize the number of medications that a patient receives. Conduct a complete drug history at each transplant visit, including prescription, non-prescription, illicit and herbal medications. Pay careful attention to adding or removing an interacting medication to a transplant regimen. It is common that doses of CNIs and mTOR inhibitors require adjustment when CYP3A inhibitors or inducers are added or discontinued. For interactions that relate to absorption, it may be necessary to adjust the timing of medication administration to minimize the interaction. In the case of pharmacodynamics interactions, carefully monitor the patient for early signs of toxicity, infection or rejection. More aggressive monitoring of drug concentrations and chemistries may be necessary in the case of all types of interactions.

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SECTION 7

Long-term Transplant Outcomes

CHAPTER 103

Long-term Outcomes after Kidney Transplantation

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Introduction

Kidney transplantation has advanced substantially in the past three decades and is now routinely accepted as the treatment of choice for most causes of end stage renal disease (ESRD). With its success, growing numbers of kidney recipients have populated the clinics, and their outcome over time has become a growing concern, supplanting short-term survival as the predominant metric for success. Indeed, the issues influencing long-term outcomes differ in several ways from the factors influencing technical and early phase success, and the data capturing these factors continues to mature. This chapter will cover the long-term results that can be anticipated for patients receiving a kidney transplant, identify the donor- and recipient-specific risk factors influencing death and graft failure, and discuss the causes of late death and graft loss.

Long-term outcomes of renal transplant recipients

Clinical barometers for measuring success after kidney transplantation include traditional end points of acute rejection rates, allograft survival, and overall patient survival. Most data derived from clinical trials is focused on short-term survival. In fact, the majority of landmark studies and pivotal trials that have led to clinical practice changes are designed based on 1-year rejection rates and survival as their primary end points. The major difficulty in attaining accurate assessments of long-term outcomes is two-fold:

- the time required to reach long-term end points, and
- maintaining accurate follow-up during the longer time frame.

One way to navigate around these problems is to make projections using Kaplan-Meier estimates from large registry data as observational or retrospective analyses. One study merged data from the US Renal Data System (USRDS) with data from the Scientific Renal Transplant Registry (SRTR) and subsequently found long-term benefits of deceased donor transplants over maintenance dialysis. These benefits were evident irrespective of genders, ages, and races. These findings re-ignited research emphasis on transplantation as a life saving procedure. Now, kidney transplantation continues to emerge as the treatment of choice for ESRD patients, with well-established benefits in patient survival over dialysis [1,2].

As longer post-transplant follow-up data becomes available, estimation of graft and patient survival after transplantation has become more accurate. Previous predictions on shorter follow-up times are now replaced with actual data based on longer follow-up times. This allows for not only actual survival calculations but also for more accurate forecasting with more available data. Our understanding of long-term graft survival has evolved from a previous overly optimistic state to a realization that improvements in patient and graft survival are driven mostly by improvement of first-year survival [3].

Progress in immunosuppression management has resulted in constant improvement in overall rejection rates and advances in surgical techniques continue to improve graft longevity. This has led to an improvement in unadjusted 1-year graft survival rates from 76% in 1987 to 93% in 2008 (OPTN/SRTR Annual Data Report 2010). Even in the early years of transplantation from 1975 to 1990, a significant increase in 1-year graft survival was seen without a corresponding increase in allograft half-life of those organs that survived past the first year [4].

The most recent analysis of long-term outcomes after transplantation indicates that most advances have resulted in dramatic improvements in first-year survival with smaller advances in longer term survival rates [5].

Acute rejection rates have seen a dramatic decline over the past 20 years, yet graft life over the long run has not been impacted as significantly. This discrepancy may partially lie in the fact that renal allografts with rejections that return to baseline renal function have similar unadjusted 6-year graft survival as those allografts that do not have rejection. However, post rejection renal function that does not return to within at least 15% of baseline function is associated with a decrease in graft survival (50.2% 6-year survival compared to 72.7% that returned to baseline function) [6].

When counseling the individual patient in the pretransplant phase, graft half-life can be a clinically useful tool to provide the patient with a reasonable expectation of survival. Yearly graft attrition rates allow assessment of survival in early versus late post-transplant as the factors relating to survival vary over time.

In analyzing cumulative half-lives based on Kaplan-Meier estimates from 1989–2005, we find that the actual half-life of deceased donor transplants has improved from 6.6 years in 1989 to 8.2 years in 2000 and is forecasted to increase to 8.8 years in 2005. Among deceased donor transplants, standard criteria half-lives improved from 6.7 years in 1989 to 8.8 years in 2000, and forecasts to 9.5 years in 2005.A more dramatic improvement is noted with expanded criteria kidney half-life with an increase from 3 years in 1989 to 5.6 years in 2000 and forecasts to 6.4 years in 2005. Living donor transplants fare better, but have seen less of an improvement over time

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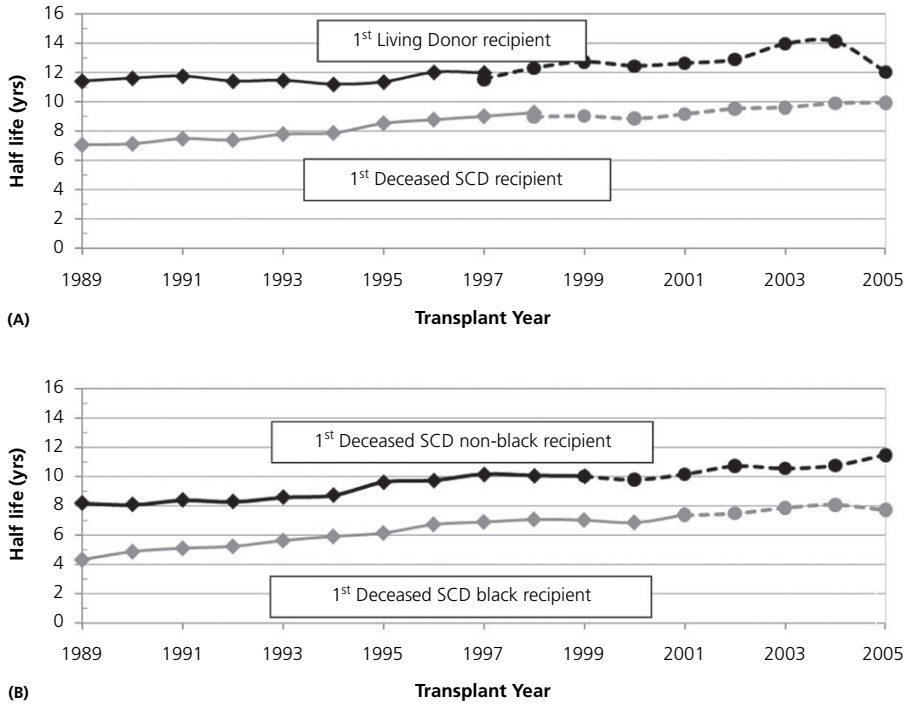

Figure 103.1. Actuarial (diamond marker, solid line) and projected (round marker, dotted line) cumulative half-lives. (A) First living donor versus standard criteria recipients. (B) First standard criteria in non-black versus black recipients. (Reproduced from [5] Lamb et al. Am J Transplant. 2011, with permission from Wiley-Blackwell).

with half-lives of 11.4 years in 1989 to a forecasted half-life to 11.9 years in 2005.

Racial disparities are known to exist with multiple studies showing black recipients having lower graft survival than non-black recipients [7,8]. In comparing half-lives of black versus non-black recipients, a similar absolute increase has been observed (4.1 to 7.4 years for black and 7.8 to 10.9 years for non-black), but not a major narrowing in the gap among black and non-black recipients (Figure 103.1).

The factors that contribute to long-term graft survival are different from short-term survival. Allograft-specific factors include chronic rejection, infections, and risks associated with long-term immunosuppression such as post-transplant lymphoproliferative disease. As an individual graft and patient life increases, more complex factors such as cardiovascular disease, weight gain, and access to care become more predominant. Indeed we are now beginning to see issues related to the aging transplant recipient that are resulting in increasing rates of death with a functioning graft.

Clinicians have struggled to find the best way to describe chronic allograft injury leading to graft loss. The term chronic rejection was initially used to describe the histologic changes of the failing allograft. The term was overly simplistic, since rejection is not the only mediator of chronic damage leading to graft loss. The term chronic allograft nephropathy (CAN) was adopted in the early 1990s as a means of emphasizing the multi-faceted etiology of progressive graft dysfunction. The characteristic lesions of CAN encompass tubular atrophy, interstitial fibrosis, glomerulosclerosis and arteriolar hyalinosis. In 2005, the 8th Banff Conference decided to eliminate the term CAN and focus on the more descriptive term chronic allograft injury. This term illustrates that a number of processes often contribute to chronic graft dysfunction. Hypertension, diabetes, infections such as BK virus, calcineurin inhibitor (CNI) toxicity, recurrent or de novo glomerular diseases, and chronic rejection can all cause chronic allograft injury and ultimately graft loss.

Organ quality and donor factors

Organ type and size

Risk factors for graft failure vary depending on the type of renal transplant. Organ quality plays a major role in outcomes [9]. Living donor transplants have minimal cold ischemia time and superior outcomes to include lower rates of acute rejection, lower incidence of delayed graft function, and longer graft life. Deceased donor transplants, regardless of the degree of HLA matching, have overall worse long-term survival when compared to living donor transplants. To illustrate this point, a living donor kidney transplant with 0 HLA matches has better graft survival than a 6 antigen match deceased donor kidney.

The quantity of functioning nephron mass in the renal allograft plays an important role in graft performance and survival. A large multi-center study of first time kidney transplant recipients determined that the ratio of kidney weight to recipient weight of less than 2.3 g/kg was an independent risk factor for graft loss at 2 years follow-up [10]. It is proposed that the same concept is relevant for kidneys from extremely young or older donors due to the smaller quantity of functioning nephrons relative to recipient need.

Donor risk index

The kidney donor risk index is an equation designed as a practical tool to assist clinicians with decision-making regarding deceased organ acceptance. This assessment takes into account donor factors that impact graft survival: donor age, height, weight, ethnicity, presence of diabetes, hypertension, death from stroke, donation after cardiac death, terminal creatinine, and hepatitis C virus infection

[11]. This calculation assigns a risk of graft failure relative to an "average" 40-year old standard criteria deceased donor kidney. While this score is useful in regards to assessing how well a kidney may do at the time of acceptance, the score does not reflect other factors that play into long-term graft survival and has not been validated prospectively.

When considering factors that predict prognosis, a distinction should be made regarding a risk factor (e.g. diabetes is a risk factor for graft failure) versus a prognostic factor, in which case the factor should reliably and predictably separate outcomes. The kidney donor risk index (KDRI) is reasonable to separate outcomes of kidneys from very high or very low risk donors, however it is not as robust in distinguishing outcomes of the majority of the "middle-range" kidneys. A risk index to reliably predict long-term graft survival that has been prospectively validated has not yet been identified.

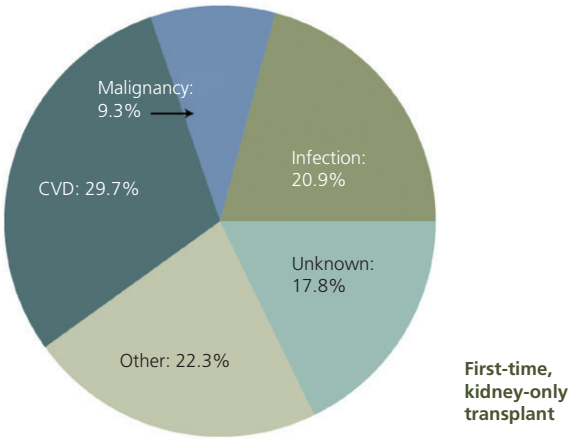

Figure 103.2. Cardiovascular disease accounts for most death with functioning renal allograft. Data from USRDS.
US Renal Data System, USRDS 2011 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2011. The data reported here have been supplied by the United States Renal Data System (USRDS). The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy or interpretation of the U.S. government.

Recipient factors

Timing of transplantation

The duration of time on dialysis is a known negative factor of allograft survival. Pre-emptive transplantation before dialysis initiation or minimal time on dialysis (<6 months) have been shown to have superior patient and death-censored allograft survival compared to and increasing time on dialysis [12]. Pre-emptive transplantation specifically is shown to significantly decrease patient mortality (relative risk [RR] 0.84 and 0.69) and graft failure (RR 0.75 and 0.73) in both living and deceased donor transplant recipients respectively. However, pre-emptive transplantation is also more likely to happen in younger, privately insured, and higher education recipients [13]. The studies showing benefit of early transplantation are mainly retrospective, and they inherently cannot avoid the preselection bias of healthier individuals with better access to medical resources receiving early transplants. Nonetheless, statistical adjustments to account for donor factors and preselection biases seem to uniformly indicate that earlier transplantation results in improved long-term outcomes. While the reasons have not been entirely elucidated, the early introduction of transplantation may reduce dialysis-related infectious and cardiovascular morbidity subsequently resulting in better long-term survival.

Death with a functioning allograft

While transplantation confers a survival advantage and net gain in life compared to dialysis, transplant recipients still have a shorter life expectancy than the general population. Cardiovascular disease is the leading cause of premature death in this patient population (Figure 103.2).

The annual incidence of death from cardiovascular causes is estimated to be 5%. Patients with advanced chronic kidney disease accumulate a significant burden of cardiovascular disease prior to transplant that further contributes to outcomes after transplant. The renal transplant recipient often has a combination of traditional and non-traditional risk factors for cardiovascular disease. The traditional Framingham risk factors include the presence of hypertension, hyperlipidemia, diabetes mellitus, tobacco use, obesity, and physical inactivity. Additional non-traditional risk factors affecting the transplant recipient are immunosuppression related side effects. Managing recipient risk factors for cardiovascular disease is of prime importance since it is the most common cause of death with a functioning allograft. This section will review each risk factor in detail.

Hypertension

Hypertension is an independent risk factor for late allograft loss. Blood pressure at one year is predictive of graft survival. There is a statistically significant reduction in graft survival for each 10 mmHg increase in systolic, diastolic and mean arterial blood pressure [14]. While there have been no prospective randomized controlled trials on blood pressure therapies and targets in the transplant population, Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend maintaining blood pressure <130/80 mmHg.

Hypertension, defined as a blood pressure exceeding 140/90, is prevalent in the majority of renal transplant recipients. It can be a consequence of pre-existing hypertension, obesity, or other transplant related factors. Graft dysfunction from acute or chronic causes is also associated with the development of hypertension.

Immunosuppressive therapy itself directly causes hypertension. Corticosteroids have been used for immunosuppression since the early years of transplantation and contribute to hypertension through mechanisms of salt and water retention. In the early 1980s, 50–60% of transplant recipients had hypertension. This prevalence has increased to 75–90% in the modern era of CNI use [15]. Many transplant programs are now employing steroid sparing and CNI avoidance protocols in attempt to minimize this adverse effect, as it is a major contributor cardiovascular disease and death with a functioning allograft.

Hyperlipidemia

In the first transplant year at least half of patients will have lipid abnormalities. Hyperlipidemia, especially elevated low-density lipoprotein (LDL), is an independent risk factor for cardiovascular disease. KDIGO guidelines recommend checking a lipid panel within the first three months post-transplant and annually thereafter. Certain immunosuppressive agents such as corticosteroids, CNIs and mammalian target of rapamycin (mTOR) inhibitors can exacerbate or cause de novo hyperlipidemia. Therefore, one may

consider modifying immunosuppression to help improve the lipid profile. Sirolimus is most associated with elevations in both total cholesterol and triglyceride levels. Among the CNIs, cyclosporine has the greatest impact on worsening of total cholesterol and LDL. The Assessment of Lescol in Renal Transplantation (ALERT) trial was a prospective, randomized control trial investigating the impact of statin therapy on cardiovascular outcomes of renal transplant patients. Over 2000 patients were randomized to fluvastatin or placebo. The statin group had a statistically significant reduction in the secondary endpoint of non-fatal myocardial infarction and cardiac death and a non-statistically significant trend toward reduction of cardiac events [16]. Based on this evidence, it is recommended that statins are the first-line pharmacologic therapy for hyperlipidemia in renal transplant patients. While attempts at lifestyle modification may be helpful, most patients will require pharmacologic therapy. When selecting a statin, one must be aware of the potential for drug interactions with the CNIs. Since both classes of drugs are metabolized via the cytochrome P450 system, some statins will need to be dose reduced when used in combination with CNIs. In general, fluvastatin, atorvastatin and pravastatin have the better safety profile for use in transplant patients. Due to the higher risk of cardiovascular disease in transplant recipients, KDIGO recommends LDL goals of less than 100 mg/dL. For diabetic transplant recipients or those with a known history of cardiovascular disease, some clinicians favor more aggressive target of less than 70 mg/dL.

Tobacco use

Up to one-third of kidney transplant patients smoke tobacco products on a regular basis Smoking at the time of transplant is an independent risk factor for all-cause mortality, ischemic heart disease, stroke, congestive heart failure and peripheral vascular disease. In a single center study, active tobacco use was associated with a two-fold increase in cardiovascular death compared to non-smokers. Additionally, data from the USRDS system indicates that incident smoking *after* transplantation increases the risk of death-censored graft loss and overall patient death. This is independent of pre-existing lung disease prior to transplantation [17]. Since patients have a forced abstinence during the transplant hospital admission, this represents an opportunity for interventions and counseling. Counseling should include the "5 A's":

1 ask about tobacco use,
2 advise to quit through clear and personalized messages,
3 assess willingness to quit,
4 assist in quitting, and
5 arrange follow-up and support.

There are a number of pharmacotherapy options available including nicotine replacement gum, lozenges and patches, the nicotinic receptor partial agonists, and anti-depressants.

Diabetes mellitus

As one of the leading causes of ESRD in the US, diabetes mellitus is present in one out of every five patients at the time of renal transplant. Among renal transplant recipients without pre-existing diabetes, 24% will develop new onset diabetes by 3 years after transplant. As in native kidney disease, diabetic nephropathy can affect the renal allograft and lead to graft dysfunction, although it is rarely the sole cause for graft loss. A retrospective study of renal transplant patients in the USRDS demonstrated that new-onset diabetes after transplantation (NODAT) was associated with increased graft failure and patient mortality. They identified a number of key risk factors for NODAT including age, African American and Hispanic ethnicity, obesity, and hepatitis C [18]. Transplant providers are encouraged to use the American Diabetes Association criteria to identify patients with impaired fasting glucose and diabetes. Impaired fasting glucose defined as a fasting glucose between 100–125 mg/dL without diabetes is also associated with higher rates of cardiovascular events in the renal transplant population. Education about lifestyle modification with diet and exercise should be included, especially in patients with obesity. Pharmacologic therapy should be prescribed with the goal of achieving HbA1c 7–7.5% per KDIGO guidelines. Low dose aspirin therapy is recommended for primary prevention of cardiovascular disease events in all renal transplant patients with diabetes.

Modern-day immunosuppressive therapy again can directly contribute to the development of NODAT. CNIs, especially tacrolimus, impair insulin secretion from pancreatic beta cells while corticosteroids contribute to insulin resistance. Therefore, providers should consider customizing immunosuppression based on the presence of or risk factor for diabetes.

Obesity

As in the general population, a large number of renal transplant recipients are affected by obesity. In fact, a retrospective review of the United Network for Organ Sharing (UNOS) database noted a 50% prevalence of obesity in renal transplant recipients. Utilizing the body mass index calculation (BMI), obesity is defined as a BMI greater than 30 and morbid obesity the BMI is greater than 35. Morbid obesity is independently associated with higher rates of delayed graft function and decreased overall graft survival [19]. In addition to its impact on renal allograft outcomes, obesity is linked to the development of insulin resistance, diabetes mellitus and atherosclerotic heart disease. Since appetite improves after transplant and dietary restrictions are lifted, transplant patients should be counseled on the importance of physical exercise and nutrition. This should be incorporated into each routine clinic visit.

Cardiovascular disease

It is important to realize that cardiovascular disease involves a spectrum of various endpoints including ischemic heart disease, congestive heart failure, peripheral vascular disease and stroke. These are all important outcomes of interest that contribute to premature death in renal transplant patients. The clinician should have a low threshold for screening. Revascularization procedures such as percutaneous angioplasty and coronary artery bypass grafting are available options in kidney transplant patients yielding 2-year patient survival rates of approximately 80% [20]. While there are risks of acute kidney injury related to the procedure, the rates of graft loss as a consequence are low.

CNI nephrotoxicity

CNI, cyclosporine and tacrolimus, have served as the backbone for maintenance immunosuppression for decades. These potent agents are largely responsible for the high rates of early graft survival seen in kidney transplantation. The weakness of CNIs is their associated nephrotoxicity. Thrombotic microangiopathy, arteriolar hyalinosis and tubular injury with isometric vacuolization have been described with CNI-related renal toxicity. Nephrotoxicity can present clinically as an acute azotemia and also as slower, chronic injury occurring with long-term use. Dihydropyridine calcium channel blockers

are believed to have a protective effect against the vasoconstrictive properties of CNIs. In a prospective study of 107 cyclosporin (CsA) treated patients, those receiving nifedipine had lower rates of delayed graft function, higher measured glomerular filtration rate and less interstitial fibrosis on biopsy at 12 months [21]. The hypothesis is that calcium channel blockers not only cause vasodilation of the afferent arteriole, but they also block the influx of intracellular calcium and this may protect the graft from ischemia-reperfusion injury. In addition to the direct effect of CNIs on afferent arteriole constriction, CNI therapy significantly contributes to cardiovascular risk by having higher incidences of hypertension, diabetes, and hyperlipidemia as mentioned above.

CNI-sparing regimens have long-been sought after with very few immunosuppression therapies showing longer allograft survival in the long term. Belatacept is the only recent exception, with better documented renal function at three years post-transplant compared to recipients maintained on cyclosporine [22]. Recipients maintained on belatacept without a CNI also seem to have better metabolic risk profiles with decreased incidence of hypertension, hyperlipidemia, and diabetes compared to cyclosporine treated recipients [23]. Long-term follow-up data is still needed, however the projection of improved renal function in patients treated with belatacept as opposed to cyclosporine appears promising.

Recurrent or de novo glomerular disease

The risk of graft failure from recurrent glomerulonephritis (GN) is highest in patients whose ESRD was from focal segmental glomerulosclerosis (FSGS), membranoproloferative glomerulonephritis, and IgA nephropathy. Patient at low risk for graft failure from recurrent GN include patients with lupus nephritis and anti-glomerular basement membrane disease. In patients with a history of idiopathic FSGS, an estimated 30–50% of patients will experience disease recurrence in the allograft. A history of rapid progression to ESRD within three years of diagnosis and previous failed transplant due to recurrent FSGS are the strongest predictors of recurrent disease.

One retrospective analysis compared 167 renal allograft recipients with recurrent or de novo glomerular disease to 4746 recipients without glomerular disease.

Patients with GN had a median graft survival of 1360 days compared to 3382 days in recipients without recurrence or de novo disease ($P < 0.0001$). The 5-year survival rate for those with recurrent or de novo disease was 39.8% versus 67.6% without disease [24]. Recurrent disease is covered in additional depth in Chapter 77.

Immunologic injury

Transplant recipients with higher panel reactive antibody (PRA) values at the time of transplant are known to have worse long-term survival. A retrospective analysis of 4000 HLA-identical sibling transplant recipients showed a 72.4% 10-year survival in recipients with no PRA pretransplant as compared to 63.3% with 1–50% PRA, and 55.5% in >50% PRA [25]. Some studies also suggest that patients maintained on lower doses of immunosuppressive therapy develop a higher prevalence of chronic allograft nephropathy as another risk for earlier graft loss. These data indicate that chronic and subclinical immune-mediated injury clearly plays a role in long-term graft survival. Identifying non-HLA immunity continues to pose a challenge in improving chronic allograft injury.

The impact of sensitization and donor specific antibodies

Sensitized patients are those individuals who have detectable HLA antibodies in the serum prior to transplantation. Pregnancy, previous blood transfusion and prior transplants are all sensitizing events, which may lead to HLA antibody formation. When present in sufficient quantity, these donor-specific antibodies (DSAs) will cause positive crossmatch studies. Some transplant centers in the US report successful transplantation of individuals with a positive flow cytometry crossmatch after the use of desensitization protocols. These protocols combine pretransplant plasmapheresis with intravenous Ig to lower the titers of DSA prior to transplant. DSA at the time of transplant has been associated with early antibody mediated rejection and shorter graft half-life. Additional detail regarding DSA and its detection can be found in Chapter 77.

Non-sensitized patients can experience antibody-mediated rejection (AMR) through the development of de novo DSAs. A multi-center, prospective trial by demonstrated that 20% of patients with a functioning renal allograft developed HLA antibodies [26]. These antibodies were associated with significantly higher rates of graft loss one year after detection. This association was true for those sensitized at the time of transplant as well as those who developed de novo DSA post-transplant. DSAs may appear months to years prior to the development of graft dysfunction. A prospective study of 300 first time renal transplant recipients with no detectable HLA antibody at the time of transplant found a 15% prevalence of de novo DSA development after the first six months post-transplant. The two strongest predictors for de novo DSA development were HLA-DR mismatch and medication non-adherence. In ten-year follow-up, graft survival was 57% in the de novo DSA group compared to 96% in the group without DSA [27]. Another single center study of 347 renal transplant patients followed prospectively over a three year period 18% of recipients developed de novo DSA, with DQ antibody being the most prevalent [28]. Patients with DQ antibody in conjunction with other DSA had worse three year graft survival (52%) compared to HLA-DQ alone, other HLA antibodies alone, or no antibodies (92–94%). This supports the pervasive belief that class II HLA antibodies have a dominant role in late graft failure. The following diagram (Figure 103.3) depicts the proposed natural history of de novo DSA and its impact on the allograft.

The spectrum of antibody mediated injury

HLA antibodies are the major players in the development of AMR. The entity of AMR traditionally compromises a wide spectrum of pathology from hyperacute rejection to acute and chronic AMR and ultimately transplant glomerulopathy. Late-onset acute AMR with or without detectable DSA is often a signal of medication non-adherence.

A variety of effective therapies for acute antibody mediated rejection are employed, including plasmapheresis, immunoadsorption, and intravenous immunoglobulin (IVIG), rituximab, bortezomib, and eculizumab. These therapies are discussed in detail elsewhere in this textbook.

While targeted therapy can often reverse the acute AMR episode with improvement in renal function, the occurrence of AMR negatively impacts long-term graft survival. Despite improvement in clinical parameters, patients with AMR may progress to develop transplant glomerulopathy. Transplant glomerulopathy is believed to be a consequence of immune mediated endothelial injury, and is highly associated with inferior graft outcomes.

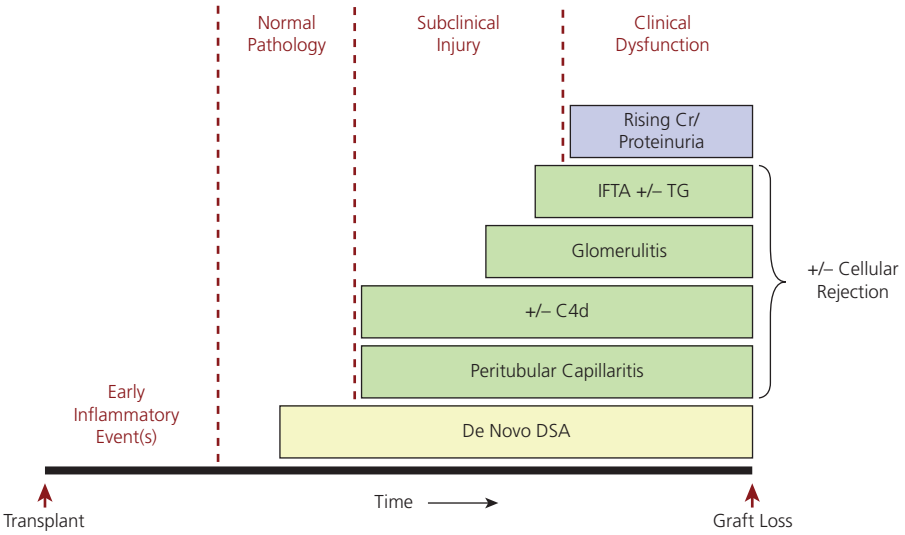

Figure 103.3. Proposed natural history of de novo donor specific antibodies (adapted from [27] Wiebe et al. Am J Transplant. 2012, with permission from Wiley-Blackwell).

Chronic antibody mediated rejection (CAMR) has been a major cause of late graft failure. Histologically, CAMR is associated with the presence of multi-lamination of the glomerular and tubular basement membrane, transplant glomerulopathy, and peritubular capillaritis with or without C4D deposition. CAMR often is accompanied with significant tubular interstitial fibrosis and tubular atrophy as well as microvascular changes. Since antibodies initiate their insult by adhering to the endothelium of the microvasculature, this is common. Despite effective immunosuppression and a variety of strategies to treat acute humoral rejection, there are no effective strategies for the management of CAMR. Clinically, CAMR may manifest as subacute, progressive renal allograft dysfunction and proteinuria. Patients may also have peripheral edema and hypertension. The presence of pretransplant DSAs, highly sensitized state and de novo DSA formation are all associated with the development of CAMR. Non-adherence is a major contributing factor and should be suspected in patients with serial subtherapeutic immunosuppression drug levels.

Infection and post-transplant malignancy

Transplantation continues to be hampered by infectious and malignant morbidity and mortality. While additional detail regarding the general topics of late infectious and malignant disease can be found in Chapters 94 and 95, respectively, the prevailing features of these complications are summarized below.

Cytomegalovirus infection

CMV mismatch (donor positive/recipient negative) status at the time of transplant is associated with decreased graft survival and increased patient mortality. CMV disease post-transplant has also been shown to be of a higher incidence in recipients that received zero match HLA-DR kidneys [29]. This single-center retrospective review of 470 transplanted recipients also found decreased graft survival of recipients with CMV disease and zero matched HLA-DR (5-year survival was 16.2% for zero matches compared to 87.5% for two matches). The predisposition to earlier graft loss may in part be due to direct morbidity from CMV infection, and also may be from the longer-term effect of decreasing maintenance immunosuppression. Although allocation based on CMV sero-status matching along with HLA has been considered in the transplant community, the current organ shortage in the US favors less waiting time over serological matching. After transplant however, most centers continue 6–9 months of extended prophylaxis in recipient CMV-negative transplants with the intent to minimize CMV disease and subsequently improve longer outcomes.

BK polyoma virus

In transplant recipients, increased immunosuppression burden is associated with active BK infection leading to tubular-interstitial nephritis, ureteral stenosis, and cystitis. Diagnosis is usually made by screening and mostly occurs within the first year after transplant, although it can occur much later. Since first-line treatment involves reduction of immunosuppression, adverse effects on the allograft may be three-fold:

- direct viral tissue injury may occur with complications in the renal parenchyma and urinary tract,
- long-term chronic immunological injury can result from immunosuppression reduction, and
- faster decline of renal function after inflammation and fibrosis may be observed.

Actual data on graft loss effects after detection of BK nephropathy are limited to very small studies with a wide variety of immunosuppression reduction strategies, making the actual impact of late BK difficult to assess. In an analysis of 41 renal allograft recipients with BK nephritis, 3- and 5-year survival was significantly reduced from 83% and 76%, respectively in those without BK down to 58% and 47%, respectively. Forty six percent of BK recipients experienced graft loss. Of those that recovered renal function after management of BK, the time to stabilization of renal function was 112 days [30]. BK virus nephropathy presents a significant threat to both short and long term graft survival.

Epstein-Barr and post-transplant lymphoproliferative disease

Post-transplant lymphoproliferative disease (PTLD, covered in detail in Chapter 96) occurs in an estimated 1% of transplanted

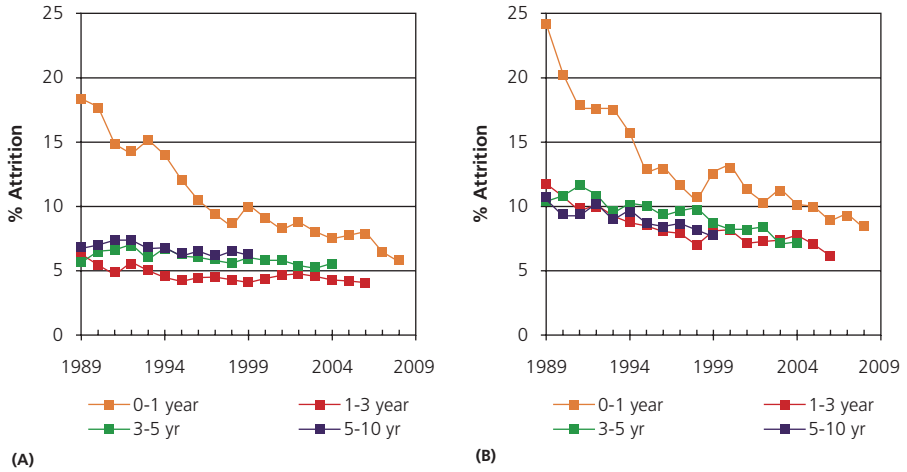

Figure 103.4. Cumulative graft failure yearly attrition rates of first kidney transplants. (A) Non-black deceased SCD donor, and (B) black deceased SCD donor. (Reproduced from [5] Lamb et al. Am J Transplant. 2011, with permission from Wiley-Blackwell.)

patients, but confers a high risk of death. It is classically associated with Epstein-Barr virus (EBV) induced B cell proliferation, although EBV-negative PTLD is well documented. It is the most common malignancy of transplant recipients excluding non-melanoma skin cancers. The traditional thought has been a higher risk of PTLD is conferred by EBV seronegative status at the time of transplant and immunosuppression burden. Recent data showing a higher incidence of PTLD in EBV negative patients on the CLTA-4 inhibitor belatacept suggests that risk of PTLD is more related to T cell suppressive therapy [22].

Most diagnoses of PTLD are made in the first 18 months post-transplant with an estimated 1% incidence over 10 years. Although a relatively rarer complication, patient survival is significantly reduced to 51% at 1-year and 39% at 5-years [31]. An OPTN database analysis of PTLD diagnosis estimated the risk of death with graft function to be 17.5 times higher with diagnosis of PTLD, and the risk of death-censored graft failure to be 5.5 times higher [32].

Social and racial determinants of health

Significant but still poorly characterized effects of socioeconomic factors on long-term outcomes of transplant recipients are becoming increasingly recognized.

Black recipients experience worse graft and patient survival rates than other races. Variations in HLA polymorphisms, immunosuppression requirements, and pharmacokinetic variability have been described as biological factors influencing higher rejection rates and graft [7,33]. In the recent era of immunosuppression advancement, evidence suggests that some biological barriers are giving way to more socioeconomic influences that result in poorer outcomes. Lower graft survival is known to be associated with low income, low education, less insurance coverage, and access to care barriers. However it is also notable that the graft survival racial gap narrows when factors such as income and education are accounted for. Many socioeconomic barriers are found in a higher prevalence and exert a more significant effect in black recipients as compared to non-black recipients.

When analyzing long-term outcomes, graft yearly attrition rates can serve as a valuable tool to distinguish the early versus late post-transplant period. In this way, we note that a greater impact on the reduction of long-term graft attrition rate of black recipients has been made (Figure 103.4). Not only is there a steeper reduction in the first year attrition rate, but the subsequent years appear to have a modest yet perceptible reduction in the 1–3, 3–5, and 5–10 year attrition rates.

The multifactorial nature of chronic allograft loss poses a significant challenge in the current transplant era. Control of the immunological response drives short-term survival, however the interventions that protect the graft early post-transplant ironically contribute to long-term attrition. Eventually, predisposition to cardiovascular disease, infectious complications, and PTLD becomes the predominant threat to both graft and patient survival.

Chronic disease states coupled with socioeconomic burdens make it difficult to pinpoint specific intervention points on a large population-based level. Improving long-term survival now requires a long-term follow-up process on each individual patient level. An emphatic approach of modifying risk factors and preventive health care from even prior to transplant surgery will eventually be necessary. As the transplant recipient is now living longer and little room is left for short-term success improvement, our focus is becoming increasingly directed at modifying chronic disease and social barriers.

Summary

Kidney transplantation remains a highly satisfactory alternative to dialysis. However, it remains a cure for kidney failure in that it imposes a chronic condition of immunosuppressive drug use that adversely influences long-term patient and graft survival. The great successes in early phase survival have now provided an opportunity to tackle the challenges of prolonged immunosuppressive drug therapy, and the chronic co-morbidities that are associated with renal failure. A thorough understanding of these issues will be required for clinicians and researchers hoping to improve transplant care in the future.

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CHAPTER 104

Long-term Outcomes after Liver Transplantation

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Introduction

Liver transplantation is now the standard of care and only cure for end stage liver disease (ESLD). However, the first human liver transplantation was reported only 50 years ago, in a three patient series whose first recipient died on the operating table, the second died three weeks after transplant, and the third died one week post-transplant [1]. Up until as recently as 1980, less than 40% of patients undergoing liver transplantation survived to 1-year after the transplant [2]. In contrast, the 2009 SRTR annual report lists graft survival as 86% at 1-year, 70% at 5-years, and 53% at 10-years, and patient survival as 90% at 1-year, 75% at 5-years, and 60% at 10-years [3].

Long-term graft and patient survival following deceased donor liver transplantation have been steadily improving over time, and much of the improvement occurred between 1980 and 1995 (Figure 104.1A and B). This has largely been attributed to refinements in surgical, immunosuppressive, and organ preservation techniques during the first three decades of our human liver transplantation experience. These are covered in depth in Chapters 56, 66, and 21, respectively. The argon beam coagulator was invented in 1988 and intraoperative blood salvage devices (cell-saver) came into use in the 1990s, as well, completely changing the magnitude of blood loss in the operating room. The conversion to tacrolimus in the early 1990s also contributed to improving long-term outcomes by decreasing not only the incidence of rejection, but also the side effects associated with high dose steroids and T-cell depleting therapy needed to treat rejection episodes. In the late 1980s, University of Wisconsin solution (Viaspan) was developed originally for pancreas preservation, but soon replaced Eurocollins as the standard for liver preservation, because of better outcomes; it remains the gold standard preservation solution today. Improvements in intensive care have also played a role in decreasing postliver transplant mortality; this is covered in depth in Chapter 43. Since the number of living donor liver transplants was well under 100 per year for most of the 1990s, long-term graft and patient survival statistics are erratic during that decade, but since 1999, when over 200 living donor liver transplants were performed, succeeding years have also shown small gains in survival (Figure 104.2A and B; see also Chapters 24 and 57).

It is interesting to note that the slopes of the survival curves from one era to another are quite similar after the three month time point, with the greatest decrease over time in liver recipient deaths occurring during the first 0–90 days post-transplant (Figure 104.3). Thus, the apparent improvement in long-term outcomes is actually the result of improved short-term outcomes following liver transplantation. Although we have made great strides in the factors that contribute to perioperative mortality, as discussed above, we have made less progress with the long-term challenges of liver transplantation, many of which are the result of underlying patient disease and the side effects of chronic immunosuppression. This chapter outlines some of the late causes of recipient death and graft loss, factors that affect long-term outcomes after liver transplantation, and late complications occurring after liver transplantation.

Causes of late deaths and graft failure

Over half of liver transplant recipients who die, do so with a functioning graft. Isolated graft failure, as a cause of death, is reported to account for only 9–43% of late deaths (beyond 1–3 years after transplant) in various single and multi-center studies in North America [4–9]. In contrast, early deaths (within three months of transplant) are overwhelmingly the result of infection, graft dysfunction, and technical complications.

Recurrent disease and chronic rejection

Graft failure results primarily from recurrent disease, followed by chronic rejection, the former almost entirely due to recurrent hepatitis C virus (HCV) infection, with a small percentage due to relapses of alcoholic hepatitis. Interestingly, in the UK, graft failure from all causes accounts for barely 10% of post-transplant deaths [10]. Of note, only 21% of liver transplants in England were done for HCV in 2008, up from 10% in 1996 [11]. Over a similar period, liver transplants done for HCV in the US rose from 21% to over 40% [12]. An in depth treatment of recurrent disease can be found in Chapter 78.

Malignancy and infection

Other major causes of death include malignancy and infection. As complications of immunosuppression, they might justifiably be considered graft-related, even if they do not represent actual graft failure. Nine to 24% of deaths are due to de novo malignancy, with an approximately equal percentage due to recurrence of the malignancy that was the indication for transplantation, making malignancy the single largest cause of late death in liver transplant recipients [4,6,7,9,10]. Post-transplant lymphoproliferative disease (PTLD) accounts for a very small proportion of these deaths, since the 15 year cumulative incidence of PTLD is only 4.7% and is

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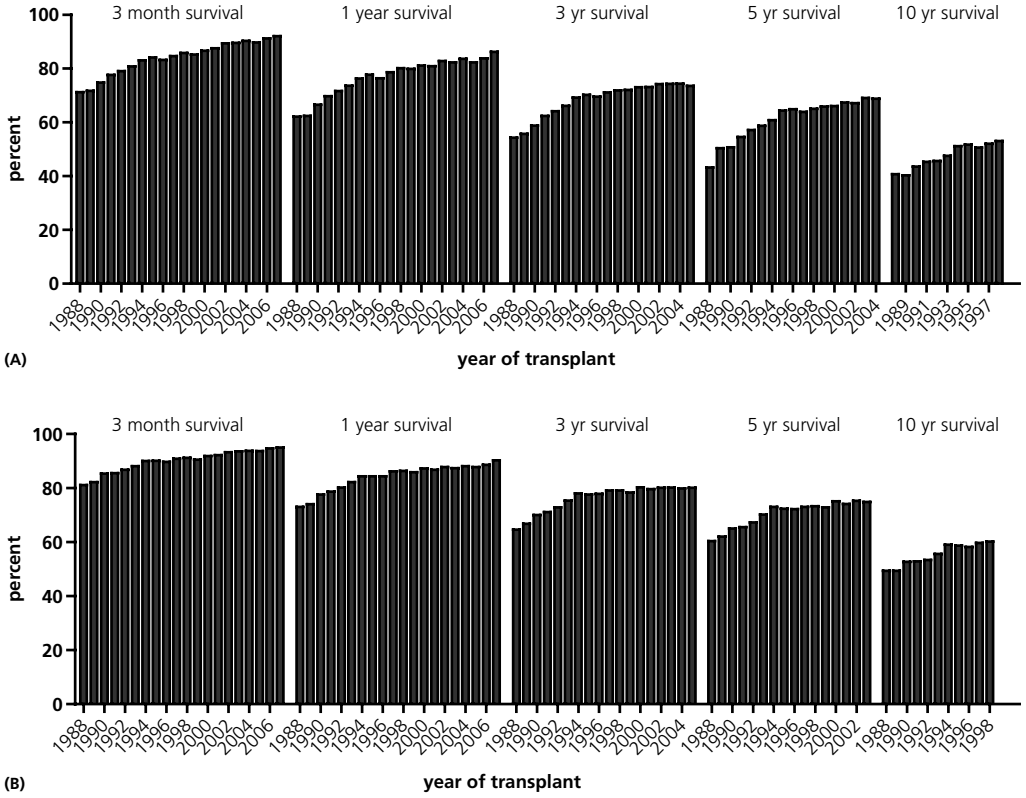

Figure 104.1. (A) Adjusted graft survival after deceased donor liver transplantation. (B) Adjusted patient survival after deceased donor liver transplantation.

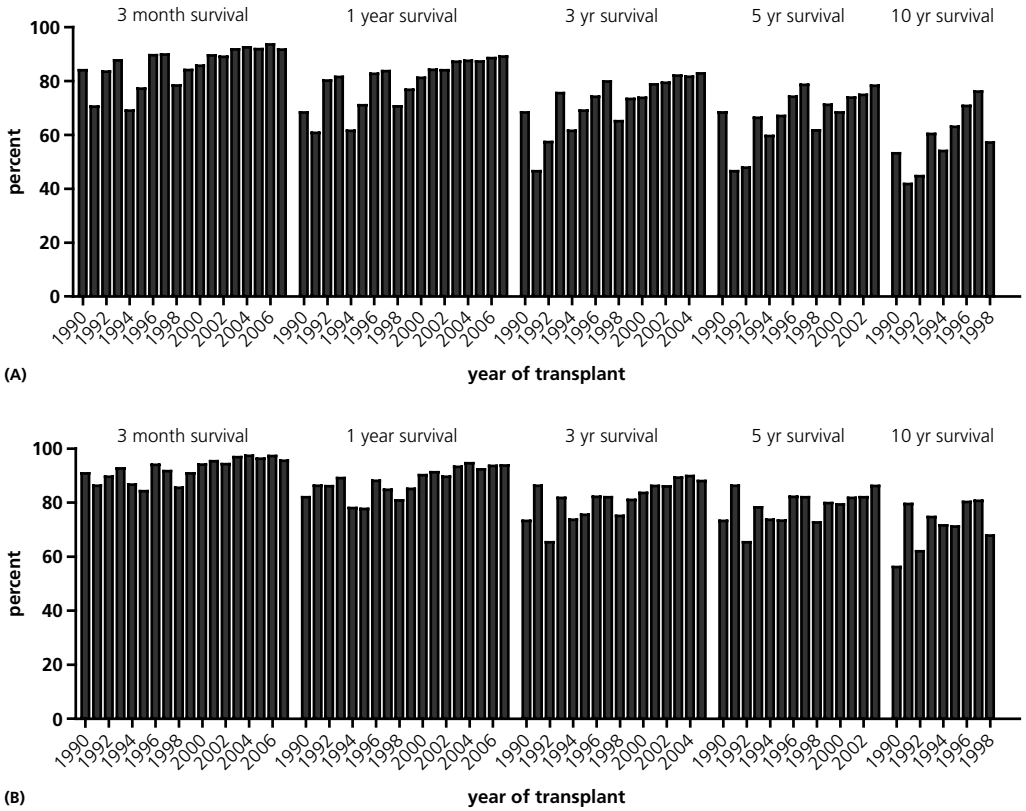

Figure 104.2. (A) Adjusted graft survival after living donor liver transplantation. (B) Adjusted patient survival after living donor liver transplantation.

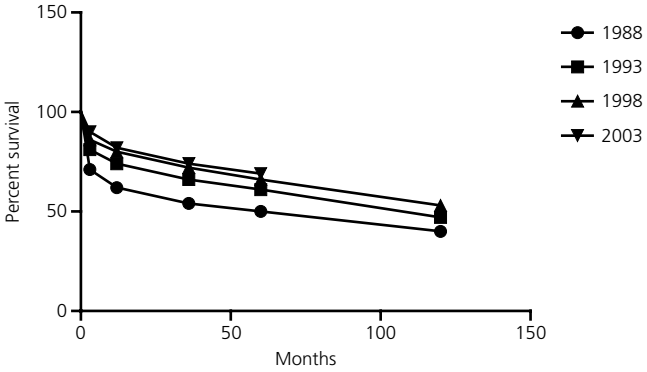

Figure 104.3. Survival of three groups: Survival proportions.

associated with only a 10–15% increase in risk of death [13]. Additional treatments of these topics are found in Chapters 95 and 96.

Infection, the most obvious side-effect of immunosuppression, is listed as the cause of death in 8–20% of cases [4,5,8,9], although as much as an additional 10% of "multi-system organ failure" reports may be related to infection, as well. Not surprisingly, series with a higher rate of death due to infection have a lower rate of death due to chronic rejection and vice versa. General discussions on infections are found in Chapter 94.

Cardiovascular disease

The primary non-graft related cause of death is cardiovascular disease (see also Chapter 88), accounting for approximately 20% of late deaths in most series [4–9,14]. Although cardiovascular disease is sometimes considered a side effect of steroids and calcineurin inhibitors, based on their well-documented association with risk factors associated with cardiovascular disease (hypertension, diabetes, hyperlipidemia), studies suggest that those who die of cardiovascular disease had risk factors or even known coronary artery disease prior to transplant [4]. One series from Canada found that there was no statistical difference in the incidence of cardiovascular disease between liver transplant recipients and the age matched general population [5].

Cardiovascular disease is likely to become the primary cause of death overall as non-alcoholic fatty liver disease (NAFLD) assumes an increasing share of the underlying diagnosis in liver transplant recipients. NAFLD is predicted to become the leading indication for liver transplantation over the next 5–10 years [15], and the risk factors associated with NAFLD are the same as those predisposing to cardiovascular disease: diabetes, hyperlipidemia, hypertension, and obesity. Indeed, deaths from cardiac events occur more frequently in those transplanted for NAFLD than in those transplanted for primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), alcoholic liver disease (ALD), or HCV [16].

Recipient factors that affect long-term patient outcomes

Primary diagnosis

Given the fact that recurrent malignancy is a major cause of death following liver transplantation, it is not surprising that malignancy as a primary diagnosis for transplant is associated with the highest risk of death. Studies estimate that transplantation for hepatocellular carcinoma (HCC) incurs twice the risk of death as transplantation for non-malignant indications [17]. SRTR reports similar or better survival at three months and 1-year after transplantation for malignant neoplasms when compared to other diagnoses, but lower at 5- and 10-years by approximately 10% and 20%, respectively. One single center study reported the gap at their institution to be over 10% at 1-year, and over 40% at 5-years and later [18]. Five and 10-year survivals following transplantation for malignant neoplasms are only 67% and 50%, respectively [3].

Liver transplantation for fulminant hepatic failure also carries higher risk of death than for non-malignant chronic liver disease, with 5- and 10-years survivals of only 71% and 58% [3]. Unlike for malignant disease, lower survival is evident within three months after transplant, after which the drop-off rate is similar to recipients transplanted for non-malignant chronic disease [3]. This is consistent with the high incidence of sepsis/multi-organ failure and neurologic complications as a cause of death post-transplant, much of which were present going into transplant [19]. Pretransplant renal failure is the strongest predictor of post-transplant mortality [19].

Although transplantation for HCV was initially thought to result in similar outcomes to other diagnoses [20], this included patients transplanted in the early 1980s and 1990s, when overall survival after liver transplantation hovered just above 70% after 1-year. In the meantime, survival after transplantation for non-HCV diagnoses has improved, but survival after transplantation for HCV has shown less improvement [21–23], and more recent studies suggest that HCV is associated with a 23% higher risk of death and 30% higher risk of graft failure than non-HCV diagnoses [24]. Part of the failure of long-term outcomes to improve over time as much as short-term outcomes may be related to the increasing percentage of transplants performed for HCC and HCV, both of which are associated with poorer long-term survival than other diagnoses.

On the other end of the spectrum, biliary atresia and metabolic disease are associated with the best long-term survival. Although biliary atresia and metabolic disease are the two leading indications for liver transplantation in children, together they comprise less than 1% of total transplants performed in the US. Pediatric liver transplantation is covered specifically in Chapter 114. Among the more common indications for transplant, transplantation for cholestatic disease, including PBC and PSC, is associated with the best outcomes [3,18].

Hepatopulmonary syndrome and portopulmonary hypertension

Hepatopulmonary syndrome (HPS) is thought to result from intrapulmonary dilation and shunting, returning unoxygenated blood to the left ventricle and causing hypoxemia. Estimated to occur in 8–32% of patients with ESLD [25,26], the only known treatment is liver transplantation. However, patients with HPS tend to fare worse after transplant than patients with normal oxygenation [26,27], with 90-day mortality in some series approaching 30% [26], although select patients may do as well as those without HPS [27]. Death after transplant is associated with a larger shunt fraction, whether measured by room air $PaO2 < 60$ mmHg, A-a gradient, or macroaggregated albumin scan [26]. Reversal of shunting post-transplant can take weeks to months.

In contrast, liver transplantation is not standard treatment for portopulmonary hypertension, not because of any viable alternative therapy, but because outcomes after liver transplantation are so poor in severe cases. Portopulmonary hypertension occurs in 4–16% of cirrhotics, more frequently in those with severe ascites [28], but the mechanism is not clear. High pulmonary vascular

resistance is thought to arise from an imbalance of vascular mediators in ESLD that lead to vasoconstriction, endothelial damage with vascular remodeling due to excessive shear stress in the setting of vasoconstriction, and finally, smooth muscle proliferation, microvascular thrombosis, and right heart failure [28]. Depending on how far remodeling has progressed, the situation may or may not resolve after transplantation.

Overall mortality following liver transplantation in the setting of portopulmonary hypertension approaches 40% during the transplant admission, nearly one-third of which are intraoperative [27]. Studies stratifying recipients by mean pulmonary artery pressure (MPAP) demonstrate perioperative mortality similar to that of recipients without portopulmonary hypertension if MPAP = 25–35 mmHg. For those with MPAP = 35–50 mmHg, perioperative mortality is over 50%, and 100% for those with MPAP >50 mm Hg [29]. Good outcomes have also been demonstrated in patients whose initial MPAP >50 mmHg, but were able to be lowered to <35 mmHg with prostacyclin [30]. The ability to decrease MPAP with vasodilators is considered to be an indicator of reversibility and a good prognostic sign.

Age, gender, and race

Although some groups have reported that patients over the age of 60 have worse outcomes than those who are younger [31–37], in reality, there is not an abrupt change in median survival at age 60. In general, younger patients, especially preadolescent children, do better than older patients after 1-year post-transplant, with three month survivals being fairly close in all age groups (Figure 104.4).

Recent studies of older cohorts suggest recipients over the age of 70 do just as well as recipients less than 60 years of age. However, the older patients tended to have lower MELD scores, creatinine levels, and rates of pretransplant intensive care unit stays, or higher albumin levels than their younger counterparts [38,39], suggesting that use of more stringent selection criteria in older candidates is important in ensuring good post-transplant outcomes. Not surprisingly, both studies found that the risk of cardiovascular complications post-transplant was higher in the septuagenarian population, indicating that screening for cardiovascular risk factors and disease is especially important in older recipients.

Twice as many men as women have received liver transplants, but gender does not appear to influence overall patient survival post-transplant [3,40]. Of note, as a sub-group, female, HCV+ recipients have a 14–15% greater risk of death at 1-, 5- and 10-years post-transplant than male, HCV+ recipients [40].

Older studies found that Asians and African Americans had worse outcomes than Whites and Hispanics [41]. Over time, outcomes among Asians have improved to the point that the group now has the highest survival rates after liver transplant [3,42]. This may be due, in part, to improving treatment for Hepatitis B virus (HBV) associated cirrhosis, which is the leading indication for liver transplantation among Asians, as opposed to HCV, which is the leading indication for liver transplantation in non-Asians [42]. Initially, outcomes after transplantation for HBV were so poor that it was considered a contraindication to liver transplant. With the advent of HBIg and then the nucleoside analogs lamivudine, tenofovir, and entecavir, HBV recurrence can be prevented, and HBV patients who do not recur have some of the best survivals post-transplant [43–46].

However, African Americans continue to suffer from poorer outcomes. It has been noted that African Americans experience higher rates of chronic rejection [47]. At the same time, they have a higher likelihood of HCV recurrence that is less likely to respond to therapy [48]. African Americans are also more likely to develop post-transplant diabetes, which has been associated with increased morbidity and mortality [49].

Body mass index

Approximately 3% of liver transplants are performed in patients with body mass index (BMI) >35 kg/m^2 [50]. Although single center studies did not find any difference in long-term survival

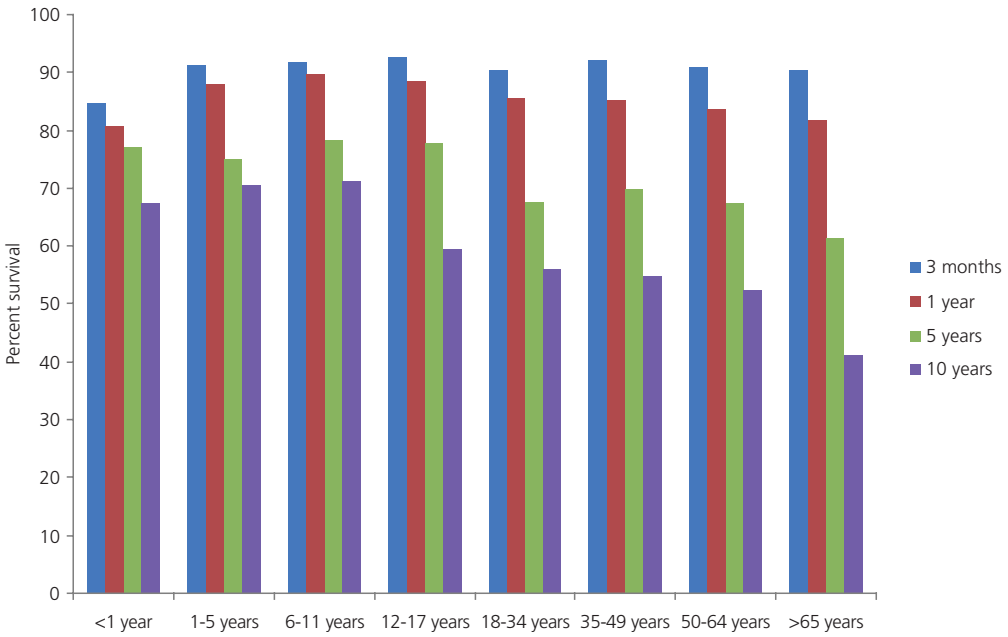

Figure 104.4. Patient survival after liver transplantation by age group.

among the obese (BMI 27–32 kg/m^2) and severely obese (BMI >32 kg/m^2) when compared to normal BMI recipients, they nevertheless found that obese patients had higher rates of postoperative respiratory failure and longer hospital stays and costs [51]. Severely obese recipients are also more likely to experience graft dysfunction, possibly due to longer warm ischemia times resulting from technical issues associated with the obese body habitus [52]. Furthermore, larger studies demonstrate that recipients with BMI >35–40 kg/m^2 experience as much as a 40% higher risk of death, generally due to cardiovascular events and within the first post-transplant year [50,52].

The disparity between the results of large and small studies is generally attributed to statistical issues, with small studies being insufficiently powered to detect survival differences. However, some authors suggest that there is truly no difference in survival if BMI's are corrected for ascites. As many as 20% of patients move into a lower obesity category once their weight is corrected for ascites, and the authors point out that each liter of ascites is associated with a 7% increase in mortality [53]. It is possible that obesity itself is not a strong predictor of post-transplant mortality, but associated co-morbidities that are more common in the morbidly obese, whether occult or recognized, play a role in increasing the risk of death [54].

As an alternate to post-transplant survival as a measure of the value of liver transplantation, some studies look at survival benefit, arguing that long post-transplant survival times are meaningless if survival times without transplant are equally long. When considering survival benefit, as determined by the hazard ratio of death with transplant to death without transplant, there is no difference among BMI categories stratified in intervals of 5 kg/m^2 [55].

On the other end of the spectrum are recipients with BMI <18.5 kg/m^2, who constitute approximately 2% of the transplant population [50]. Although they are generally considered to be high–risk candidates, there are few studies published on outcomes in the severely underweight. Those few indicate that they have a 55–60% higher risk of death after transplant, mostly due to hemorrhagic and cerebrovascular complications [50,51]. Again, most deaths appear to occur during the first year after transplant [50]. Their survival benefit from liver transplantation is slightly lower than for other BMI categories (0.14 vs. 0.14–0.17), but this difference is not statistically significant [55].

Multi-organ transplantation in ESLD

Of the various combinations of multi-organ transplants performed, simultaneous liver-kidney transplants is the most common, numbering over 300, and sometimes over 400, a year since 2005 [56]. This has been substantially influenced by allocation policy in the US, with renal failure increasing a recipient's MELD score substantially. The indications for combined liver-kidney transplant are beyond the scope of this chapter, but studies suggest that there is no penalty in survival for recipients who receive a kidney at the time of primary liver transplantation than for those who receive a liver only [57,58]. In fact, a subset of ESLD patients with renal insufficiency fare better with concomitant renal transplantation than liver transplantation alone, although the severity of renal failure and length of dialysis that defines this subgroup varies among studies [58–60]. At the same time, multiple studies have demonstrated that pretransplant renal failure is a predictor of increased mortality post-transplant [61,62], suggesting that including a kidney with the liver reverses the detrimental effects of renal failure in liver transplantation. Of note, simultaneous liver-kidney transplantation results in better renal allograft survival compared to kidney after liver transplantation [63], due in part to the removal of donor specific antibodies by the liver [63–65], although this protection from humoral rejection of the kidney is not absolute [66].

The need for any other concomitant abdominal organ transplant besides kidney is associated with much poorer outcomes than liver alone. However, this is presumably balanced by an even higher risk of mortality without transplant. Approximately 100 liver-intestine (a handful including pancreas and/or kidney) transplants are performed a year in the US. In spite of significant improvement in survival after intestine transplantation over the last ten years, 1-year patient and graft survival following combined liver-intestine transplantation hovers around 70%, much lower than for either liver alone or intestine alone [67]. Three and 5-year patient and graft survival are just above and just below 60%, and 10-year patient survival is less than 50% [67]. Only a handful of liver-pancreas (with or without kidney) are done a year [56], with 3- and 5-year survival reported to be 71% and 64% [68]. Outcomes after liver-intestinal and multivisceral transplants are covered in Chapter 109.

Combined liver-thoracic organ transplantation is similarly rare. In 2009, a record high of 11 liver-heart transplants (with an additional liver-heart-kidney) and ten liver-lung transplants (with an additional liver-lung-heart) were performed [56]. Although uncommon, outcomes after liver-heart transplantation are only slightly worse than for liver alone and certainly no worse than for heart alone, with patient at 1-, 3- and 5-year survival reported to be 85%, 77%, and 75% [69,70]. This may be due, in part, to the fact that the leading indication for simultaneous liver-heart transplantation is familial amyloidosis, in which the recipients rarely have any pretransplant liver dysfunction except for mutant transthyretin production [69–71]. In addition, the mean age of all reported liver-heart transplant recipients was only 46 years old when last calculated in 2007 [69]. As in simultaneous liver-kidney recipients, combined liver-heart transplantation appears to provide some protection against rejection for the heart allograft over heart transplantation alone [70].

Liver-lung transplantation is done most commonly for cystic fibrosis, followed by alpha-1-anti-trypsin disease [72]. In 2008 1-year patient survival was calculated to be less than 70% [72,73], and there remains a great deal of controversy over when and if a liver, lung, or dual organ transplantation should be performed, especially in the case of cystic fibrosis [73–75]. Triple organ transplantation with liver, lung, and heart is reported to results in 1-year survival of 50% and 5-year survival of 37.5% [74].

Retransplantation

When a primary graft fails, the only rescue therapy available is retransplantation. There has been a decline in the percentage of donor livers used for retransplantation and post retransplant survival has been improving over the past 20 years, but over 15% of liver transplants are currently performed to replace a failed previous graft, and survival rates after retransplantation remain inferior to those after primary transplantation. The most recent SRTR analyses put 1-year patient survival after retransplantation between 71–77% [76–79], significantly lower than the 90% 1-year survival after primary transplantation [3]. Single institution studies with longer follow-up estimate their five year patient survival to be 48–62% [80–82]. The best retransplant outcomes in the literature are from a single institution series in Germany, reporting 70%

patients alive 1-year after liver retransplantation and 67% at 5-year [83]. However, other European studies find their 1-year survival to be in the low- to mid-60% range [81,84]. Results of small series of 3rd, 4th, or 5th liver transplant patients demonstrate even higher mortality than after 2nd transplant [78]. One year graft survival after retransplantation is only 66% [76,79].

Not surprisingly, high MELD, ventilator dependence, renal insufficiency, and ICU status are all poor prognostic factors after retransplantation [80,83–86]. Most large studies suggest that HCV seropositivity is associated with increased risk of graft failure and patient death [79,85,87,88], and this effect is magnified as the MELD at time of retransplant increases. In one SRTR analysis of patients MELD>30, HCV-positive patients had only 42% 1-year survival (as compared to 55% in HCV-negative patients) and 21% 5-year survival (47% in HCV-negative) [85]. PNF as a reason for retransplantation has been variously reported to adversely affect survival [88], have no effect [81], or improve survival [88]. Patients undergoing retransplantation 7–30 days after their primary transplant do worse than those who receive their retransplant within seven days or after months/years for chronic graft failure [80,84]. Advanced donor age [44–54] and cardiac death donors negatively influence patient survival [80,83,89]. Of interest, the presence of preformed antidonor class I HLA antibodies is associated with approximately 20% poorer survival, but only in adult recipients and not pediatric recipients [90]. This study did not document that graft failure (and patient death) in antibody positive recipients was due to rejection.

ABO and HLA matching

Initially, ABO incompatible liver transplantation was associated with rates of graft loss and either death or need for retransplantation as a high as 50% in the first 30 days [91]. Graft failure resulted from antibody induced disseminated intravascular coagulation in the liver [91], arterial thrombosis, cholangiopathy, and rejection [92,93]. Outcomes were especially poor for retransplant and O blood group recipients, whose risk of death was 2.46 and 3.43 times higher than primary transplant and non-O blood group recipients of ABO incompatible organs, respectively [92,94].

As experience grew with modulation of antibody production, varying combinations of plasma exchange, IVIg, plasmapheresis, Rituximab, splenectomy, Cytoxan, and T-cell depleting induction therapy were used in ABO-incompatible liver transplantation, primarily in infants, who tend to have lower anti-A and anti-B titers [95]. However, older children and adults were included, and graft and patient survival in small series (6–14 patients) were reported to approach that for ABO-compatible transplants [96–99]. Although rates of rejection and cholangiopathy varied from study to study, overall, rejection episodes tended to be steroid responsive, and the incidence of biliary lesions was much decreased in the setting of intense immunosuppression and strategies for antibody depletion [96–99]. Interestingly, antibody titers after the immediate post-transplant period (two weeks) were not associated with rejection, either cellular or antibody mediated, and some patients continued to have high titers in spite of antibody depleting treatment while others had low to undetectable titers for many months after stopping treatment [96].

Small series (6–10 patients each) of A2 organs transplanted into O recipients without antibody depleting therapy have been reported with equally good outcomes [100,101]. This has been explained by the fact that organs from A2 donors have lower cell surface expression of A antigens because A2 subtype transferase is less efficient than the A1 transferase at converting the precursor antigen (H) into the A antigen [102]. From an allocation perspective, this is justified by the median wait time for blood group O candidates being twice that for blood group A and AB candidates and nearly five times longer than for blood group AB candidates [103].

Overall, ABO incompatible transplantation accounts for 2–4% of pediatric transplants and <1% of adult transplants in the last decade [104]. Graft survival in infants and children after ABO incompatible transplantation appears to be equivalent to ABO compatible transplantation [104,105], but in adults, graft survival is approximately 10% lower at 1-, 3-, and 5-years, evening out at 10-years [104]. In fact, for grafts surviving beyond three months, long-term survival was equivalent for ABO compatible and ABO incompatible transplants [104].

HLA matching does not appear to have any effect on graft or patient survival after liver transplantation [106,107]. HLA matching in pediatric living related transplantation is associated with a lower rate of rejection during the first 6 weeks post-transplant [108], and anti-HLA antibodies and C4d deposition can be found during episodes of rejection [109,110], this does not appear to have any long-term consequences. However, there are reports that a positive T-cell cross match is associated with a higher risk of acute rejection [111], and 10% lower graft survival at 1-, 3-, and 5-years [112]. Pretransplant cross-matching is not routinely performed in the US, but retrospective cross matches could be used to guide immunosuppression management in patients who may be at higher risk of rejection and graft loss.

Donor factors that affect patient and graft survival

Short-term donor risk factors

The majority of donor factors affect graft and patient survival in the first 90 days post-transplant, manifesting as prolonged graft dysfunction or primary non-function. Donor age is one of the most important, each decade adding a 15–20% increased chance of graft failure until livers from donors >70 are 1.65 times as likely to fail as livers from donors <40 years of age [113]. Each hour of cold ischemia time (CIT) adds only a 1% increase in risk of graft failure [113], but CIT >12 hours interacts with donor age >45 years to increase liver failure [114] and older grafts do particularly poorly in HCV+ recipients. Interestingly, regional sharing increases risk of failure by 11% and national sharing by 28%, independent of CIT [113]. Stroke as a cause of death carries a 16% greater risk of graft failure over death from anoxia or trauma [113]. Livers from African American donors are approximately 1.2 times more likely to fail than livers from white donors [113]. Split livers, or partial grafts, are 50% more likely to fail [113].

It is not clear whether steatosis adversely affects liver allograft function because it is a marker of recent or ongoing hepatocyte injury, or whether the fatty infiltration is directly toxic and contributes to ischemia-reperfusion injury. Microvascular steatosis of any degree is not associated with poor function and outcomes [114], but macrovascular steatosis of >25% incurs a 62% rate of delayed function, 12% rate of primary non-function, and shorter patient survival [114]. In other studies, grafts with ≥30% steatosis experience delayed function with elevated AST/ALT and INR, but 5-year graft survival is not statistically significant [115,116]. Another series showed 1-year graft survival to be 82% with no steatosis, 73% with 0–10% steatosis, 74% with 11–30% steatosis, and 62% with

31–60% steatosis [117]. Very few centers transplant grafts with steatosis >60%. Of note, HCV recurrence is earlier and more frequent in HCV+ recipients who received grafts with >30% steatosis [117]. Steatosis can reverse after transplantation [118] and a number of investigators are studying approaches to "de-fat" steatotic livers pretransplant [119].

Living donor liver transplantation

Unlike in kidney transplantation, where living transplantation confers better recipient and graft survival than deceased donor transplantation (DDLT) [120], living donor liver transplantation (LDLT) does not carry this clear advantage, while subjecting the donor to much higher risk of morbidity and mortality. Post-transplant patient survival in the US is no better following LDLT than DDLT, even though LDLT recipients tend to be younger and have lower MELD at time of transplant [121,122]. At 10 years, there is a slight trend towards better survival, but this has not yet been statistically significant [122], and in fact, HCV+ recipients may have a 12–15% lower survival following LDLT by 1–3 years post-transplant [122]. Acute rejection episodes are more common in DDLT [122], and allograft failure rates following LDLT are higher than for DDLT [123].

Technical complications are more likely to occur after LDLT than DDLT. During the first 90 days, these are predominated by biliary leaks, occurring almost three times more often in LDLT recipients that DDLT (10–32% vs. 8–12%) [124,125]. Later, biliary strictures develop, occurring 2–3 times more frequently in LDLT (28–32% vs. 5–15%) [125–127]. Hepatic artery thrombosis occurs approximately three times as often (6.5% vs. 2.3%) and portal vein thrombosis in 3% of LDLT, being very uncommon in DDLT [126]. Center experience is important in decreasing technical complications post LDLT [124].

However, studies comparing patient survival on an intent-to-treat basis find that LDLT recipients have approximately half the mortality rate of similar transplant candidates who remain on the wait list for a DDLT [128–130]. The LDLT advantage is even greater in wait list candidates with MELD < 15 who have a high risk of mortality that is not represented by their MELD score [130]. This effect is due almost entirely to death on the wait list of candidates without a living liver donor [128–130]. In short, LDLT does offer significant survival benefit to patients who would otherwise die on the wait list without a DDLT, in spite of the risk of developing complications or the need for retransplant. Not unexpectedly, LDLT is utilized more commonly in areas of the country where wait times are longer and the risk of dying on the wait list is higher [131].

Donation after cardiac death

The number of donors after cardiac death (DCD) has increased by over 10-fold in the last 15 years [132,133]. Although the use of DCD livers expands the donor pool, their use is not without risk. Graft failure following transplant with DCD grafts occurs anywhere from 50–85% more often than with donors after brain death (DBD) grafts [132,134], corresponding to 3-year graft survivals of 10–15 percentage points lower [132,133,135,136], even though DCD livers tended to be from younger donors with shorter cold ischemia times [135]. The gap in graft survival was less significant in "low risk" recipients, as determined by young age, primary transplant, normal kidney function, and good medical condition [133,136], suggesting that recipient selection plays an important role in DCD outcomes. DCD livers with less than 30 minutes of warm ischemia time and less than 10 hours of CIT tend to have better outcomes [136]. Patient survival is lower following DCD liver transplantation, as well [133,137].

Ischemic-type cholangiopathic lesions occur more frequently in DCD grafts (20–40%) than DBD grafts (5%) [138–140], although in DBD grafts their incidence correlates with CIT [141]. These biliary strictures, unlike anastomotic strictures, often cannot be managed endoscopically or with surgical revision, and up to 50% require retransplantation [142]; only 1–2% of anastomotic strictures require retransplantation [143].

Hepatitis B and Hepatitis C positive donors

In the US 15% of donors are HBV core antibody positive (HBVcAb+) [144] but surface antigen negative, indicating an exposure to HBV, with ambiguous viral load. The risk of viral transmission from HBVcAb+ donors to recipients is 25–95% without viral prophylaxis, and can be associated with severe hepatitis and graft loss [145]. However, with various combinations of HBIg, lamivudine, adefovir, and/or entecavir, HBVcAb+ livers can be transplanted into even surface antibody negative recipients with a risk of transmission of less than 5% [145–147] and similar graft and patient survival at 30 days, 1-year, and 5-years [144,145].

Similarly, multiple studies have shown no difference in patient or graft survival among HCV+ recipients, whether they receive grafts from HCV+ or HCV− donors [148–152], if the grafts are otherwise normal. However, HCV+ grafts from older donors (age ≥50 years) are nearly six times more likely to fail and result in recipient death than HCV+ grafts from younger donors (age <50 years), an age effect which is much larger than in HCV− grafts, and largely due to HepC related fibrosis [152], Among grafts from donors age ≥50 years, HCV+ grafts are almost three times as likely to fail as HCV− grafts, an effect that is not seen in younger grafts. Even with occasional retransplantation, patient survival followed similar trends [152].

Late complications of liver transplantation

Renal failure

The incidence of Stage IV and V (estimated GFR <29 L/min) chronic kidney disease (CKD) at 1-, 3-, 5-, and 10-years following liver transplantation has been estimated to be 8%, 14%, 18%, and 25%, respectively [153]. The most common cause of end-stage renal disease (ESRD) post liver transplant is calcineurin inhibitor (CI) toxicity. Other pathologic findings include diabetic nephropathy, thrombotic microangiopathy, acute tubular necrosis, focal segmental glomerulosclerosis, and presumed unrecovered hepatorenal syndrome [154,155].

As expected, advanced age, diabetes, hypertension, acute kidney injury, and calcineurin-inhibitor use increase the likelihood of developing chronic kidney disease, with pre-existing kidney disease as the major predictor of post-transplant CKD [153,155]. HCV infection is also a risk factor for developing CKD, probably because of HCV's association with immune complex glomerulonephritis [156] and diabetes [157,158]. Conversion to sirolimus, in order to minimize CI exposure, can sometimes result in improvement in GFR [159–162].

In all non-renal transplant patients, ESRD is associated with nearly five times the risk of death [153], and in one series of liver transplant patients in particular, the 13-year survival in patients with ESRD was only 28%, while in those without kidney disease, it was 55% [154]. As in other non-renal transplant patients [155], kidney transplantation was associated with a 71% 6-year survival

after developing ESRD, compared to only 27% in those who remained on renal replacement therapy [154].

Post-transplant diabetes mellitus

The reported incidence of new-onset diabetes after liver transplantation, both Type 1 and 2, ranges from 5–27% [163–166]. Many more patients require insulin transiently, while on high-dose steroids. Age >45 is thought to double the risk developing diabetes [163], while HCV positivity increases the risk 2–6 times, depending on the series [163,164,166,167]. Even in non-transplanted patients, HCV infection is associated with a higher risk of diabetes than HBV infection [164], and this is thought to be due to changes in insulin sensitivity resulting from cytokines produced in response to infection as well as the virus itself [168]. Insulin resistance increases with the level of viremia [168,169], partially through HCV-core protein induced degradation of insulin signaling proteins (insulin receptor substrate-1) [170,171], and decreased expression of insulin growth factor binding protein-1 [170]. Maintenance immunosuppression with tacrolimus, which decreases insulin secretion by β-cells and is directly β-cell toxic [172], is associated with up to three times the risk of diabetes when compared to cyclosporine [163,164]. Interestingly, receiving a graft with >10% steatosis on biopsy has also been associated with over three times the likelihood of developing diabetes [163].

Infections, renal failure, neurologic, and cardiovascular complications occur more frequently in those with de novo diabetes than in non-diabetics [173,174]. Some authors find similar patient and graft survivals among patients with new-onset diabetes and patients without diabetes [163,165], while others find the risk of death to be over three times higher in diabetics [167]. Some of this disparity may be due to the mix of type 1 and type 2 diabetes in the study populations, as studies distinguishing type 1 from 2 find that patients who develop type 1 diabetes suffer from approximately 40% lower patient and graft survivals, while patients who develop type 2 diabetes have equivalent survival rates as non-diabetics [175].

De novo malignancy

Depending on the series, de novo malignancy is estimated to occur in 3–55% of post liver transplant patients [173,174]. Varying by the type of cancer, this corresponds to relative risk of up to 70 times higher (non-melanoma skin cancer) than in the general population [173], while breast and urogenital cancers appear to occur at similar or lower rates as in the general population [176]. Compared to immunocompetent individuals, the chances of developing malignancy is higher in patients who are in an immunosuppressed state for any reason, whether it be genetic, pharmacologic, or infectious. This is thought to result from failure of T cells, macrophages, and natural killer cells to clear oncogenic viruses and/or malignant cells with aberrant protein expression. Many immunosuppressive medications, including calcineurin inhibitors and anti-metabolites, are also thought to have direct oncogenic activity [174].

As in non-transplant patients, smoking and a history of alcohol are associated with greater likelihood of developing cancer, especially oropharyngeal tumors [176,177], which occur as much as seven times more frequently after liver transplantation as in the general population [173]. Among primary diagnoses, primary sclerosing cholangitis was associated with the highest risk of non-skin malignancies, most often colorectal carcinomas [174,176], although the prevalence of ulcerative colitis (UC) in the PSC patients of those studies is not known. It is noteworthy that in non-transplant patients, the presence of PSC with UC further increases the risk of colorectal carcinoma beyond that associated with UC alone [178], but little is known about the risk of colorectal carcinoma in non-transplants patients with only PSC. Overall, colorectal carcinoma occurs as much as 12 times more frequently post liver transplant as in matched non-transplant patients.

Not only is the risk of developing cancer higher, the diagnosis is likely to carry a worse prognosis after liver transplantation than in the general population. Studies in solid organ transplant patients demonstrate that 5-year survival after diagnosis of colorectal cancer is less than half in transplant patients than in stage matched population controls [179,180] of outcomes following colorectal cancer solid organ. The probability of death in liver transplant patients five years after being diagnosed with any non-skin malignancy is less than 50% [176]. It is not clear whether the observed poor prognosis is the result of transplanted patients having more aggressive disease with different tumor biology, or simply the immunosuppressed state again providing poor defense against a tumor that is otherwise comparable to that in a non-transplant patient. In either case, liver transplant patients are counseled to undergo rigorous skin surveillance for new lesions and some authors speculate that colorectal screening should be performed as often as every three years even in patients under 50 [180], while annual colonoscopy is recommended for all PSC patients [181].

Operational tolerance

Operational tolerance in liver transplant recipients, loosely defined as normal graft function in the absence of exogenous immunosuppression, was first reported among five non-compliant patients who had stopped taking their immunosuppression one year or later after transplantation, yet continued to have normal graft function by clinical parameters and laboratory testing for up to 13 years after cessation of immunosuppression [182]. Subsequent studies of deliberate immunosuppression withdrawal, starting years after transplantation, for side effects such as infection, malignancy, or less commonly, renal dysfunction, report success rates as high as 23% for follow-up periods of as long as 10 years [183–185]. By and large, those that developed abnormal liver function tests and/or biopsy confirmed rejection, were easily treated with re-institution of calcineurin inhibitor (CI)therapy, or the addition of low-dose steroids, with return to baseline liver function and histology. Patients with recurrent HCV disease had stabilization or even improvement of histologic disease after stopping immunosuppression [186]. Immunosuppression withdrawal is not recommended for patients who have an underlying autoimmune etiology for their primary liver disease.

However, there have been reports of chronic rejection developing several years out from cessation of immunosuppression [183,187], although when caught early by protocol biopsy (in the setting of normal liver function tests), those changes, too, reversed with re-institution of CI therapy [187]. This suggests that patients with normal liver function tests after immunosuppression withdrawal may benefit from surveillance biopsies every several years, which some authors have even suggested for recipients who remain on stable immunosuppression [188]. It is not clear, though, whether increasing immunosuppression levels would have any effect on the progression of chronic rejection in the latter population.

One series in which immunosuppression withdrawal was instituted early (six months after transplantation) had a much lower rate of success and more severe cases of rejection in those that failed,

requiring high-dose steroids and even T cell depleting therapy for rescue [189]. Similarly, a clinical trial of immunosuppression withdrawal in pediatric recipients of parents living donor liver transplants has also found that the 60% of patients who have successfully been weaned off suppression had a longer time period between transplant and weaning than those who failed (100 months vs. 73 months) [190].

The search for additional predictors of operational tolerance has included a number of retrospective studies reporting that successfully weaned patients have lower levels of Th1 cytokines [191] and higher frequencies of CD4+CD25+ (presumably regulatory) T cells and gamma-delta T cells [192,193], and B cells [192]. Gene expression analysis reveals that operationally tolerant recipients down-regulate stress and inflammatory response genes and up-regulate DNA repair and cell cycle control genes, when compared to normal controls and patients who failed immunosuppression withdrawal [193,194]. One recent prospective study, profiling graft tissue prior to immunosuppression withdrawal, found that subsequent tolerance was associated with a higher average level of hepcidin antimicrobial protein (HAMP) mRNA [195], which is up-regulated in hepatocytes in response to iron deposition, but is also expressed in neutrophils and macrophages and appears to play a role in innate immunity. Likewise, transferrin receptor (TFRC) mRNA, which is expressed at lower levels in recipients who will experience successful immunosuppression withdrawal [195], is responsible for hepatocyte uptake of transferrin bound iron, but is also up-regulated on T and B cells in response to antigen stimulation. As exciting and provocative as these findings are, the wide, overlapping range of expression levels in patients who do and do not require immunosuppression [195] means that measuring mRNA levels is not yet clinically useful.

Summary

Liver transplantation is now unarguably the best long-term option for patients with ESLD. However, numerous conditions conspire to limit long-term survival. These include issues related to the primary indication and complications associated with the requirements for immune suppression. The future for recipients of a liver transplant remains favorable relative to recipients of other organs, but imperfect when compared to the general public. As such, refinements in the procedure, methods for maintenance immunosuppressive therapy, and improved anti-viral and anti-neoplastic therapies remain paramount in improving long-term outcomes.

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CHAPTER 105

Long-term Outcomes after Heart Transplantation

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Introduction

Despite advances in pharmacologic and device treatment of chronic heart failure, long-term morbidity and mortality remain unacceptably high with many patients progressing to end-stage heart failure. The 5-year mortality for patients with symptomatic heart failure approaches 50%, and may be as high as 80% at 1-year for end-stage patients [1]. Over the last four decades, cardiac transplantation has become the preferred therapy for select patients with end-stage heart disease. Approximately 2400 heart transplants are performed annually in the US. According to the registry of the International Society of Heart and Lung Transplantation (ISHLT), the median survival of patients post-transplantation is currently 10 years, up to13 years for those surviving the first year post-transplant [2] (Figure 105.1), a significant improvement over that of medical therapy for heart failure.

Critical to the success of heart transplantation are the continual investigational efforts to optimize immunosuppressive regimens. Improvements in immunosuppression, donor procurement, surgical techniques, and post-transplant care have resulted in a substantial improvement in the survival of transplant recipients. Nonetheless, problems still exist and the purpose of this chapter is to provide an overview of long-term complications of heart transplantation in the current era.

This chapter will review the major causes of mortality and morbidity after transplantation. The major causes of mortality include rejection, infection, malignancy, and transplant coronary artery disease. Due to the importance and complexity of transplant coronary artery disease, this topic is discussed in another chapter (Chapter 79). The major causes of morbidity include renal dysfunction, hypertension, diabetes, dyslipidemia, gout, and osteoporosis.

Long-term outcomes after heart transplantation

Survival

Survival after heart transplantation has steadily improved in the past three decades. In the 1980s, 1-year survival was 70% and the conditional half-life, the time at which 50% of patients who survived the first year are still alive, was 9.4 years. In the most recent report from the ISHLT registry [2], 1-year survival is almost 90% with a conditional half-life of 14 years. Notably, the mortality rate beyond the first year after transplant has improved only marginally for patients who received allografts after 1992, and there has been no statistically significant improvement in the past two decades. This fairly constant annual mortality rate of approximately 3–4% is higher than that of the general population, and likely exists because the processes responsible for long-term mortality, including cardiac allograft vasculopathy and malignancy, remain a challenge of detection and treatment. Thus, future improvements in post-transplant survival may result from interventions aimed at the detection and treatment of cardiac allograft vasculopathy and malignancy.

An in-depth analysis of risk factors for survival at 1-, 5-, 10-, 15-, and 20-years post-transplant is provided in the ISHLT registry report [2]. The strongest risk factors for one-year mortality, associated with a 50% or more increase in the risk of 1-year mortality, are mainly related to technical issues and the underlying disease responsible for transplantation, including the use of temporary circulatory support, congenital cardiomyopathy versus non-ischemic cardiomyopathy, prior transplant, pretransplant ventilatory support or dialysis. Risk factors for 5- and 10-year mortality, on the other hand, are most referable to immunological issues and toxicity related to immunosuppression, including dialysis or infection after transplant, rejection during the first post-transplant year, and lack of immunosuppression therapy with a combination of at least two of the following classes: cell cycle inhibitors, calcineurin inhibitors (CIs), and proliferation signal inhibitors.

Transplant era influences 20-year survival; those patients transplanted in 1990 have better 20-year survival than those transplanted in 1985. Other factors affecting 20-year survival include etiology for transplantation, sex, age, ischemic time, and center volume. Patients receiving retransplant and those receiving transplant for ischemic heart disease or valvular heart disease have a lower likelihood of survival past 20 years after transplant compared with patients who receive an allograft for non-ischemic cardiomyopathy. The reasons for this are unclear, and may be related to progression of the underlying disease or older age of recipients with ischemic or valvular disease versus non-ischemic cardiomyopathy. Women also have a somewhat higher risk of death compared with their male counterparts. Younger donor age, younger recipient age, lower allograft ischemic time, and higher center volume are additional factors associated with long-term survival, and these risk factors appear consistent across countries.

Quality of life

Patients not only gain increased quantity of life after transplantation, but quality of life is improved as well. Data from the ISHLT registry indicates that in the first years after heart transplant, approximately 75% of recipients report having a normal healthy

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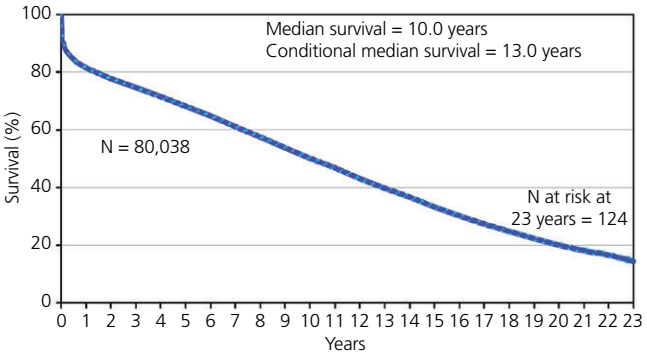

Figure 105.1. Survival for adult heart transplants performed between January 1982 and June 2009. In an analysis of over 80 000 reported heart transplants internationally, the median survival of patients post-transplantation is currently 10 years, up to13 years for those surviving the first year post-transplant. Reproduced from [2] Stehlik et al., J Heart Lung Transplant 2010;29:1089–1103. Copyright © 2010, with permission from Elsevier.

lifestyle or only few disease symptoms, an additional 15% participate in normal activities with some difficulty, and less than 10% report a higher degree of limitations [2].

In-depth analysis of a smaller cohort of transplant patients indicates that the most frequently reported symptoms long-term after heart transplantation, at moderate rates of 50–65%, were fatigue, sexual dysfunction, memory problems, bruising, and cramps [3]. These symptoms are less common in recipients who are married and those with a higher educational level.

Based on information from the international registry, many patients return to work after transplant. Among recipients aged 25–55 years old, approximately 50% were employed five years after transplantation [2]. On the basis of the functional data reviewed above, it is apparent that additional recipients could return to the workplace; however, in the US the structure of disability benefits and health insurance considerations may represent a barrier to this process.

Rejection

Diagnosis

Transplant rejection remains one of the major causes of death after heart transplantation [2]. Rejection is most frequent during the first month after heart transplantation and declines thereafter. Acute rejection may cause significant morbidity and mortality in heart transplant recipients, requiring judicious monitoring and prompt clinical intervention. Because clinical symptoms of rejection are often vague, routine testing for rejection in the absence of symptoms is standard practice. Unlike renal or liver transplantation, there are no laboratory markers for rejection in heart transplantation. The endomyocardial biopsy is the standard approach for the routine surveillance of rejection. The endomyocardial biopsy is most commonly performed via a right internal jugular venous approach under fluoroscopic guidance. This is an outpatient procedure with a low risk of complications. The most serious complications (which occur in 0.5% of cases) include tricuspid valve injury and cardiac perforation which could result in tamponade [4,5]. While the timing of biopsies will vary from center to center, in general biopsies are performed frequently early after transplantation and less frequently as time goes on. At our center, we see patients in clinic twice weekly for the first month and perform biopsies weekly for the first month. Then, the visits are spaced out with biopsies every other week in month 2, monthly biopsies in months 3 through 6, and biopsies every other month in months 8 through 12 (Table 105.1). Thus, patients will undergo approximately 13 biopsies in the first year. At our center, we do not perform routinely scheduled biopsies after the first year, as the risk of rejection is much lower. After year 1, biopsies are performed only if the heart transplant recipient develops symptoms or signs of rejection. However, we monitor such patients in clinic every six months with echocardiograms and every year with annual angiograms to assess for cardiac allograft vasculopathy (as discussed in more detail in Chapter 79).

The purpose of the endomyocardial biopsy is to assess for myocardial damage in the form of cellular or antibody-mediated rejection. The diagnosis of cellular rejection is made in accordance with the revised ISHLT grading scale, published in 2005, which simplifies the prior 1990 classification [6,7]. Biopsies are classified as:

- Grade 0 R — no rejection (no change from 1990);
- Grade 1 R — mild rejection (1990 Grades 1A, 1B and 2);
- Grade 2 R — moderate rejection (1990 Grade 3A); and
- Grade 3 R — severe rejection (1990 Grades 3B and 4).

Grade 2R or higher rejection on biopsy is considered significant and meriting treatment, as discussed in further detail below (see also Chapter 83).

The diagnosis of antibody-mediated rejection is less straightforward, but has achieved greater standardization after a consensus conference in 2010 [8]. By the proposed classification, endomyocardial biopsies are graded based on the presence of histologic and immunologic findings consistent with antibody-mediated rejection. Histologic findings include endothelial activation with intravascular macrophages and capillary destruction. Immunologic findings encompass complement and HLA deposition. The grading scheme stratifies biopsies based on:

- no histologic or immunologic evidence of antibody-mediated rejection (negative, AMR0);
- either histologic or immunologic evidence of antibody-mediated rejection (suspicious, AMR1);
- both histologic and immunologic evidence of antibody-mediated rejection (positive, AMR2); and
- a final category for severe findings of myocardial destruction, AMR3.

Moving forward with a standardized classification scheme for the diagnosis of antibody-mediated rejection will allow further study into the natural history and treatment of this less well-understood condition.

While not required for the diagnosis of antibody-mediated rejection, we also perform screening for anti-HLA antibodies post-transplantation. Antibodies are checked at months 1, 3, 6, and 12 post-transplantation and then annually (Table 105.1). The presence of donor-specific anti-HLA antibodies in high levels (usually median fluorescent intensity above 10 000 or standard fluorescent intensity above 200 000) are considered potentially cytotoxic and may merit a change in treatment, depending on the clinical situation, as discussed further on.

Although performing an endomyocardial biopsy is straightforward, the morbidity associated with this invasive procedure has led to attempts to identify other means of diagnosing rejection. Many non-invasive parameters have been assessed, including changes in the electrocardiogram, echocardiogram, or magnetic

Table 105.1. Post-transplant monitoring

	Clinic	Anti-HLA antibody screen	Echocardiogram	Endomyocardial biopsy	Angiogram
Week 1	xx		x	x	
Week 2	xx		x	x	
Week 3	xx		x	x	
Week 4	xx	x	x	x	
Week 5	x				
Week 6	x		x	x	
Week 7	x				
Week 8	x		x	x	
Month 3	x	x	x	x	
Month 4	x		x	x	
Month 5	x		x	x	
Month 6	x	x	x	x	
Month 8	x		x	x	
Month 10	x		x	x	
Month 12	x	x	x	x	
Semi-annual	x		x		
Annual	x	x	x		x

resonance imaging, as well as abnormalities in laboratory findings including B-type natriuretic peptide. However, none have offered adequate accuracy or reliability for inclusion into clinical practice until the Allomap, an 11-gene expression signature derived from peripheral blood mononuclear cells that may predict cellular rejection [9]. In a clinical trial setting, the Allomap gene expression profile has been shown to be non-inferior to the biopsy in the diagnosis of cellular rejection [10]. However, the Allomap has not yet widely been incorporated into clinical practice, mainly because of critical problems with the randomized trial and limitations of its generalized use [11,12].

The landmark randomized controlled trial — the IMAGE study — compared the Allomap gene expression profile to endomyocardial biopsies in patients 6 months to 5 years post-transplant, with 85% of patients beyond one year after transplantation. The utility of the Allomap in this population is unclear, since many centers do not perform routine biopsies after 1-year post-transplantation when the risk of rejection is low [12,13]. Furthermore, the Allomap will not detect antibody-mediated rejection, and cannot be used in patients who have received blood transfusions or hematopoietic growth factors affecting leukocytes (such as granulocyte-colony stimulating factor) within the past 30 days [9]. Thus, the widespread use of Allomap instead of endomyocardial biopsy will likely take time and require further randomized trials or, at the very least, clinical registry experience, prior to adoption by most transplant centers.

Risk factors

The risk factors for rejection vary with the time post-transplant. Risk factors associated with early rejection, within 1-year after transplant, include younger age, female donor, female recipient, positive cytomegalovirus (CMV) serology, prior infections, and OKT3 induction therapy [2]. In contrast, risk factors for later rejection include a greater number of rejection episodes in the first year after transplantation and the presence of prior CMV infections [2].

While rejection is rarer later after transplantation, prompting some centers to eliminate scheduled biopsies in such patients, it still may occur. Late rejection is often prompted by non-compliance with immunosuppressive medications, addition of medications that inadvertently lower immunosuppressive levels (such as rifampin), or discontinuation of medications that have been maintaining levels (such as diltiazem, azole antifungals, or macrolide antibiotics), or infection episodes [14]. Antibody-mediated rejection is more common later after transplantation [15] and may be associated with an increased risk of transplant coronary artery disease [16] and death [17,18].

Table 105.2. The treatment of rejection depends upon the results of the biopsy and the clinical status of the patient, with more aggressive treatments reserved for patients with more unstable clinical presentations

	Asymptomatic	Reduced EF	Heart failure/shock
Cellular rejection	• Target higher CNI levels • Oral steroid bolus + taper • Rapamune • No treatment *Or* • Oral steroid bolus/taper	• Oral steroid bolus/taper *or* • IV pulse steroids	• IV pulse steroids • Cytolytic therapy (ATG) • Plasmapheresis • IV immune globulin • Inotropic therapy • IABP or ECMO support
Antibody-mediated rejection		• Oral steroid bolus/taper *or* • IV pulse steroids +/− • IV immune globulin	

EF, ejection fraction; CNI, calcineurin inhibitor; ATG, anti-thymocyte globulin; IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation.

Treatment

The management of rejection proceeds in a step-wise fashion based on the severity of rejection detected on biopsy and the patient's presentation (Table 105.2). Rejection most often occurs early after transplantation, and treatment is similar regardless of the timing of presentation. Grade 1R cellular rejection or findings suspicious for antibody-mediated rejection, AMR1, in the absence of clinical or hemodynamic compromise generally merits no intervention. The management of AMR1 is controversial at this time, and at some centers treatment may proceed as for higher levels of rejection, as described below.

More serious findings on the biopsy, including Grade 2R or higher cellular rejection or AMR2 or higher antibody-mediated rejection, require treatment. The intensity of treatment depends on the patient's presentation. If the patient has no heart failure symptoms and normal left ventricular ejection fraction, treatment options include oral or intravenous pulse steroids, targeting higher

levels of immunosuppressive medications, switching from cyclosporine to tacrolimus [19,20], or switching from mycophenolate mofetil to a proliferation signal inhibitor [21–23]. Given the equivalent success of intravenous and oral corticosteroid therapy for the treatment of asymptomatic cellular rejection [24], an outpatient course of oral corticosteroids is often the first-line treatment for asymptomatic cellular rejection. Asymptomatic antibody-mediated rejection is more challenging. Recent studies indicate that it may be associated with poor outcomes [16–18], but it is unclear whether treatment affects outcomes. At the Cedars-Sinai Heart Institute, such patients will receive an oral corticosteroid bolus, consideration of intravenous immune globulin, and close monitoring of donor-specific HLA antibodies.

For patients with a reduced ejection fraction on echocardiogram, treatment is more aggressive. A reduction in ejection fraction in the absence of biopsy evidence for rejection may be treated with intravenous corticosteroids and cytolytic therapy with antithymocyte globulin in addition to the adjustments in immunosuppressive medications outlined above. If there is evidence of AMR2 or higher, such patients will also receive intravenous immune globulin. If donor-specific anti-HLA antibodies are present in the setting of AMR or a fall in ejection fraction, patients may receive a steroid bolus and taper or more intensive therapy with intravenous immune globulin, rituximab, or bortezomib.

Finally, in patients presenting with cardiogenic shock the results of the biopsy are less important, and aggressive treatment includes intravenous corticosteroids, cytolytic therapy, plasmapheresis, intravenous immune globulin, intravenous heparin (as patients often have thrombotic occlusion of the cardiac microvasculature on postmortem examination [25,26]), and hemodynamic support with intra-aortic balloon counterpulsation or even extracorporeal membrane oxygenation (ECMO). Such aggressive treatment with ECMO support can lead to acceptable outcomes if used in a timely fashion prior to cardiac arrest and accompanied by aggressive treatment for rejection [27].

The protocols for treatment for rejection will vary between transplant centers, as there are no randomized trials comparing strategies. However, given the relatively small number of heart transplants performed internationally and the relative rarity of rejection, such trials would be difficult to conduct or power to assess differences between treatment strategies. Thus, as clinicians, one must rely on experience and judgment to formulate the treatment plan that maximizes benefit and minimizes toxicity of these therapies.

Long-term management

While cellular rejection is often successfully treated with corticosteroids and cytolytic therapy, resulting in a resolution of heart failure and normalization of the ejection fraction [28], management of antibody-mediated rejection is often more complicated. Patients often have a persistent reduction in ejection fraction, restrictive physiology leading to recurrent heart failure symptoms, and accelerated progression of transplant coronary artery disease [28].

The management of such patients with a persistent drop in ejection fraction after treatment of symptomatic rejection is not well established. At the Cedars-Sinai Heart Institute, we often rely on therapies to reduce the levels of donor-specific anti-HLA antibodies, including rituximab and bortezomib, as well as long-term photopheresis to alter the function of T cells. In small case series, such therapies have shown benefit [29,30], although often such patients go on to require redo transplantation.

Infection

Overview

Due to immunosuppressive therapy, cardiac transplant recipients are at risk for infection in a generally predictable pattern based on time after transplantation [31] (see Chapter 94, Figure 94.1). Bacterial infections such as staphylococcus and streptococcus occur in the early postoperative period and usually involve wound infections. Opportunistic infections usually do not occur until at least several weeks following cardiac transplantation. Opportunistic infections may be caused by fungi (candida, aspergillus), parasites (pneumocystis, toxoplasmosis), and viruses (CMV).

While prophylaxis against common opportunistic infections is key in the decreased incidence of certain infections in transplant recipients, such as oral candidiasis, pneumocystis pneumonia, toxoplasmosis, and cytomegaloviruses, such infections can and do occur. Furthermore, detection of active infection in a transplant recipient is challenging as the classic signs and symptoms of infection may be blunted by the suppressed immune response. The diagnosis of infection in solid organ recipients must proceed aggressively, as infections can progress rapidly in immunosuppressed individuals. Furthermore, the treatment of infection in solid organ recipients will often start broadly, and the guidance of an infectious disease specialist well versed in transplant infectious disease is essential in guiding diagnosis and treatment.

Infection risk

As with acute rejection, monitoring for immune status and infection risk remains problematic. This has led to several investigational attempts for monitoring assays, none of which are well validated at present, and there is currently no standard approach to accurately assess a transplant recipient's risk for infection. However, an immune- monitoring assay (ImmuKnow; Cylex, Columbia, MD) performed on peripheral blood, which measures adenosine triphosphatase (ATP) release from activated lymphocytes, may offer some guidance in profoundly immunosuppressed patients [32,33]. In the largest study to date in heart transplant recipients, the average immune monitoring score was significantly lower in patients who developed an episode of infection within one month after the measurement compared with steady-state patients [34]. An immune monitoring score of less than 200 ng ATP/ml was associated with future infection.

At our center, we may target lower CI trough levels or reduce mycophenolate mofetil doses in patients with scores less than 200 ng ATG/ml, especially those with recurrent infections. However, the assay has limitations. It does little to provide guidance for finer adjustments in immune management outside of profoundly immunosuppressed patients, and over-infection itself is one of the most valid indicators of over-immunosuppression. Furthermore, whether dynamic changes in the immune monitoring score in response to adjustments in immunosuppression alters patient outcomes has not yet been determined.

Donor transmission or recipient reactivation

While rare, donor transmission or recipient reactivation of infection can be a catastrophic complication of organ transplantation. While every effort is made to screen potential donors for transmissible diseases and recipients for re-activating disease, such infections can still occur, primarily early after transplantation during the period of most intense immunosuppression.

Donor transmission can occur through infection that is latent in transplanted tissue or active bacteremia. Latent infections include

cytomegalovirus (which is why the donor exposure status is key in determining the length of recipient prophylaxis as outlined below), tuberculosis, and *Trypanosoma cruzi* (Chagas disease). Viremia or bacteremia may also be undetected at the time of organ procurement. Rarer causes of donor-transmitted infections include West Nile virus, rabies, human immunodeficiency virus (HIV), and lymphocytic choriomeningitis infection. Missed infections occur because serologic testing is not 100% since seroconversion may not occur during acute infection and not all infections are tested for. Molecular diagnostics may improve the accuracy of donor screening in the future, and augmented screening on a regional basis (with, for example, screening for Chagas disease in Texas and the southwestern US) may improve detection of potential transmissible infections. Once such an infection is detected, aggressive prophylaxis and treatment may stem the progression of disease in the recipient.

Recipient reactivation of infection is also a concern and potential solid organ transplant candidates with active infection are excluded from transplantation. However, temporally distant infection may occur post-transplantation with parasites such as Strongyloides or Chagas disease. At Cedars-Sinai Heart Institute in southern California, patients from Chagas-endemic areas are screened for exposure pretransplantation and followed closely for reactivation post-transplantation with a polymerase chain reaction (PCR) assay. Patients with a positive PCR for the *T cruzi* organism receive prophylaxis with benznidazole under the guidance of the transplant infectious disease specialist.

Fungal infections

All transplant recipients receive antimicrobial prophylaxis for oral candidiasis. At Cedars-Sinai Heart Institute, such prophylaxis is given for three months, coinciding with a drop in corticosteroid dosing to below prednisone 10 mg daily. Options for prophylaxis include clotrimoxazole troches (lozenges) or nystatin oral preparations. In patients who present a higher risk for systemic fungal infections, such as those from an area endemic for coccidioidomycosis, fluconazole or itraconazole may be prescribed prophylactically. The –azoles increase the concentration of CIs and proliferation signal inhibitors, and thus the dosage of cyclosporine, tacrolimus, sirolimus, and everolimus must be reduced up to 50% when initiating –azole therapy, with close follow-up of trough levels.

Parasites

It is standard for transplant recipients to receive trimethoprim-sulfamethoxazole (TMP-SMX) for pneumocystis for one year. TMP-SMX has virtually eliminated pneumocystis pneumonia in heart transplant recipients, and also prevents Nocardia infection and toxoplasmosis. For patients with sulfa allergies, dapsone may be used as long as the patient has no evidence of G6PD deficiency. In such patients, atovaquone is a reasonable alternative.

Viral infections

Prophylaxis against CMV depends on the patient's risk group, defined by the CMV IgG titer. The risk of CMV infection is highest in transplant recipients who have a negative CMV IgG titer (indicating no prior exposure to CMV) and are given a heart from a donor who is CMV IgG positive. At the Cedars-Sinai Heart Institute, these patients receive one year of valganciclovir therapy. The lowest risk of CMV infection occurs in cases where both the donor and the recipient are CMV IgG negative, and these patients receive valganciclovir or acyclovir (which provides inferior CMV protection but is less expensive) for three months. Other patients will receive valganciclovir for six months. Of course, after any treatment for acute rejection with high-dose steroids or cytolytic therapy, patients will receive three months of valganciclovir and TMP-SMX prophylaxis.

Vaccines

The use of vaccines in heart transplant recipients remains controversial. Live vaccines are definitely contraindicated because of the patients' immunosuppressed states. Even dead vaccines may pose a risk because they can promote activation of the immune system and cause rejection. In the Cedars-Sinai Heart Institute, we recommend dead vaccines such as the influenza or pneumococcal vaccines only to patients more than six months after transplant and with no history of rejection within the previous six months.

Malignancy

Incidence

After cardiac allograft vasculopathy (CAV), malignancy is the second most common cause of mortality in heart transplant recipients [2]. Malignancies are approximately two- to fourfold more common in heart compared with renal transplant recipients [35–38]. The ISHLT registry demonstrates that cumulative risk of malignancy is 26% by eight years, most (18%) due to skin cancer [2]. The enhanced risk of cancer among cardiac transplant recipients is thought to reflect the greater degree of immunosuppression that heart transplant recipients receive, either due to inherent immunologic requirements or the lack of a non-invasive monitoring test for rejection such as transaminases or serum creatinine in liver and kidney transplant recipients, respectively [39].

The risk of malignancy from immunosuppressive agents is due to reduced immune surveillance for neoplasia. All immunosuppressive agents are believed to contribute to the cumulative risk of malignancy, with the possible exception of corticosteroids. In Kaposi sarcoma, sirolimus has resulted in complete regression in renal transplant recipients [40] and other studies in solid organ transplant recipients indicate a decrease in the incidence and progression of malignancy with use of a proliferation signal inhibitor, sirolimus or everolimus [41–43].

Clinical presentation

Cancer in solid organ transplant recipients usually presents three to five years post- transplantation. Cutaneous malignancies are the most common type after heart transplantation, mainly squamous cell and basal cell carcinomas [44]. Compared with the general population, organ transplant recipients are approximately 65–250 times more likely to develop squamous cell carcinoma, and 10–16 times more likely to develop basal cell carcinoma [45,46]. Risk factors for the development of skin cancers include fair skin, previous history of skin cancer, geographic location (in areas of high sun exposure), and intensity of immunosuppression [47,48].

Post-transplant lymphoproliferative disorder (PTLD), most commonly a B cell lymphoma related to Epstein-Barr virus (EBV) infection, may occur after transplantation. An increase in the total burden of immunosuppression increases the risk of developing lymphoma, so that PTLD may sometimes occur within the first year after transplantation, when the level of immunosuppression is the highest [49,50]. More than 50% of patients with PTLD present with extranodal masses involving the gastrointestinal tract, lungs, skin, liver, central nervous system, and the allograft itself. Risk factors

for the development of PTLD include a higher level of immunosuppression, including the use of cytolytic therapy for induction [50] and EBV serostatus (with EBV-seronegative recipients of EBV-seropositive donors being at the highest risk) [51].

Finally, neoplasms common in the general population also occur in heart transplant recipients, including breast, lung, and prostate. Lung cancer is more common in heart and lung transplant recipients than in recipients of other solid organs, likely because smoking, a strong risk factor for lung cancer, may also contribute to end-stage heart and lung disease requiring transplantation [52].

Treatment

The most critical point of treatment of malignancies is prevention. We encourage all heart transplant recipients at our institution to undergo routine health maintenance screenings with their primary care physicians, including mammograms, pap smears, prostate exams, and colonoscopies as indicated for non-transplant patients. In addition, patients are instructed to utilize sun protection and to establish care with a dermatologist for routine skin exams. While the utility of annual chest radiograph screening has not been established in the early detection of lung cancer, we have anecdotally detected many early lung cancers in this manner, and thus perform annual chest radiograph screening at our center.

The initial approach to malignancy is reduction of immunosuppression, and this may be the only treatment required for some forms of PTLD. In our institution, we will consider switching patients with newly diagnosed malignancy to a proliferation signal inhibitor such as sirolimus or everolimus, due to its possible protective effect in malignancies, in place of a CI or mycophenolate mofetil (MMF). Compared with CI-based regimens, immunosuppression with the mammalian target of rapamycin (mTOR) inhibitors sirolimus or everolimus may reduce the risk for malignancies, including non-melanoma skin cancer, in organ transplant recipients [41–43]. An immune monitoring assay, such as the Cylex blood test described above, may offer insight into the patient's global state of immune suppression [32–34] and thus provide a target for lowering immunosuppressive agents in patients with malignancy.

Beyond reduction in immunosuppression and switching the CI or MMF to a proliferation signal inhibitor, further treatments are at the discretion of the consulting specialist. Dermatologists will often perform extensive and frequent excisions for the treatment of squamous and basal cell carcinomas. PTLD may require treatment with chemotherapeutic agents, most commonly rituximab combined with cyclophosphamide, doxorubicin, vincristine, and corticosteroids (commonly referred to as R-CHOP).

General medical management

It is essential that all heart transplant recipients receive regular care from an internist for routine health maintenance. Such patients require the same general medical surveillance as non-transplant patients, including age-appropriate cancer screening for malignancies of the cervix, breast, colon, and prostate. Since cutaneous malignancies are so common in heart transplant recipients, annual screening by an internist or dermatologist is essential, especially in fair-skinned individuals or those with a history of skin cancer. Internists may also manage the long-term complications of heart transplant recipients, including renal dysfunction, hypertension, dyslipidemia, diabetes, osteoporosis, and gout. However, it is essential to instruct transplant recipients to inform the transplant center of any new medication recommended by the internist, and there may be unforeseen interactions that should be monitored, as described below.

Renal dysfunction

Renal insufficiency is a common adverse effect of the CIs, and often worsens over time, such that up to 8% of transplant recipients will develop end-stage renal disease at five or more years post-transplant [2,53,54]. "Renal-sparing" immunosuppressive regimens are often utilized in such patients, including a reduction in CI dose or substitution of the CI with sirolimus [55]. To be successful, the timing is important; if the creatinine is too high, often above 2.5 mg/dL, the renal damage may be irreversible. When replacing CIs with sirolimus, the key to preventing rejection is to withdraw CIs gradually over a period of 2–4 weeks while awaiting therapeutic sirolimus levels, and monitoring patients with a follow-up echocardiogram and biopsy at one month after complete withdrawal of the CI [56]. While randomized trial data of renal sparing regimens are not yet available, we have found that, with these caveats, renal-sparing protocols appear efficacious, safe, and beneficial for select patients.

Hypertension

Hypertension after cardiac transplantation is primarily a result of CI use and occurs in up to 80% of patients [2]. Post-transplant hypertension is often difficult to control and often requires a combination of several antihypertensive agents [57,58]. No agent has been shown to be superior in clinical trials of renal [59–61] or heart transplant recipients [62]. However, beta blockers are often avoided in heart transplant recipients because the denervated heart relies on circulating catecholamines to maintain cardiac function during exercise, and thus heart transplant recipients often experience significant fatigue with beta blocker administration. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers may not be tolerated due to renal dysfunction or hyperkalemia. Dihydropyridine calcium channel blockers such as amlodipine and nifedipine are often effective, but may result in troublesome dependent edema and will increase levels of CIs, which should be monitored after initiation.

Dyslipidemia

Lipid abnormalities are common after heart transplantation, due to the use of steroids, calcineurin inhibitors, and proliferation signal inhibitors [63]. The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) are effective in reducing total and LDL cholesterol in heart transplant recipients. Notably, treatment initiated within two weeks of transplantation with statins is associated with a lower frequency of hemodynamically compromising rejection episodes and improved survival over the first transplant year [64]. These agents likely have an immunosuppressive effect in addition to their lipid-lowering activity. As hyperlipidemia is so common following transplantation, all cardiac transplant recipients should receive statin therapy. Pravastatin is the statin of choice, since it is not metabolized by the cytochrome 3A4 system, reducing the possible interactions with CIs [14].

Diabetes

Given that diabetes mellitus is a major cardiovascular risk factor leading to the development of end-stage heart disease, diabetes is common in patients pretransplant. Furthermore, the use of steroids and tacrolimus post-transplant may cause or worsen diabetes; up to 32% of heart transplant recipients are diabetic by 5-years

post-transplant [2]. While diabetes is associated with poorer long-term survival [64], there are few data regarding the treatment of these patients. In general, since renal insufficiency in so common in these patients, metformin is often avoided. Furthermore, thiazolidinediones are not preferred due to the risk of fluid retention. Shorter-acting sulfonylureas may be the agents of choice for many heart transplant recipients [14].

Osteoporosis

Osteoporosis resulting in vertebral compression fractures is a common and debilitating problem after heart transplantation, exacerbated by steroid use. At our institution, we recommend screening bone-density examinations by internists on an annual basis. To prevent osteoporosis, patients should receive supplemental calcium and vitamin D, engage in weight-bearing exercises, and receive bisphosphonates as recommended by the primary care physician.

Gout

Causes of gout after heart transplantation include CI use, diuretic use, and renal insufficiency. Non-steroidal anti-inflammatory agents are often avoided in heart transplant recipients due to the potential exacerbation of renal insufficiency. Colchicine may be used to treat acute attacks, although there is a risk of myoneuropathy when colchicine is given in conjunction with CIs. Thus, for acute flares, systemic or intra-articular steroids are often the treatment of choice. Allopurinol is useful as suppressive therapy, as long as the patient is not receiving azathioprine, since the combination may result in severe myelosuppression. Given the potential for adverse effects and drug interactions, consultation with a rheumatologist is often helpful in management of gout in heart transplant recipients.

Summary

Improvements in immunosuppression, donor procurement, surgical techniques, and post-transplant care have resulted in a substantial improvement in the survival of heart transplant recipients. Nonetheless, problems still exist and include rejection, infection, malignancy, transplant coronary artery disease, renal dysfunction, hypertension, diabetes, dyslipidemia, osteoporosis and gout. Newer strategies in prevention and management of these complications are ongoing, and future advances in non-invasive monitoring assays will allow better assessment of rejection and the level of immune response. This will allow clinicians to tailor current therapies to the needs of individual heart transplant recipients to maximize benefit and minimize toxicity.

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CHAPTER 106

Long-term Outcomes after Lung and Heart-Lung Transplantation

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Introduction

End-stage lung failure remains a common cause of morbidity and mortality in the United States and worldwide. For many of these patients, lung transplantation offers the only hope for a longer life. Since the first successful lung transplant in 1983, the number of programs offering lung transplantation and the volume of transplants performed has steadily increased. In particular, over the last decade, the volume of lung transplants performed annually in the US and worldwide has grown significantly. In the US alone, approximately 1700–1800 lung transplants are performed yearly with over 3500 performed annually worldwide [1,2] (Figure 106.1).

Commensurate with the increase in volume, we have seen a steady improvement in patient outcomes over time particularly when follow-up care is delivered at an established large volume transplant center [1–3]. Critical to the incremental success of lung transplantation over the years has been continual efforts to modify immunosuppressive therapy to diminish the effects of steroids on bronchial healing and other complications, improved assessment and preservation of lung grafts and improved surgical and post-transplant management. These are specifically detailed in chapters 26, 59, and 72 covering organ resuscitation and preservation, the surgical technique and perioperative immune management, respectively. While these efforts have led to improved survival, particularly short-term survival, the median survival following lung transplantation remains a disappointing five years [1]. Late (i.e. more than 1-year from transplant) graft failure and death most commonly occurs as a result of bronchiolitis obliterans syndrome (BOS) and infection, a consistent finding for the last two decades. In addition to these often fatal complications, morbidity related to renal dysfunction, hypertension, diabetes or osteoporosis remains common.

The short-term outcomes following lung transplantation are discussed in chapters 72 and 80. This chapter will review the long-term outcomes following adult lung transplantation including the major causes of mortality and morbidity. While heart-lung transplantation is performed less frequently than isolated lung transplantation, the long-term outcomes more closely resemble those of lung transplant recipients than of heart transplant recipients. For that reason, long-term outcomes following heart-lung transplantation will also be discussed. Specific consideration and coverage of pediatric lung transplantation can be found in Chapter 116.

Long-term outcomes

Survival

Survival following lung transplantation has steadily improved over the last two decades. In the 1980s and early 1990s, 1-year survival approximated 70% with a half-life of 3.9 years and a conditional half-life, the time at which 50% of patients who survived the first year are still alive, of 7.0 years [2] (Figure 106.2). In a report from the International Society for Heart and Lung Transplant (ISHLT) Registry, 1-year survival has improved to 81% for recipients of lung transplants between 2004 and June, 2010 while the half-life has increased to 5.9 years [2] (Figure 106.2). This temporal improvement in survival is demonstrated in the US data, as well [1]. The improvement in long-term survival is largely driven by the increase in early survival, as the rate of attrition following the first year from transplant has been relatively constant (Figure 106.2). Despite these improvements, survival statistics for lung transplant recipients remain comparatively lower than other solid organ transplants with heart, kidney and liver transplant recipients enjoying a half-life of greater than 10 years following transplantation.

Factors affecting survival

As in prior reports, the most recent report from the ISHLT Registry demonstrates a greater survival for recipients of a double lung transplant (DLT) compared to recipients of a single lung transplant (SLT) [2]. These data should be interpreted with caution, however, as differences in survival according to procedure type may be due to clinical factors that influenced the decision regarding procedure type (such as recipient age, comorbidities and diagnosis as well as donor issues). Many investigators have examined survival by procedure type in conjunction with recipient diagnosis [4–9]. Meyer et al. found that recipients of a bilateral lung transplant for chronic obstructive pulmonary disease (COPD) had a higher median survival than single lung COPD recipients (6.7 vs. 4.6 years, respectively); although there was no statistically significant survival benefit in recipients older than 60 years [10]. Additionally, others have suggested that procedure type may impact risk of BOS. Hadjiliadis et al. reported that COPD recipients of a DLT were more commonly free from BOS than SLT recipients at both 3 years (57.4% vs. 50.7%) and 5 years (44.5% vs. 17.9%) after transplant [5]. While conjecture, this may be partially explained by additional lung reserve in DLT recipients.

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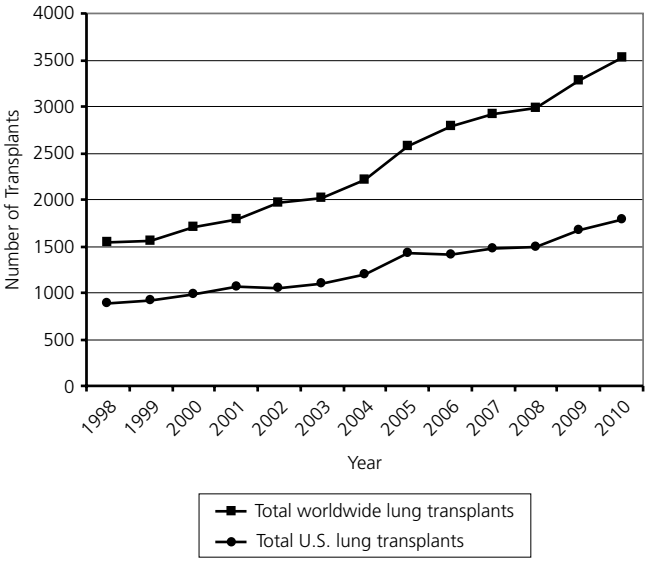

Figure 106.1. Number of yearly lung transplants performed worldwide and in the US between 1998 and 2010. The International Society for Heart and Lung Transplantation (ISHLT) registry reports that 3519 lung transplants (2599 double and 920 single) were performed worldwide in 2010 (the highest yearly total to date). In the US, the Annual Report of Organ Procurement and Transplantation network (OPTN) reported that 1785 lung transplants (1246 double and 539 single) were performed in 2010 in the US. Figure generated from data from the ISHLT registry and the 2011 Annual Report of OPTN.

Among recipients with a diagnosis of interstitial pulmonary fibrosis (IPF), it was reported in the 2011 ISHLT Registry Report that unadjusted survival with a DLT is better than that of SLT three years or more after lung transplantation [6]. However, in an analysis of the UNOS registry that included 3327 patients with IPF, survival was not significantly different between DLT and SLT following adjustment for recipient and donor baseline characteristics [7]. Weiss et al. analyzed the survival of high-risk IPF patients after the introduction of the Lung Allocation Score (LAS) in the US. DLT was associated with a 14.4% decrease in mortality at 1-year, suggesting that it may be advantageous over SLT [8]. Regarding pulmonary arterial hypertension (PAH), many reports suggest that DLT is favorable in recipients with a diagnosis of PAH [9].

The number of transplants being performed in patients 65 years or older has increased dramatically over the last decade. In 2001 in the US, 3.4% of lung transplants were performed in recipients 65 years or older while in 2011 this had increased to 26.6% [1]. This trend is also seen in the ISHLT Registry [2]. With this demographic shift in mind, it is also clear that survival rates after lung transplantation differ by recipient age. In particular, in recipients 65 years and older survival half-life is 3.6 years while that in recipients 35–49 years old is 6.5 years [2]. The main reason for the difference in outcome appears to be late events leading to worse long-term survival as 1-year survival appears comparable between selected recipients older than 65 and younger recipients [2].

Disease-specific differences in survival are apparent but may be confounded by differences in severity of illness, co-morbidities,

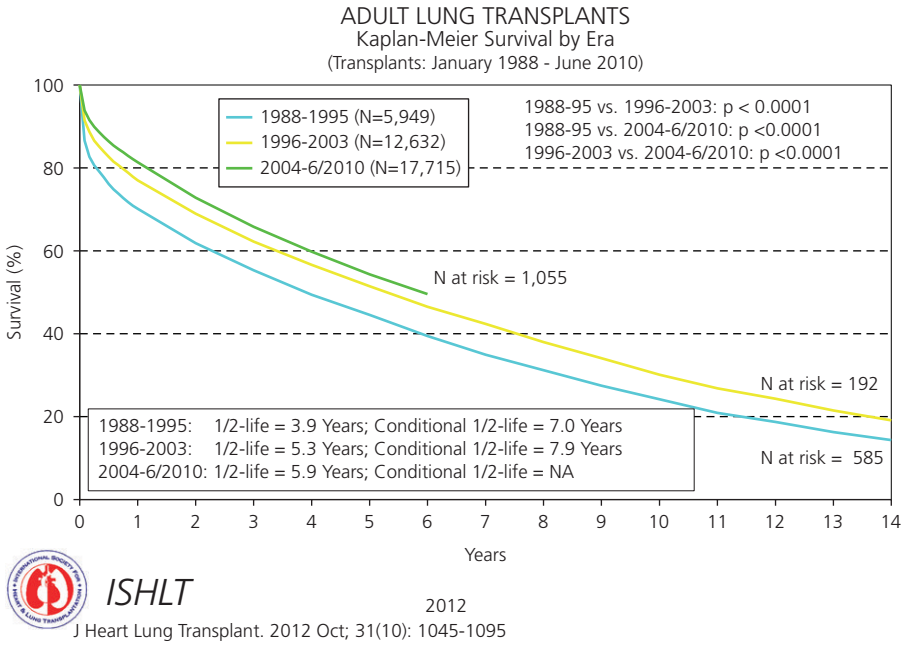

Figure 106.2. Adult lung transplant survival by era calculated using the Kaplan-Meier method. This incorporates information from all transplants for whom any follow-up has been provided. Since many patients are still alive and some patients have been lost to follow-up, the survival rates are estimates rather than exact rates because the time of death is not known for all patients. Survival rates were compared using the log-rank test statistic. No adjustments were made for multiple comparisons. (Source: International Society for Heart and Lung Transplantation, accessible from http://www.ishlt.org/registries/slides.asp?slides=heartLungRegistry)

and average age among disease populations. In addition, certain diagnoses carry higher risks of operative complications and primary graft dysfunction. Among recipients surviving at least 1-year, there were better conditional half-lives after transplant for the primary diagnoses of cystic fibrosis (CF) with an average half-life of 10.4 years, PAH with an average half-life of 10.0 years, sarcoidosis with an average half-life of 8.4 years, and α_1-antitrypsin deficiency with 8.6 years, compared with both COPD and IPF at 6.8 years [2]. Recipients with a primary diagnosis of COPD have the highest 1-year survival and recipients with PAH have the lowest 1-year survival. Lung transplant recipients with IPF have lower 1-year and 10-years survival than others.

It is important to note that most of the published survival data on US outcomes are for recipients who were transplanted under the umbrella of the previous organ allocation system, in which priority was given to recipients with protracted wait-list times. In May of 2005, the new lung allocation score (LAS) System was initiated, prioritizing patients on the basis of the risk of death, *(urgency)*, and the likelihood of 1-year survival post-transplant, (*utility*) [11]. The LAS system is, in part, a scoring system based on the severity of illness, which may have a significant impact on the characteristics of patient selection as well as survival outcomes [12]. Immediately after initiation and implementation of the LAS System, wait-list times and wait-list deaths showed a modest but significant decrease. Over time, the mean LAS score for transplant recipients has increased [13] and, while controversial, some data suggest that a high LAS score may be associated with increased morbidity and mortality after lung transplantation [14,15]. How this will impact outcomes and organ allocation in the US is unclear at this point. As part of the LAS system, wait-list deaths and post-transplant survival will be continually monitored and the LAS system modified as needed. The impact of the LAS on waitlist management is covered in Chapter 40.

An in-depth analysis of risk factors (in addition to those discussed previously) for mortality following lung transplantation is included in the most recent ISHLT Registry Report [2]. The strongest categorical risk factors for 5-year mortality include recipient use of intravenous inotropes (relative risk [RR] 2.13) and recipient dialysis (RR 1.79). Continuous risk factors associated with 5-year mortality, conditional on surviving 1-year, include low and higher recipient age and low transplant center volume.

Heart-lung transplantation

The volume of adult heart-lung transplants performed annually is significantly less than that of lung transplants with a worldwide volume of 86–114 yearly since 2003 [2]. The predominant indication for heart-lung transplantation continues to be congenital heart disease and PAH with a diminishing number being performed for CF. Heart-lung transplantation survival rates differ from lung transplantation survival rates with a relatively higher rate of early mortality in heart-lung transplantation recipients. Heart-lung transplantation recipient survival rate is 63% at 1-year; however, the survival rate following the first year is relatively good with a conditional half-life of post 1-year survival of 10.0 years. As with lung transplantation, survival following heart-lung transplantation has improved significantly in every decade between 1982 and 2010. In heart-lung transplantation recipients, pretransplantation diagnosis is also associated with survival outcomes. Eisenmenger syndrome is associated with the best overall survival when compared to recipients with a primary pretransplant diagnosis of PAH and other congenital heart diseases [2].

Quality of life

The goal of lung transplantation is not only to extend survival of those afflicted with end-stage lung disease but to also improve health-related quality of life (HRQL). While outcomes analyses of survival and lung function following transplantation are common, in depth analyses of HRQL following lung transplantation have been relatively infrequent. The ISHLT Registry does compile functional status post-transplant and, although limited due to incomplete reporting, data suggest that more than 85% of surviving patients at 1-, 3- and 5-years have no activity limitations and that at 5-years post-transplant, 35% of surviving recipients are working either part- or full-time while 46% are not working and 19% are retired [2]. In a recent thematic analysis of published studies of HRQL in lung transplant recipients, Singer et al. found several important insights [16]. First, their analysis found that the literature supports the assertion that lung transplantation results in clinically meaningful and significant improvements in HRQL with greatest improvement in the physical health and functioning domains. Second, they found that lung transplant recipients may derive greater HRQL benefits than other solid organ transplant recipients when comparative data, although limited, is analyzed. Third, lung transplant recipients manifest substantial residual impairments in HRQL compared to population norms suggesting that the significant benefits in HRQL is attributable to the extremely poor HRQL in patients awaiting lung transplantation. Lastly, their data supports that HRQL following lung transplantation remains an understudied field. While they identified 73 studies on HRQL following lung transplantation published between January 1, 1983 and December 31, 2011, a similar search yielded 1131 HRQL articles published in heart transplantation, 1291 in liver and 1689 in kidney. This undoubtedly will be an area of increasing investigation in the future.

Acute rejection

Despite the application of new immunosuppressive medications in lung transplantation, acute and chronic rejection remain common. Indeed, the burden of rejection is perhaps greater in lung transplant recipients that in other solid organ recipients with the possible exception of small bowel recipients. Exposure of the lung to the external environment (including infection) and the rich immune milieu in the lung are felt to be contributing factors (which may also be true for the small bowel). Historically, acute cellular rejection (ACR) was felt to be the predominant form of acute rejection in the lung; however, antibody-mediated rejection (AMR) is now also felt to play a role, as it does in other solid organ transplants. The leading cause of mortality after the first post-transplant year is BOS and remains the main barrier to long-term survival. These issues are discussed in the following sections. Where pertinent differences exist between lung transplant recipients and heart-lung transplant recipients, they are discussed.

Acute cellular rejection

The clinical and histopathological features of ACR are presented in their entirety in Chapters 72 and 84. However, ACR remains an important factor in subsequent outcome and is thus presented here in that context. Lung transplant patients with ACR can be asymptomatic or present with a wide range of symptoms and findings. Non-specific symptoms can include cough, dyspnea and sputum production while non-specific physical exam findings can include hypoxemia, fever and abnormal breath sounds that can be difficult

Table 106.1. Revised classification and grading of pulmonary allograft rejection

Category of rejection	Grade	Severity	Histological finding
Grade A:	0	None	Normal lung
Acute cellular vascular rejection	1	Minimal	Scattered, infrequent mononuclear perivascular infiltrates
	2	Mild	More frequent perivascular infiltrates identifiable at lower scanning magnification, eosinophils may be present
	3	Moderate	Easily recognizable, dense perivascular infiltrates with extension into peribronchial septa and airspaces commonly associated with endothelialitis, eosinophils, and neutrophils may be present.
	4	Severe	Diffuse perivascular, interstitial, and air space infiltrates with prominent pneumocyte damage and endothelialitis.
Grade B:	0	None	No evidence of bronchiolar inflammation
Acute cellular airway rejection	1R	Low grade	Infrequent and scattered lymphocytes (or forming a circumferential band); no epithelial damage or intra-epithelial infiltration
	2R	High grade	Larger and activated lymphocytes in bronchiolar submucosa with more frequent eosinophils and plasmacytoid cells; intra-epithelial lymphocyte infiltration associated with epithelial damage
	X	Ungradeable	Tissue ungradeable
Grade C:	0	None	Obilterative Bronchiolitis absent
Chronic airway rejection	1	Present	Obilterative Bronchiolitis present
Grade D:			Accelerated graft vascular sclerosis
Chronic vascular rejection			

R, denotes revised grade to avoid confusion with the 1996 scheme.
Based on the 2007 revision of the working formulation for the standardization of nomenclature (21).

to differentiate from symptoms and signs of infection [17]. Similarly, findings on imaging studies and pulmonary function studies (PFTs) are not sensitive or specific and typically cannot be reliably differentiated from infection [18,19]. As a result, bronchoscopic transbronchial biopsy (TBBx) has been and remains the gold standard for the diagnosis of ACR. The risks of TBBx including bleeding and pneumothorax have prompted many investigators to attempt to correlate protein or cellular markers in bronchoscopic-guided bronchoalveolar lavage (BAL) fluid with ACR. To date, however, no single BAL marker has proven to have sufficient sensitivity or specificity to supplant TBBx in the diagnosis of ACR [20]. Similarly, although there is some promise, surrogate cellular and molecular markers in peripheral blood have, thus far, proven insufficiently strong to diagnose ACR reliably.

ACR is graded pathologically and is subdivided into acute cellular vascular rejection (Grade A ACR) and acute cellular airway rejection (Grade B ACR) with various grades of severity within each category [21] (Table 106.1). ACR is common following lung transplantation with at least one-third of lung transplant recipients experiencing ACR within the first year post-transplant. After the first year post-transplant, the frequency of ACR declines significantly. The management of lung transplant recipients, while controversial, includes the routine use of surveillance bronchoscopy with TBBxs at many lung transplant centers [22]. While some lung transplant programs perform TBBxs only when dictated by clinical circumstances [23], many believe that surveillance TBBxs (at least for the first 18–24 months post-transplant) provide valuable information that allows appropriate treatment of asymptomatic rejection and fine tuning of immune suppression [24]. There are no data, however, to demonstrate that a protocol including surveillance TBBxs impacts BOS or survival.

While in its severe form ACR can be fatal, ACR is usually treatable and is responsible for less than 2% of deaths following lung transplantation [2]. ACR remains a major focus of post-lung transplant care, however, and is the most significant risk factor for development of BOS. Studies suggest that higher grade ACR, increased frequency of ACR , recurrent high grade ACR and Grade B ACR (lymphocytic bronchiolitis) independent of Grade A rejection are risk factors for BOS [25–27]. In addition, minimal Grade A1 ACR has been identified in some studies as a risk factor for BOS [28] as has large airway inflammation [29].

Although the risk factors for ACR are presented in detail in Chapter 72, a brief recounting here is necessary given the link between ACR, BOS and long-term outcomes. Reported categorical risk factors for ACR include donor : recipient HLA disparity, type of immune suppression, recipient factors and infection. Several large studies have examined the association between HLA disparity and ACR risk and, while the exact relationship requires further study to define, it appears clear that increasing HLA disparity worsens lung transplant outcomes and appears associated with risk of ACR [30,31]. With regards to immunosuppression, unadjusted data from the ISHLT Registry suggests that induction with an IL-2R antagonist is associated with significantly lower ACR rates in the first year post-transplant as is use of a tacrolimus-based maintenance regimen [2]. While results have varied in several randomized comparative trials of cyclosporine versus tacrolimus-based maintenance immunosuppression, the most recent and largest randomized trial found that tacrolimus was associated with a lower cumulative incidence of BOS despite no difference in the rates of ACR [32]. Recipient factors that appear to be related to ACR risk include genotypes that have been associated with either a reduced or heightened ability to mount an immune response [20] as well as gastroesophageal reflux disease that could enhance alloimmune responses against the graft leading to greater ACR [33,34]. Finally, various viral and bacterial infections have been associated with enhanced risk of ACR and/or BOS and worse long-term outcomes [35].

While most lung transplant recipients are treated lifelong with a maintenance triple drug immunosuppression regimen consisting of a calcineurin inhibitor (CI), an antimetabolite and steroids, there is a lack of consensus regarding the optimal regimen and considerable variability in the use of induction agents [2,36]. Similarly, there is no standardized approach between centers to the treatment of ACR and a paucity of randomized trial data to support an evidence-based approach to selection of agents. There is, however, general consensus that rejection episodes of ACR Grade 2 or higher require treatment. Initial therapy is commonly with pulse dose steroids (500 mg to 1000 mg/day) for 3–5 days followed by an oral steroid

taper and/or increase in other maintenance immunosuppressive agents [36]. With higher grades of acute rejection associated with graft dysfunction, some centers choose to utilize polyclonal or monoclonal anti-lymphocyte preparations. Persistent or recurrent ACR is generally treated with a repeat course of steroids, a change in maintenance immunosuppressive agents (e.g. change in CI or addition of a mTOR-inhibitor) and/or polyclonal or monoclonal anti-lymphocyte preparations [36]. Other approaches reported in small series have included the use of alemtuzumab [37], inhaled cyclosporine [38], total lymphoid irradiation [39] and extracorporeal photophoresis [40]. Although, as discussed above, Grade A1 and Grade B (lymphocytic bronchiolitis) acute rejection have been associated with the development of BOS, there is inconsistency between programs as to whether to treat and whether treatment alters the risk of later BOS. In a recent US survey, 80% of respondents would treat symptomatic Grade A1 rejection while only 35% would treat asymptomatic Grade A1 rejection. Furthermore, 12% of respondents stated that they would not treat lymphocytic bronchiolitis [22]. To complicate matters further, there was significant variability in the grading of lymphocyte bronchiolitis.

Antibody-mediated rejection (AMR)

The contribution of anti-HLA alloantibody to rejection in renal and cardiac transplantation has been recognized for some time, and is discussed in depth mechanistically and clinically in Chapters 6 and 89, respectively. Its importance in lung transplantation, outside of hyperacute rejection, has become increasingly evident over only the last 10–15 years but it now seems well established that pulmonary AMR exists and that the consequences of pulmonary AMR can include persistent and refractory ACR and BOS [41]. Based on the recent ISHLT Pathology Council consensus publication [41], the classic diagnostic components that comprise lung AMR include:

1 the presence of clinical allograft dysfunction,
2 circulating donor-specific antibodies (DSA), and
3 pathologic findings [41].

The authors emphasized that the histopathologic findings, covered in Chapter 84, in AMR are non-specific patterns of injury that are seen in a variety of disorders and also noted that capillary C4d immunoreactivity while commonly seen in AMR with circulating DSA can also be seen in the absence of such antibodies. They also emphasized that, based on current definitions and the experience of most transplant pathologists, AMR is a relatively infrequent finding and that diagnosis of AMR should rely on the interaction of pathologist, immunologist and clinician. They also encouraged centers to that develop protocols to routinely assess for DSA in conjunction with clinical visits or at the time of surveillance bronchoscopy.

There are no randomized controlled trials evaluating treatment of established AMR in lung transplantation — or kidney transplantation, for that matter. Existing data regarding therapeutic approaches comes mostly from single center reports. Plasmapharesis (to remove circulating antibodies), immunoglobulin (IVIG — which causes B cell apoptosis, blocks binding of donor reactive antibodies and inhibits complement activation) and rituximab (an anti-CD20 antibody) are the common agents utilized alone or in combination [20]. Two new agents that are now being used by some programs include bortezomib (an inhibitor of the 26s proteasome that causes plasma cell apoptosis) and eculizumab (an antibody against complement component C5 that results in blockade of terminal complement activation) [20]. Adjunct therapies have typically included polyclonal anti-lymphocyte preparations and mycophenolate mofetil for its anti-B cell proliferative effects. Many unanswered questions remain regarding components of therapy, length of therapy and effects on clinical outcome (including risk of BOS).

Questions also remain regarding patients who have discordance in the classic diagnostic components of AMR and how to manage those patients. For example, many patients have been found to have circulating DSA in the absence of demonstrable histologic findings or C4d staining and with or without allograft dysfunction. Whether this is a marker of inadequate immunosuppression resulting in T cell activation and early alloantibody production or something else is unclear at this point. Additionally, humoral autoimmunity to the self-epithelial antigen K alpha-1 tubulin has been associated with development of BOS and, in at least one study, the development of DSA preceded the development of anti-K alpha-1 tubulin antibodies suggesting that DSA mediated injury may have augmented development of autoimmunity [42,43]. Much work remains to elucidate these complex interactions, the clinical consequences and the efficacy of potential therapies.

Chronic lung allograft dysfunction

Historically, chronic rejection of the lung was felt to result from cellular alloimmunity resulting in fibroproliferative narrowing and occlusion of small airways and culminating in allograft dysfunction. Because the diagnostic yield of transbronchial biopsies identifying lesions characteristic of chronic rejection was felt to be low [44], a clinical grading scale of obstructive decline in forced expiratory volume in 1-second (FEV1) of at least 20% compared with the best postoperative values in the absence of other identifiable factors was developed and termed BOS. BOS was felt to be an obstructive, irreversible decline in pulmonary function resulting from fibrotic occlusion of bronchioles. Recently, investigations have suggested that not every decline in FEV1 meet the criteria for BOS and the term chronic lung allograft dysfunction (CLAD) has been proposed as an overarching name with BOS encompassing one phenotype. Two other emerging phenotypes of CLAD, neutrophilic reversible allograft dysfunction (NRAD) and restrictive allograft syndrome (RAS), have subsequently been proposed with what appear to be distinct clinical patterns and therapeutic response rates. This section provides an overview of these entities.

BOS

The most common form of CLAD is BOS and is the leading cause of death after one year from transplant. In the 1–3 and 3–5 year intervals following lung transplantation, BOS is the leading cause of death at 26% and 29.2%, respectively [2]. BOS also causes significant morbidity, reduction in HRQOL and escalates cost in lung transplantation. The incidence of BOS, as reported from the ISHLT Registry, is 48% by 5 years and 76% by 10 years after transplantation, while that of BOS during first year post-transplant is 9.6% [2].

BOS is defined as a sustained (>3 weeks) decline from the best baseline FEV1 in the absence of alternative causes of graft dysfunction (e.g. infection, acute rejection, and airway stenosis, anastomotic stricture) [45]. The best baseline is defined as the average of the two best FEV1 values measured post-transplant at least 3 weeks apart. A decline from baseline of at least 20% is defined as BOS with progressive loss of function defined in progressive stages of BOS (Table 106.2). The typical pathological findings of BOS, when available, include:

Table 106.2. Bronchiolitis obliterans syndrome classification

	Original classification 1993		Current classification 2001
BOS 0	$FEV_1 > 80\%$ of baseline	BOS 0	$FEV_1 > 90\%$ of baseline and $FEF_{25-75} > 75\%$ of baseline
		BOS 0-p	$FEV_1 > 81–90\%$ of baseline and/or $FEF_{25-75} \leq 75\%$ of baseline
BOS 1	$FEV_1 > 66–80\%$ of baseline	BOS 1	$FEV_1 > 66–80\%$ of baseline
BOS 2	$FEV_1 > 51–65\%$ of baseline	BOS 2	$FEV_1 > 51–65\%$ of baseline
BOS 3	$FEV_1 < 50\%$ of baseline	BOS 3	$FEV_1 < 50\%$ of baseline

BOS, bronchiolitis obliterans syndrome; FEF_{25-75}, mid-expiratory flow rate; FEV_1, forced expiratory volume in one second.
Modified from Estenne M, Maurer JR, Boehler A, et al. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. J Heart Lung Transplant 2002; 21:297.

- submucosal lymphocytic inflammation and disruption of the epithelium of small airways in early phase,
- ingrowth of fibromyxoid granulation tissue into the airway lumen, resulting in partial or complete obstruction, and
- granulation tissue in a cicatricial pattern and resultant fibrosis, which finally obliterates the airway lumen.

Typical symptoms are non-specific and include; dyspnea, cough, sputum production, and limitation of exercise capacity. Chest computed tomography (CT) might show bronchiectasis with peribronchial thickening and air trapping. In patients with RAS (see further on), chest CT typically demonstrates interstitial opacities, upper lobe fibrosis, ground glass opacities and honeycombing. BOS is most commonly diagnosed between 1.5 and 4 years post-transplant [46]. The course is highly variable and may be insidious in onset and progression or result in a gradual decline in function over months to years or may result in an abrupt, severe decline in function over weeks [47]. Initial deterioration with subsequent stabilization at a lower level has also been described [47]. Early onset BOS and high-grade onset BOS [2,3] predict worse survival [46].

The pathogenesis of BOS is complex and can involve alloimmune and non-alloimmune mechanisms acting independently or in concert. Classically, BOS is felt to be the result of alloimmune-mediated injury to the graft. Supporting this thesis, as discussed previously, is the relationship of ACR to BOS with both Grade A ACR (persistent/refractory, high grade and late onset) and Grade B ACR (lymphocytic bronchiolitis) being strong risk factors for BOS. Similarly, and as discussed previously, humoral alloimmunity to HLA is associated with development of BOS, as well. Much work over the last decade has also focused on the role of autoimmunity rather than alloimmunity as a cause of BOS [42,48]. Data suggest that epitopes of collagen type V exposed during lung injury (such as during ischemia reperfusion injury around the time of transplantation) and K-alpha1 tubulin can lead to autoimmunity resulting in graft injury and BOS. Much ongoing work is focused in this area and potential therapeutic interventions.

Abundant evidence has demonstrated other risk factors for BOS that are seemingly not alloimmune related. These include primary graft dysfunction, gastroesophageal reflux and various infections including cytomegalovirus (CMV) infection and community-acquired viral infection (CARV) as well as others. While the mechanism inciting development of BOS is not clear, some have suggested that graft injury from these events results in a heightened inflammatory state in the lung leading to enhanced alloreactivity while others have suggested other pathways including aberrant healing following injury [49,50].

Primary graft dysfunction, (PGD) is a risk factor of BOS that is independent of acute rejection, lymphocytic bronchitis, and community-acquired respiratory viral infections, and this risk is directly related to the severity of primary graft dysfunction [51]. A retrospective series by Whitson et al. reported that bilateral lung recipients with Grade 3 PGD had worse long-term survival, higher incidence of BOS, and compromised pulmonary function [52]. The mechanisms of this co-relation between PGD and BOS are thought to be oxidative stress, reduced nitric oxide production, and up-regulation of HLA class II antigens in ischemia reperfusion injury.

Gastroesophageal reflux (GERD) has been suggested to occur with a frequency in lung transplant recipients as high as 73% in one single center study [53]. Much evidence suggests that GERD is associated with the development of BOS and data suggest that early surgical intervention (gastric fundoplication) for lung recipients with GERD can result in greater freedom from BOS compared to patients treated medically [53,54]. Much work continues in this area with many investigators hoping to conduct a randomized clinical trial.

CMV infection has long been considered a risk factor of the development of BOS [55]. While this result was not confirmed in a single-center study [56], a second single-center cohort study demonstrated that treated CMV pneumonitis within the first 6 months after transplantation significantly increased the risk for BOS [57]. Similarly, in single center reports, CARV [58], human herpesvirus-6 [59] and *Chlamydophila pneumonia* [60] infection were associated with development of BOS. In a single-center analysis of 161 cases, Valentine et al. found that pneumonia and airway colonization with gram positive bacteria, gram negative bacteria, and fungal pathogens were independent determinants of chronic allograft dysfunction [61]. Additionally, Weigt et al. reported that *Aspergillus* colonization was strongly associated with BOS and BOS related mortality [62].

To date, there is no proven treatment for BOS. Historically, many agents and therapeutics have been evaluated in single center studies but the results of many of these studies are viewed with skepticism due to small sample sizes, lack of suitable controls and endpoints such as stabilization of FEV1 or reduction in rate of FEV1 decline (as opposed to improvement) that can occur during the natural history of BOS. Approaches have included alteration of maintenance immunosuppression converting from cyclosporine to tacrolimus as well as converting from azathioprine to mycophenolate mofetil [63,64]. Cytolytic therapy with an antilymphocyte or antithymocyte preparation or with alemtuzumab (a humanized CD52-specific antibody) also has been reported to have an effect in some patients [65,66].

Perhaps the approaches with the greatest evidence are azithromycin, extracorporeal photopheresis (ECP) and surgical fundoplication (discussed previously) in those patients with GERD. Gerhardt et al. first reported the use of azithromycin (250 mg three times per week) in BOS patients in a small pilot study in 2003 [67]. In the study, 5 of 6 patients demonstrated a significant improvement in FEV1 resulting in much interest in azithromycin as a therapy for BOS. Most subsequent studies have also suggested that a subset of patients with BOS respond to azithromycin with improved FEV1 and many studies have also suggested that those likely to respond have BAL fluid (BALF) neutrophilia [68].

This has led many investigators to suggest that the group of patients with BALF neutrophilia represents a distinct phenotype of CLAD; accordingly they have termed this neutrophilic reversible allograft dysfunction (NRAD).

ECP is performed via a closed loop intravenous system and involves removal of approximately 700 ml of the patient's blood and isolation of leukocytes with subsequent exposure to ultraviolet light in the presence of 8-methoxypsoralen (8-MOP) [69]. The induced bonding between 8-MOP and DNA pyrimidine bases results in lymphocyte apoptosis. ECP is safe and, since US Food and Drug Approval in 1998, has been used to successfully treat cutaneous T cell lymphoma as well as acute and chronic graft versus host disease and a variety of other diseases including acute cellular rejection in heart transplant recipients [70,71]. Although the exact mechanism of action is not clear, it has been suggested that ECP causes immunomodulation, possibly via the induction of Tregs [72]. The first successful use of ECP in lung transplant recipients was reported in 1995 where it was used to treat acute rejection [73]. Since then, several studies have suggested efficacy in lung transplant recipients with refractory acute rejection or BOS. Interpretations of results and enthusiasm have been limited, however, due to small sample size, non-comparable adjuvant immunosuppression and short-term follow-up, as well as expense and need for intravenous access. Recently, however, Greer et al. published a retrospective series of 65 well-characterized patients with BOS, 64 of whom had deteriorated despite azithromycin therapy. With a median follow-up of 503 days, this study demonstrated efficacy of ECP in 54% of treated patients and identified three subgroups of CLAD (restrictive allograft syndrome, rapidly progressive and non-neutrophilic) that were significantly less likely to respond to ECP [69]. This study is likely to generate renewed enthusiasm for studying ECP as a treatment for CLAD.

Lastly, retransplantation can be life saving for patients with BOS; however, retransplantation remains controversial due to limited number of donor lungs as well as the generally lower survival rates and higher rate of BOS that that following primary transplants [74].

Strategies to prevent BOS (and other forms of CLAD) have been partially discussed previously. Given the strong link between acute rejection and BOS, monitoring strategies including use of surveillance bronchoscopy and TBBx are used by many centers with the hope of preventing and aggressively treating acute rejection. There is no clear evidence, however, that surveillance biopsies and TBBxs have any impact on BOS or survival. Clouding this issue, however, is the potential for TBBx sampling error [75] and interobserver agreement between pathologists on ACR grading [76] to result in under diagnosis of acute rejection. In the ISHLT Registry, the use of induction therapy is associated, in unadjusted analysis, with longer survival [2]. The limited clinical trial data, however, is indeterminate and further multicenter studies are needed. The use of tacrolimus as compared to cyclosporine is also associated with improved survival in ISHLT Registry data [2] and, as discussed previously, randomized trial data supports a lower incidence of BOS and perhaps ACR [32]. In 2011, Vos et al. published a randomized trial of azithromycin prophylaxis started at discharge from the hospital after lung transplantation [77]. At 2 years following transplantation, BOS occurred significantly less frequently in the azithromycin group compared to the placebo group (12.5% vs. 44.2%, $P = 0.0017$). The azithromycin group also demonstrated a better FEV1 and lower BALF neutrophilia.

Neutrophilic reversible allograft dysfunction and restrictive allograft syndrome

Following the initial publication of azithromycin treatment of BOS by Gerhardt et al. [67], numerous studies corroborated the finding while some did not. The reason why some patients did not respond was not initially clear but later studies correlated BALF neutrophilia and high BAL IL-8 levels with response to azithromycin [69,78]. Investigators characterized the phenotype of responders as having a high percentage of neutrophils in BALF (>15%), CLAD (obstructive physiology) developing early after transplant and they had an improvement in FEV1 of at least 10% after 3–6 months of azithromycin. This group appears to have a good prognosis with azithromycin treatment.

Restrictive allograft syndrome is a term that was coined by the Toronto Lung Transplant Program and describes lung transplant recipients that suffer from a persistent decline in FEV1 (>20% compared with the best postoperative baseline) and a restrictive pattern of pulmonary mechanics (defined as a decline in total lung capacity [TLC] of >10% compared with baseline) [79]. This pattern is observed in about 25–30% of lung transplant patient suffering from CLAD. Diagnosis is made with PFTs and high-resolution chest CT which demonstrates interstitial opacities, upper lobe fibrosis, ground glass opacities and honeycombing compared to patients with BOS or NRAD [79]. Patients with RAS have a lower survival rate after diagnosis with a median survival of 8 months in a series from Belgium and a median survival of 16 months in the Toronto series (compared with 35 months and 46 months for patients with obstructive BOS, respectively) [79,80]. No beneficial therapy has been identified to date and the general impression is that RAS patients do not respond to azithromycin. The landscape is made a bit more complex in that some investigators have noted that some patients first develop a classical obstructive BOS pattern (no change in TLC) and then later develop findings of RAS (decline of >10% in TLC). Further studies are needed to delineate these patterns and to better understand the pathophysiology.

Infection

As with all solid organ transplant recipients, and as covered generally in Chapters 92–94, recipients of lung transplants are at increased risk of infection due to the requisite immunosuppressive therapy. However, lung transplant recipients are generally thought to be more susceptible to infection than most other solid organ recipients, and thus, infection is presented with specific reference to lung transplantation here. The factors exacerbating the risk of infection in lung transplant recipients include the generally higher level of immunosuppression required as well as impaired local defense mechanisms (loss of lymphatic drainage, diminished mucociliary function, and reduced cough) and a constant environmental exposure to organisms inhaled through the tracheobronchial tree. As a result, infection remains a common cause of morbidity and mortality following lung transplantation. Indeed, non-CMV infection is second only to graft failure as the most common cause of reported death in the first year following transplantation (20% of deaths) and secondary only to BOS as the most common of cause of reported death after the first year [2]. Bacterial and viral infections predominate following lung transplantation, however, fungal and mycobacterial infections also occur at an increased frequency. Prophylaxis against opportunistic organisms such as CMV, fungi and *Pneumocystis jirovecii* is common in

clinical practice and reduces infection risk for these organisms but infections with these organisms still occur.

The diagnosis of pulmonary infection in the lung transplant recipient can be quite vexing. Symptoms and signs include fever, shortness of breath, sputum production and hypoxemia and can be difficult to distinguish from acute rejection. While a positive sputum culture and/or a positive BAL culture with a negative biopsy for ACR can be highly suggestive or definitive, there are few other definitive tests and an amalgam of findings and diagnostic studies is frequently necessary to guide therapy. The Cylex Immune Cell Function Assay (ImmunoKnow; Cylex, Inc., Columbia, MD) was developed to assess global immune function in solid organ transplant recipients and to aid in the diagnosis of infection or rejection. The assay measures ATP production in stimulated peripheral blood T cells and correlates with both infection and rejection in kidney, liver, heart and small bowel transplant recipients [81]. Although there appears to be some correlation between very low ATP levels and infection and very high ATP levels and ACR, the test remains inadequate to diagnosis infection or ACR and guide therapy [82,83].

Bacterial infection

Bacterial infection, especially gram-negative bacteria, is the most common cause of pneumonia after lung transplantation [84]. The majority of gram-negative bacteria as a cause of pneumonia are: *Pseudomonas aeruginosa*, *Acinotobacter* species, *Enterobacteriaceae*, *Stenotrophomonas* species, and *Burkholderia* species. Colonization of the airway before transplantation must be considered, especially in patients with cystic fibrosis, because it may result in postoperative complications, such as pneumonia. Regarding the multi-drug resistance of colonized bacteria, *Burkholderia cepacia* may merit heightened watchfulness and investigation. *Burkholderia cepacia* was previously known as Pseudomonas cepacia and consists of nine distinct species. Recent studies suggested that a specific species of *Burkholderia cepacia*, *B. cenocepacia* (genomovar III), is associated with increased post-transplant mortality [85]. As a result, many lung transplant programs will not offer transplantation to patients (most commonly with cystic fibrosis) with *Burkholderia cepacia* colonization. The multi-drug resistance of *Pseudomonas aeruginosa* in the pretransplant period is not considered a risk factor of mortality [86]. Conversely, the post-transplant colonization of *Pseudomonas aeruginosa* is associated with the high incidence of BOS and a shorter period of BOS-free survival [87].

Viral infection

CMV infection is the most significant viral infection and the second most common infection overall, after bacterial infection [88], in lung transplant recipients. As a result, lung transplant programs focus attention on the prevention of CMV as a routine regimen. The lung transplant recipient is at increased risk of significant CMV viral load when compared to other sold organ transplants. Without aggressive prevention, it is estimated that CMV disease occurs in 38–75% of lung transplant recipients [89].

Risk factors for CMV infection include the serostatus of the donor and recipient; the combination of seropositive donor and seronegative recipient is at the highest risk. According to Zuk et al. 94.9% of centers use prophylaxis against CMV for donor positive, recipient negative patients [90]. Regarding the duration of the prophylaxis, the 2010 Transplantation Society International CMV Consensus Group guidelines recommended at least 6 months of prophylaxis for CMV D+/R− recipients, and three to six months in CMV D+/R+ and D−/R+ individuals [91]. In a recent multicenter randomized trial, extended CMV prophylaxis (12 months) in lung transplant patients at high risk for CMV infection resulted in a significant decrease in the incidence of CMV disease compared to recipients who received prophylaxis for 3 months [92]. CMV infection accounts for less than 1% of deaths more than 1 year after transplantation, however, as previously discussed, CMV infection appears to be a risk factor for development of BOS [57] and, thus, may contribute to the mortality of recipients with the development of BOS.

In addition to CMV, numerous other viruses are increasingly being recognized as having a potentially important impact on short- and long-term outcomes in lung transplant recipients. As discussed previously, community-acquired respiratory viral infections are common in lung transplant recipients and have been demonstrated to be a common trigger for acute rejection and/or BOS [93]. Previous exposure to varicella-zoster virus is common and reactivation of latent virus can cause painful shingles and rarely wide spread cutaneous or visceral involvement. The seroprevalance of human herpes virus 6 (HHV-6) and human herpes virus-7 (HHV-7) are high in adults and reactivation following solid organ transplantation common [94]. Reports have linked HHV-6 to acute and chronic rejection in liver transplant recipients [95]. Studies thus far in lung transplant recipients have revealed an association between HHV-6 and interstitial pneumonia but have not demonstrated an association with acute rejection or BOS [96,97].

Fungal infection

While bacterial and viral infections are more common, fungal infections occur in 15–35% of lung transplant recipients and are associated with a higher mortality [98]. *Aspergillus* and *Candida* species are the major causes of fungal infection in lung transplant recipients with the prevalence of *Candida* infections declining. Other less common fungal pathogens that cause infection in lung transplant recipients are *Fusarium*, *Scedosporium* and *Zygomycetes*. Invasive infection with these organisms is associated with 34–78% mortality despite antifungal therapy [99].

Forty-four percent of all invasive fungal infections in lung transplant recipients are due to *Aspergillus* and lung transplant recipients have the highest incidence of invasive aspergillosis (IA) among all solid organ transplant recipients [100], perhaps because *Aspergillus* is pervasive in the environment and is acquired via inhalation. As a result, much attention is focused on *Aspergillus* in lung transplant recipients. Isolation of *Aspergillus* from the tracheobronchial tree may be an indication of colonization (absence of disease), tracheobronchitis with airway anastomotic infection or invasive pulmonary or disseminated disease (i.e. IA). *Aspergillus* tracheobronchitis has become less common than in previous eras and typically develops around a mean of 50 days following transplantation [99,101]. Complications include airway anastomotic dehiscence, stenosis and bleeding. *Aspergillus* tracheobronchitis is associated with a mortality of 20% and without antifungal therapy may progress to IA with a greater risk of death.

The mean time to development of IA is currently longer than in previous eras, occurring around 500 days post-transplant [102]. This temporal shift may be due to the frequent use of fungal prophylaxis post-transplant (see further on). Risk factors for invasive aspergillosis include colonization before or after transplant, single-lung transplantation, CMV infection, hypogammaglobulinemia, environmental exposure, chronic rejection and the net state of immunosuppression [99]. The treatment of IA is reduction of

immune suppression followed by an anti-fungal agent (voriconazole is first-line therapy) according to the 2008 Infectious Diseases Society of America guidelines on the treatment of aspergillosis [102]. Due to advances in management, mortality from IA has declined from 81% in previous eras to 38% [98]. Despite a lack of evidence demonstrating efficacy, prophylaxis against fungal infection is commonly practiced. In a recent worldwide survey, 59% of lung transplant programs stated that they use universal antifungal prophylaxis in the first 6 months post-transplant with 97% targeting *Aspergillus*. The agents used were variable with most programs employing voraconazole alone or in combination with inhaled amphotericin. Most agreed that antifungal prophylaxis guidelines are needed [103].

Malignancy

Solid organ transplantation is a significant risk factor for malignancy and is discussed generally in Chapter 95. The mechanism of developing malignancy is thought to be decreased immunologic surveillance due to chronic immunosuppression in the postoperative period. Malignancies account for a small percentage of mortality in the first year after transplantation and beyond the first year, 9.1–15.9% of all deaths are due to cancer [2]. Non-melanoma skin cancer is the most prevalent malignancy overall but post-transplant lymphoproliferative disease (PTLD) is the m*ost common malignancy among 1-year survivors of lung and heart-lung transplantation* and is second most common overall.

Post-transplant lymphoproliferative disorders

Post-transplant lymphoproliferative disorders (PTLD) are defined as lymphoid and/or plasmacytic proliferations, which occur in both of solid organ transplant and allogeneic hematopoietic cell transplantation. The development of PTLD is mediated by the activation of Epstein-Barr virus (EBV). As a result of reduced suppression of T-cell response, EBV induces B cell proliferation, which leads to simple lymphoid hyperplasia or non-Hodgkin's lymphoma-like aggressive lesion. General features of PTLD are presented in Chapter 96.

Risk factors for development of PTLD include; amplified T cell immunosuppression and recipient EBV seronegative status, primary CMV infection, male-gender, and older recipient age [104]. According to a single center study by Kremer et al. the incidence of PTLD is 4.8% and has decreased recently [105]. The authors suggested that the earlier usage of anti-thymocyte globulin (ATG) as an induction therapy before 2000 might be correlated to the incidence of PTLD.

PTLD within the first year after surgery tends to present in the thorax including allograft or mediastinal lymph nodes. Late PTLD has an extrathoracic presentation with the gastrointestinal tract a common site of the disease. The time to diagnosis of PTLD after lung transplant is 22.5–40 months [105–107].

Important factors in preventing PTLD are identification of high-risk EBV-naïve patients, routine surveillance of EBV viral load, antiviral prophylaxis, and a reduction of immunosuppression in patients with seroconversion of EBV status and rising EBV viral load. The treatment of PLTD includes reduction of immunosuppression, antiviral therapy, anti-CD20 (Rituximab, monoclonal B cell inhibitor), and sometimes chemotherapy with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone). In cases presenting with large masses or abdominal obstruction, surgical intervention can be one option of treatment. Anti-CD20 is reported to have a 66% response rate in PTLD lung transplant recipients [108]. Similar results of anti-CD20 treatment have been reported [109]. Chemotherapy with CHOP is selected for patients presenting with disseminated disease, clinical progression on rituximab, or non B cell PTLD. One clinical trial of rituximab followed by chemotherapy of CHOP for PTLD demonstrated a 60% clinical remission in solid organ patients and extended survival on average of 6.6 years [110].

Skin cancers

Skin cancers represent approximately 40% of all malignancies in organ transplant recipients [111,112]. The most common skin cancers in recipients are squamous cell carcinoma, basal cell carcinoma, melanoma and Kaposi's sarcoma. Risk factors for squamous cell carcinoma and basal cell carcinoma include intensity and duration of immunosuppressive treatment, older age at transplantation, as well as ultraviolet exposure and geographic location. Although it remains unclear if a specific type of immunosuppressive agent contributes to malignancies in recipients, recent evidence indicates that mycophenolate mofetil may be linked to a lower risk for skin cancer than azathioprine-based immunosuppression [113]. Sirolimus was reported to reduce the risk of malignancy and non-melanoma skin cancer in renal transplant recipients in a prospective, randomized controlled trial [114] while cyclosporine and azathioprine use has been correlated with development of skin cancers in solid organ transplant recipients in several studies [115,116]. These agents are suggested to have carcinogenic effects [117,118]. Regarding other medications commonly used in lung transplant recipients, prolonged usage of voriconazole has been reported to be associated with non-melanoma skin cancer in renal transplant recipients and in lung transplant recipients [119,120]. The prevention of skin cancers in recipients requires patient education and counsel on the hazards of sun exposure. The effect of retinoid as a prophylaxis has been not conclusive.

Other medical morbidities

Morbidity related to immunosuppressive drug toxicity on other organ systems are common following lung transplantation as they are following other solid organ transplants. Common morbidities include hypertension, renal insufficiency, hyperlipidemia, diabetes mellitus and osteoporosis. Many of these complications have onset in the first year following transplantation but, despite the gradual tapering of immunosuppressive medications over time after that, the cumulative prevalence continues to rise. The most recent ISHLT data suggests that the incidence of hypertension in the first year post-transplant is 52% and rises to 83% of surviving recipients at 5-years post-transplant [2]. Similarly, the incidence of diabetes at 1-year post-transplant is 26% and increases to 41% at 5-years while that of hyperlipidemia is 25% at 1-year and rises to 58% at 5-years. It is important to manage these medical issues well as all three are major risk factors for cardiovascular disease.

Renal insufficiency can be quite troubling following lung transplantation as chronic kidney disease can severely negatively impact HRQOL and dramatically escalates mortality risk [121]. According to ISHLT data, the incidence of severe renal dysfunction among survivors at 5-years post-transplant is substantial with 4% of recipients requiring dialysis or having undergone renal transplantation while 15.5% have an abnormal creatinine above 2.5 mg/dL [2]. At 10-years post-transplant, the percentage of survivors requiring dialysis or having received a renal transplant rises to 14.4% [2]. In

general, lung transplant programs have tried to utilize nephron-protective strategies such as aggressive weaning of calcineurin agents and/or conversion to a sirolimus based immunosuppressive regimen. Similarly, post-transplant osteoporosis related, at least in part, to immunosuppressive medications is common and can lead to fractures that negatively impact HRQOL [122].

Summary

Significant improvements in recipient and donor selection, surgical technique, immunosuppression and post-transplant care have facilitated the growth and application of lung transplantation resulting in improved HRQOL and survival for many patients. Despite these advances, significant difficulties and challenges remain. Survival has improved largely due to enhanced 1-year survival with persistent and constant late attrition largely due to chronic rejection and infection resulting in disappointing long-term survival. In addition, the ravages of immunosuppression including infection, malignancy, hypertension, hyperlipidemia, renal insufficiency, diabetes and osteoporosis are common. With a better understanding of the mechanisms that lead to chronic lung graft injury and, hopefully, better and more non-invasive methods to tailor immunosuppressive therapy, results will improve.

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CHAPTER 107

Long-term Outcomes after Pancreas Transplantation

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Introduction

Pancreas transplantation offers select patients with type 1 diabetes (T1D) the potential for normal glycemic control without the need for exogenous insulin. In patients with concomitant end-stage nephropathy, simultaneous pancreas-kidney transplantation (SPK) represents the optimal long-term therapeutic approach. Successful SPK transplantation liberates patients from dialysis and re-establishes normoglycemic regulation. This therapeutic benefit is balanced against the morbidity related to the operation and the adverse effects of life-long immunosuppression; two significant barriers that often limit the number of potential surgical candidates. Over the last four decades, there has been a significant improvement in both the early and long-term outcomes after pancreas transplantation [1–4]. Advancements in surgical technique, progress in immunosuppression, and refinements in post-transplant clinical care have all contributed to enhanced graft and patient survival.

Appropriate allocation remains a challenge as recipient risk must be individualized to regional waiting times and balanced against the relative benefits of reversing uremia versus reversing diabetes in patients requiring both kidney and pancreas transplantation. The availability of living kidney donors raises the real issue of whether some patients are better served with living donor kidney transplantation (LDK) first, followed by pancreas transplantation (PAK). Recent updated data on the natural history of patients receiving SPK has been analyzed and these long-term outcomes should inform the decision-making process for diabetic, uremic patients selecting optimal therapy.

In the following chapter we describe the evolution of the surgical technique involved in pancreas transplantation, discuss the data on the long-term outcomes of this procedure, the associated complications, the effects of pancreas transplantation on secondary diabetic complications, and novel ways to expand the donor pool. The surgical technique itself is covered in depth in Chapter 60. Finally, this chapter will summarize the current recommendations for optimizing pancreas allograft and patient survival. Additional information on the recognition and treatment of pancreas rejection is covered in Chapter 73, and consideration of the criteria for candidacy for pancreas transplantation, and its investigational alternative, islet transplantation, is considered in Chapter 32.

Evolution of surgical technique in pancreas transplantation

A complete discussion of long-term outcomes after pancreas transplantation requires acknowledgement of the evolution in surgical technique. Pancreas transplantation with long-term allograft and patient survival was dependent on the development of improved immunosuppression, but also on pioneers who modified the operation. Kelly et al. performed the first pancreas transplant on December 17, 1966 using a duct-ligated segmental graft implanted simultaneously with a kidney from a deceased donor in a 28 year old recipient [5]. This milestone was followed by a series of pancreas transplants using whole pancreatic duodenal allografts with and without the spleen attached, duct-injection strategies, and segmental grafts with the main pancreatic anastomosed to the ureter. These efforts uniformly failed to provide long-term function or acceptable patient survival as a result of technical complications and immunologic rejection. One-year graft survival was reported at 21% and 1-year patient mortality was 39% (International Pancreas Transplant Registry; 1980).

The method of preparing the pancreaticoduodenal allograft and the technique for surgical implantation has been previously described [6–9]. At the University of Wisconsin, the pancreas and liver are removed en bloc in donor recoveries performed by the transplant team (see Chapters 21 and 22 for detailed descriptions of the abdominal organ recovery procedures). Using this technique, the pancreas is separated from the liver and the allograft is subsequently prepared on the back-table in ice-cold UW solution (ViaSpan, Bristol Myers Squibb, Garden City, NY). When several different recovery teams are present for multi-organ recoveries, en-bloc removal is often not performed and the organs are separated in situ. Care must be taken to preserve the pancreatic vasculature and parenchyma, both of which can be easily damaged. The back table preparation of the allograft continues to be the most critical step with respect to the technique of pancreas transplantation. Careful inspection of the pancreas includes examination for fat content, fibrosis, the texture of the pancreatic parenchyma, size and quality of the pancreatic vasculature as well as the donor vessels retrieved for transplant, and the presence of anomalous anatomy [6,9]. Splenectomy is performed by dissecting the pancreatic tail away from the spleen and selectively ligating the distal splenic arteries and proximal splenic veins. The duodenal segment is shortened

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

with oversewing of the proximal and distal staple lines. Neurolymphatic tissue in the area of the superior mesenteric artery (SMA) and splenic artery is carefully removed. Ligation of small venous branches allows lengthening of the portal vein, and arterial reconstruction is routinely performed with an iliac Y-graft [6]. In situations where the procurement will yield a pancreas and a separate intestinal allograft, the mesentery of the small bowel must be divided while maintaining the proximal SMA and superior mesenteric vein (SMV) and their tributary arcades that supply the pancreatic head and duodenum with the pancreatic allograft [9].

The pancreas is often transplanted on the right side with the venous anastomosis performed to the distal inferior cava or common iliac vein (Figure 107.1). Recent reports continue to confirm that no survival advantage is conferred by either portal venous or systemic venous drainage techniques [2,10]. Venous extension grafts are not routinely used and the duodenal portion of the allograft is usually directed cephalad. The arterial anastomosis is performed to the proximal right common iliac artery. Although the bladder-drainage technique was developed at the University of Wisconsin and is still performed in certain centers, enteric drainage of the pancreas is preferentially performed by sewing the donor duodenal segment to the recipient jejunum, ~20–60 cm distal to the ligament of Treitz, using a two-layer hand sewn anastomosis technique. The kidney is implanted into the left iliac fossa, with vascular anastomoses performed to the left iliac vein and iliac artery. Although the pancreas is often transplanted first at the authors center with no observed increase in the frequency of thrombosis, other centers have reported some advantage to reducing the risk of pancreatic thrombosis with placing the kidney first when more than one operative team is involved and more than one operative exposure is required [11]. Current analysis of the largest repository of pancreas transplant recipients demonstrated that the majority of current pancreas transplants performed in the US have changed from bladder drainage to primary enteric drainage and that the donor portal vein is more commonly anastomosed to the systemic venous drainage in the recipient [2]. Approaches that have reinvigorated the use of certain historical techniques such as the use of segmental donor allografts in living donor pancreas transplant [12] and portal-gastric (donor jejunum-recipient gastric) anastomosis [13] have continued to advance the field but are not routinely performed across US centers.

Long-term outcomes after pancreas transplantation

More than a decade ago, Ojo et al. demonstrated that pancreas transplantation in patients with T1D and resultant diabetic neuropathy increased patient survival in comparison to deceased donor kidney transplantation alone or dialysis [14]. Recent data from several comprehensive reports have described the most current long-term outcomes after pancreas transplantation (Table 107.1).

Registry data and large multicenter data

The International Pancreas and Islet Transplantation Registry (IPITR) founded in 1980 and housed at the University of Minnesota under the direction of Professor David E.R. Sutherland, maintains the most comprehensive database for all reported

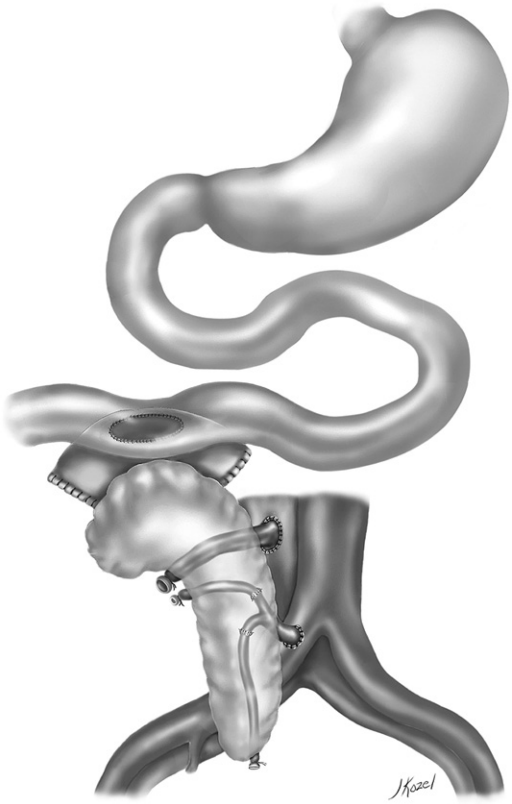

Figure 107.1. Enteric drainage technique for pancreas transplantation. Reprinted from [4] Sollinger HW, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. Ann Surg 2009, 250(4):618–630, with permission from Wolters Kluwer Health.

Table 107.1. Studies describing long-term outcomes after pancreas transplantation from large single-center analyses, regional collaboratives, and registry data

Source [Reference]	Most up to date analysis from the IPTR [2]	Analysis of 18 159 IPTR pancreas transplant [1]	European Registries [20,21]	Largest series of SPK patients worldwide [4]	Updated annually (www.srtr.org) OPTN-SRTR	Most comprehensive non-US study [15]	Analysis in 13 selected European countries [19]	Largest series from Latin America (Brazil) [16]	Survival outcomes from largest series in Europe (Portugal) [17]	Survival outcomes from largest series in Europe (Sweden) [18]
Registry/ collaborative/ single center	International Pancreas Transplant Registry (IPTR)	IPTR	Registry	Single Center (University of Wisconsin)	OPTN-SRTR	Registry/ Collaborative	Registry/ Collaborative	Colloborative	Single center	Single center

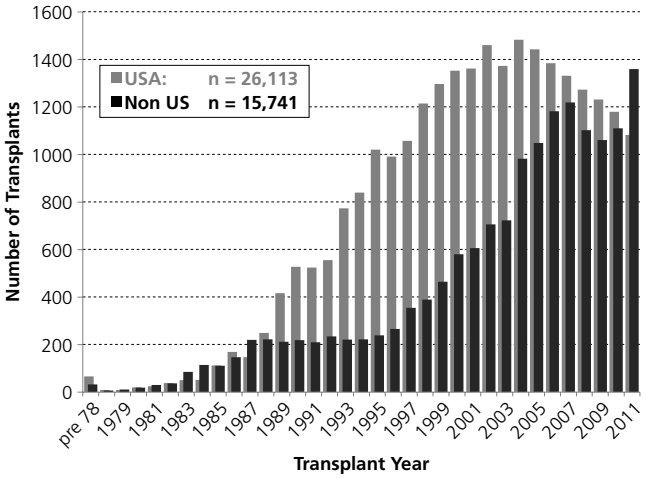

Figure 107.2. Annual number of US and rest of World pancreas transplantations reported to UNOS/IPTR, 1966–June 2013. Reproduced with permission of Professor Angelika Gruessner.

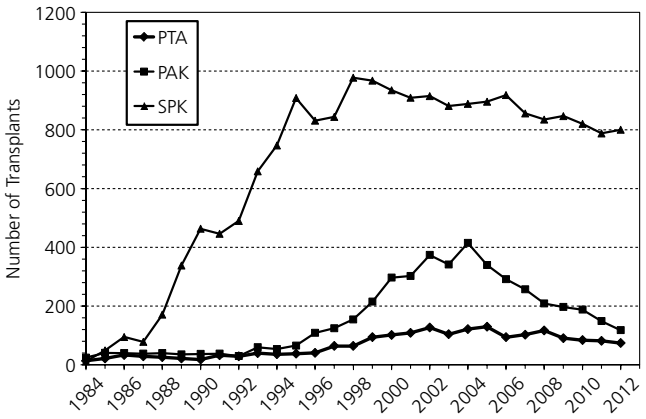

Figure 107.3. Annual number of US pancreas transplantations for the major recipient categories, 1988–2012. Reproduced with permission of Professor Angelika Gruessner.

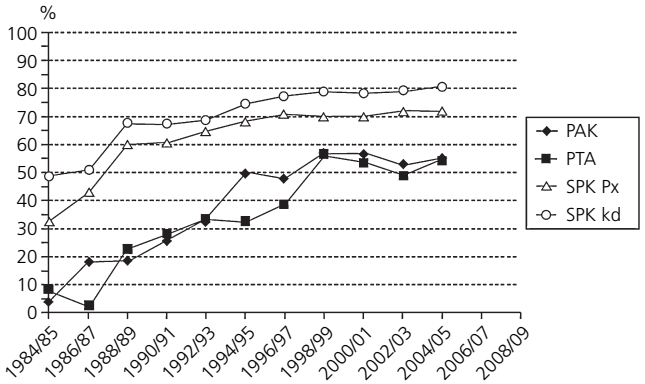

Figure 107.4. Rates of 5-year pancreas and pancreas/kidney graft function performed between January 1, 1985 and December 31, 2005. Reprinted from [1] Gruessner AC, Sutherland DE, Gruessner RW: Long-term outcome after pancreas transplantation. Curr Opin Organ Transplant 2012, 17(1):100–105, with permission from Wolters Kluwer Health.

pancreas transplants worldwide (www.iptr.umn.edu). Their recent analysis of 18 159 pancreas transplants performed across eras from July 25, 1978 to December 31, 2005 demonstrated improved short-term and long-term graft survival over the last three decades. In the US, the number of pancreas transplants being performed demonstrated a constant increase from 1966 to 2005, and a decrease in the overall number of cases since then, with the most significant reduction seen in the SPK cohort [1–3] (Figure 107.2 and Figure 107.3). Recent 5-, 10-, and 20-year graft function rates were 80%, 68% and 45%, respectively, for SPK; 62%, 46% and 16%, respectively, for pancreas after kidney (PAK); and 59%, 39%, and 12% , respectively, for pancreas transplants alone (PTA) [1–3] (Figure 107.4 and Figure 107.5). Five-year patient survivals after transplantation were 87%, 83% and 89% in SPK, PAK, and PAT patients, respectively [2]. As of December 2010, more than 37 000 pancreas transplantations have been reported to the IPTR (25 000 in the US; 12 000 outside the US) [2]. Analysis of trends over the last three decades demonstrated a change from bladder to enteric drainage, evolution of immunosuppression protocols from predominantly cyclosporine/azathioprine to tacrolimus/mycophenolate mofetil based with patients currently receiving primarily anti-T cell induction, a significant increase in the age of pancreas transplant recipients across all three pancreas categories, an increased focus on utilizing younger donors with shorter ischemic preservation times, and a significant decrease in rates of early technical and immunological graft loss [2].

Reporting of outcomes outside of the US to the IPTR is not mandatory and the IPTR registry data regarding the non-US experience is often incomplete. The non-US experience has been described largely in multi-institutional series and registries [2,15–18]. Perosa and colleagues published the most comprehensive report of non-US pancreas transplants. In this analysis of 10 108 reported transplants, the majority were performed in Europe (n = 6766), followed by Latin America (n = 1945), Canada (n = 671), Oceania (n = 499), Asia (n = 222), and Africa (n = 5) [15] (Figure107.6). This study also demonstrated that previous IPTR analyses have under-reported the number of non-US transplants performed [3] and that the number of non-US transplants performed reaches approximately 1100 annually, with over 10 000 pancreas transplants performed in total outside of the US [15]. Interestingly, in Perosa's study period a higher proportion of SPK transplants were performed in non-US countries (85% compared to 67%) [15,19]. In a comparative analysis of 13 selected European countries versus the US, differences in pancreas transplant rates were shown to be independent of deceased donor activity rates [19]. Some of these data described by Perosa et al. were derived from collaborative databases. These registries often do not have detailed information on allograft survival and patient outcomes. For example, Eurotransplant® is responsible for the allocation of donor organs in Austria, Belgium, Croatia, Germany, Luxembourg, the Netherlands, and Slovenia [20]. This international collaborative includes all transplant hospitals, tissue-typing laboratories and hospitals where organ donations take place. Available data from the Eurotransplant agency includes number of donors used for pancreas transplantation, type of pancreas transplant performed, and wait-list information, but long-term outcomes and the fate of the transplanted organs are not reported. Similarly, the Council of Europe which is based in Strasbourg, France, has diverse interests including maintaining human rights, social justice, cultural identity

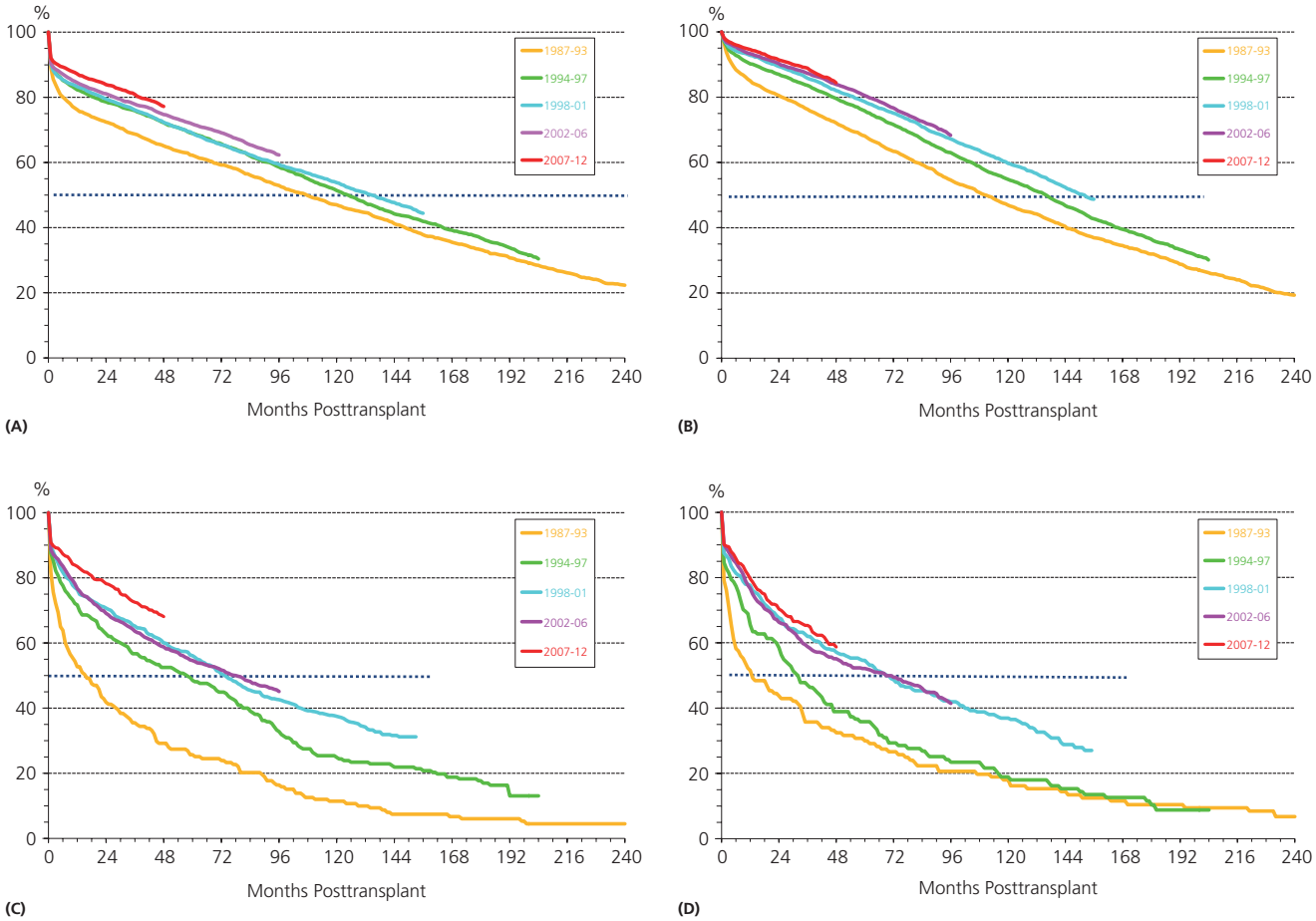

Figure 107.5. Primary deceased donor graft function over five eras for simultaneous pancreas kidney (SPK): pancreas graft (A); SPK kidney graft (B); pancreas after kidney (PAK) pancreas graft (C); and pancreas transplant alone (PTA) pancreas graft (D). Reproduced with permission of Professor Angelika Gruessner.

and also gathers and publishes an annual newsletter that contains data on the number of transplants performed in the European Union (EU). In 2010, 769 pancreas transplants were reported to be performed from 9206 deceased donors in the EU (n = 27 countries) [21]. In contrast, European single-center studies often analyze their own long term outcomes and have published 10-year survival results after SPK of 94%, 62%, and 69% for patient, kidney and pancreas allograft survival, respectively, that are not dissimilar from those noted by the IPTR in the US [17,18].

In Latin America, a recent study of 500 pancreas transplants represents the largest reported series from this continent. One-year patient survivals were 82%, 93%, and 95% for SPK, PAK, and PTA, respectively, while 1-year pancreas graft survivals were 70%, 86%, and 77% for SPK, PAK and PTA, respectively [16]. Interestingly, these data indicated a lower survival for SPK patients. As discussed by the authors, this trend reflected the survival of recipients in an impoverished socioeconomic region where the leadership and centers decided to expand the donor and recipient selection criteria in order to enable transplantation [16].

Simultaneous pancreas kidney (SPK) transplantation

In patients with T1D and resultant end-stage nephropathy, therapeutic options include dialysis, deceased donor kidney transplant (DDK), living donor kidney transplant (LDK), simultaneous pancreas-kidney transplant (SPK) or pancreas after living or deceased donor kidney transplant (PAK). Mortality is high for patients maintained on hemodialysis alone without kidney transplantation with a patient survival of 9.6–11.2% at 10 years (USRDS Data; Ten Year Survival Probabilities: Incident hemodialysis patients).

Cumulative studies with long-term follow-up have demonstrated that simultaneous pancreas-kidney transplantation in T1D patients with diabetic nephropathy increases patient survival compared with live-donor kidney or deceased donor kidney transplantation and remains the optimal therapeutic approach in these patients [2–4]. Based on IPTR data for 2010, SPK transplants accounted for 72% of pancreas transplants performed in the US [2]. Wait-time analysis performed for patients who actually received a transplant demonstrated that median time until a PTA patient received a pancreas transplant was 110 days, and 243 days for a SPK patient [2]. Enteric drainage was utilized in 91% of SPK patients. Era analysis showed that one year primary SPK pancreas graft function increased from 77.2% in 1987–1993, to 85.5% in 2006–2010. SPK transplants have been noted to have a 72% and 80% 5-year function rate for the pancreas and kidney, respectively and these rates of survival have not seen a significant improvement since 1996 [1]. Five-year patient survival after SPK has been noted to be ~87% [2,4].

At the University of Wisconsin, we have focused on transplantation with SPK since 1985 and we have recently reported our outcomes from 1000 SPK transplants with a 22-year follow-up [4]. In this series which represents the largest published SPK experience in the world, we demonstrated that the 5-, 10-, and 20-year patient survival for SPK recipients was 89%, 80%, and 58%, respectively [4], and these rates are similar to registry data at the 5- and 10-year mark [2]. A significant observation derived from our study is that patient survival after SPK was shown to exceed even that of diabetic uremic patients receiving LDK (Figure 107.7), an observation that contradicts some of the data in previous reports. In a study by Young et al. [22], 1-year patient survival was higher in the living kidney alone cohort compared to SPK (97% vs. 95%), however, at 6 years, patient survival was noted to be higher in the SPK cohort (85% vs. 80%). Other studies have also noted a survival advantage with SPK and this modality continues to be the most durable and efficient therapy for achieving normoglycemia [23–25]. The notion that the pancreas allograft can exert a protective effect on either the kidney allograft or the patient is not new and Kleinclaus et al. have previously shown that kidney function in PAK patients is superior to that seen in patients who have received LDK transplant alone [26]. Together, these data suggest that improved metabolic control potentially confers a survival advantage over correction of uremia alone. However, cumulative evidence from studies that describe a significant change in the progression of secondary complications such as diabetic retinopathy, cardiac deterioration, and vascular disease after SPK, are limited and insufficiently controlled [27–29]. As such, the causative factors underlying these significant observations remain undetermined.

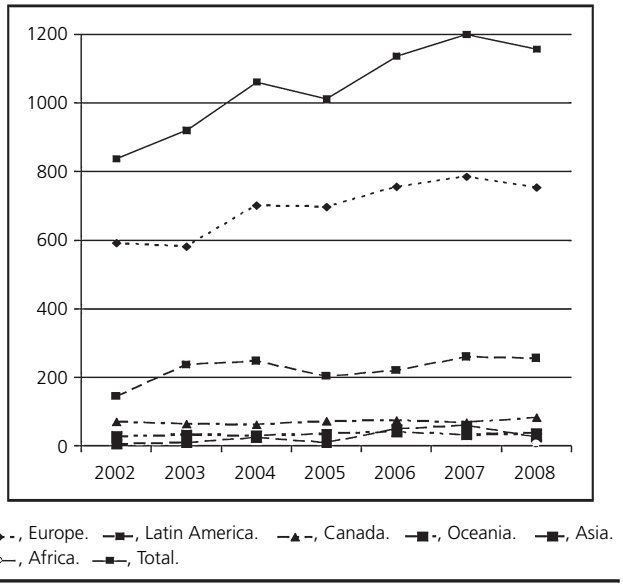

Figure 107.6. Patient survival in type 1 diabetic patients. Reprinted from [4] Sollinger HW, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. Ann Surg 2009, 250(4):618–630, with permission from Wolters Kluwer Health.

The survival advantage conferred by SPK transplant is dependent on a technically successful pancreas transplant. Preserved function at 1-year has been shown to be an important factor as early graft loss was associated with decreased patient and kidney graft survival [24,30]. Careful selection of recipients and matching with appropriate donor organs continues to be of paramount importance in SPK transplantation. Historical factors that have contributed to reduced patient and pancreas survival include donor obesity [31], recipient obesity (BMI $> 30\,kg/m^2$) [32], older donor age (>45 years) [33] and older recipient age (>50 years [34,35]. However, recent studies have suggested that acceptable outcomes are achieved if selected older recipients, obese donors, or obese recipients are judiciously considered [36–39].

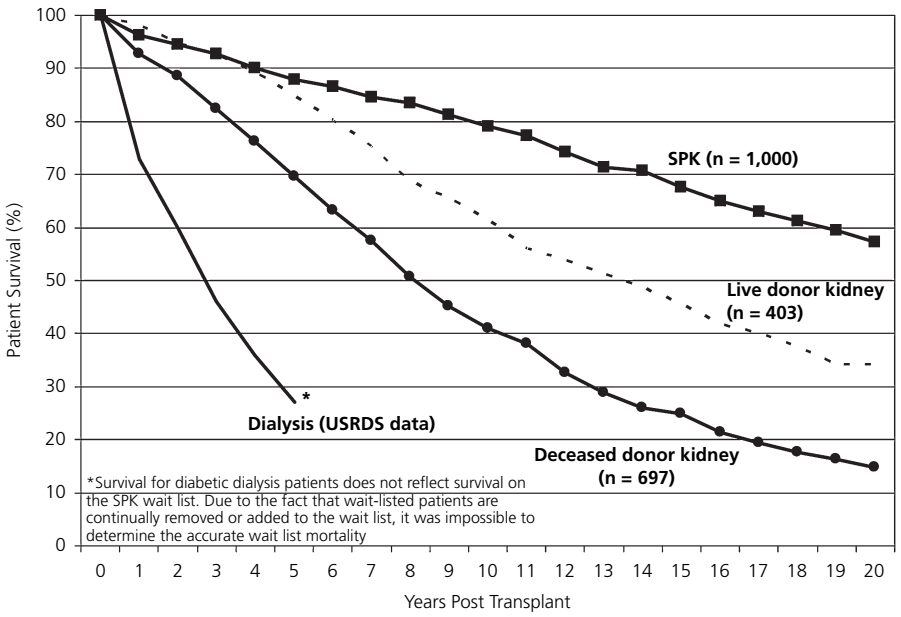

Figure 107.7. Number of pancreas transplants performed per year in non-US areas. Reprinted from [15] Perosa M, Boggi U, Cantarovich D, Robertson P: Pancreas transplantation outside the USA: an update. Curr Opin Organ Transplant 2011, with permission from Wolters Kluwer Health.

Improvements in graft and patient survival have led to increased numbers of patients presenting with failure of either the kidney or the pancreas allograft after SPK. In a recent analysis, pancreatic allograft survival was examined after subsequent kidney retransplantation in primary SPK patients [40]. Pancreas survival rates were 78%, 49%, and 40%, respectively at 1-, 5-, and 10-years. Although this represents a reasonable risk to the pancreas allograft (20–25% patients may experience pancreas allograft loss), the risks of reinitiating dialysis and the relative success of renal retransplantation, should allow medically appropriate patients to pursue retransplantation [40]. Repeat SPK after primary SPK has also been described [41]. The majority of patients underwent transplant nephrectomy (89%) or transplant pancreatectomy (78%) either prior to or at the time of re-transplant [41].

Pancreas after kidney transplantation

Diabetic end-stage renal disease (ESRD) patients that receive a kidney from a living donor have a unique opportunity to minimize or avoid exposure to dialysis. From a pragmatic perspective, these live donor transplants also decrease demand of organs from the deceased donor kidney pool [42]. Allocation rules and different geographical waiting times may limit SPK transplantation such that pancreas after living donor kidney transplantation becomes the primary approach for certain patients. These differences in pancreas and kidney allograft availability are important considerations for patients deciding between waiting for SPK versus proceeding with living donor kidney transplantation and subsequent pancreas transplant. Specific discussion on organ allocation policy can be found in Chapter 137. In addition, differences in long-term pancreas allograft survival between PAK and SPK recipients represent another significant consideration. While the gap is closing, pancreatic allograft survival in PAK recipients is still inferior to graft survival in SPK (80% vs. 86%) [2,25,34,43]. The differences in outcome could be partially attributable to a higher rate of rejection and increased rates of 3-year graft loss due to immunological causes in PAK (14%) compared to SPK (5%) [34].

The fate of the kidney allograft after subsequent pancreas transplantation continues to be a concern in the published literature and represents a challenging decision for kidney transplant recipients considering a subsequent pancreas transplant. Cumulative evidence suggests that cardiovascular risk stratification and careful assessment of kidney allograft function at the time of PAK are two important considerations in the appropriate selection of candidates for successful PAK transplant. Poor outcomes for the kidney allograft after PAK may be related to surgical risk of the operation, recipient co-morbidities, immunological events, and/or medication induced nephrotoxicity [44]. Rigorous analyses performed in a prospective, randomized and controlled manner comparing kidney allograft outcomes for patients undergoing PAK to kidney allograft outcomes in diabetic patients who have only undergone kidney transplant are unlikely, given the inherent selection bias; not all patients after kidney transplantation are eligible to undergo PAK. As a result, many existing studies do not provide a "fair" assessment of outcomes. However, Browne et al. recently approached this analytical dilemma by adjusting their analysis accordingly using a PAK propensity score and utilizing time-dependent and time-varying variables in multivariate models [42] rather than restricting the analysis to only those patients wait-listed for PAK as in prior studies [43,45,46]. Their results demonstrated a higher risk of kidney allograft failure in PAK recipients compared to kidney only transplant recipients during the first two weeks after PT, with the risk equalizing at 143 days, and after 366 days the risk reversed with PAK recipients demonstrating a significant improvement in long-term kidney survival [42]. These findings corroborate the increased risk of perioperative death after pancreas transplantation that has been previously reported [47] and emphasize the importance of appropriate patient selection for PAK. More importantly, Browne et al. demonstrated a 69% 10-year kidney graft survival and that long-term renal function could be preserved. They determined that PAK represents a safe and viable approach. The conclusions drawn from this report contrasted with a prior study of 126 PAK recipients that cautioned against subsequent pancreas transplantation in kidney transplant recipients with a pre PAK-glomerular filtration rate (GFR) <45 mL/min, an interval >1-year between kidney to pancreas transplant, kidney rejection episodes prior to PAK, and proteinuria [44]. In both studies, decreased pre PAK-GFR (<40 mL/min/1.73 m^2 or <45 mL/min/1.73 m^2) is associated with subsequent kidney allograft failure. Although in Browne's study this effect was not apparent until three years after PT. Adequate long-term follow-up is required for accurate analyses transplant outcomes and although the nature of the study was retrospective, our recent 20-year follow-up analysis is unique in the reported literature. Recent findings reported by Poommipanit et al. suggest that pancreas after living kidney transplantation (PALK) is associated with improved patient survival compared with SPK [43]. In these data, survival analysis was performed for up to five years post-transplant. The authors acknowledge that the major difference in survival was seen in the early post-transplant period with a 2.3% and 3.7% survival difference seen at 3 months and 12 months, respectively. Among their patients who had a functioning kidney graft at 12 months, they found no difference in survival between PALK and SPK in the subsequent four years. Patient survival was also similar in PALK and SPK when the pancreas transplant was considered the index date [43]. The differences in reported survival at 1-year were small (99.24% vs. 95.55%; PALK vs. SPK) and largely reflect the early advantage conferred by LDK. In our series, small variations were also noted when SPK was compared to LDK survival in T1D patients in the early post-transplant period [4]. However, the major differences in survival in our series were detected at 5-years and thereafter with >20% survival difference noted more than 10-years after transplant (Figure 107.7). The 20-year longitudinal follow-up in our series provides significant long-term analysis that is absent from studies with shorter follow-up. Post PAK rejection of the kidney or pancreas was associated with increased kidney and pancreas allograft loss and patient death [44]. Interestingly, the association between the interval of kidney and pancreas transplant and subsequent kidney allograft failure [44] was not observed in other studies [42,48]. Together, these data suggest that the mortality risk of PAK transplant and the risk for subsequent kidney allograft failure may be minimized in this most current era and support pancreas after living kidney transplantation as a safe alternate strategy in regions where SPK transplantation is infrequent or if the patient is already on dialysis (Table 107.2) [49]. If SPK is available as a primary mode of therapy and the patient is not yet on dialysis, the treatment of choice for an appropriate T1D diabetic recipient with nephropathy is SPK transplant.

PTA

The evidence that PTA provides a benefit to long-term patient survival and ameliorates secondary complications associated with diabetes in patients with T1D without pre-existing diabetic nephropathy remains debatable. Successful PTA in this patient

Table 107.2. Summary of advantages and disadvantages of transplant options for diabetic kidney disease

	Advantages	Disadvantages
Living donor kidney transplant alone (LDKA)	**1** Minimizes waiting time/time spent on dialysis **2** Low early morbidity and mortality	**1** No normalization of blood glucose **2** Inferior patient survival over time when compared with SPK recipients with functioning grafts
Simultaneous pancreas-kidney transplantation transplant (SPK)	**1** Normoglycemic regulation **2** High quality deceased donor kidney transplant	**1** Higher earlier morbidity and mortality compared to kidney transplant **2** Early failure of pancreas (<1 year), outcomes worse versus LDKA
Pancreas transplantation after living donor kidney transplant	**1** Normoglycemic regulation	**1** Two separate surgical procedures, increased mortality postoperatively following pancreas transplant **2** Potential risk to kidney transplant

(Adapted from [49] Wiseman AC: Pancreas transplant options for patients with type 1 diabetes mellitus and chronic kidney disease: simultaneous pancreas kidney or pancreas after kidney? Curr Opin Organ Transplant 2012, 17(1):80–86 with permission from Wolters Kluwer Health)

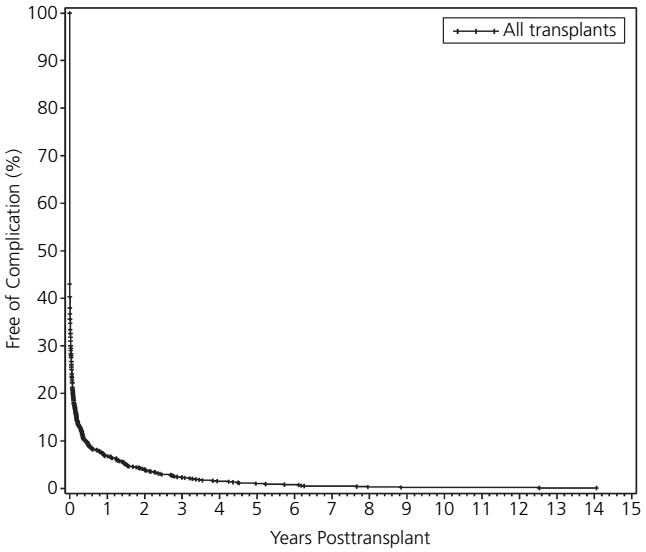

Figure 107.8. Overall complications following SPK transplantation from 1985–2007. Reprinted from [4] Sollinger HW, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. Ann Surg 2009, 250(4):618–630, with permission from Wolters Kluwer Health.

population largely represents an improvement in quality of life and has not been demonstrated to offer a survival advantage in large, controlled studies. Selection of PTA recipients is usually limited to patients with:

1 A history of severe metabolic complications (hypoglycemic unawareness, hyperglycemia, ketoacidosis) requiring medical attention.
2 Failure of insulin-based management [50].

From an immunological standpoint, PTA still represents the highest risk across the three pancreas categories with 1-year graft loss rates noted to be 6.0% in PTA compared to 3.7% in PAK, and 1.8% in SPK [2]. Recent studies report a 5-year patient survival of 90–98% after PTA [51], a 5-year graft survival of 73–89% [1,52], with a pancreas graft half-life of ~9 years [53]. There does not appear to be a clear survival advantage for PTA in comparison with patients on the waiting list [46,47,54], and the improvements in outcome for microvascular diabetic complications are not based on prospective, randomized studies [55]. Interestingly, Boggi and colleagues recently reported a significant decrease in hemoglobin A1C and lipid profiles in pre versus post-transplant PTA recipients [52]. However, adequate controls were missing with no comparisons made to untransplanted patients controlled with insulin therapy, nor with patients who were non-candidates for PTA. More importantly, the potential deleterious effects of calcineurin inhibitors (CIs) and other nephrotoxic drugs may offset the beneficial effects of glycemic control conferred by PTA. Various authors have demonstrated nephrotoxicity from long-term immunosuppression with 32% of patients undergoing PTA experiencing a ≥40 mL/min/1.73 m^2 decrease in estimated GFR and up to 9.9% patients required subsequent renal transplantation [56]. In a cohort of patients undergoing solitary pancreas transplant (both PTA and PAK), the rate of renal failure after transplantation requiring dialysis or kidney transplantation was 8% [45]. Data from protocol biopsies corroborated these adverse effects on the kidney allograft from cyclosporine and tacrolimus [57,58].

Complications after pancreas transplantation

Early technical failure after pancreas transplantation

The IPTR definition of technical failure includes graft thrombosis, pancreatitis, anastomotic leak, infection, and bleeding [3]. Technical failure accounts for approximately 50% of the causes of graft loss in the first six months after pancreas transplantation [59]. In a recent analysis of 6282 SPK recipients, early pancreas graft failure was associated with subsequent reduction in kidney allograft and patient survival [60]. Prevention of early graft failure involves utilization of standardized selection criteria and protocolized post-transplant chemoprophylaxis regimens [61]. Overall complications after SPKT are high (Figure 107.8) [4], but have been greatly reduced over the years at our center and others due to improvement in immunosuppression and refinements in our post-transplant management. Meticulous care to avoid early complications is critical to long-term success and avoidance of early reoperative surgery is directly associated with improved outcomes [62]. Patients with early technical failure are still candidates for pancreas retransplantation and the long-term outcomes for pancreas allograft survival, patient survival, and rate of acute rejection are not dissimilar to primary SPK [63].

Bleeding

The most common cause of post-transplant relaparotomy continues to be postoperative bleeding. In contrast to graft thrombosis, postoperative bleeding does not typically result in graft loss (<0.3% of pancreas grafts) [3]. After pancreas transplantation, bleeding can develop in the intra-abdominal compartment, in the gastrointestinal tract, or within the bladder in bladder-drained patients. Perioperative anticoagulation is associated with a significant proportion of cases of intra-abdominal bleeding [64]. The clinical management of a significant intra-abdominal hematoma involves cessation of anticoagulation, correction of any underlying or ongoing coagulopathy, and prompt re-exploration if hemodynamic instability is

encountered or if a washout is indicated to evacuate a potential source of secondary infection within the hematoma. Early gastrointestinal bleeding after pancreas transplantation often originates from the duodeno-jejunal anastomosis [64,65]. Late bleeding is often caused by gastritis, gastric or duodenal ulcers, colitis, or Dieulfoy's lesion, aorto-enteric fistula and mycotic aneurysms [64,66]. Late bleeding often requires intervention and represents a significant and morbid event. The development of an aorto-enteric fistula or a pseduoaneurysm can be a threat to graft viability and potentially patient survival. A high index of suspicion is required for prompt endovascular or operative management [66].

Thrombosis

Arterial and venous thrombosis can develop after pancreas transplantation but venous thrombosis is more common by 2-fold [67]. Thrombotic events can be classified as early or late, and partial versus complete. Total venous or arterial occlusion often results in allograft loss requiring pancreatectomy, although graft salvage has been reported with early thrombectomy [66]. Many centers utilize some form of anticoagulation following transplantation including aspirin, unfractionated or low-molecular weight heparin, with warfarin less frequently employed [66,67]. In our experience, the rate of bleeding requiring re-exploration has been minimized by avoiding systemic anticoagulation with heparin or dextran while also maintaining a very low rate (<1%) of pancreatic graft thrombosis [68]. This rate of thrombosis is lower than the rate reported by others (10–20%) [69–71]. The incidence of pancreas graft thrombosis based on data from the IPTR seems to be fairly consistent (4–8%) over the last several decades [1]. These registry data also indicate that SPK recipients have less pancreas graft thrombosis compared to PAK or PTA recipients [3]. This observation may partially be explained by the anticoagulant effects of uremia and the lower incidence of rejection seen in SPK recipients [67]. Other groups, including Troppmann et al. have previously identified risk factors for thrombosis and these include donor age, cardiocerebrovascular causes of donor death, the use of an aortic Carrel patch, arterial pancreatic graft reconstruction using a splenic artery to superior mesenteric artery anastomosis, interposition graft between the splenic artery and the superior mesenteric artery, portal vein extension graft, left-sided implantation into the recipient, and graft pancreatitis [69]. More recent data have also demonstrated that use of histidine-tryptophan-ketoglutarate (HTK) is associated with increased thrombosis rates, particularly when the cold time exceeded 12 hours [72]. Early after transplantation, the causes of vascular thrombosis are multifactorial, but beyond the two week time frame, thrombotic events are predominantly caused by inflammation and acute rejection [67]. An important caveat is whether portal venous thromboses were symptomatic or asymptomatic. Incidentally identified partial thromboses may be amenable to system anticoagulation [73]. Common factors associated with successful non-operative management included the absence of symptoms and the presence of collateral venous drainage [74]. In our experience, we also believe that not using a venous interposition graft to extend the portal vein is critical in reducing the risk of pancreas graft thrombosis and this practice is supported by others [69]. Graft thrombosis was a very infrequent cause of graft loss in our long-term experience. Less than 3.6% of recipients with pancreatic allograft loss were caused by thrombosis in our series [75]. This rate is low in comparison with other published reports (3–17%) [3,71,76]. Certainly, once thrombosis is identified, there is a high risk of graft loss. Although early exploration is associated with a higher negative laparotomy rate (43%), timely re-exploration may permit salvage thrombectomy in 45% of grafts [77].

Anastomotic leak

Anastomotic leaks represent less than 0.5% of all causes of graft loss [1,3]. The rate of anastomotic leak was higher in BD SPK (pancreaticocystotomy, 22.8%) compared to ED-SPK patients (pancreaticojejunostomy, 5.7%) [4]. Recipients with leaks from ED pancreas transplants present with drainage of enteric contents and potentially peritonitis while leaks from BD pancreas transplants often follow a more benign clinical course. Increase systemic amylase and lipase, reflecting peritoneal uptake of enteric drainage can be an early sign and should prompt suspicion. Given the effects of immunosuppressive medications, peritoneal signs can be remarkably unimpressive despite frank intraperitoneal soilage. Surgical repair was more favorable and more likely to succeed in ED SPK where the use of Roux-en-Y diversion was frequently employed. In BD SPK, repair was less successful and management with Foley drainage sometimes resulted in closure of leaks. Ongoing sepsis should prompt graft pancreatetomy.

Cardiovascular complications

Cardiovascular events represent the major cause of death in SPK patients (7.2%) [4]. These events, usually myocardial infarction, were documented either by autopsy, clinical presentation or laboratory diagnosis. As a result, stricter preoperative screening criteria were utilized in the recent era of our program at the University of Wisconsin. Stringent SPK patient selection criteria (see Chapter 32) may have contributed to the differences observed at our center compared to others. Cardiovascular mortality represents an important contributor to overall patient outcome. The frequency of pretransplant cardiac interventions in this series was low and possibly reflects a low overall cardiac risk profile of these patients. This in turn may contribute to the higher perceived survival in this group. Other causes of death in our series include infection (3.4%), stroke (1.8%), renal failure (1.5%), malignancy (1.3%), and bleeding (0.8%) [4]. A large number of deaths (n = 57) occurred where accurate diagnosis was not possible and/or autopsy was declined or inconclusive.

Enteric drainage, bladder drainage and enteric conversion

After 390 bladder-drained SPKs, our center (University of Wisconsin) began performing pancreas transplants using enteric drainage [6]. We analyzed kidney graft survival and pancreas graft survival between enteric-drained (ED) and bladder-drained (BD) SPKs. Interestingly, we found no statistical difference in survival for either the kidney or pancreas allograft between the two groups (Figure 107.9 and 107.10). This represented an unexpected result given the high frequency of rejection and infectious complications that occurred in BD patients and the subsequently improved immunosuppression with mycophenolate and tacrolimus in the ED patients.

Although there was no difference in allograft survival between ED SPK versus BD SPK, our experience shows that ED pancreas transplantation improves quality of life compared to BD. Complications related to BD include metabolic acidosis, high incidence of urinary-tract infections, hematuria, urethritis, urethral disruption and dysuria. In more than half of our surviving BD patients, enteric conversion was performed to correct serious urinary tract infections and intractable metabolic acidosis. Within 15 years after

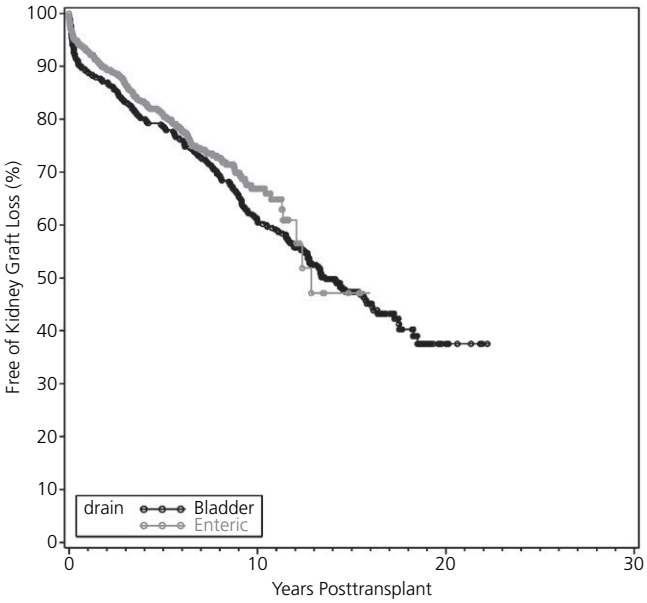

Figure 107.9. Kidney graft survival, bladder versus enteric drainage in kidney-pancreas patients 1985–2007. Reprinted from [4] Sollinger HW, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. Ann Surg 2009, 250(4):618–630, with permission from Wolters Kluwer Health.

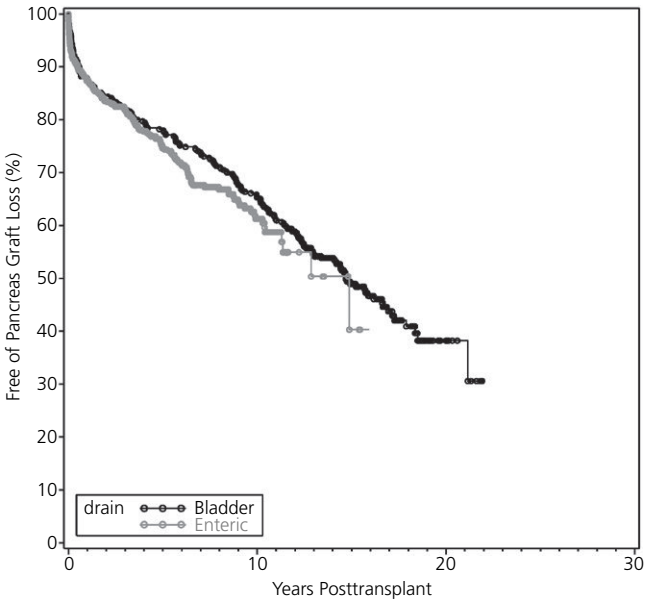

Figure 107.10. Pancreas graft survival, bladder versus enteric drainage in kidney-pancreas patients 1985–2007. Reprinted from [4] Sollinger HW, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. Ann Surg 2009, 250(4):618–630, with permission from Wolters Kluwer Health.

transplantation approximately 50% had undergone enteric-conversion [4]. Our experience revealed a higher complication rate from anastomotic leak when conversion was performed late after transplantation due to the increased friability of the duodenal segment. Although comparable pancreatic graft and patient survival rates at 1-, 5-, and 10-years were reported in a recent study between EC and primary ED SPK patients [78], it should also be noted that the authors observed a significant early complication rate after EC (surgical complications — 31.3; re-operation — 25%; and early graft loss — 6.3%). Enteric conversion should be offered judiciously in these patients and when performed, the use of Roux-en-Y loop is indicated in these late conversions. It is now our current practice to always perform ED at the time of primary transplant.

Proponents of BD argue that the ability to measure urine amylase levels as a method of detecting early rejection offers an advantage to ED. Results from our own center (University of Wisconsin) with the use of ED in solitary pancreas transplants (SP-ED) do not support this notion. In this study, acute rejection was confirmed by pancreas biopsy and the rates of rejection and allograft survival were compared between SP-ED recipients versus SPK recipients [79]. We hypothesized that if BD is critically important in the pancreas transplant setting then one would expect a lower graft salvage rate following acute rejection in SP-ED patients where monitoring of urinary amylase is not possible. In comparison with SPK, where kidney allograft function would enhance the detection of rejection, we demonstrated that the graft salvage rate following acute rejection was not different between solitary and combined pancreas transplants [79]. These data confirm that diagnosis and treatment of graft rejection based on allograft biopsies can be preserved in SPK without the need for BD. More complications requiring relaparotomy are seen in the early postoperative period in ED recipients compared to BD recipients. Complications in 24% of ED SPK transplants occur in the first 30 days after transplant compared to 15% in BD SPK. However, the majority of complications (76%) requiring reoperation in BD SPK occurs late (>1 year) with many related to the need for enteric conversion [4].

Recurrent T1D after transplant

Dr. Sutherland first described recurrence of T1D after pancreas transplantation with his series of HLA-identical siblings receiving pancreas allografts at the University of Minnesota [80–83]. Recurrent T1D was observed 4–8 weeks after transplantation in the absence of immunosuppression and was characterized by insulitis with mononuclear cell infiltration and selective β-cell destruction in the absence of pancreas rejection [83]. Based on these initial studies and other observations, the utility of immunosuppression was proposed to be 2-fold; suppression of rejection in non-HLA identical transplants and inhibition of autoimmunity in the pathophysiology of recurrent insulitis. However, as the field advanced, recurrent T1D was identified as a clinical entity even in patients with adequate immunosuppression to prevent rejection [84,85]. Cardinal features of recurrent disease include clinical symptoms of hyperglycemia without rejection, biopsy-proven insulitis with selective β-cell loss, persistence or reappearance of auto-antibodies (GAD, IA2), preserved exocrine function, and the presence of circulating autoreactive T cells [83]. These features are important to document, as post-transplant hyperglycemia can develop [86] and may or may not represent autoimmune T1D recurrence. A key finding is that autoantibody conversion, as opposed to persistence or negativity, is associated with a high risk of developing recurrent diabetes. The rate of recurrent disease was noted to equal

the incidence of chronic rejection in a series from the University of Miami, and accounted for up to 5% of patients. Post-transplant hyperglycemia from chronic allograft rejection may be challenging to differentiate from recurrent T1D. In these studies by Pugilese et al. their pancreas recipients were transplanted with the technique of bladder drainage and this was instrumental in their ability to document preserved exocrine function. Enteric drainage has become the predominant approach in the US [2], and measurement of urinary amylase is not possible in these patients. Improvements in diagnosis, prevention, and therapy are required to appropriately manage this clinical entity. An additional discussion of recurrent disease in pancreas transplantation can be found in Chapter 77.

Effect of pancreas transplantation on secondary complications of diabetes

Restoration of both endogenous insulin secretion and renal function has been demonstrated to have beneficial effects on the secondary complications associated with diabetes and evidence has shown that transplantation prolongs life expectancy in T1D. However, many studies addressing this topic are not prospective, adequately powered or controlled with regard to relative medical co-morbidities, stage of diabetic disease, and variability in controls where comparative groups are often derived from non-transplanted patients or patients with failed transplants. Additionally, measured parameters are often utilized as surrogate markers for the complex secondary complications of diabetes and in certain cases it remains debatable if these measures are correlative or clinically relevant.

Nephropathy

The pathophysiological lesions of diabetic nephropathy are characterized by thickening of the basement membrane, mesangial matrix accumulation, hyalinosis of glomerular arteries, and glomerular sclerosis [55,87]. Studies performed by Bohman et al. demonstrated that the development of diabetic glomerulonephropathy was prevented in patients undergoing successful pancreas transplantation [88,89]. Markers of diabetic glomerulopathy including glomerular basement membrane width, total mesangial and total mesangial matrix volumes were unchanged at 5-years but became markedly decreased at 10-years suggesting that diabetic glomerular and tubular lesions in humans are reversible. More recent studies have shown that these lesions of diabetic nephropathy could be reversed in the native kidneys of non-uremic recipients of pancreas transplants alone [58,90,91]. However, the lessons learned in the PAK patient population remain relevant as nephrotoxicity from long-term immunosuppression complicates the balance. Additionally, although long-term morphological changes related to diabeteic nephropathy have been shown to improve or stabilize, it remains debatable as to whether a clinically significant difference in renal function as measured by GFR reduction has been shown in a carefully designed, controlled analysis. An additional discussion of recurrent diabetic nephropathy can be found in Chapter 77.

Retinopathy

Most of the studies showed little impact on progression of retinopathy. Ramsay et al. demonstrated that retinal events occurred in up to 30% of patients during the first three years following successful pancreas transplant and remained stable thereafter [92]. However patients with failed grafts continued to have retinal events. These data have been substantiated in recent studies [27,93]. Giannarelli et al. analyzed 33 patients that underwent pancreas transplant and compared them to 36 T1D patients who were not transplanted and at 30-months follow-up, amelioration or stabilization of retinopathy occurred in 91% of transplanted patients but only in 43% of non-transplanted patients [93]. These results need to be considered with the parallel observations that retinopathy can deteriorate immediately post- transplant in 10–35% of patients with unstable eye disease [55] and that steroid and calcineurin use may exacerbation cataracts and glaucoma [94].

Neuropathy

Several studies have demonstrated an improvement in peripheral nephropathy in pancreas transplant recipients. Solders et al. showed that neuropathy improved in patients receiving a pancreas transplant whereas progression occurred in those with no transplant or with a failed graft. Kennedy et al. evaluated diabetic autonomic and sensory motor neuropathy progression in pancreas-transplanted patients by comparing them with the patients undergoing intensive insulin treatment [95]. The two groups were compared at 12-, 24- and 42-months after transplantation and progression was noted in both sensory motor and autonomic neuropathy in insulin-treated patients, whereas an improvement was demonstrated in transplanted recipients. Recent studies by Martinenghi et al. confirmed these findings by showing that increases in nerve conduction velocity occurred in combined pancreas-kidney transplanted patients with a functioning graft compared with those with failed pancreas transplant [96]. The drawback to these studies involves the difficulty with measuring neuropathic progression. Symptoms are often subjective and may be challenging to quantify while improvement in conduction velocity may or may not be clinically relevant.

Peripheral vascular disease

Overall, diabetics have a 15 times higher risk of lower extremity amputation than non-diabetic individuals [97]. Returning patients to normoglycemia would in theory reduce the risk of peripheral vascular disease. However, there continues to be a limited number of well-designed, sufficiently powered and adequately controlled analyses on the outcomes of peripheral vascular disease after pancreas transplantation [98–100]. Morrissey et al. demonstrated that peripheral vascular complication rate (defined as amputation, ischemic ulcer requiring treatment, and lower extremity bypass surgery or angioplasty) appeared to be accelerated early after transplant in SPK recipients compared to diabetic patients that underwent kidney transplantation alone (KTA) [101]. Although, peripheral vascular complications before transplantation were comparable between groups ($P = .94$), after transplantation, there were 35 new vascular complications in 18/39 SPK recipients versus 32 events in 20/65 KTA recipients ($P = .005$). These data were limited by patient numbers and also by the controls as patients who underwent KTA were not adequately controlled for their candidacy for pancreas transplant, relative medical co-morbidities, renal function, and degree of hyperglycemic regulation. The more recent study by Biesenbach et al. noted a reduction in peripheral vascular complication rate in SPK compared with KTA patients [99]. However, only 12 SPK patients and 10 diabetic subjects with KTA were included in the study and the difference in outcomes observed for myocardial infarction (16% vs. 50%), stroke (16% vs. 40%) and amputations (16% vs. 30%) must be considered with caution.

In the analysis released by the Centers of Disease Control, the age-adjusted rate of leg and foot amputations fell from 11.2 per 1000 individuals with diabetes in 1996 to to 3.9 per 1000 in 2008 [102]. The incidence of T1D patients requiring amputation after

pancreas transplantation have been reported to be ~8–9.5% [100,103,104]. In comparison, amputation rates for type II DM patients have been reported to be 6.1–19% [105–107]. In patients with T1D, the cumulative probability of having a non-traumatic lower extremity amputation was 11.0% for women and 20.7% for men [108]. Many studies have utilized amputation rates as a surrogate for peripheral vascular disease or as a primary measure of outcome. However amputations are the net result of multiple factors including neuropathy and repetitive injury to the foot, followed by impaired wound healing, impaired immune response to bacterial infection, and progressive intimal vasculopathy. Amputation rate alone cannot be an adequate measure of peripheral vascular disease in this population. In patients with T1D receiving pancreas transplants, the protective effects associated with improvement in metabolic regulation and correction to normoglycemia should be weighed against the physiological progression of age-related vasculopathy, pre-existing disease and the adverse effects of immunosuppressive medications on worsening arteriopathy and further studies are required to assess the net effect on peripheral vascular disease.

Expanding the donor pool

Donation after cardiac death and pancreas transplantation

At the University of Wisconsin, we have routinely accepted select donor after cardiac death (DCD) donors for SPK and solitary pancreas transplantation [109]. We apply our customary criteria for assessing the appropriateness of donation for pancreas transplantation and use an index warm ischemia time of 30–45 minutes. In our series, 8.6% of SPKs were recovered from DCD donors [4].

Patient survival rates at 10-years for SPKT from DBD and DCD donors are similar (80% vs. 84%, respectively; $P = 0.97$) [4]. Pancreas allograft survival at 5- and 10-years is also similar between DBD versus DCD donors (5-yrs: 76% vs. 75%; 10-yrs 64% vs. 60%; $P = 0.57$). Kidney allograft function using GFR and creatinine as markers were similar in DBD versusDCD SPK recipients ($P = 0.89$ and $P = 0.78$, respectively) even though DCD kidneys exhibited a higher rate of delayed graft function. These data are consistent with other reports in the literature that have compared extended donors versus conventional donors in pancreas transplantation [110].

Living donor pancreas transplantation

Living donor pancreas transplantation is offered in very few centers. The incentive for using segmental transplantation of the pancreas allograft from suitable living donors derives from the advantage in shortening the time to transplant for recipients waiting for deceased donor transplantation and for allowing patients who are allosensitized to HLA antigens access to transplant. In a recent report, the University of Minnesota published their cumulative experience in which Sutherland et al. describe a significant decline in their technical failure rate as the learning curve was negotiated over the last few decades, with equivalent graft survival rates between living and deceased donor pancreas recipients, and the prediction that this technique will expand as more pancreas transplant recipients are added to the waiting list [12]. Currently, the technical expertise required restricts candidates to a few, select centers and the attendant risks related to surgical complications and postdonation diabetes limits the pool of potential surgical candidates.

Influence of donor- and recipient-specific factors on outcomes

Much of the challenge in modern transplantation lies in matching the donor with the appropriate recipient. The idea of a donor-risk index has been established in the liver transplantation literature by Feng et al. In the arena of pancreas transplantation, emerging reports have sought to characterize donor-specific and recipient-specific factors that may guide allocation and influence outcome [111]. Axelrod et al. have recently published a pancreas donor risk index (PDRI) that predicts pancreas transplant graft outcome at the time of organ offer [112]. Increased PDRI was associated with a significant reduction in 1-year pancreas graft survival. The appropriate application of such a risk index would vary by region and center and the authors themselves conclude that this continues to be a work in progress. While most pancreas transplant centers prefer to procure their own donor allografts, the use of imported pancreatic allografts from beyond the region should be considered a safe and viable way to enable transplantation [113]. Increased utility of these organs will require close monitoring of cold-ischemic times and attention to the appropriate donor-recipient risk factors. In separate studies, other risk factors identified by Luan et al. include recipient race as they recently demonstrated that African American SPK recipients had 38% and 47% higher risk for late death-censored kidney and pancreas graft failure, respectively, and African Americans were twice as likely to lose their allografts from rejection [114].

Role of immunosuppressive medications

Initially, routine maintenance immunosuppression for pancreas transplantation at our center consisted of cyclosporine A in combination with azathioprine, and prednisone. In 1995, azathioprine was replaced by mycophenolate mofetil and tacrolimus was introduced. Our current maintenance regimen includes tacrolimus, myfortic, and low-dose prednisone. Induction therapy evolved from antithymocyte globulin, muromonab, daclizumab, Campath-1H, to basiliximab. General discussions on induction and maintenance immunosuppressive therapy can be found in Chapters 65 and 66, respectively.

The introduction of MMF represented a paradigm shift for us (University of Wisconsin) as our incidence of acute rejection reduced dramatically for both the kidney (34% vs. 75%, $P = 0.001$) and the pancreas (7% vs. 24%, $P = 0.003$) allograft, and also decreased the incidence of steroid-refractory rejection episodes requiring antilymphocyte rescue therapy (15% vs. 52%, $P = 0.01$) [6]. Others have published a similar experience with antithymocyte globulin induction even conferring a negative effect (vs. daclizumab), while fewer complications were observed with tacrolimus combined with MMF in direct comparison with tacrolimus combined with rapamycin or cyclosporine compared combined with MMF [111].

Our recent experience using Campath-1H (alemtuzumab) demonstrated that there was no survival advantage in comparison to induction with IL-2 receptor antibodies [115]. Moreover, there was a statistically higher incidence of cytomegalovirus infection and viremia in the alemtuzumab treated group. The consensus within our group is that Campath-1H does not offer a substantial benefit and we currently use basiliximab induction for patients receiving their primary transplant.

Summary

Selecting the appropriate therapy for a recipient with T1D with renal failure continues to be a critical decision for programs offering pancreas transplantation. A variety of therapeutic options including dialysis, DDK, LDK, SPK, or PAK exist. The principles and guidelines at our center (University of Wisconsin) are driven by the benefit of the SPK transplant needing to outweigh the increased morbidity of the surgical procedure and the use of lifelong-immunosuppression. Cumulative evidence from the literature suggests that correction of the metabolic derangements associated with hyperglycemia through combined pancreas-kidney transplantation may provide a survival advantage over kidney transplantation alone. Appropriate selection should be based on the morbidity inherent in each individual organ recipient and the variable waiting times particular to each region. A summary of the findings and our recommendations for clinical practice are as follows:

- Current 5-year pancreas graft function rates are 80%, 62%, and 59% in SPK, PAK, and PTA, respectively. Five-year patient survivals after transplantation are 87%, 83% and 89% in SPK, PAK, and PAT patients, respectively. SPK should be offered as the primary option in T1D patients with diabetic nephropathy who are not yet on dialysis as it represents the treatment option that provides a survival advantage to the allograft and patient. Living donor kidney transplant may be an option for T1D patients that have already initiated dialysis and if their region has long wait times for SPK. Meticulous surgical technique, especially with regard to back-table preparation of the allograft, is linked to long-term success and graft survival. Appropriate selection of patients prior to pancreas transplantation is required to avoid interruption of renal allograft function in PAK candidates, with careful consideration of two important pre-operative parameters: cardiovascular status and renal allograft function.
- Early technical failure after pancreas transplant results in decreased renal allograft and patient survival.
- Graft thrombosis and cardiovascular events represent the most morbid complications after pancreas transplant. While routine use of systemic anticoagulants varies amongst centers and no regimen has been demonstrated to be superior, attention to surgical technique and donor graft quality continue to be important factors in the development of graft thrombosis.
- Enteric drainage is the recommended approach for managing exocrine secretions in pancreas transplantation and is currently the most widely practiced option. Enteric conversion from bladder drainage should be considered in patients with overt symptoms, metabolic derangements, and alterations in quality of life. Careful selection and decision-making is required in patients who present distant to their primary operation.
- Some improvement in secondary complications of diabetes is seen with pancreas transplantation but the data for these studies are often lacking in prospective design, power, and adequate controls.
- Use of donor after cardiac death organs or imported allografts should not deter transplantation if careful and judicious selection of donor-recipient matches are made

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CHAPTER 108

Long-term Outcomes after Islet Transplantation

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Introduction

Remarkable progress has occurred in the past 12 years in outcomes and activity of clinical islet transplantation [1–6]. Once just a curiosity, this established, minimally invasive treatment provides reliable, robust glycemic control for selected patients with unstable type 1 diabetes mellitus (T1DM) that cannot otherwise be stabilized by alternative means. Currently over 1000 patients have undergone islet transplantation in approximately 40 international sites (752 islet allografts and 333 autografts were registered with the Collaborative Islet Transplant Registry (CITR) at the time of this writing) [5,7]. Interestingly, in contrast to solid organ transplantation, access to deceased donor pancreas organs has not been the only impediment limiting further expansion of activity. Restricted availability of clinical islet isolation facilities with the required clinical good manufacturing practice (cGMP) ultra-clean room environment, skills and expertise involved in islet manufacture, general inefficiencies in procuring islets from available organs, combined with marked restrictions in clinical research funding also are rate limiting factors. In the US, plans are underway to secure a Biological Licensure Application (BLA) with the Food and Drug Administration (FDA), and when this occurs, Medicaid, Medicare and other third party reimbursement will likely have major impact on further expansion.

In the longer term, a limited islet supply and need for intensive immunosuppression are perceived as the two drawbacks that restrict broader application in T1DM [3,7]. The risks of poorly controlled diabetes and immunosuppression are well defined. Many in the diabetes community have a hyperinflated perception of the transplant and immunosuppressive risks, while those caring for islet transplant patients on a daily basis may conversely perceive heightened risks from ongoing complications of diabetes. As islet transplantation becomes more routine, and immunosuppressive management more streamlined, it is likely that the risks of transplantation and life-long immunosuppression will balance favorably with those of even the most stable insulin-controlled diabetes. This would broaden the procedural indications and would open up the therapy to include application in the pediatric population [8]; the earlier successful islet transplantation is applied in the course of diabetes, the greater the potential protective impact in preventing secondary complications. Conversely, the longer the lifetime duration of immunosuppression, the greater will be the accrued risks of malignancy, infection and renal injury [9–12]. Only carefully controlled trials that randomize patients with T1DM to insulin or transplantation and provide lifelong follow-up on an intent-to-treat basis in both arms will define when such a balance has been met.

In the meantime, acknowledgement of a lack of immediate proximity of transplantation tolerance, stem cell-derived and xenogeneic islets for routine clinical delivery has led several funding organizations to explore alternative ways of stabilizing glycemic control, including high technology insulin pumps with closed-loop sensors to serve as bridge or destination therapies, perhaps with avoidance of transplantation altogether [13]. It is our premise and bias that sustained normoglycemia cannot be achieved by exogenous subcutaneous insulin depot delivery, and that even the best pumps and sensor approaches will fall short of the precise moment-to-moment control provided by islets within the native pancreas, or within an adequate mass of transplanted islets. This chapter discusses clinical outcomes currently achieved in clinical islet transplantation, and takes on the challenges of the next steps required to further transition this therapy in preparation for matched randomized controlled trials.

Risks of diabetes and alternative intervention strategies

T1DM is an emotive and pervasive disease that affects both children and adults, cannot be prevented presently, and is increasing in incidence; furthermore, insulin therapy, while life-saving, is not curative. The incidence varies from 17–40 per 100 000 population, with the highest incidence observed in Finland and Sardinia, and this risk is accelerating rapidly [14]. There are an estimated 490 100 children under the age of 15 with T1DM [15]. While mortality rates vary considerably between first and third world countries, even in wealthy countries the mortality rates for both children and adults with T1DM are at least twice that of people of similar age without the disease [16,17]. In the US, diabetes is the seventh leading cause of death, is the leading cause of renal failure, non-traumatic limb amputations, and new cases of blindness amongst adults, and consumes more than $174 billion in estimated healthcare costs [17,18]. While the Diabetes Control and Complications Trial (DCCT) clearly demonstrated that improved glycemic control from intensive insulin substantially lowered risk of secondary complications with lower glycosylated hemoglobin (HbA1C), the risks of hypoglycemia increased markedly [19–21].

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Hypoglycemia has been long-recognized as a complication of excess insulin therapy, and indeed the early definition of one "Unit" of insulin was that amount required to induce fatal hypoglycemia in a rabbit [22]. The subcutaneous site for glycemic monitoring and depot delivery of insulin represents an imperfect approach, with response lag inevitably failing to provide complete physiologic glycemic control. Efforts to correct HbA1C and further reduce risk of secondary complications can exacerbate risk of troublesome hypoglycemia, especially in a subset of more susceptible individuals [21,23,24]. Car drivers both in the US and in Europe with T1DM experienced twice as many collisions as their non-diabetic spouses, in part related to neuroglycopenia, retinopathy and peripheral neuropathy [25–27].

The "dead in bed" syndrome remains one of the most feared complications of unrecognized hypoglycemia from insulin therapy in T1DM, where patients are typically found dead in the early morning, and accounts for 4.7– 7.3% of unexplained deaths overall, and for 6% of deaths overall in T1DM patients younger than 40 [27–30]. Figure 108.1 demonstrates the glucose parameters of two patients wearing continuous subcutaneous glucose monitoring

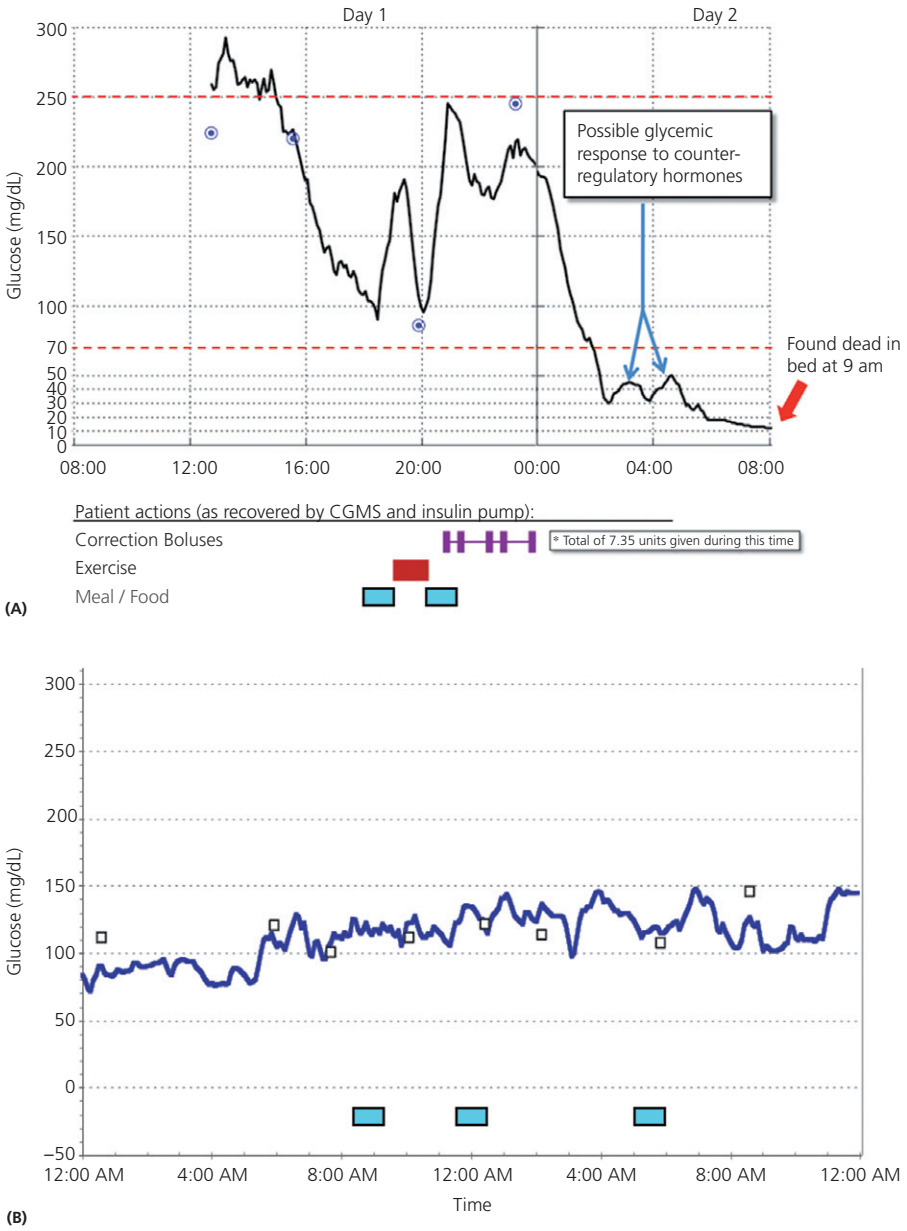

Figure 108.1. Continuous subcutaneous glucose monitoring systems (CGMS) records from: (A) A 23-year old man with T1DM using an insulin pump, complicated by severe hypoglycemia, was found convulsing by his mother, taken to the Emergency department, and treated with orange juice. The following morning he was discovered by his family at 9am unresponsive and pulseless. CGMS records and autopsy confirmed death by hypoglycemia (dead in bed syndrome). (B) A 44-year old woman with previous T1DM treated with two islet transplants at the University of Alberta, with sustained normoglycemia as demonstrated by CGMS, and with HbA1C of 5.5%, and not requiring insulin for 3 years.
Figure 1A modified from the original published in reference [31], and generously provided by Professor R.J. Tanenberg with kind permission. Reprinted from Endocrine Practice, Vol 16, Confirmation of hypoglycemia in the "dead-in-bed" syndrome, as captured by a retrospective continuous glucose monitoring system, p.246, Copyright (2010), with permission from the American Association of Clinical Endocrinologists.

systems (CGMS), where in (A), a 23 year old male died from fatal hypoglycaemia, dead in bed syndrome despite use of an insulin pump (HbA1c 6.4%), as recorded by CGMS [31], and (B), a 44 year old female with previous severe hypoglycemia and lability achieved sustained normoglycemia without exogenous insulin with HbA1C of 5.5% after islet transplantation.

Refined technological advancements in improved insulin delivery algorithms and glucose-sensing technologies offer potential to prevent hypoglycemia while correcting HbA1C, thus broadening the therapeutic window of tolerability for insulin [32,33]. However, the hysteresis lag in current sensor monitoring, delivery and absorption of infused insulin in the subcutaneous site will remain persistent limitations of this technology. The low glucose suspend (LGS) automated insulin shut-off on insulin pumps linked to closed loop sensors are one key advance that may mitigate risk of severe hypoglycemia [34–37]. Ongoing limitations of this technology include suboptimal accuracy, reliability and sustainability beyond a few days with continuous glucose monitors and time-lag delays associated with subcutaneous insulin delivery. The use of insulin alone, without additional counter-regulatory glucagon, is also seen as an additional shortfall in this approach.

Advances in both short and long-acting insulin formulations have led to improvements in glycemic control. The initial switch from beef and porcine to recombinant synthetic human insulin led to a concerning increase in hypoglycemia and unawareness, with reduced counter-regulatory responses [38–40]. The more recent introduction of a long-acting basal micro-crystalline insulin analogue, glargine (Lantus, Sanofi-Aventis Inc., Paris France) with a more prolonged 18–26 hour duration profile, has generally been associated with lower risk of severe hypoglycemia when compared with neutral protamine Hagedorn (NPH) insulin, but still may not resolve severe recurrent hypoglycemia in susceptible patients [41–44]. Development of inhaled insulin was initially promoted as a promising and innovative approach for more rapid vascular absorption, and seemed attractive as it avoids a need for repeated injection. In practice, controlled trials failed to demonstrate superiority of the approach, and Pfizer withdrew manufacture of Exuberta in 2007, and other companies followed suit, with the exception of MannKind Inc., which is still undergoing FDA review. Concerns relating to a slightly increased risk of lung cancer, lack of superiority, and considerable and unjustified increased expense, will certainly hamper ongoing developments with this approach [45,46].

Efforts aimed at prevention and early intervention in T1DM have met with disappointment, despite enormous promise generated by data generated in mouse models of autoimmune diabetes. Over 463 interventional treatments have been shown to prevent or reverse autoimmune diabetes to varying degrees in non-obese diabetic (NOD) mouse models [47]. Efforts focused on translation of several of the most promising approaches from mice to T1DM have met with frustration, despite enormous investment and a series of large-scale recent clinical trials [48]. This has brought in to question the utility of this mouse model as a surrogate of the human disease [49]. Encouraging preliminary clinical pilot data suggested that a non-Fc binding CD3 antibody [50–53], mycophenolate mofetil (MMF) + daclizumab [54], rituximab B-lymphocyte depletion [55,56], a soluble NBI-6024 altered insulin peptide ligand [57], vitamin D3 [58], nicotinamide [59], parenteral insulin, oral insulin, nasal insulin, and the elimination of cow's milk from infant feeding could potentially mitigate diabetes onset, or at the very least prolong endogenous C-peptide and sustain the honeymoon period [48,59–61]. To date, none of these approaches have proven robust benefit when subjected to adequately powered randomized clinical trials [61]. Perhaps the most promising approach to date has been the use of intravenous non-myeloablative autologous hematopoietic stem cell transplantation after mobilization with granulocyte colony-stimulating factor, and conditioning with thymoglobulin and cyclophosphamide, as first described by Voltarelli et al. in Brazil [62–67]. This approach of "immunological reset" was designed to eliminate both autoreactive and non-autoreactive lymphocyte clones, with subsequent immune reconstitution. Updated reports indicated that 20 new onset T1DM patients achieved insulin independence, and 12 maintained this state for a mean of 31 months, a remarkable achievement without mortality, but with nosocomial pneumonia in two cases, and oligospermia in nine cases [63,68,69]. The mechanism underpinning this approach appears to be restoration of apoptosis-related gene deregulation that may have contributed to breakdown of immune tolerance and T1DM [70]. Ongoing trials are much needed to further define the risk-benefit and sustainability of this interesting approach.

Thus, standard or intensive insulin therapy currently remains the gold standard therapy for the majority of patients with T1DM, with a clear understanding that tighter glycemic control lessens the risk of secondary diabetic complications, but substantially increases the risk of troublesome and occasionally life-threatening hypoglycemia. Insulin pumps, CGMS and improvements in insulin crystalline structural design represent considerable improvements for many T1DM patients. For a substantial minority however, a life with frequent hypoglycemia or inexorable threat of progressive secondary complications represents an intolerable burden. For these individuals, islet transplantation represents an increasingly attractive option. Whole pancreas transplantation offers the potential to restore euglycemia, and can generally resist the metabolic strain with far greater reserve than an islet transplant. Both require similar levels of immunosuppression, but islet transplantation represents a more elegant solution (Figure 108.2). Replacement of the endocrine without the exocrine component in islet transplantation is readily accomplished through a non-surgical and exceedingly low risk approach. In contrast, the risks and outcomes of whole pancreas

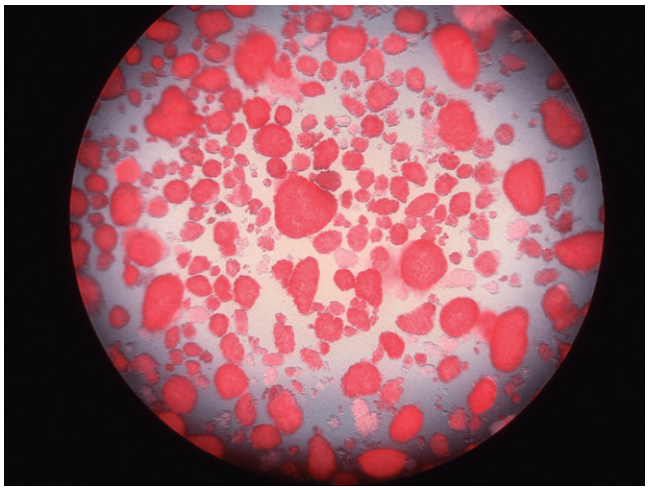

Figure 108.2. Purified human islets in the final preparation, stained with dithizone for quantification. Islets vary in size between 50–500 μm, and the final islet mass is estimated as Islet Equivalents (IEQ), with a preparation of >5000 IEQ/kg recipient weight generally being sufficient for transplantation.

Table 108.1. Indications for islet transplantation

A. Islet transplant alone (ITA)
Type 1 diabetes, duration >5 years
Age >18 years, weight <90 kg, insulin requirement <1.0 U/kg/day
Absence of malignancy or untreated infection
Ability to comply with immunosuppression and close follow-up
Refractory hypoglycemia or lability despite:
1 Optimal intensive insulin or insulin pump with appropriate monitoring
2 Supervision of a diabetologists of endocrinologist
3 Evidence of increased hypo risk:
I. Clark Score ≥4
II. HYPO Score ≥1000
III. Lability Index (LI) ≥400
IV. Combined HYPO ≥400 and LI ≥300
B. Islet after kidney (IAK)
Type 1 diabetes, successful prior renal allograft
Tolerating maintenance immunosuppression
Prednisone ≤5 mg/day
Absence of BK virus, or other active opportunistic infection
Non-sensitized (PRA < 20%)

For Clarke Score, see ref [80].
For HYPO Score and Lability Index, see ref [81].

transplantation have improved substantially over the past quarter century, and more than 30 000 have been performed internationally [71–74]. From a patient's perspective, avoiding potential risks of laparotomy, pancreatitis, graft thrombosis, surgical site infection and occasional mortality in pancreas transplantation make islet transplantation the more attractive option, provided that similar metabolic reserve is sustained over time [75–78].

Patient selection and procedural indications for islet transplantation

Patients are selected for clinical islet transplantation based on indications summarized in Table 108.1. Additional discussion of patient selection for and management of patients awaiting islet or pancreas transplantation can be found in Chapters 33 and 41.

Islet transplant alone

The indications for islet transplant alone (ITA) require evidence of T1DM (C-peptide negative), with sufficient duration (>5 years) to justify that all reasonable alternative attempts have been made by an independent diabetologist or endocrinologist to correct and stabilize refractory glycemic control, including optimized intensive insulin or insulin pump therapy combined with frequent glycemic monitoring [79]. A series of objective scores are applied that involve a detailed patient questionnaire (the Clark Score) [80], and stringent review of detailed glycemic records allows for calculation of the hypoglycemia score (HYPO) and lability index (LI), as originally developed by Ryan et al. [81]. The threshold values summarized in Table 108.1 represent currently accepted indications for islet transplantation used in the major centers or in the CIT Consortium trial centers [79]. Selection of patients with a duration of T1DM >5 years provides assurance that adequate opportunity for optimized medical management has been provided. The restriction of age >18 years reflects general consensus that a lifetime commitment to immunosuppression should be avoided in the pediatric population, except in exceedingly rare instances where the risk of death or irreversible brain injury from hypoglycemia cannot be avoided by alternate means [8]. Avoidance of subjects of weight >90 kg or with daily insulin requirement >1.0 U/kg avoids futility of transplantation where there is marked insulin resistance (Table 108.1A).

Islet after kidney

The indications for islet transplantation in the setting of islet after kidney (IAK) transplantation are much more straightforward, as patients are already subjected to the chronic risks of immunosuppression (Table 108.1B). In IAK transplantation, patients must have a well-functioning prior kidney transplant and be able to tolerate standard maintenance immunosuppression. Corticosteroids are used by some centers for maintenance therapy in kidney transplantation, and although there are concerns regarding both insulin resistance and islet toxicity from prednisone, any negative impact is perceived to be negligible if the dose is ≤5 mg per day, and indeed islet transplant rejection has been reversed successfully by pulsed steroid therapy [82–85]. Kidney transplants are more susceptible than other organs to infection with BK virus, so-named after the initials of the first renal transplant patient to have this polyoma virus isolated from urine in 1971 [86]. Exacerbation of BK nephropathy can be especially problematic, and can be potentiated by depletional therapies, and therefore avoidance of additional risk by screening for BK virus in potential IAK subjects is appropriate [87]. Finally, avoiding subjects with broad HLA sensitization and high panel reactive antibody (PRA) from previous kidney transplant rejection, blood transfusion or pregnancy may have important bearing on the success of an upcoming islet transplant [88,89]. Furthermore, recognizing that kidney transplants have a limited half-life of 8–15 years [90,91], and that kidney retransplantation may soon be the commonest indication for kidney transplantation (up to 20% of patients on kidney transplant lists are due to failed previous transplants), further increased PRA from additional islet sensitization could be especially problematic in this population [92–94]. Therefore an initial PRA <20% is felt to be a reasonable cut-off for IAK transplantation.

Risks of islet transplantation

Procedural risks

Islet transplantation is especially attractive as this cellular therapy can be delivered safely into the intrahepatic portal venous system by a simple percutaneous transhepatic approach that avoids need for surgery. In fact, in experienced high volume islet transplant centers, this procedure is currently far safer than for any other solid organ transplantation. Nonetheless, there are potential risks of the intraportal approach including intraperitoneal bleeding and portal venous thrombosis, both of which are avoidable if the procedural details of catheter tract ablation and intraportal and therapeutic heparinization are adhered to closely [95]. The technical details of islet transplantation are discussed in depth in Chapter 61.

The commonest complication is of mild pain or discomfort either at the catheter insertion site, or as referred pain to the right shoulder tip, is transient, occurs in up to 50% of patients, is easily controlled by standard analgesic medications, and generally resolves fully in 24–48 hours.

Bleeding

Despite the use of therapeutic anticoagulation with heparin (70 units per kg recipient weight intraportally followed by intravenous infusion at 3 units per kg per hour adjusted to PTT of 60–80 seconds), significant bleeding requiring surgical intervention is

now an extremely rare occurrence in our hands, with a current frequency of <1% (3 of 342 cases at the University of Alberta) [95,96]. Use of a 4 or 5 French catheter leaves a narrower tract in the hepatic parenchyma, and ablation of this tract with Avitene paste or D-STAT for a track length of at least 4 cm effectively prevents intraperitoneal bleeding [97–99]. Using this approach, we have not needed to give either a blood transfusion, laparoscopic or surgical intervention for intraperitoneal bleeding in the most recent 150 procedures in Edmonton [95,96]. In one unusual case we encountered bleeding within the right chest cavity, which was recognized early and readily controlled by angiographic coil ablation of an intercostal artery followed by chest tube insertion. Such an injury would not be controlled by track ablative techniques. The use of ultrasound guidance to facilitate portal access, and avoiding the inferior rib or costal border with the initial puncture, may help to avoid such an injury [100–102].

Portal venous thrombosis

Complete thrombosis of the entire portal venous system is one the most feared complication of intraportal islet transplantation [103–105]. However, complete thrombosis with sequelae of chronic portal hypertension has now proven to be an exceedingly rare complication in the current era of low-endotoxin collagenase, continuous gradient purification, routine islet culture, close portal pressure monitoring, therapeutic heparinization and limiting the infused islet tissue mass to ≤5 cc packed cell volume [106–115]. At the University of Alberta, we have yet to encounter complete thrombosis of the main portal vein following islet transplantation now in 342 separate percutaneous intraportal infusions in 164 patients (0% incidence). Furthermore, none of our patients have ever manifested signs of portal hypertension with up to 13 years of follow-up [95,96].

Branch occlusion of segments of the portal tree on the other hand is a rare but entirely benign finding occasionally observed in our hands, has not been associated with propagation into the main tree, and is not associated with portal hypertension. Branch portal occlusion has been documented in 3.7% of cases previously, but the frequency has decreased markedly in the past five years since the introduction of therapeutic heparinization [95,116]. Treatment with heparin followed by a vitamin K antagonist (warfarin) for three months has been often used, but this is probably not necessary as the inciting factor (islet deposition) is a finite event. Patients with an underlying thrombophilia including protein C, S, antithrombin 3 deficiency, Factor V Leiden mutation may be at increased risk of this complication, and screening of patients with a prior thrombotic history may lower this risk [117].

Gallbladder puncture and inadvertent biliary cannulation

The percutaneous transhepatic approach can potentially lead to inadvertent puncture of the gallbladder, but this is an entirely avoidable complication if ultrasonic guidance is applied routinely [97,99,100]. Intraparenchymal cholangiography is anticipated with the 22 gauge seeker Chiba needle, and generally does not lead to bile leak [118].

Transient elevation in liver function, and hepatic steatosis

Transient mild elevation in liver transaminase has been described previously after intraportal islet transplantation, occurred in up to half of the cases, may be up to fivefold increased in 27%, and normalize completely by one month without intervention [119]. Interestingly, with routine adoption of islet culture protocols, improved collagenase enzymes, therapeutic heparinization and the use of anti-inflammatory agents (infliximab, etanercept, Anakinra, aspirin) both the incidence and severity of transaminitis has decreased markedly in our more recent experience.

Hepatic steatosis has also been observed on ultrasound, magnetic resonance imaging (MRI) and in liver biopsies of islet allograft and autograft recipients in up to 20% of cases [120–124]. The fat deposits are usually macrovesicular, focal, and likely reflect local high insulin release from functioning islets. These changes are reversible when islets are rejected [120,122]. These changes have not been associated with non-alcoholic steatohepatitis (NASH) in islet recipients, but their long-term consequence will be defined by additional follow-up.

Risk of liver cancer

Concern has been expressed previously regarding potential transformation of hepatocytes to hepatocellular carcinoma (HCC) in the longer term when islets are embolized to the liver. Dombrowski et al. initially described high rates of hepatic adenomas and HCC in streptozotocin-induced diabetic rats receiving low islet engraftment mass, and subsequently observed similar changes in spontaneously diabetic BB/Pfd rats, attributing this increased risk to a series of trophic factors released locally by intrahepatic islet transplants [125,126]. This is an observation that appears to be unique to the rat. Clinical islet auto- and allo-transplants have been carried out in over 1500 humans since 1977, and there has yet to be a single case report of this complication, thus presumably the risk of malignant transformation after clinical islet transplantation is negligible [7,127–129].

Transmissible infection, and contaminated islet preps

The risk of donor-derived serious infection (bacterial, fungal, viral or prion-related) is well described in solid organ transplantation. Notable examples include transmission of hepatitis C, human immunodeficiency virus (HIV), lymphocytic choriomeningitis from a donor's pet hamster fatal in three organ recipients, rabies virus in a donor bitten by a bat with fatal outcome in four recipients of kidneys, liver and an arterial segmental graft [130–133]. Fortunately donor-derived transmissible infection has been rare in islet transplant recipients. A fatal case of untreated West Nile viral encephalitis was described in a 45 year old woman who received two remote islet transplants three years earlier [134]. In this case, there was a history of a recent mosquito bite at a horse stable, and the infection was clearly not of donor origin [134]. Careful screening of donors, avoidance of high-risk donors for non-lifesaving transplants, and protracted additional testing of donor tissue during islet culture can further lessen the risk of donor-derived infection. Nucleic acid testing (NAT) for HIV, hepatitis B and C has been advocated in selected, high-risk cases to shorten the detection window in donor infection, and we delayed several islet transplants from high-risk donors from proceeding until NAT testing has been released and found to be negative [135]. More thorough consideration of donor derived infectious risks can be found in Chapter 92.

When islets are isolated and stored in culture, there is a potential for bacterial contamination of the preparation. Addition of antibiotics to the culture media may reduce this risk. A Gram stain of the final islet product must be negative, the endotoxin content must be low (<5 EU/kg recipient weight), and a sample of the islet product that has not been exposed to antibiotics must be set aside for post hoc bacterial and fungal culture. High endotoxin content from

impure collagenase enzymes used prior to 1998 may have contributed to poor islet function in the recipient [136,137]. Current collagenase blends have low entotoxin content.

Prophylactic broad-spectrum antibiotic treatment of the recipient at the time of islet transplantation is advised in all cases. Nonetheless, despite inadvertent infusion of contaminated islet products in rare cases, there have been no sequelae of intrahepatic abscess or systemic infection observed; this is clearly reassuring [138]. This risk is probably low because the hepatic blood-flow is high, and the liver contains active phagocytic Kupffer cells in the sinusoidal linings.

Challenges of immunosuppression

Control of autoimmunity and rejection without islet toxicity

Generalized immunosuppression required to suppress the allograft response is associated with a host of well-characterized risks and side effects, most notably an increased risk of opportunistic infection and malignancy. These risks occur with increasing frequency depending on the type, dose and combination of agents used. In clinical islet transplantation, the combination therapy must be sufficient to suppress both potent autoreactivity from recurrent T1DM as well as alloreactivity. Furthermore, islet rejection with progressive hyperglycemia tends to be a late and usually irreversible hallmark of endocrine failure, in contrast to solid organ liver or kidney transplantation where graft dysfunction is detectable at an earlier stage [3,83,139]. Islet rejection has only rarely been reversed in the clinic with corticosteroid therapy [84], and therefore adequate and sustained prophylaxis from the outset is seen to be a critical component of success [1]. Recurrence of autoimmune T1DM clearly occurs after whole pancreas transplantation, despite the use of potent immunosuppressive combination therapies, and may be the underlying explanation for the 50% insulin independence rate persisting by five years after pancreas-alone transplantation [140–143]. Recurrent autoimmunity has also been clearly established in liver biopsy specimens after clinical islet transplantation with selective beta cell destruction [144], and is the most likely explanation for the inexorable loss of insulin independence observed after five years in the original Edmonton Protocol series [145–147]. Roep and colleagues have developed an elegant series of in vitro assays for detection of autoreactivity following islet transplantation, and have recently applied HLA Qdot nanotechnologies to more precisely define islet epitope responses to preproinsulin, islet antigen 2, glutamic acid decarboxylase 65, islet amyloid polypeptide and other targets [148–152]. It is clear from these and other studies that effective control of both autoreactivity and alloreactivity is essential for the long-term functional survival of islet allografts in T1DM [153–155].

While potent immunosuppression is therefore required, it is also increasingly apparent that many of the key agents used are also specifically toxic to beta cells [156]. This adds a unique challenge for islet transplantation, as it cannot be assumed that immunosuppressive strategies tuned for optimal solid organ transplantation success will also be appropriate in islet transplantation [82]. Using lineage tracing techniques, Nir et al. demonstrated that both tacrolimus and sirolimus, drugs used in the Edmonton Protocol, are potent inhibitors of beta cell regeneration in mice [157]. Ricordi et al. highlighted early direct toxicity of tacrolimus on human islet function in immunodeficient mice, and more recent studies by Rostambeigi et al. suggest that both insulin secretion mitochondrial density are decreased [158,159]. It is known that portal levels of immunosuppressive agents expose islets to particularly high local drug levels, especially in the early post-transplant period [160,161]. Interestingly, sirolimus was shown to improve insulin secretion in culture and in vivo, and the anti-inflammatory effects of this agent could potentially improve islet survival in culture [162,163]. Conversely, Zhang et al. observed reduced islet engraftment and impaired beta cell function in a marginal mass mouse islet transplant model [164]. Merani et al. observed that the long-acting glucagon-like peptide 1 (GLP-1) analogue was protective against immunosuppressive toxicities from both tacrolimus and sirolimus in a human islet mouse model [165]. Newer agents such as the sphingosine-1-phosphase receptor modulator FTY720 lack beta cell toxicity, and looked promising in marginal mass islet transplant models both in mice and primates, but the Achilles' heel proved to be accelerated rates of macular degeneration in early clinical trials, an unfortunate side effect for patients with T1DM and pre-existing retinopathy [166–171]. Taken together, there is no ideal immunosuppressive drug for use in islet transplantation, and a compromise that provides reliable protection from both auto and alloreactivity, while at the same time does not excessively impair islet function, is the current pragmatic approach.

A practical approach to immunosuppression in islet transplantation

Chapters 65, 66, and 67 provide a general review of induction, maintenance and rescue immunosuppressive management strategies. We herein focus on current practical approaches to immunosuppression as they relate to clinical islet transplantation.

Induction therapies — daclizumab, basiliximab, thymoglobulin and alemtuzumab, and risk of CMV, EBV and PTLD

The anti-IL2R monoclonal antibodies (mAb) daclizumab and basiliximab have been used widely in islet transplantation since 2000 [1,2]. Daclizumab is no longer being made available for commercial sale, a fiscal rather than biological decision by its licensing company. Although well tolerated without side effects, these agents add only marginal potency to immunosuppressive regimens, and have minimal impact on T1DM autoimmunity. Indeed, large-scale clinical interventional trials using daclizumab and mycophenolate mofetil failed to preserve beta cell function or extend the honeymoon period in new-onset T1DM [54]. We therefore advocate use of lymphocyte depletional approaches in clinical islet transplantation, as first introduced by Hering et al., especially for initial islet transplants [4,172–174]. A wealth of preclinical studies suggests that lymphocyte depletion is a strong adjunctive strategy in autoimmune regulation and tolerance [151,152,175–177]. Bellin et al. have recently analysed data from both Minnesota and from the CITR CITR, demonstrating a profound impact of initial depletional therapy on 5-year insulin independence outcomes, regardless of the maintenance immunosuppressive regimen, and especially in combination with tumor necrosis factor-alpha (TNF-alpha) blockade (vide infra, Figure 108.3) [172]. Thymoglobulin (rabbit ATG, Genzyme in the US or Fresenius outside of the US) is given as a cumulative dose of 6 mg/kg by peripheral intravenous infusion over 2–3 days, with the goal being to infuse at least 2 mg/kg prior to islet infusion while islets are maintained in culture, to avoid transplantation during the depletional cytokine storm [178]. In Edmonton, we have favored depletion with alemtuzumab 30 mg by peripheral

intravenous infused over 3 hours, based on potency, tolerability, sustained effect and cost. Predosing for alemtuzumab includes acetaminophen 650 mg oral, together with intravenous diphenhydramine 50 mg and solumedrol 250 mg. Calne et al. were the first to develop alemtuzumab induction for clinical organ transplantation with their *prope* tolerance approach in combination with low dose cyclosporine [179–181]. We began using alemtuzumab for islet transplantation in 2002, initially in combination with sirolimus monotherapy, but observed high rejection rates, similar to the early experience seen in kidney transplantation [182–185]. After switching to tacrolimus (target trough $\geq$10 ng/mL for 3 months, followed by 8–10 ng/mL thereafter and mycophenolate mofetil up to 2 g daily as tolerated in divided dose, we have found that this approach is best tolerated by patients and provides superior insulin independence at 5 years (Shapiro 2012, personal observation). Tan et al. used alemtuzumab induction with sirolimus and tacrolimus maintenance in seven IAK recipients in Fuzhou, China, with 4/7 patients independent of insulin at one year [186]. Froud et al. from Miami used alemtuzumab induction with short-term calcineurin inhibition, using tacrolimus and sirolimus for 3 months followed by sirolimus and mycophenolate mofetil thereafter, with insulin independence observed in 2 of 3 subjects treated [187]. Over two thousand patients have received alemtuzumab induction in solid organ transplantation, suggesting the apparent safety of this approach despite profound and sustained lymphocyte depletion [74,188–204]. Ongoing trials at the University of Alberta and elsewhere are currently exploring the role of alemtuzumab in control of autoimmunity in islet transplant recipients. We have reported increased rates of CMV transmission and reactivation in islet recipients receiving depletional therapies with either thymoglobulin or alemtuzumab, especially at later time points beyond the period of initial CMV prophylaxis [205–207]. Fortunately these episodes have been largely sub-clinical, and detectable only by serial PCR monitoring, and have not led to specific organ related disease, and have been readily treatable with intravenous ganciclovir or oral valganciclovir (Roche Canada) [205].

While OKT3 and ATGAM have been previously linked to increased rates of EBV-related post-transplant lymphoproliferative disorder (PTLD), the risk appears to be lower with thymoglobulin and alemtuzumab [208,209]. The incidence of PTLD in a meta-analysis of 2246 kidney and heart transplant recipients receiving thymoglobulin was 0.98% at 5 years, and was significantly lower (0.63%) where antiviral prophylaxis with valganciclovir was given [210,211]. Kirk et al. reviewed the incidence of PTLD in 59 560 kidney transplant recipients in a large US database, and found a significantly lower risk of PTLD with alemtuzumab (0.37%) than with thymoglobulin (0.67%) [212]. The use of mTOR inhibitors overall was strongly associated with PTLD in this analysis, leading the authors to conclude that lymphocyte depletion per se is not an independent risk factor for PTLD, rather the overall intensity of immunosuppression is the more correlative metric [212]. Interestingly, alemtuzumab has been used effectively to treat PTLD in the setting of bone marrow transplantation and hematological malignancy [213,214]. In the setting of bone marrow and stem cell transplantation after lymphoablation, the use of alemtuzumab has been associated with very low rates (<1%) of PTLD despite EBV reactivation in this high-risk group [215]. In solid organ transplantation there have been very few anecdotal reports of PTLD after alemtuzumab induction. Currently, there have been no reports of PTLD following islet transplantation in any of the major trials, irrespective of the induction and maintenance regimen, and although a report from Cure et al. found late EBV reactivation in three IAK recipients treated with tacrolimus and sirolimus, none transformed to PTLD [1,2,101,118,146,216,217].

Maintenance immunosuppression

Selection of synergistic maintenance immunosuppression that is universally tolerated has been especially challenging in islet transplantation. A desire to use "islet-friendly" medications that lack beta-cell toxicity does not necessarily mesh well with a need to provide potent and sustained protection from both auto- and alloimmunity [7,156]. High-dose sirolimus (levels 12–15μg/L for the initial three months, then reduced to 10-12μg/L thereafter) combined with low-dose tacrolimus (4-6μg/L) was advocated in the Edmonton Protocol as a means to reduce calcineurin-inhibitor exposure in patients with underlying overt or covert T1DM nephropathy [1,218]. While this approach led to unprecedented high rates of one-year insulin independence in the three leading islet transplant centers [219], it became apparent that such a combination was difficult to titrate when extended to the setting of an international multicenter trial [2,220]. High dose sirolimus was perceived as the major culprit in this regimen [221], with intolerable rates of mouth ulceration [105,118], ovarian cysts and amenorrhea in female patients [222–224], fatigue, diarrhea, occasional severe small bowel ulceration [225], pneumonitis [226], peripheral and retroperitoneal edema [123,227] and accelerated proteinuria due to sirolimus, that was reversible after drug withdrawal [228,229]. Although sirolimus remains an integral component of the CIT registration trials in the US, the University of Alberta has abandoned the routine use of this agent in islet transplantation, based on the above experience of side effects. The more standard transplant combination of tacrolimus and mycophenolate mofetil has been tolerated with a far superior side effect profile and compliance in our hands. The substantial increase in 5-year insulin independence rates observed with depletional induction and high-dose tacrolimus and mycophenolate mofetil maintenance suggests that despite theoretical concerns regarding islet toxicity, in practice this combination can provide effective prophylaxis from rejection and recurrent autoimmunity. The downside of this approach is a need to exclude patients with underlying diabetic renal impairment at baseline, with current recommendations requiring evidence of adequate renal reserve, with a baseline estimated glomerular filtration rate $>$60 mL/min/1.73 m^2 and albumin excretion rate $<$300 mg/g creatinine.

Calcineurin-inhibitor avoidance in islet transplant

Since diabetes is the commonest indication for renal transplantation, approaches that provide equivalent immunoprophylaxis without side effects, but avoid calcineurin inhibitors (CNI), are seen as a major priority for the field. While the combination of sirolimus and mycophenolate mofetil can provide effective short-term prophylaxis, it is only generally effective when used in combination with depletional induction therapies in islet transplantation, and it remains unclear whether complete elimination of CNI will impact the 5-year insulin independence rates [172,187]. Posselt et al. have explored two different induction strategies that may accomplish these goals [230]. They found that depletion induction with thymoglobulin and induction and monthly maintenance costimulation blockade infusions of belatacept facilitated a CNI-free regimen requiring only sirolimus or mycophenolate mofetil

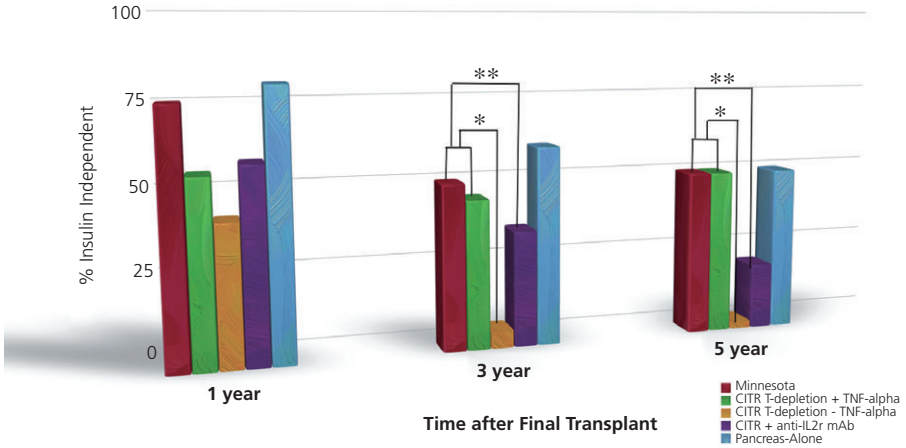

Figure 108.3. Five-year insulin independence rates comparing pancreas alone transplantation (PTA) with different induction and anti-inflammatory strategies in clinical islet transplantation. This data from the American Journal of Transplantation (reproduced from [172] Bellin et al. 2012, with kind permission from Wiley) demonstrates outstanding 5-year insulin independence rates of 50% when T-depletional therapies + TNF-alpha blockade (etanercept) are given in combination. These results equate to the 50% insulin independence rates of PTA observed at five years from the International Pancreas Transplant Registry Data.
* $p < 0.01$; ** $p \leq 0.05$.

[230]. Alternatively, they tested an anti-leukocyte functional antigen-1 antibody (anti-LFA1 mAb, efalizumab) in place of costimulation blockade [230]. While most encouraging, these early observations are currently limited to five subjects per group, and the anti-LFA1 antibody has unfortunately been withdrawn from the market because of exceedingly low rates of progressive multifocal leukoencephalopathy (PML) seen in psoriasis-treated patients (three confirmed cases and an additional case suspected in >46 000 treated) [231–233]. The ability to combine belatacept with depletional therapies in ongoing trials may be further restricted by the licensure trials and post-market surveillance of belatacept therapies in transplantation, which may necessitate a low-risk tolerance approach by Bristol Myers Squibb. Therefore an ideal CNI-free approach in clinical islet transplantation is not readily available for more widespread and long-term testing at present.

Adjunctive peritransplant prophylactic management

Antibacterial, antifungal, and antiviral prophylaxis generally follows local practice guidelines, in concert with a transplant infectious diseases specialist. Generally we recommend that broad-spectrum antimicrobial prophylaxis be administered before intraportal islet transplantation.

Anticoagulation

The anticoagulation regimens are outlined in detail in the techniques chapter 61. In brief, heparin (70 units per kg recipient weight) is delivered intraportally with the islets, and a heparin infusion is initiated in the radiology department at 3 units per kg per hour, then titrated to maintain a PTT of 60–80 seconds, and continued for 48 hours post-transplant. This approach, combined with intensive insulin therapy, has improved our single donor islet engraftment success [116]. We then continue low-molecular weight heparin (enoxaparin 30 mg twice daily s.c.) for 7 days, and aspirin 81 mg enteric coated for 14 days post-transplant, provided there are no concerns regarding bleeding or thrombosis on the doppler ultrasound examinations.

Anti-inflammatory medications

Concomitant medications may include vitamin E (800 iU oral pre-transplant then for 7 days post), anti-TNF-alpha blockade with etanercept (50 mg intravenous pre-transplant, then 25 mg s.c. on days 3, 7 and 10 post, as described by Hering et al.) [4,174]. Farney et al. initially described this approach in mice [234]. McCall et al. from our group recently found remarkable potentiation of marginal mass human islet engraftment in immunodeficient mice when the combination of etanercept and an anti-IL1 beta receptor antibody, anakinra was used [235]. Preliminary clinical data from Matsumoto et al. suggested that this combination might be beneficial in a small series of islet recipients [236]. Encouraging data from Bellin et al. suggests that potent anti-inflammatory, anti-TNF alpha strategies given at the time of islet transplantation with etanercept or infliximab may have lasting positive impact on 5-year insulin independence rates, and especially when combined with depletional induction therapies (Figure 108.3) [172]. We generally give pentoxifylline 400 mg orally tid for 7 days post-transplant, following the original Giessen protocol [237].

CMV prophylaxis

CMV and EBV prophylaxis using valganciclovir is recommended for all subjects receiving depletional therapies, irrespective of donor and recipient CMV status, starting at 450 mg once daily for 14 days, then increased to 900 mg once daily for 12 weeks post-transplant [205]. We further recommend sulphamethoxazole 400 mg — trimethoprim 80 mg once daily for 6 months for *Pneumocystis jiroveci* prophylaxis post-transplant. If allergic to sulphonamide, then pentamidine 300 mg by inhalation may be substituted, but dapsone should be avoided as it interferes with HbA1C determination [238].

Other long-term risks in islet transplanted patients

Death

The risk of death following islet transplantation has fortunately been exceedingly rare. Currently at the University of Alberta, our

actuarial survival rate is 96% with 13 years of follow-up (6/164 subjects). We have encountered no deaths as a direct or indirect result of immunosuppression, to the best of our knowledge. Of six deaths, four were cardiovascular from underlying cardiac microangiopathy, coronary occlusion or arrhythmia, one was from fatal hypoglycemia in a patient with a failed islet transplant and off immunosuppression for a protracted period, and one resulted from an inadvertent methadone overdose in a patient that had severe ocular pain, had been taking escalating doses of methadone previously, stopped taking the drug for two years, then resumed at his former dose by self-administration.

Data from the most recent CIT International Registry (including the six deaths in Edmonton above) currently documents a total of 18 deaths, or 1.3% overall, with 15 of the 18 deaths occurring in patients transplanted between 1999–2003 [239]. Of these cases, three were possibly related, and one definitely related to islet transplantation or immunosuppression. While robust comparative data has not been collected in an appropriate non-islet transplant control group in formal analysis, we have encountered a similar number of deaths from severe hypoglycemia in patients on waiting lists for islet transplantation, providing accumulating evidence of equipoise for islet transplantation versus optimal medical insulin therapy for a head-to-head trial.

Malignancy

In terms of malignancy, in the CITR there have been 16 cases of basal or squamous cell carcinoma of the skin arising in 13 patients, with an overall rate of 2.3% [239]. Most of these are presumably related to chronic immunosuppression, and were fully treated by excision. Additionally in the CITR there were two patients with breast cancer, two lung cancers and two thyroid cancers (0.35% incidence respectively) [239]. Sirolimus may have a beneficial protective role in reducing the incidence and severity of several malignancies in transplant populations, with a growing body of evidence [240–249].

HLA sensitization

The risk of HLA sensitization has been highlighted as a concern, as patients receiving islet transplants from more than one donor with inadequate immunosuppression may become highly sensitized from rejection, and this may preclude them from future successful islet, pancreas or renal transplantation. Campbell et al. from Edmonton demonstrated that the presence of preformed HLA antibodies are linked to worse islet transplant survival, emphasizing the need for detailed high-resolution, standardized HLA antibody screening and prospective cross-matching where indicated, before proceeding with transplantation [88]. We have previously reported high rates of broad HLA sensitization with high panel reactive antibody (PRA) in subjects that became C-peptide negative with failed islet transplants, but this sub-group represents only a small fraction of the total numbers of subjects treated, with an overall risk of 16% [89]. Complete withdrawal from immunosuppression may have been the inciting factor, and the possibility that sensitization could be prevented by continuation of low-dose maintenance monotherapy remains in question. Interestingly, the more potent alemtuzumab tacrolimus and mycophenolate mofetil protocol has been associated with exceedingly low rates of PRA sensitization (unpublished data, Shapiro Edmonton). The Geneva-GRAGIL network have reported low rates of HLA sensitization (10.8% risk) in IAK recipients, despite the use of multiple donors, in rates equivalent to kidney transplantation alone [250]. Review of the CITR database for risk of HLA Class I sensitization suggests that a post-transplant rise in PRA by $\geq$20% is associated with a 3.6-fold risk of graft failure [251]. Rickels et al. have made clear recommendations to minimize risk of sensitization, including avoidance of multiple islet transplants, waiting for negative cytotoxic cross-match by high resolution techniques before proceeding, and avoiding donor-recipient antigen mismatches where there is an identifiable alloantibody present prior to transplant [252].

Long-term outcomes

Current detailed data accrued in 570 patients in the most recent CIT registry, and from a series of recent presentations from the leading islet transplant centers indicate substantial improvement in 5-year insulin independence rates [172,239]. The original Edmonton Protocol series reported on inexorable loss of insulin independence by five years post-transplant, with only 15% of patients remaining completely free of insulin (Figure 108.4A) [2,7,145,146]. Although the Edmonton longer-term results fell short of their target, profound stabilization in glycemic control was sustained in 73% of subject followed beyond ten years with correction of HbA1C to less than 6.5% with complete protection from recurrent hypoglycemia. Similar results have been reflected in the CITR database [253]. This remarkable stabilization in glycemic control is rarely if ever achieved by insulin pumps and CGMS monitoring, but requires life-long immunosuppression to sustain, and have met with mixed opinions [254–259]. Explanations for deteriorating insulin reserve have been varied, but are clearly multifactorial. Recurrent autoimmunity, rejection, and inhibition of beta cell proliferative capacity are likely the dominant culprits, adding additional stress to an injured, inflamed and under-dosed initial islet engraftment mass (Figure 108.4B) [124,260–264]. Metabolic overstimulation of a marginal islet transplant mass can trigger endoplasmic reticulum linked islet cell apoptosis and with amyloid protein deposition likely deleterious to islet functional survival [265–268]. Over 80 clinical trials are currently registered with ClinicalTrials.gov that will address many of these opportunities, with the goal being to restore a full, physiological initial islet engraftment mass in a non-injured and non-inflamed state as an essential starting point for future tolerance trials that will obviate a need for a lifetime of immunosuppression.

Currently, at least five independent islet transplant centers are reporting 50–70% insulin independence at five years post-transplant (Edmonton, Minnesota, Geneva-GRAGIL and Lille) using different induction and maintenance strategies (International Islet and Pancreas Transplant Association meeting, Prague, 2011) [4,172–174,269,270]. The results for islet-alone transplantation represent a substantial advance, and outcomes now approximate results of whole pancreas transplant alone (PTA) at five years, according to the International Pancreas Transplant Registry [71,172]. The implications of this equivalency in outcome will take time to resound amongst both patients and transplant communities. The attraction of a simple, percutaneous cellular implant for islet transplantation with avoidance of major surgery and life-threatening complications must to be offset by a current need for more than one islet donor, an 50% failure rate during islet processing, and the fact that islet transplantation, although desirable, is restricted to limited centers with cGMP islet manufacturing skills, and probably more importantly is not universally reimbursable at present. Recent impressive data from the San Francisco group indicate an 82% processing success rate in 45 pancreas organs from donors under age 50 and

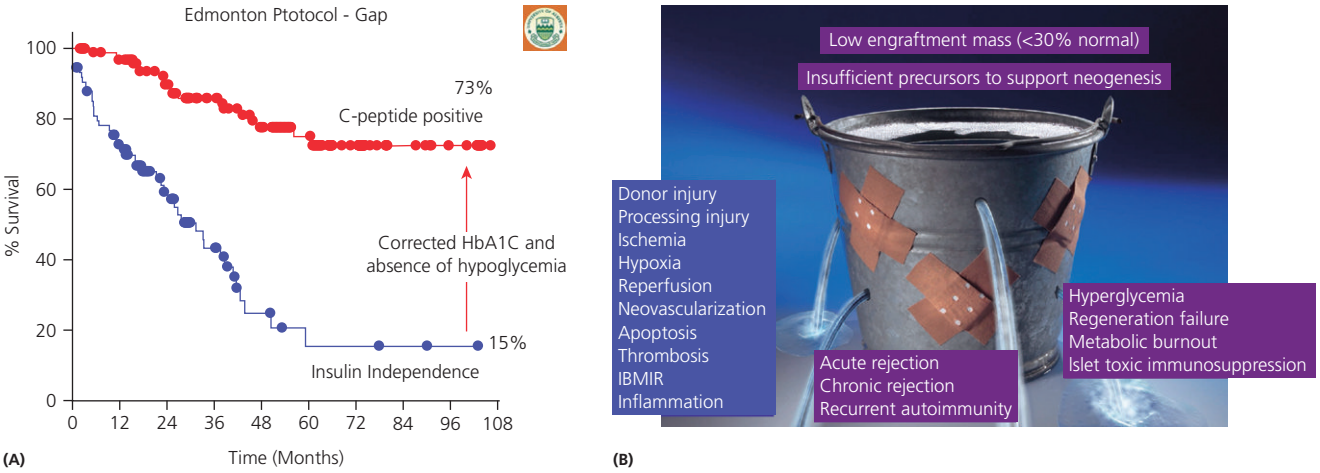

Figure 108.4. (A) Inexorable loss of insulin independence at 5 years in the original Edmonton Protocol, but with maintenance of regulated C-peptide secretion, correction of HbA1C and prevention of hypoglycemic episodes in 73% of cases. Data from [2,7,145,146].
(B) Possible multifactorial explanations for the gradual loss of insulin independence in these patients. A limited initial islet engraftment mass, failure of regeneration, and especially recurrent autoimmunity represent the dominant challenges that must be overcome in future islet transplant trials. Background image; www.tsamedien.de.

mean BMI of 34, with a mean islet isolation mass of 630 000 IEQ (Gregory Szot, personal communication). If this level of islet isolation success can be transferred to other centers, this will have major bearing on pancreas allocation paradigms with younger, more optimal donors.

CIT consortium trials, and other government funding

The CIT consortium is a $75 million initiative begun in 2004, funded jointly by the National Institute of Allergy and Infectious Diseases (NIAID) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) from the National Institutes of Health (NIH) designed to advance clinical islet transplantation [5,271]. A total of ten islet manufacturing sites and 12 clinical centers are active participants, including centers in the US, Canada and Sweden. The major goals of the consortium have been to develop two Phase III registration trials (CIT-07, islet alone IAT, and CIT-06, IAK) that will be used by the FDA to justify a Biologic License Application (BLA) and subsequent third-part, Medicaid and Medicare reimbursement for islet transplantation in the US [5]. This will be a critical step, as it will free up limited research funding to focus on the detailed refinements needed to take islet transplantation to the next stage, without having to fund the routine, clinical and manufacturing components of the treatment. A BLA requires submission to the FDA of complete, standardized details of the islet manufacture process, together with a comprehensive account of the medical effects of the biologic product. To this end, the CIT consortium group has developed standardized procedures for islet manufacture, clinical patient care, and rigorous end-point definitions for impact and risk in the two different ITA and IAK populations [5]. It is anticipated that the CIT-07 subjects reach their primary one-year end-point of correction of HbA1C and protection from hypoglycemia by late 2012, with follow-up completed in 2013, with submission of a final study report in 2014–2015. This final study report will form the basis for the BLAs.

Other CIT center Phase I/II trials are currently investigating approaches to improve islet engraftment by inhibition of the instant blood mediated inflammatory reaction (IBMIR), safer, CNI-free immunosuppression using costimulation blockade with belatacept, deoxyspergualin, or with combined B cell and T depletional therapy, or exenatide (GLP-1) therapy [5].

Table 108.2. National reimbursement for islet transplant

Country	Agency	Year
Canada	Provincial Reimbursement Government of Alberta	2001
UK	National Institute of Clinical Excellence (NICE)	2008
Australia	Government of Australia Juvenile Diabetes Research Foundation (joint funding)	2011
Switzerland	Swiss Government GRAGIL Consortium	2000
Nordic Network	Government of Sweden	2008
USA	FDA Biological License Application (BLA)	pending 2014–2015

Several other countries have successfully engaged government support, most notably in Canada (Provincial funding through the Government of Alberta), the UK (through the National Institute for Health and Clinical Excellence — NICE), Switzerland, and in Australia, with joint partnership between the Government of Australia and the Juvenile Diabetes Research Foundation (JDRF) (Table 108.2).

Islet-alone versus islet-after-kidney transplantation — similar outcomes

In contrast to whole pancreas transplantation where only 18% of transplants are carried out as PTA, 84% of islet transplants have been ITA in the past decade, with only 16% carried out as IAK [71,73,239]. This reflects a general perception that T1DM with end-stage renal failure is best managed surgically with a combined or sequential solid organ transplant, whereas T1DM patients with brittle control and good renal function are better served with an islet approach, as outcomes have been less stellar, and risk higher with PTA than with SPK transplantation [71,77,272]. Simultaneous islet-kidney (SIK) transplantation has been carried out successfully

in a limited number of cases, but requires considerable logistics to achieve this with the same cadaveric donor, and with a potential 50% islet isolation failure has not been popular [186,273,274].

Further consideration of IAK transplantation seems appropriate, as the prior kidney transplant takes the risk-benefit balance of immunosuppression out of the equation. IAK transplantation is a major parallel current focus of the CIT consortium BLA trials in CIT-06 [5]. In the early days, there was concern that an islet transplant would increase the immunological risk to the transplanted kidney through donor sensitization, but this has not been the case in the current era, and indeed the presence of functioning islets has been protective to the kidney [250,273,275–278].

Islet transplantation and impact on secondary diabetic complications

Compelling data has been accrued in whole pancreas transplantation clearly demonstrating that near-perfect glycemic control has major protective benefit in stabilizing and reversing coronary artery disease, intimal carotid thickness, diabetic glomerulopathy, neuropathy, retinopathy and other secondary complications of T1DM, and can decrease cardiovascular mortality [279–291]. Equally compelling data is beginning to emerge in clinical islet transplantation to suggest a similar protective impact of sustained euglycemia in stabilization and reversal of secondary diabetic complications. The Milan group has demonstrated consistently that clinical islet transplantation with positive c-peptide can stabilize both macro and microangiopathic changes in T1DM, irrespective of insulin independence status [263,277,292–296]. Thompson et al. from the Vancouver group conducted a prospective, cohort crossover study comparing the intervention of islet transplantation against optimal medical therapy [297]. There was a reduced progression of retinopathy in the islet transplant group, and a trend towards improvement in nerve conduction velocity [298]. Furthermore, despite the addition of CNI immunosuppression, there was a reduced decline in glomerular filtration rate in the islet transplant group compared to medical therapy [297]. Further large-scale randomized control trial data without cross-over will be needed to further corroborate these important positive findings, but may be difficult to fund and implement.

Single-donor islet transplantation

The use of multiple donor islet preparations in the 2000 Edmonton Protocol series (median 2, range 2–4) provided an adequate engraftment mass to sustain short-term (1–3 year) insulin independence, but raised concerns about the practicality of this approach as well as potential risk for HLA sensitization [1,88,89]. The concept that a single pancreas could provide sufficient islets to treat multiple recipients was first demonstrated in rats by Payne et al., and subsequently in large animal canine islet autografts by Griffin et al. [299,300]. While such an approach would have major bearing on islet supply and impact in the broader treatment of T1DM, it has yet to be demonstrated clinically. Perhaps the closest demonstration has been a successful living donor islet allotransplant in a mother to daughter transplant in Japan, where the T1DM recipient was rendered insulin independent using only a distal 50% pancreatectomy [301].

The Minnesota series therefore represented a substantial advance, as high rates of single-donor engraftment were consistently achieved when a non-Fc-binding anti-CD3 antibody was used for induction, together with optimized gradients for islet purification using Opti-prep, islet culture in the presence of insulin-like growth factor I, transplantation under the cover of etanercept anti-TNF alpha blockade, and peritransplant insulin and heparin [4]. Unraveling the secrets of consistent single donor success has been complicated by potential bias in donor and recipient selection for factors possibly favoring high islet potency, yield, insulin requirement and resistance in recipients, but clearly this is a highly desirable outcome [302]. Several other centers and approaches have reported variable rates of single donor engraftment success (Table 108.3). Markmann et al. similarly achieved single donor islet engraftment success in 5 of 7 cases with an Edmonton-like approach using higher BMI donors [303]. We confirmed the utility of Hering's approach with intensive peritransplant insulin and heparin as an adjunctive engraftment-promoting strategy, and found that our single donor insulin independence rate rose from 10% to 40% with this approach, as an encouraging clinical demonstration of the IBMIR effect [116,304,305]. The Emory and San Francisco groups both described high rates of single donor islet engraftment in the presence of efalizumab (anti-LFA1) in a CNI-free strategy, but no longer clinically available (discussed earlier) [230,306,307]. Until we have precision islet potency assay that is universally applied across trial sites, it will be difficult to define which specific components of which protocols contribute most to single donor engraftment success. Efforts by the CIT consortium to incorporate islet oxygen consumption rates, glucose-dependent oxygen consumption rates and laser-scanning cytometry for mitochondrial and beta cell viability, will help to provide comparative data, as well as predictive potency assays that the FDA requires for quality control testing of the manufactured islet product [308–310].

Table 108.3. Single donor islet protocols

Author	Center	Approach	Year [Reference]
Hering *et al*	Minnesota	anti-CD3 + etanercept	2005 [4,172]
Markmann *et al*	Pennsylvania	Edmonton-like	2003 [303]
Turgeon *et al*	Emory	Efalizumab + MMF	2010 [306]
Posselt *et al*	San Francisco	ATG + Efalizumab + SRL or MMF	2010 [230]
Posselt *et al*	San Francisco	ATG + Belatacept + SRL or MMF	2010 [230]
Koh *et al*	Edmonton	Peritransplant insulin + heparin	2010 [116]
Matsumoto *et al*	Kyoto	Living donor islet transplant	2005 [301]
Matsumoto *et al*	Baylor	ATG + anakinra + etanercept	2011 [236]
Ghofaili *et al*	Vancouver	Exenatide	2007 [316]
Faradji *et al*	Miami	Exenatide	2009 [311]
Gangemi *et al*	UIC Chicago	Exenatide	2008 [317]

GLP-1 analogues in clinical trial

The use of a short-acting GLP-1 analogue exenatide has been evaluated extensively by the Miami, Vancouver and University of Illinois groups as a means to facilitate both single donor and supplemental islet engraftment [311–317]. While exenatide clearly has both positive direct and indirect effects on islet function, therapy is needed continually to sustain marginal mass graft function. Up to 30% of patients cannot tolerate exenatide because of severe nausea. An alternate, long-acting GLP-1 analogue, liraglutide, has been associated with much lower rates of nausea and clinical intolerance. Merani et al. found that liraglutide improves marginal mass human islet engraftment in mice, and protects against immunosuppressant

beta cell toxicity in vitro and in vivo [165]. When tested in a rapidly growing large animal pig islet autotransplant model, the presence of liraglutide helped to resist metabolic graft failure over time [318]. When added to the islet culture media, liraglutide significantly improved human islet survival, reduced apoptosis, and facilitated marginal mass engraftment in mice [319]. Based on this data, Novo Nordisk is currently conducting a major international trial at 16 sites (Shapiro, principal investigator), for a randomized placebo-controlled comparison of outcome of liraglutide in clinical islet transplantation [320].

Alternative strategies in current clinical trials

Control of IBMIR

The Swedish group has had a longstanding interest in innate IBMIR-islet injury triggered in minutes following intraportal islet transplantation through the increased expression of tissue factor, platelet aggregation and inflammation [304,321]. High circulating levels of thrombin-anti-thrombin complex and C-peptide released from dying islets confirm that this is a clear clinical entity, and the detectable loss of labeled human islets by positron emission tomography imaging in patients undergoing intraportal islet transplantation confirm that this is a major for islet protection in the peritransplant period [322,323]. Peritransplant insulin and heparin can partially modify this response and increase single donor engraftment rates [116]. The surface binding of heparin to islets is one effective approach tested in pig islet transplant models, and awaits further evaluation in the clinic [305]. Furthermore, anchoring of surface-immobilized vascular endothelial growth factor on the islet surface similar to heparin can potentially stimulate islet angiogenesis [324]. Several strategies will likely be further tested clinically, including low molecular weight dextran, direct thrombin inhibitors (e.g. hirudin, desirudin, melagatran, dabigatran) and other high-specificity inhibitors of the extrinsic coagulation pathway. Balancing a positive impact on islet survival while avoiding excessive risk of bleeding with these approaches will be critical.

Control of apoptosis

Our basic laboratory has focused on temporary inhibition of islet apoptosis since our initial studies demonstrated the damaging effect of this pathway in human islets. Emamaullee et al. demonstrated that ex vivo viral transduction of islets with x-linked inhibitors of apoptosis proteins (XIAP) led to substantial improvement in marginal mass islet graft survival [325–327]. A fluoromethylketone (FMK) agent called ZVAD-FMK was also highly effective, but too toxic for potential clinical application [328]. Nakano et al. demonstrated that a FMK-derivative caspase-3 inhibitor was protective of human islets in culture, but the lack of pan-caspase inhibition may have lowered the potency of this approach [329]. We further explored a potent pan-caspase inhibitor called EP1013, and found that just 10–30% of the usual marginal islet mass was required to reverse diabetes in mice with mouse or human islets, and furthermore this strategy was highly effective as a facilitator in costimulation-blockade induced tolerance induction in mice [330,331]. EP1013 lost patent and status, and is no longer available for trials. We therefore tested a similar pan-caspase (IDN-6556, Conatus Pharmaceuticals Inc., San Diego) inhibitor in mice, with human islets and subsequently in a large animal marginal mass pig autograft model, all with similar compelling data [332]. This agent is now undergoing preliminary Phase I/II safety and efficacy studies in clinical islet transplant patients in Edmonton. While there are potential theoretical concerns relating to malignant transformation when apoptosis is inhibited especially in combination with potent immunosuppression, short-term (two-week) temporary therapy should hopefully offset this risk.

Immunological tolerance

Complete restoration of self-tolerance with elimination of autoreactive T cell clones active in T1DM, together with means to permanently eliminate alloreactivity, would be the key transformative step that could move islet transplantation from the status of treatment to cure. Achieving dual tolerance to these separate but interlinked pathways of attack has been phenomenally difficult to translate to the clinic, although such tolerance can be attained routinely in mice, and occasionally in large animal non-autoimmune primate transplant models [3]. Chapters 11 and 76 address the mecanisms of tolerance and specifics related to its clinical translation respectively and in detail, and will not be reiterated here. Costimulation blockade was a promising approach to induce peripheral tolerance induction in mice, but due to different maturity, exposure, avidity binding, receptor expression and the confounding effects of heterologous immunity, costimulation (using either CTLA4-Ig or belatacept) has failed to induce clinical tolerance when used as a sole strategy [333–340]. The more potent strategy of anti-CD40L (Hu5c8) met with considerable excitement in primate renal transplantation, failed to yield similar result in the clinic, but furthermore led to fatal thromboembolic events in patients [341]. An alternative chimeric anti-human CD40 antibody lacks thromboembolic risk, and also has potent immunosuppressive properties [342]. Adams *et al* demonstrated marked synergy of anti-CD40 with belatacept in a cynomolgus monkey islet allograft model, an approach that could potentially be translated to the clinic [343].

While costimulation blockade has not at this point shown clinical tolerogenic effects, myeloablative and non-myeloablative conditioning approaches with HLA-matched allogeneic bone marrow infusion have certain shown tolerance and promise in combined kidney and bone marrow transplantation in patients with hematological malignancies, provided potent conditioning strategies (usually involving total lymphoid irradiation) are given [344–350]. Translation of similar strategies to islet transplantation will likely occur over time, as further knowledge on bone marrow enrichment, dosing and conditioning are more fully developed. A recent collaborative study of the Fuzhou and Miami groups from The Cure Alliance (www.thecurealliance.org) and the Diabetes Research Institute (DRI) Federation have evaluated the potential of autologous mesenchymal stem cells (MSC) as an inductive strategy in clinical living-related renal transplantation [351]. A total of 159 patients were randomized to receive autologous MSC's dosed at 1–2 $\times$ 10^6/kg, with standard or low dose CNI maintenance, and were compared with control subjects receiving standard anti-IL2R plus CNI. The salient findings were a lowered the risk of rejection, decreased risk of opportunistic infection and improved renal transplant survival [351]. Similar approaches combined with T depletion have demonstrated remarkable regenerative potential in new onset T1DM with insulin independence (vide supra) and if combined with this approach could provide the missing link for both elimination of autoreactivity and control of alloimmunity. Peripheral infusion of regulatory T cells, alone or in combination with MSC's could further facilitate such an approach.

In addition, a recent collaborative study between Ildstad group in Louisville and the Leventhal group in Chicago reported successful establishment of durable chimerism and donor-specific tolerance in renal transplant recipients, using a non-myeloablative conditioning of the recipients [352]. Should this approach be confirmed also in autoimmune conditions it could become transformational not only for solid organ and islet cell transplantation, but also for restoration of self-tolerance in intervention trials for patients with T1D.

Summary

This chapter has thoroughly reviewed the current risks and status of T1DM, and the alternative strategies currently being developed to further improve glycemic control. Islet transplantation offers the potential to restore euglycemia, completely protect against hypoglycemia and lability in a way that exogenous insulin is unable to do, and with far less risk than whole pancreas transplantation. Remarkable strides have occurred since the Edmonton Protocol was introduced in 2000, and currently over 1000 patients have undergoing islet auto or allotransplantation in up to 40 international sites. While the initial five year results showed a sharp loss of insulin independence but continued protective reserve, recent reports from five independent clinical islet centers, the CITR registry and most notably the Minnesota center, are reporting insulin independence rates exceeding 50%, which now match the registry results for whole pancreas transplantation. Steady progress has occurred in single donor islet transplantation, and will likely accelerate with a series of current exciting clinical trials. A BLA for islet manufacture in 2014–2015 will have major impact on reimbursement and activity for centers in the US, as trials currently move forward within the CIT consortium. Parallel clinical trials aimed at restoration of self-tolerance and regulatory T cell or MSC infusion trials will take us several steps closer from refined treatment to robust cure for T1DM.

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CHAPTER 109

Long-term Outcomes after Intestinal and Multi-visceral Transplantation

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Introduction

Intestinal transplantation is a life-saving procedure for patients with complicated intestinal failure. Although its results lag behind those of most other solid organs, its potential for sustained success is manifest by the existence of several patients alive more than 20 years after transplantation who are nutritionally independent, with restored health and social life. The primary problems impeding ideal outcome after intestinal transplantation are related to a comparatively unrelenting risk of graft loss or mortality due to rejection, disease recurrence or infection. These likely relate to the complex interplay between the gut barrier and immune function of the donor organ with the alloimmune response of the recipient. Inclusion of the liver and other components of a multivisceral graft appear to mitigate this somewhat, but the inclusion of an intestinal allograft continues to place an additional immune burden in play for the patient and physician. This chapter will review the long-term outcomes of intestinal transplantation, highlighting the known factors of influence.

Long-term graft and patient survival

Although progress in intestinal transplantation is continuously being made and outcomes have improved, long-term results still lag behind the results that have been achieved in kidney, liver and heart transplantation; they resemble the results of lung transplantation. According to the Intestinal Transplant Registry, as of the 12th International Small Bowel Transplantation Symposium (2011), 2611 intestinal transplants had been performed worldwide, more than half (55%) in children (covered explicitly in Chapter 117). Five-year patient survival is only about 50%, with some improvement for patients who received a transplant after the year 2000. With time, the survival rate shows continuous decline: 35% of the patients are alive at ten years, with no difference between children and adults. Composite grafts (multivisceral and composite liver-intestinal grafts) have better long-term outcomes than isolated intestinal grafts (55% and 65% versus 45% at 5-years post-transplant, respectively). Risk factors included age <1 year of age at the time of transplantation, lack of induction immunosuppression and the patient's condition at the time of transplant, with location of the patient in the hospital — particularly ICU — being a foreboding indicator. Improved outcomes are noted for patients who are at home at the time of transplant and being transplanted in a center with high volumes. Receiving a liver as part of a composite graft is associated with improved survival in all eras with a hazard ratio of 0.68 ($P = 0$) [1,2].

Similar results were found when analyzing the outcomes of adult recipients who survived >5 years after intestinal transplantation at our center [3]. From 1994–2010, 115 adult patients received 126 transplants, with 79 patients transplanted during 1994–2005. Thirty-six patients (42%) survived longer than 5 years. Median follow-up period was 9.2 ± 3.2 years. All survivors were off parenteral nutrition (PN). There was no difference between long-term survivors and non-survivors in regards to induction immunosuppression, donor age, donor/recipient HLA matching (number of loci) or cross matching (negative/positive). However, patients receiving a liver-containing graft (liver-intestine or multivisceral) had significantly improved survival rates 5 years post-transplant versus patients who received non-liver containing grafts (modified multivisceral and isolated intestine) (Figure 109.1). Additionally, there was a significant difference in pretransplant condition: 52% of long-term survivors were at home at the time of transplant versus 78% of non-survivors ($P = 0.015$) [3] (Figure 109.2). It appears that pretransplant condition not only affects short-term outcomes but is a contributing factor in long-term outcomes as well.

In a recent presentation before the American Surgical Association, investigators from the University of Pittsburgh, site of the oldest and largest single-center series worldwide, reported 5-year patient and graft survival of 60% and 50% respectively. Conditional patient survival beyond 5 years was 75% and 61% at 10 and 15 years respectively. Corresponding graft survival was 59% and 50% [4]. Leading causes of death were rejection, infection and renal failure. The latter was more common among adults than children. Leading cause of graft loss was rejection.

The study firmly corroborated previous standing observations:

- The vast majority of the long-term survivors (92%) achieved nutritional independence with regular diet.
- The vast majority of the long-term survivors (92%) had intact graft function quantified with physiologic and anthropometric measures.
- Quality of life is significantly better in patients who had transplants versus patients on parenteral nutrition.

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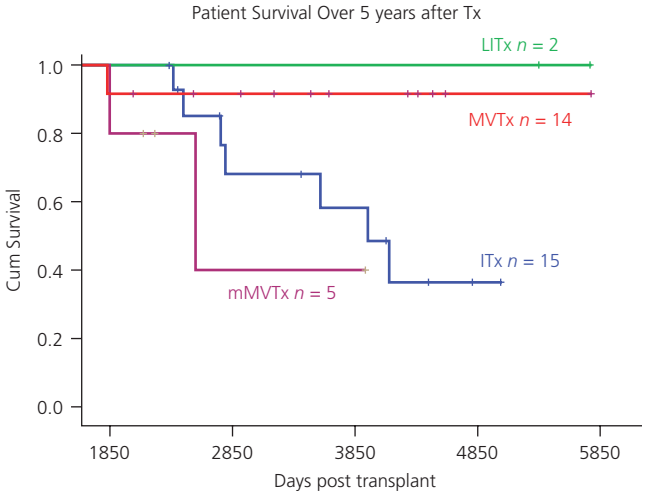

Figure 109.1. Long term survival for patients who served at least 5 years from the University of Miami Miller School of Medicine showing the attrition of survival by organ type. Shown are progressively worsening results for patients who received a liver/intestine transplant (LITX), multivisceral transplant (MVTX), intestinal transplant alone (ITX), and modified multivisceral transplant (mMVTX).

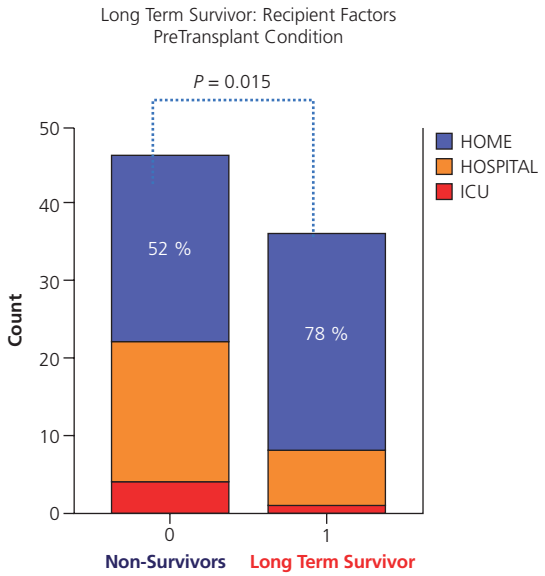

Figure 109.2. Characteristics of patients who are long-term survivors following intestinal transplantation stratified by pretransplant location. Long-term survival is associated with patients who were healthy enough to be at home when called in for transplantation.

- There was a high incidence (greater in children) of long-term neuropsychiatric and behavioral disorders such as depression, anxiety and impaired cognitive functions.
- Paradoxically, in spite of the high incidence of these disorders, these patients have an extraordinary ability for social adjustment. The majority of the children attend school and the majority of the adults maintain their families and have a productive occupation.

The study showed for the first time that the most important risk factor for long-term survival is the lack of social support, testament to the rigors of coping with the myriad complications of this procedure and its associated therapies. The non-inclusion of the liver in the graft emerged as a second-most significant risk factor for long-term survival. Other predictors included early rejection, recipient age and sex, type of immunosuppression, splenectomy and HLA mismatch.

Chronic renal failure is a common complication after intestinal transplantation with an overall reported incidence of 21.3% at 5 years. The requirement for renal replacement treatment in long-term survivors is also significant and reported to range from 5–9%. Renal failure may be a fatal complication after intestinal transplantation because lack of venous access can be prohibitive for dialysis or even kidney transplantation. Similarly, there is an increased incidence of malignancies, mainly of lymphoid origin post-transplant lymphoproliferative disorder (PTLD). Other, common malignancies (skin, colon, etc.) seem to be more frequent in other solid organ transplantation.

Clearly the leading cause of death for long-term survivors, rejection, should be preventable or treatable. The diagnosis is usually based on biopsies (see Chapter 86) following clinical signs of rejection (abdominal distention, diarrhea or ileus, bloody enteric output). Specific details of the clinical presentation and management of intestinal rejection can be found in Chapter 74. Most of these patients live far from the transplant centers. With the stoma closed, there is no easy access for endoscopy.

We have shown that decreasing serum citrulline levels may be useful for diagnosis. They stabilize at 3 months post-transplant. A decrease from baseline of more than 25% or an absolute value of less than 13–15 m/L in the absence of concurring serious infections are both strong indicators of rejection [5]. Additional research is being carried out at our center and others to create a panel of non-invasive markers of rejection.

There is also increasing evidence suggesting that panel reactive antibody and disease- susceptible antigen levels correlate with the development, severity and response to treatment of acute rejection [6]. Their role in the long-term prognosis is uncertain and currently also the target of scrutiny.

Pediatric growth and development

Children receiving an intestinal transplant despite being weaned off parenteral nutrition, often require prolonged enteral nutrition for catch-up growth beyond the first 2–5 years post-transplant [7–10]. This is likely due to long-standing food aversion and anorexia in some cases.

In a report by investigators from Paris, data on 31 pediatric patients free from PN showed that 84% of children remained PN free, while 45% continued to require enteral nutrition [10]; persistent fat malabsorption made higher energy intake by means of enteral supplementation necessary in order to attain normal growth [10].

Intensive nutritional support that provides 20–30% more calories than the recommendation (by age) will be the key for suitable growth [11].

Patient function, quality of life and graft failure

Rejection is the most common cause of graft loss in intestinal transplantation. Early detection of rejection requires judicious implementation of screening protocols including endoscopy and a few available, yet non-specific, biomarkers (citrulline, calprotectin).

Acute rejection

After intestinal transplant, late-onset acute rejection continues to be an issue in late survivors. One large single-center review, including 36 adult survivors >5 years after intestinal transplant, reported incidence of late acute rejection at 14%. All of these patients had received an isolated intestinal graft and were treated with thymoglobulin [3].

Late acute rejection is commonly associated with non-compliance or follows an episode of gastroenteritis [12,13]. Although late acute rejection is rare, it is often severe, leading to graft loss.

Chronic rejection

Early, recurrent and/or severe episodes of acute rejection predispose a patient to chronic rejection. In addition, recipients of isolated intestinal transplants are at an increased risk for chronic rejection as compared to recipients of liver-containing grafts. A full-thickness biopsy is required for accurate diagnosis of chronic rejection and as such, its clinical diagnosis is often made based only on clinical signs, with the diagnosis confirmed at time of retransplant enterectomy, or sadly, at autopsy. The reported incidence of chronic rejection by many centers is around 15% [12–14]. There is currently no treatment for chronic rejection other than retransplantation.

Retransplantation

Graft failure, defined as a necessity for graft removal, often leads to retransplantation. Reports in the Intestinal Transplant Registry [1,2] showed very poor results for early recipients of intestinal retransplantation. More recently, better outcomes have been reported, with a patient survival of 71% [15]. Similar results were reported in 500 adult and pediatric recipients [16].

Infection

Infection is the most common complication in intestinal transplant recipients and the leading cause of death [1]. Infections are more common early after transplant with 50% occurring within the first 3 months, 25% during months 3–12, and only 25% after 12 months post-transplant [17]. However, even after the early post-transplant period infections account for 60% overall mortality [1]. Late-onset infectious enteritis poses a difficult challenge because presentation resembles acute rejection at a time when stomas are usually closed and immediate access to endoscopy is not available. This becomes even more critical since infectious enteritis can trigger development of rejection [18–20].

Neoplastic disorders

De novo malignancies occur in a significant number of intestinal transplant patients, with a reported incidence of 15% [21]. Of these, 13% were PTLD, and only 3.2% non-lymphoid cancers. PTLD is covered in depth in Chapter 96. Given the higher level of immunosuppression needed to maintain an intestinal transplant free of rejection, and given the large amount of lymphoid tissue present in an intestinal graft, the risk of PTLD is highest in intestinal transplant recipients when compared to other solid organ transplants (SOT). The majority of cases of PTLD are Epstein-Barr virus (EBV) driven with abnormal B cell lymphoproliferation but T cell, Burkitt's lymphoma and EBV negative subtypes have also been described [22]. The most common site of PTLD involvement is the intestinal graft. Overall cumulative risk was 8% at 6 months, 11% at 1 year, 16% at 5 years and 27% at 10 years post-transplant [21]. Primary immunosuppression, recipient age, splenectomy and treatment of rejection were identified as significant risk factors for the development of PTLD. Pediatric recipients are at a higher risk for developing PTLD since the majority of children are EBV-naïve at time of transplant, though the incidence of PTLD has decreased to 7% in most recent cohorts [21]. Management of PTLD has also improved due to early detection by means of blood EBV quantitative polymerase-chain-reaction assay and the use of rituximab [23].

The cumulative risk of non-lymphoid cancer is 4% at 5 years and 10% at 10 years with adults being more commonly affected [21]. Non-lymphoid cancers most commonly included non-melanotic skin cancer. Other malignancies have been described including lung, testicular, breast, uterine, colorectal, vulvar and prostate. Lastly, after intestinal transplant for Gardner's syndrome, desmoid tumors can recur, likely related to immunosuppression.

Graft-versus-host disease

The abundance of donor-derived lymphoid tissue in intestinal grafts accounts for a higher incidence of graft-versus-host disease (GVHD) in intestinal transplant recipients compared to other SOT. Incidence of 5–10% has been reported in intestinal transplant patients as compared to 1–2% of other SOT recipients [24,25]. Mortality is quite high after development of GVHD, especially if the patient fails initial steroid therapy [26]. Most of the deaths are due to ensuing infection and multi-organ failure. Aggressive, proactive monitoring of donor chimerism levels in the peripheral blood of intestinal recipients may allow for an earlier diagnosis just as symptoms arise and prove to be an important longitudinal tool in determining treatment success.

Renal dysfunction

Several studies have shown that intestinal transplant patients appear to have the highest risk of chronic renal failure compared to all other SOT recipients. Ojo et al. [27] were the first to demonstrate this, reporting a 21.3% risk at 5 years post-transplant. These patients tend to have multiple episodes of dehydration before transplant as well as after transplant, and calcineurin inhibitors and episodes of dehydration contribute heavily to the development or worsening of nephrotoxicity.Predictors of eventual renal dysfunction include preoperative eGFR <75%, preoperative ICU location and high-dose FK- immunotherapy. GFR <75% post-transplant was a predictor of poor patient survival [28]. Researchers from the Thomas E. Starzl Institute in Pittsburgh reported that 9% of long-term survivors >5 years post-transplant required renal transplant [4].

Summary

Intestinal transplantation remains a challenging procedure the results of which lag behind most other transplanted organs. While the types of allospecific and protective immune complications are similar in type as those seen across the spectrum of SOT, their magnitude and frequency remains high, and their occurrence persistent. Appropriate patient selection, with particular attention toward family social support and patient cognizance of the risks, is critical for optimal outcomes. Regardless of the challenges, intestinal transplantation remains the best available option for patients in selected circumstances.

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CHAPTER 110

Long-term Outcomes after Vascularized Composite Allograft Transplantation

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Introduction

Vascularized composite allotransplantation (VCA) is a developing field for the reconstruction of multi-tissue defects unable to be ameliorated with autologous tissue. Thus far, these transplants have been used to provide form and function to over 90 patients worldwide. The most common VCAs reported to the international registry include transplantation of the hand(s) and/or digit(s) (54 patients), larynx (16 patients), abdominal wall (9 patients), and face (7 patientss). Other transplants, including knee, uterus, femoral diaphysis, and lower limb have also been reported [1].

VCA is possible today due to advancements in microsurgery and transplantation. The technical aspects of VCA are essentially those of free tissue transfers, techniques that have been extensively used in reconstructive surgery for several decades. Advancements in immunosuppression, after the introduction of cyclosporine, are now being applied to VCA. Similar to other organ transplants, today, the individual immunosuppressive regimens vary amongst the centers performing VCA. However, most patients are currently maintained on multi-drug regimens typically inclusive of tacrolimus, mycophenolate mofetil, and steroids. Considering that VCA is a quality of life (QoL) transplant, a barrier for its more widespread application is the side effects and toxicities related to these conventional immunosuppressive agents.

Acute and chronic rejection in VCA

At the time this chapter is written, acute rejection episodes have been observed in up to 85% of VCA recipients within the first year [2]. There are several theories as to why the incidence of rejection episodes is higher than in solid organ transplantation. First, the dermis and epidermis are rich in dendritic cells, imparting a high degree of immunogenicity to the skin. Secondly, the number of acute rejection episodes diagnosed may partially reflect the fact that we are constantly able to observe the transplanted skin and this may result in detection of rejection episodes that in a solid organ transplant might go unnoticed. Third, the number of tolerance trials in VCA may condition for sub-therapeutic levels of immunosuppression. Finally, VCAs are in constant contact with the environment and the skin can show changes due to environment exposure (temperature, sun exposure, allergens) that may grossly be mistaken for rejection.

In 2005, in an effort to standardize reporting, facilitate prospective data collection and collaboration amongst the various centers performing VCAs, and advance the understanding of VCA pathology, an international consensus group developed the Banff working group. This was published the 2007 working classification of skin-containing composite allograft pathology [3], and now serves as the basis for ongoing reporting of rejection-related histology in VCA.

Chronic rejection and antibody-mediated rejection (AMR) has been reported in VCA and the numbers will likely increase as the case volume and graft survival times accumulate. Possible features of chronic injury in VCA proposed by the Banff 2007 consensus include vascular narrowing, adnexal loss, atrophic changes to skin and muscle, deep tissue fibrosis, nail changes, and myointimal proliferation [3]. To date, there is one report of suspected chronic rejection in VCA in the western countries. This patient, who underwent transplantation in Louisville under the immunosuppression regimen of alemtuzumab induction and mycophenolate mofetil/tacrolimus maintenance therapy, experienced severe graft ischemia necessitating amputation of his transplanted hand at nine months. Examination revealed severe intimal hyperplasia confined to the arteries of donor origin as the cause of graft ischemia. At the time of graft loss, superficial punch biopsies did not show histologic rejection, although deeper tissues contained cellular infiltrates. Class I and II donor-specific antibodies, not detected previously, became positive following immunosuppression cessation and graft excision [4]. Additional information on the clinical features of VCA rejection can be found in Chapter 75, and a more comprehensive treatment of the histopathological presentation of rejection can be found in Chapter 85.

Complications in VCA

Immunosuppression complications

Complications related to immunosuppression include metabolic disturbances, malignancy, and opportunistic infections [5]. Other chapters in this text review the incidence of immunosuppression complications in various solid organ transplants. Reports specific to immunosuppression-related and psychosocial complications in VCA recipients are common and are reviewed in this chapter. VCAs are considered QoL transplants and as such, the risk benefit ratio

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(potential risks: improvement of life) necessitates close examination of additional factors compared to a life-saving transplant.

This chapter will aim to focus on the long-term outcomes of VCA, which to date have been largely undefined. We will define long-term as the period beyond one year post-transplant. As with most emerging fields, quantifying the outcomes of VCA is challenged by the relatively small number of transplants performed to date and, consequently, the very small number of patients available for long-term follow-up. Additionally, the follow-up methods differ greatly amongst the centers performing these transplants, which further hinder the ability to collect unifying data. We must look to the experience of solid organ transplantation for guidance in monitoring long-term outcomes and gathering objective data that can be compared across multiple centers. Renal transplants are commonly monitored by serial serum creatinine, graft failure signified by the need for return to dialysis, and death with and without functioning allografts. Similarly, cardiac and pulmonary transplants are followed functionally by ejection fraction and pulmonary function testing, respectively. As the field moves forward, it will be necessary to standardize objective, functionally significant outcome measures to report. Because the specific risks and methods of follow-up vary greatly among the tissue(s) transplanted, we will now focus on some specific types of VCAs to allow more specific discussion of what are, at least functionally, widely variable transplants.

Hand transplantation

Background

Hand transplantation is technically feasible, with the surgical technique and technical complications (e.g. arterial thrombosis, venous congestion, etc.) similar to those associated with microsurgical replantation of an autologous limb, and covered in detail in Chapter 64. To our knowledge, at the time this chapter is written, 45 patients have received hand transplantation(s) in the western world (23 unilateral and 22 bilateral). However, only 21 unilateral and 18 bilateral patients in western countries have been reported to the International Registry [1]. Furthermore, details have been published in the scientific literature of only 13 unilateral and 14 bilateral patients.

Patient selection

Appropriate patient selection, screening, and a multidisciplinary evaluation are critical; their importance cannot be overemphasized. Due to the novelty of this therapy, candidates may display eagerness to take the risk, but they might not be suitable for the procedure. Candidates may be medically appropriate, but short of proof of compliance, social support, or a methodical follow-up commitment. Patient recruitment should include education, information about international outcomes, standard-of-care options including prosthesis and surgical reconstruction with autologous tissue, immunosuppression, rehabilitation, complications, and the overall transplant process [6].

Debate continues as to whether unilateral amputees should be offered hand transplantation. Rationale for limiting potential candidates to bilateral amputees includes the reality that the QoL after bilateral hand loss is poor even with use of prosthesis, and that bilateral amputees may be more apt to follow rehabilitation programs [7]. It has been suggested that there is a higher propensity for patient dissatisfaction in unilateral transplants because the patient may compare the function of the transplanted hand to that of their native hand [5]. However, based on the reports of patient satisfaction documented in the literature, this hypothesis has not been supported. Furthermore, the replantation literature generally supports efforts at unilateral upper extremity replantation, demonstrating that patients who have unilateral replantations are more likely to have "excellent" or "good" long-term outcomes (by Carroll score) than those patients who undergo revision amputation and use prosthetic devices [8].

The Louisville Instrument for Transplantation (LIFT) analyzes the amount of risk an individual deems acceptable for various VCAs. LIFT utilizes both time trade-off and standard gamble techniques to assess health risks [9,10]. It has shown, in both healthy controls and kidney transplant recipients, that the level of acceptable risk is equivalent for bilateral hand and kidney transplantation. In addition, the risk acceptance for transplantation of a unilateral hand is significantly higher than that for a foot [9].

Outcomes of hand transplantation

Patient mortality

A patient death has been reported two months following combined bilateral hand and face transplant. Contributing to this patient's death was pseudomonas infections of both his hand and face allografts, leading to eventual cardiac arrest and anoxic injury due to airway occlusion [11]. A second death took place within the first week after a bilateral transplantation (personal communication between Martin Iglesias and Linda Cendales, September 2010). No further data has been made available at this time. This translates to a mortality rate of 4.5% among hand transplant recipients.

Allograft survival

Thus far, four patients have experienced allograft loss requiring amputation within one year of transplant. The first was a perioperative loss of a unilateral transplant due to arterial thrombosis [12]. The second patient experienced graft ischemia due to severe intimal hyperplasia at nine months [4]. The third and fourth patients, though not yet reported in the academic literature, have been confirmed by the surgical teams and via media press releases to have lost their hand allografts (one of these performed in conjunction with a facial allograft) within the first week after transplant [13]. Therefore, the rate of hand transplant recipients experiencing graft loss within one year is estimated as 8.9%.

Of the 41 patients in the western world whose hand allografts survived beyond a year, one patient who underwent transplantation in 1998 is reported to have experienced subsequent graft loss. The graft loss was the result of patient non-compliance with his prescribed immunosuppression regimen, leading to graft rejection and the patient's eventual request to have the transplanted hand amputated after 29 months [14]. Therefore, the rate of hand transplant recipients experiencing hand allograft loss after one year is currently 2.4%.

Functional outcomes of hand transplantation

Sensorimotor function

Sensorimotor recovery following hand transplantation is multifactorial. Neural regeneration is critically important and it appears that this regeneration is accelerated in allotransplantation when compared to replantation due to the use of the calcineurin inhibitor tacrolimus, which has been shown to promote axonal regeneration [7]. The time required for muscular reinnervation is one of the reasons that the expected functional outcomes may be correlated to the level of transplantation, with some cases evidencing a faster and more complete return of function with more distal transplants

[15]. This may be, in part, due to the length of time the intrinsic hand muscles remain denervated, resulting in some degree of muscle atrophy following transplantation. After replantation of amputated limbs, functional outcomes vary with the level of injury; the likeliness of regaining useful control increases with more distal amputations [8,16,17]. However, less functional recovery is expected for replantations at certain levels of injury within the hand (Zone II of the fingers) [18].

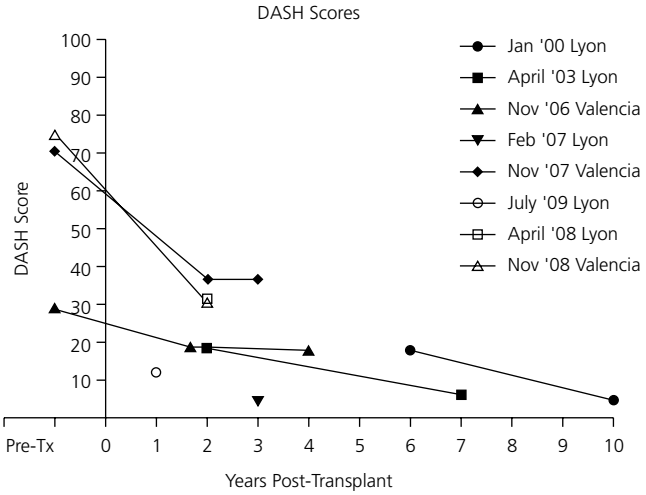
In addition, functional recovery is undoubtedly related to the mechanism of injury, surgical reconstruction, and therapy programs required of all hand transplant recipients, with some authors citing intense rehabilitation and patient compliance as the two most important determinants of success in limb transplantation [19]. Likewise, the upper extremity replantation literature supports compliance with rehabilitation and the psychosocial disposition of the patient as important prognostic indicators for functional outcomes [17]. The time to commence, intensiveness, splinting, modalities (e.g. electro-stimulation, ultrasound), and length of rehabilitation specific for limb transplantation have not yet been established. Currently, every center has adopted a rehabilitation plan following its own experience with reconstructive procedures.

The long-term functional assessment of hand transplant patients is a challenging process, currently being approached in various ways by the teams performing these complex procedures. The majority of measures of limb/hand function were designed to quantify upper extremity disability or for evaluation after replantation. Some of these measurement tools are now being applied to the evaluation of hand transplant patients.

The Carroll test is one of the objective upper extremity scoring devices now being applied to follow functional recovery in hand transplantation. Only three unilateral and one bilateral hand transplant patients (all from the same transplanting center) have had their Carroll scores formally reported in the literature. Besides purely functional measurements such as the Carroll test, several tools attempt to include the impact of hand function on a patient's life. For example, some hand transplant centers are using the Chen score, a measure commonly used to assess hand function after replantation. To stratify function into four grades, the Chen score takes into account range of motion, muscular power, sensibility recovery, and ability to achieve independence and/or return to work. A change in Chen score can be used to compare pre and post-transplant functional status. However, the Chen score has not been tested for validity, responsiveness, or reliability; and at any given time, a patient may not neatly fall into a single category [20]. Tamai's and Ipsen's scores are similar scoring systems to Chen's, with modifications primarily related to the level of detailed evaluation and the number of digits examined [21]. Among the five hand transplant patients who have had their Chen scores reported in the literature, all patients have achieved Chen Grade II status post-transplant [15,20,22].

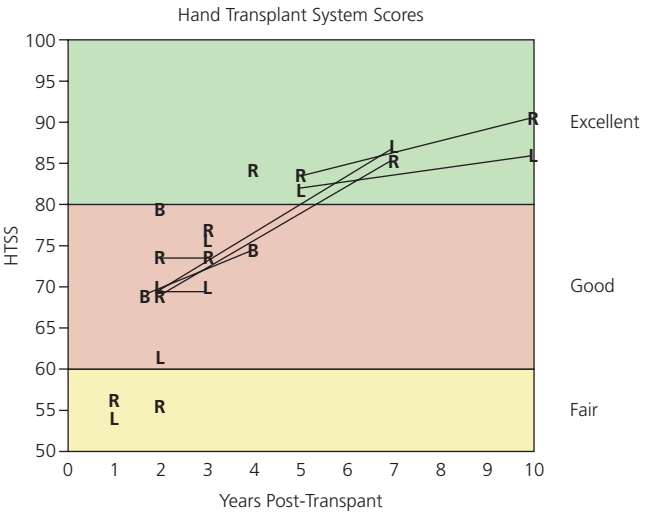
The DASH (Disability of the Arm, Shoulder, and Hand) Score is a validated 30-item patient-reported disability measure of the upper limb in which a higher score reflects a greater level of disability (Figure 110.1). It asks patients to score on a five point scale their ability to perform various tasks, limitations posed by their upper extremity deficit, pain level, and interference with self-image and social activities [23]. One benefit of the DASH score is that it can be used to comprehensively compare the amount of disability before and after transplantation.

The International Registry of Hand and Composite Tissue Transplantation put together a Hand Transplant Score System (HTSS) in 2007 (Figure 110.2). This score system proposes a tool for reporting of functional results amongst the various centers but has not yet been validated. The HTSS provides a weighted score (maximum 100 points) evaluating six aspects of hand function: movement; sensibility; appearance; psychological and social acceptance; daily activities and work status; and patient satisfaction and general well-being [2]. One limitation of the HTSS is that it is solely a measure of post-transplant function and therefore, the clinician is unable to directly compare a patient's outcome to their pretransplant baseline. Of the nine patients whose teams have reported their HTSS scores, the longest available follow-ups have most patients falling within the "good" range [14,20,22] (Table 110.1).



[13, 19]

Figure 110.1. DASH scores reported in hand transplantation.



[13, 14, 19, 21, 29]

Figure 110.2. HTSS reported in hand transplantation.

Psychosocial outcomes

Given that the primary goal of hand transplantation is to improve the QoL of the recipients, patient satisfaction with their transplanted limb is perhaps one the most important outcomes

Table 110.1. Hand transplantation score system

Appearance (maximum 15 points)		
Skin color and vascularization	Normal	3 points
	Abnormal	0 points
Skin texture	Normal	3 points
	Abnormal	0 points
Hair growth	Normal	3 points
	Abnormal	0 points
Nail growth	Normal	3 points
	Abnormal	0 points
Matching with contralateral hand (monolateral tx: size, color, texture)	Excellent	3 points
	Good	2 points
	Fair	0.5 points
	Poor	0 points
Matching with upper limb/body (bilateral tx)	Excellent	3 points
	Good	2 points
	Fair	0.5 points
	Poor	0 points
Movement (maximum 20 points)		
Active ROM		
Forearm (combined pronation-supination)		
	>150	2 points
	>120	1 point
	>90	0.5 points
Wrist (combined flexion-extension)		
	>90	2 points
	>45	1 point
	> 25	0.5 points
Thumb & long fingers (total digital ROM of contralateral or normal hand – %)		
	>50	2 points
	>25	1 point
	>10	0.5 points
Strength (Jamar dynometer)		
Grip	>10kg	2 points
	>5kg	1 point
	>2.5kg	0.5 points
Pinch	>2kg	2 points
	>1kg	1 point
	>0.5kg	0.5 points
Intrinsic muscle activity		
	Clinically useful	6 points
	EMG detectable	3 points
	None	0 points
Cortical reintegration of the hand		
	Yes	4 points
	No	0 points
Psychological and Social Acceptance (maximum 15 points)		
Social behavior (1 point each behavior)		
Holding/shaking hands		
Feeling well in a group		
Overcoming sense of embarrassment		
Sense of being accepted		
Ability to create new relationships		
Being able to overcome handicap		
Satisfactory global social acceptance		
Affectiveness (1 point each aspect)		
Caressing		
Hugging		
Touching		
Sense of intimacy with partner		
Satisfactory global affectiveness		
Body Image (1 point each aspect)		
Sensation of having a complete body		
Self-confidence in personal appearance		
Use of jewelry (watch, etc) on hand(s)		
Sensibility (maximum 20 points)		
Tactile sensation (Semmes-Weinstein Monofilament testing)		
Median nerve	Green (1.65–2.83)	3 points
	Blue (3.22–3.61)	3 points
	Purple (3.84–4.31) 2 points	
	Red (4.56)	1 point
	Red (6.65)	0 points
Ulnar nerve	Green (1.65–2.83)	3 points
	Blue (3.22–3.61)	3 points
	Purple (3.84–4.31) 2 points	
	Red (4.56)	1 point
	Red (6.65)	0 points

(*Continued*)

Table 110.1. (*Continued*)

Protective sensation (hot-cold-pain)		
Yes (median-ulnar)		5 points
Yes (median)		2 points
Yes (ulnar)		1 point
No		0 points
Radial nerve		1 point
Discriminative sensation		
Median nerve	S2PD-grade S4	3 points
	S2PD-grade S3+(7–12 mm)	2.5 points
	S2PD-grade S3 (>15 mm)	1.5 points
	S2PD-grade S2 (none)	0 points
Ulnar nerve	S2PD-grade S4 (2–6 mm)	3 points
	S2PD-grade S3+(7–12 mm)	2.5 points
	S2PD-grade S3 (>15 mm)	1.5 points
	S2PD-grade S2 (none)	0 points
Sweating	Normal	2 points
	Abnormal	0 points
Daily activities and work status (maximum 15 points)		
Activities of daily life (1 point for each activity)		
Driving/riding a bicycle		
Combing hair/personal hygiene/shaving		
Grasping glass		
Pouring water from bottle		
Using cutlery/chopsticks		
Brush teeth		
Holding hands		
Writing		
Symmetrical use of hands		
Work Status		
	Employed	6 points
	Unemployed	0 points
Patient satisfaction and general well being (maximum 15 points)		
Patient satisfaction		
	Very satisfied	5 points
	Satisfied	3 points
	Unsatisfied	0 points
Well-being	Physically and mentally healthy	5 points
	On pharmacological treatment for side effects	0 points
	Permanent side effects/pathologies from drugs	−5 points
Quality of life	Improved a lot	5 points
	Improved	3 points
	Same	0 points
	Worsened	−3 points
	Worsened a lot	−5 points

Reproduced from [2] Lanzetta M, Petruzzo P, Dubernard JM et al. Second report (1998–2006) of the international registry of hand and composite tissue transplantation. Transplant Immunologyl 2007;18:1–6, Copyright 2007, with permission from Elsevier.

measured. Nonetheless, few transplant centers have reported formal QoL measures [12]. However, of the hand transplant patients reported in the scientific literature (13 unilateral and 14 bilateral), over 51% are independent in daily activities and/or have returned to employment after transplantation.

In addition to the patient who requested amputation after medication non-compliance, thus far there is only one report in the literature of a patient being unsatisfied with the achieved functional result. This patient experienced a burn to his transplanted limb, which resulted in ankylosis of the right elbow [14].

Cortical reorganization after hand transplant

Cortical plasticity in VCA, particularly hand transplantation, is an area of interest being studied by several groups. The primary motor cortex (M1) contains the motor homunculus, a somatosensory "map" of individual body segments oriented along the central sulcus [24]. Non-invasive modalities to investigate cortical changes include functional MRI (fMRI) and transcranial magnetic stimulation (TMS). It is known that the brain is an ever-evolving structure and cortical changes begin to occur in as little as 24 hours following amputation of an upper extremity [25]. Hand transplantation offers the opportunity to study the reversibility of these cortical changes (sometimes years after amputation), the extent to which allografted limbs can integrate into a recipient's M1, and the potential prognostic, rehabilitative, or functional implications derived from such information. It has been demonstrated in a limited number of patients that the cortical reorganization that occurs in amputation can be reversed following hand transplantation and that newly transplanted intrinsic muscles are capable of acquiring representation within the recipient motor cortex [26,27].

Complications reported in hand transplantation

Immunosuppression complications

The most common metabolic complications reported in hand transplantation are renal dysfunction, hyperglycemia, and dyslipidemia (Table 110.2). Viral infections have included at least five patients with cytomegalovirus and one patient each with herpes simplex virus I, Ebstein-Barr virus, and varicella zoster virus

Table 110.2. Complications reported in hand transplant recipients

Transplant date, location	Complications reported in unilateral hand transplant recipients			Citations
	Metabolic	Infectious	Other	
September 1998 Lyon, France	hyperglycemia, increased creatinine	HSV	non-compliance resulting in graft loss at 29 months	[14,15,65]
January 1999 Louisville, USA	none reported	CMV	none reported	[4,66]
Feb 2001 Louisville, USA	diabetes	none reported	osteonecrosis (bilateral hips), non-compliance resulting in rejection episodes	[4,66]
April 2006 Wroclaw, Poland	transient hyperglycemia	none reported	none reported	[12,22]
November 2006 Louisville, USA	hyperlipidemia	CMV (colitis)	severe neutropenia, forearm seroma requiring debridement and skin grafting, marginal zone lymphoma (unusual B and T cell clones in blood)	[4,66]
January 2008 Wroclaw, Poland	none reported	none reported	perioperative graft loss due to arterial thrombosis	[12]
July 2008 Louisville, USA	none reported	none reported	graft loss at 9 months (intimal hyperplasia)	[4]
September 2008 Wroclaw, Poland	hyperglycemia	none	none reported	[12]
November 2008 Louisville, USA	none reported	none reported	none reported	[4]
October 2009 Wroclaw, Poland	none reported	CMV infection	none reported	[12]
January 2000 Lyon, France	hyperglycemia	none reported	serum sickness	[14,15]
March 2000 Innsbruck, Austria	increased creatinine, dyslipidemia, diabetes	CMV	bronchospasm/hypotension in response to thymoglobulin, AV fistulas (requiring ligation)	[5,67–69]
February 2003 Innsbruck, Austria	none reported	CMV	none reported	[28,67]
April 2003 Lyon, France	none reported	osteomyelitis (left ulna)	perioperative thrombosis of left ulnar artery	[14,15]
November 2006 Valencia, Spain	hypertension, diabetes, dyslipidemia	VZV reactivations	psychiatric factitious disorder manifesting as acute vision loss, mouth ulcers, basal cell carcinoma (nose)	[20,29,70,71]
February 2007 Lyon, France	none reported	EBV	none reported	[14]
November 2007 Valencia, Spain	increased creatinine, hypertension	mixed/fungal cutaneous infection	cytokine release syndrome (rash, hypotension, resistance to mechanical ventilation) in response to alemtuzumab, mouth ulcers	[20,72]
November 2008 Valencia, Spain	increased creatinine, worsening of hypertension, diabetes, dyslipidemia	none	anemia, headache	[20,73,74]
July 2009 Lyon, France	hyperglycemia	none	neutropenia-related oral cellulitis, thrombosis of right radial and left ulnar arteries on POD 5	[14]
April 2009 Paris, France*	none reported	pseudomonas infection of allografts	cardiac arrest, death	[11]
August 2010 Louisville, USA	none reported	none reported	perioperative arterial thrombosis, ischemic necrosis requiring amputation of thumb through IP and small finger through DIP	[4]
June 2010 Wroclaw, Poland	polyuria with normal renal function	none reported	perioperative arterial thrombosis, epidermolysis and necrosis of distal left phalanges requiring amputation	[12]
May 2011 Boston, USA*	none reported	pneumonia	perioperative bilateral hand allograft loss	[13]

CMV, cytomegalovirus; VZV, Varicella zoster virus; EBV, Ebstein-Barr Virus; POD, postoperative day; * simultaneous facial allograft

[4,5,12,14,20,28]. Bacterial infections of the allografts have been reported [11,13,14]. There is one report of cutaneous fungal infection [20]. There are two reports of oncologic complications in the form of basal cell carcinoma of the nose and a marginal zone lymphoma [4,20].

Psychosocial complications

At the time this chapter is written, non-compliance with immunosuppression has been reported as the causative factor in the loss of one unilateral hand allograft [14]. Factitious disorder, manifested as acute vision loss, has also complicated the care of one patient [29].

Face transplantation

Background

The first face transplant was performed in 2005 and to date, 18 facial allografts have been performed worldwide [1,30,31]. However, only seven of these transplants have been reported to the International Registry [1] and only eleven transplants (representing eight transplant teams) have published their outcomes in the scientific literature. The limited number of facial allografts reported shows that the procedure is technically feasible and has the potential to restore functional and aesthetic properties that may not be attainable through traditional reconstruction. However, this potential comes at a significant risk including death.

Several studies have been conducted to determine the relative risk that people would accept in order to receive a face transplant. It has been demonstrated that both healthy subjects and patients who have already received a kidney transplant (thereby already susceptible to immunosuppression risks) would accept a higher level of risk to receive a face transplant than a kidney transplant [9]. The highest level of risk was accepted for a full-face transplant as compared to five other proposed vascularized composite allografts and kidney transplantation [9]. In a population of people with facial disfigurements, 77% would undergo face transplantation after being presented with 20 potential immunosuppression side effects and 71% would undergo a face transplant if the chance of rejection was 50% in the first year [10].

Outcomes of face transplantation

Patient mortality

There have been two deaths in recipients of face transplants, bringing the percentage of fatal outcomes to greater than 11%. The first

Table 110.3. Sensorimotor outcomes of face transplantation

Transplant date, location	Motor outcome	Sensory outcome	Citations
November 2005 Amiens, France	1 week – able to eat/drink 6 months –complete labial contact 18 months – symmetric smile	6 months – normal temperature and light touch	[34]
2006 Xi'an, China	2 years- able to eat, drink, and talk but facial nerve not fully functional. No symmetric smile. Patient death at 27 months.	3 months –discriminatory sensation restored 8 months – temperature sensation restored	[32,38]
January 2007 Paris, France	POD 10 – Able to speak/eat EMG: 3 months-minor motor response to facial nerve stimulation (left orbicularis oculi) 6 months: activity during voluntary contraction of left orbicularis oculi and bilateral orbicularis oris 9 months: spontaneous mimicry 12 months: motor reinnervation of facial and trigeminal territories (motor responses to nerve stimulation and involuntary blink reflex)	3 months – mechanical and temperature sensation present	[11,39]
December 2008 Cleveland, USA	Can eat/drink, smell, purse lips.	Not reported	[32]
March 2009 Paris, France	POD 24 – intelligible speech 8 months: complete mouth closure	Deep pressure sensitivity regained	[11]
April 2009 Paris, France	N/A – patient death at 2 months	N/A – patient death at 2 months	[11]
April 2009 Boston, USA	Immediate speech improvement. POD 3 – oral intake 1 year: nasal breathing, able to smell, symmetric smile	By 6 months – sensation to light touch, pinprick, cold; 2PD = 15 mm	[41]
August 2009 Valencia, Spain	16 months – able to swallow (transplant included tongue)	Not reported	[75]
August 2009 Paris, France	POD 20 – intelligible speech 8–12 months: complete mouth closure	Not reported	[11]
January 2010 Seville, Spain	Improved speech, able to eat By 6 months: Motor recovery of levator labi and buccinator muscles.	3 months – sensory recovery began 6 months – near complete sensation to pain and temperature	[35]
March 2010 Barcelona, Spain	10 weeks – soft diet 120 days – EMG showed initial signs of muscle activity.	4 months – sensation recovery in forehead, eyelids, cheeks, and intraoral mucosa. No sensation to lips.	[40]

of these deaths is presumed due to immunosuppression non-compliance and multi-organ failure 27 months post-transplant [32]. The second death occurred two months following combined facial and bilateral upper extremity transplantation. This death resulted from anoxic cardiac arrest following tracheostomy occlusion, resulting in an irreversible brain injury [11,31]. However, the pathology leading to this series of fatal complications was an overwhelming pseudomonas infection of the allografts.

Allograft survival

At the time this chapter is written, no cases of facial graft loss have been reported. The consequences of a facial allograft loss are potentially profound when considering the limited options for reconstruction. As graft loss is to be anticipated in at least some sub-set of recipients at some point post-transplant, the management of facial allograft loss is certain to be a critical topic in determining the risk-benefit ratio of this procedure.

Functional outcomes of face transplantation

Sensorimotor function

The goal of face transplantation is both functional and cosmetic. From a functional standpoint, the ideal outcome would allow for emotional expression through animated facial expression, effective mastication allowing for normal eating, improved speech, ability to control oral secretions and breathe nasally, and allow for protection of the eyes. All of these functions are dependent on the regeneration of peripheral nerve function within the allograft. Several invasive and non-invasive modalities have been used to evaluate motor and sensory recovery after face transplantation. However, at this time there is no standard battery of tests to evaluate function following face transplantation and this makes the objective comparison of outcomes across centers extremely difficult (Table 110.3).

Psychosocial outcomes

The social importance of an aesthetically acceptable face should not be underestimated. The goal is an outcome superior to what could be obtained through conventional reconstructive techniques. A severely disfigured face is detrimental to a person's ability to interact normally with society and negatively impacts self-esteem and QoL [30]. The impact of severe facial disfigurement on QoL has been shown to be comparable to serious medical conditions such as HIV, diabetes mellitus type I, and end stage renal disease (ESRD) requiring hemodialysis [33]. It has been shown in a sample of the general population that if afflicted with a severe facial deformity, they would be willing to accept a 34% risk of death and would trade 12 years of their life to undergo face transplantation [33]. Most of the reported outcomes regarding QoL following face transplant are qualitative, with teams reporting patients returning to work and reintegrating into their premorbid social lives [11,34,35]. At this point, few quantitative measures of QoL following face transplant are reported in the literature.

Aesthetic outcomes

One of the ethical concerns raised in face transplantation is the potential transference of donor appearance to the recipient. Anatomic studies have been conducted in cadaveric models of subsuperficial musculoaponeurotic system face transplantation and demonstrate that the transplanted faces more closely resemble the recipient than the donor for some anthropometric measurements (skull base width, craniofacial height); while other aspects

(intercanthal width, middle third depth) are approximately equally similar to donor and recipient appearance [36]. Cadaveric study of osteocutaneous face transplantation has demonstrated grossly visible similarity to the donor face [37]. The presence or degree of appearance transfer in facial transplants containing both bone and soft tissue is an ongoing area of inquiry [30].

No formal instrument is in place to measure aesthetic improvement after face transplantation, but the subjective improvement is in the published reports. At the time this chapter is written, there are no reports of patient dissatisfaction with their esthetic result and there are numerous reports of patients being satisfied with their appearance, resuming normal social lives, and returning to work (Figure 110.3) [11,34,35,38–41].

Complications reported in face transplantation

Immunosuppression complications

Of the 11 patients with reported outcomes, two have experienced renal dysfunction and two have developed new-onset diabetes after transplantation [34,35,38,41]. Six of the 11 patients have had infectious complications [11,34,38,42]. Viral infections have included two patients with cytomegalovirus, two patients with herpes simplex virus I, and one patient with a poxvirus [11,34,42,43]. Bacterial infections of the allografts, lungs, and gastrointestinal tract have been reported [11,41,43]. Oral candidiasis has also been reported in one patient [44]. There is one report of oncologic complication in the form of carcinoma in situ of the cervix detected 50 months after transplant [43].

Psychosocial complications

Non-compliance with immunosuppression has contributed to the death of one face transplant recipient (Table 110.4) [32].

Abdominal wall transplantation

Background

Abdominal wall transplantation is carried out in conjunction with small bowel or multivisceral organ transplantation in patients with either loss of abdominal domain or severely damaged abdominal walls due to multiple surgeries, enterocutaneous fistulae, or tumors. The functional goal of the transplanted abdominal wall is to allow closure of the abdomen after multivisceral transplant and to provide ongoing coverage and support for underlying organs. Therefore, the immunologic considerations and follow-up of abdominal wall transplants is unique because so far, the recipients have required lifelong immunosuppression for their primary transplants and the role of the VCA is supporting.

Outcomes of abdominal wall allografts

At the time this chapter is written, there are 15 abdominal wall transplants in a total of 14 patients reported in the literature, with only nine of these cases having been formally reported to the international registry [1,45]. Of these 14 patients, four have been pediatric recipients. All patients underwent another transplant in conjunction with the abdominal wall (nine intestine, four multivisceral, two multivisceral without liver). Two patients received their abdominal wall allografts in a second surgery days after their visceral transplant. All patients received induction immunosuppression with alemtuzumab (anti-CD52) and maintenance with tacrolimus monotherapy, except one child who received daclizumab induction and tacrolimus and methylprednisolone for maintenance [45].

Two of the 15 reported abdominal wall transplants have been lost to vascular thrombosis within one week of transplant (one patient underwent subsequent retransplantation with a second abdominal wall), bringing the rate of perioperative graft loss to 13%. One patient was subsequently retransplanted with a second abdominal wall [45].

Of the 14 patients whose allografts survived the immediate postoperative period, nine patients have died due to sepsis, multi-organ failure or, in one case, lymphoproliferative disease. No deaths have been attributable to the abdominal wall allograft. Of the five surviving patients, one abdominal wall allograft failed. As of the most recently published reports, the longest follow-up of an abdominal wall allograft is 7.1 years (Figure 110.4) [45].

There is also one reported case of a vascularized posterior rectus sheath fascia transplanted to allow abdominal wall closure in a pediatric liver and double kidney recipient. In this case, a 10×20 cm segment of posterior rectus fascia was procured in continuity with the falciform ligament and received blood supply via the single hepatic artery anastomosed as part of the orthotopic liver transplant. Immunosuppression consisted of steroid induction and maintenance with steroids, tacrolimus, and daclizumab. No signs of rejection were noted on tissue biopsies. However, the patient died due to fungal septicemia of a presumed respiratory source 51 days following transplant [76].

Laryngeal transplantation

Background

Conventional methods to restore laryngeal function after traumatic or oncologic loss are not ideal and patients without a functioning larynx experience impairment of their QoL [47]. Ability to speak is lost, inability to protect the upper airway results in tracheobronchial infections, and taste and smell may be diminished. Similar to issues raised with other QoL transplants, debate exists whether these functions justify surgical and immunosuppression risks of transplant.

Several studies have examined QoL with regard to laryngeal transplant [48–50]. Twenty percent of healthy volunteers, if faced with laryngeal cancer, would choose radiation therapy over surgery in order to retain natural speech, even though this choice imparts a 20–30% lower survival rate at three years [50]. In one study of laryngectomees, 75% would undergo laryngeal transplantation under ideal conditions and this number was not significantly lower in situations where a stoma had to be maintained or a prolonged hospital stay was necessary. Furthermore, one-half of laryngectomees would accept transplantation even if it did not result in a normal voice but less than 20% were willing to risk their lives for this procedure [48]. Laryngectomees as a group are much less risk-accepting than kidney transplant recipients and healthy controls [49]. Despite this risk aversion, over one-half were still interested in laryngeal transplant after being presented with the side effects of immunosuppression and over one-third would pursue a transplant with a 50% chance of rejection [49]. The vast majority of patients who undergo laryngectomy have cancer. The appropriateness of transplant immunosuppression in oncology patients is controversial, especially in QoL transplants.

Sixteen laryngeal transplants have been reported to the International Registry, the first performed in Cleveland, Ohio, in 1998 and the remainder from a team in Colombia [1].

However, of these16 known laryngeal transplants, only one patient's outcomes have been formally reported in the literature

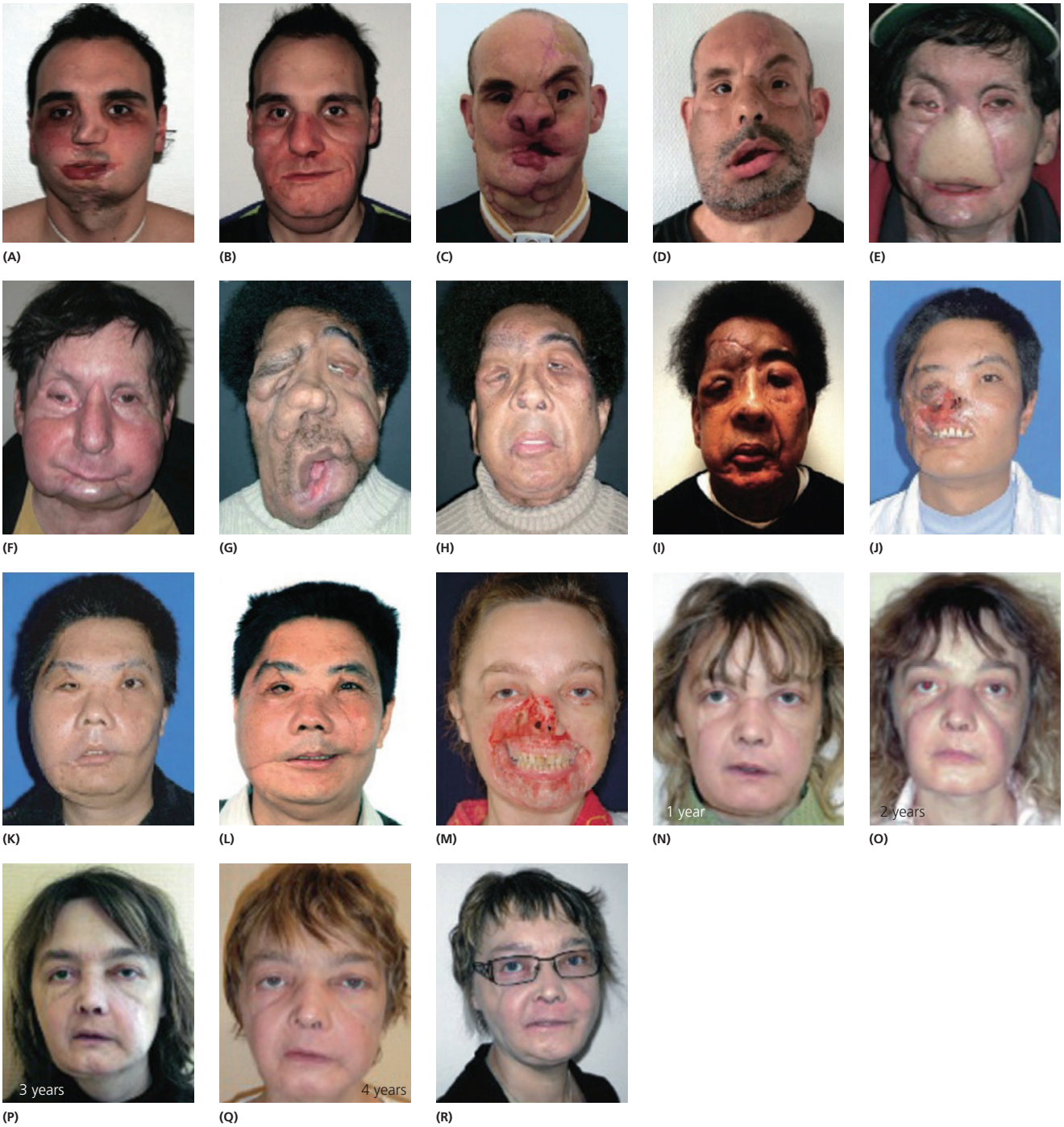
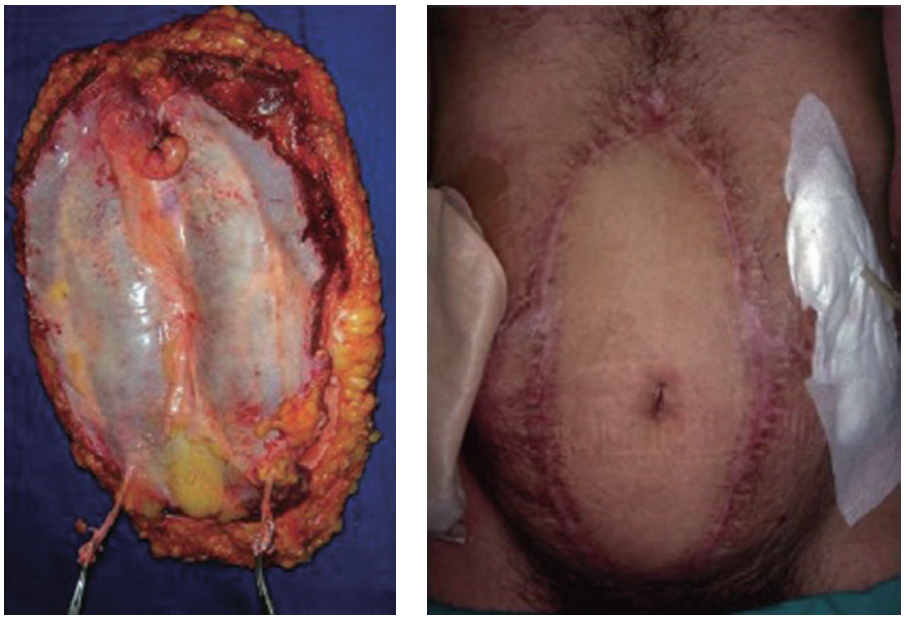


Figure 110.3. Aesthetic outcomes of face transplantation. (A) Pre-op. (B) 11 month follow-up. (C) Pre-op. (D) 1 year follow-up. (E) Pre-op. (F) 1 year follow-up. (G) Pre-op. (H) 1 year follow-up. (I) 3 year follow-up. (J) Pre-op. (K) 20 month follow-up. (L) 2 year follow-up. (M) Pre-op. (N) 1 year follow-up; (O) 2 year follow-up; (P) 3 year follow-up; (Q) 4 year follow-up; (R) 5 year follow-up. (A–D, I) Reproduced from [11] Lantieri L et al. with permission from John Wiley and Sons. (E,F) Reproduced from [41] Pomahac et al. with permission from John Wiley and Sons. (G,H) Reproduced from [39] The Lancet ,Vol 372. Lantieri L, Meningaud JP, Grimbert P et al. Repair of the lower and middle parts of the face by composite tissue allotransplantation in a patient with massive plexiform neurofibroma: a 1-year follow-up study. Lancet, 2008;639–645. Copyright 2008 with permission from Elsevier. (J,K,L) Reproduced from [38] The Lancet, Vol 372. Guo S, Han Y, Zhang X et al. Human facial allotransplantation: a 2-year follow-up study. Lancet 2008;631–638, Copyright 2008 with permission from Elsevier. (M) Reproduced from [44] The Lancet, Vol 368. Devauchelle B, Badet L, Lengelé B et al. First human face allograft: early report. Lancet, 2006;368:203–209, Copyright 2006 with permission from Elsevier. (N–R) Reproduced from [43] Petruzzo P, Testelin S, Kanitakis J et al. First human face transplantation: 5 years outcomes. Transplantation, 2012;93:236–240, with permission from Wolters Kluwer Health.

Table 110.4. Complications reported in face transplantation

Transplant date, location	Complications reported in face transplant recipients			Citations
	Metabolic	Infectious	Other	
November 2005 Amiens, France	Thrombotic microangiopathy (thrombocytopenia, hemolytic anemia, acute renal failure, hypertension), renal dysfunction, hypertension, dyslipidemia	HSVI (lips), Molloscum contagiosum (cheeks), Candida stomatitis Bilateral bacterial pneumopathy	Carcinoma in situ of cervix, cholangitis	[34,43,44]
2006 Xi'an, China	diabetes	none	non-compliance, death	[32,38]
January 2007 Paris, France	none	CMV viremia	transient steroid induced confusion	[11,39]
December 2008 Cleveland, USA	none	CMV viremia, C.difficile and Aeromonas diarrhea	neutropenia	[42]
March 2009 Paris, France	none	Pseudomonas pneumonia	None	[11]
April 2009 Paris, France	none	Pseudomoas infection of allografts	cardiac arrest, death	[11]
April 2009 Boston, USA	diabetes	none	rosacea transferred from donor	[41]
August 2009 Valencia, Spain	none	none	benign pseudosarcomatous spindle-cell postsurgical nodule (tongue)	[75]
August 2009 Paris, France	none	HSVI (lips)	None	[11]
January 2010 Seville, Spain	renal dysfunction	none	None	[35]
March 2010 Barcelona, Spain	none	none	acute oro-cutaneous fistula, parotid sialocele	[40]



(A) (B)

Figure 110.4. Abdominal wall allografts. (A) Abdominal wall allograft with two epigastric pedicles. (B) Abdominal wall allograft three months after transplant. Reproduced from [64] with permission from John Wiley and Sons.

[51,52]. The recipient of this transplant was a 40-year-old man who had been aphonic for 20 years due to crush injury sustained in a motorcycle accident. The transplant included larynx, trachea, pharynx, and thyroid and parathyroid glands. Immunosuppression included cyclosporine, mycophenolate mofetil, methylprednisolone, and steroids. Tacrolimus replaced cyclosporine after 15 months. Additionally, he received muromonab-CD3 for the first week after transplant [51]. No further follow-up has been reported.

Outcomes of laryngeal transplantation

In the first case, first speech occurred on postoperative day 3. By 4 months, the right vocal cord assumed midline position and by 6 months the left vocal cord was para-midline. Reinnervation of bilateral vocal cords and cricopharyngeus muscles was confirmed by EMG. By 36 months, all speech characteristics fell within normal range. No thyroid abnormalities were encountered. After 14 weeks, return of an adequate swallow allowed for oral feeding without aspiration. Olfactory and taste senses have returned. The patient reported QoL improvement and is employed as a motivational speaker [51].

Complications reported in laryngeal transplantation

Immunosuppression complications

Hypertension and increased creatinine responded to oral antihypertensive agents and cyclosporine dose reduction. Infectious complications included oropharyngeal thrush, tracheobronchitis, and Pneumocystis carinii pneumonia [51].

Table 110.5. Outcomes of knee joint transplantation

Transplant date	Function achieved	Complications	Allograft/limb outcome	Citations
April 1996	6 months – complete osseous integration 12 months – full ROM	Vascular pedicle occlusion, implant infection after TKA	Rejection at 15 months. TKA. AKA.	[53]
November 1996	12 months – stable joint, able to walk and participate in sports, returned to work	Non-compliance with immunosuppression leading to graft loss, implant infection after TKA	Rejection at 36 months. TKA. AKA.	[53]
December 1996	N/A	Surgical site infection	Allograft removal at 5 weeks. Arthrodesis.	[53]
July 1997	Stable joint, able to walk, returned to work (after TKA)	Fatigue fracture at 2 years, implant infection after TKA	Rejection at 24 months. TKA. AKA.	[53]
February 1998	Full ROM after TKA	Fatigue fracture at 14 months, infection after re-injury following TKA.	Rejection at 14 months. TKA. Arthrodesis	[53]
April 2002	8 weeks – partial weight bearing 6 months – returned to work 1.5 years – good ROM, full weight bearing 4 years – full weight bearing, satisfactory ROM	Infection resulting from necrosis/ischemia from vessel changes attributed to rejection	Rejection at 56 months. AKA.	[53,54]

TKA, total knee arthroplasty; AKA, above knee amputation; ROM, range of motion.

Psychosocial complications

An episode of patient non-compliance with the prophylaxis regimen resulted in the aforementioned pneumonia [51].

Knee joint transplantation

Background

Knee joint allotransplantation attempts to reconstruct a functional joint damaged by extensive trauma or infection. The International Registry records six knee transplants performed at a single center in Murnau, Germany [1]. All of the recipients had suffered loss of bone, cartilage, and experienced deficient extensor mechanisms of the knee joint due to trauma or infection [53]. No operative complications were reported and cold ischemia times ranged between 18 and 25 hours [53]. Five of the knee transplant patients received induction therapy with thymoglobulin, cyclosporine, azathioprine and steroids and two-drug maintenance immunosuppression with cyclosporine and azathioprine. The sixth knee transplant patient received induction therapy with thymoglobulin, mycophenolate mofetil, tacrolimus, and steroids and maintenance immunosuppression with mycophenolate mofetil, tacrolimus, and steroids [53,54].

Outcomes of knee joint transplants

No patient deaths subsequent to vascularized knee joint transplantation have been reported. One knee allograft was lost due to surgical site infection at 5 weeks. The remaining five allografts were lost to complications related to rejection at 14, 15, 24, 36, and 56 months [53,54]. Of note, the graft loss at 36 months was subsequent to patient non-compliance with immunosuppression [54]. Clinical signs of rejection included joint instability, decreased range of motion, and swelling [54]. Four patients have eventually required above knee amputation and the remaining two underwent arthrodesis [53,54].

A significant problem encountered in the first five knee transplants was an inability to immunologically monitor the allograft since there was no visible portion and no defined biomarker has been identified. The transplant team used local signs of inflammation to herald rejection, but this likely resulted in undiagnosed episodes of rejection throughout the clinical course [55]. To better monitor the transplant with a visible transplanted tissue, the sixth patient received a vascularized skin segment in conjunction with the knee, which was used for immunologic monitoring. Important contributions to VCA made by the group's experience are the blood vessel changes observed in some of the patients. The first patient in this series experienced vascular pedicle occlusion at 15 months, which when considered in conjunction with perivascular lymphocytic infiltration, has retrospectively been deemed rejection-related [56]. The final patient in this series also experienced graft loss due to vessel occlusion. Predicated by superficial skin necrosis of the sentinel skin graft, biopsies from the synovial and soft tissues of the allograft revealed perivascular infiltrates, intimal proliferation, and concentric obliteration or occlusion of arteries. These vessel changes led to graft necrosis, deep infection, and eventual graft loss. The transplant team suggest this myointimal proliferation as a sequelae of ongoing rejection [54].

No metabolic or oncologic side effects of immunosuppression were reported. However, 100% of the knee allograft recipients had their clinical courses complicated by infections [53–55]. Given that all patients eventually experienced allograft failure requiring amputation, more investigation is taking place relative to the acute, antibody-mediated, and chronic alloimmune injury (Table 110.5 and Figure 110.5) .

Femoral diaphysis transplantation

Background

The International Registry records three femoral diaphysis transplants performed at a single center in Murnau, Germany [1]. The bone defects ranged from 12–33 cm and were due to osteomyelitis in two cases and chondrosarcoma with a 5-year period of disease-free survival in one case. No operative complications were reported and cold ischemia times ranged between 16 and 25 hours. Immunosuppression was initiated with four-drug therapy of cyclosporine, azathioprine, anti-T lymphocyte globulin, and methylprednisolone. Patients were converted to two-drug maintenance therapy with cyclosporine and azathioprine after one week [57].

Outcomes of femoral diaphysis transplants

No patient deaths have been reported subsequent to vascularized knee joint transplantation. Two patients achieved bone healing [56]. The third patient necessitated allograft removal due to

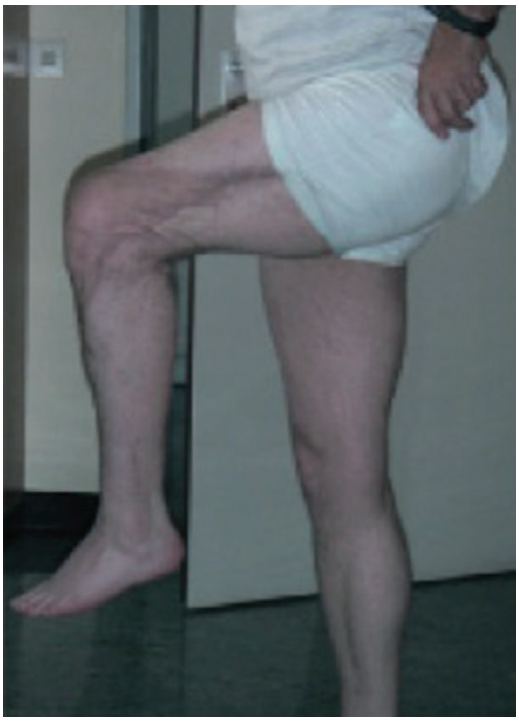


Figure 110.5. Range of motion 1.5 years after vascularized knee joint allotransplantation. Reproduced from [53] with permission from John Wiley and Sons.

recurrent deep infection at 18 months and underwent autologous bone graft reconstruction [55]. All three patients are reported to be fully-weight bearing with unencumbered flexion of knee and hip joints [57].

Other VCAs

Lower extremity transplantation

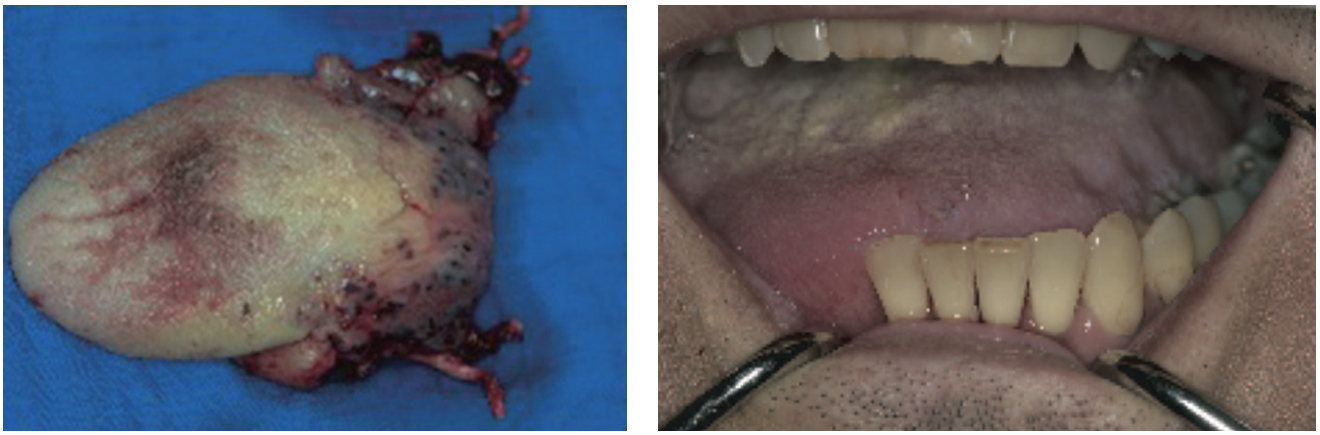
There is one report of a unilateral lower limb transplantation from one ischiopagus twin, afflicted with an unsurvivable cardiac anomaly, to her twin. This transplant was performed in 2003 on a three-month-old patient, who has now been followed for over 6 years. By 30 months after transplant, voluntary leg movement and gross sensation was observed. Improvement continued and has resulted in a normal tone limb with good strength in all movements other than reduced dorsi- and plantar flexion of the foot. Patellar and plantar reflexes are absent. The transplanted limb is shorter than the native leg and this results in a pelvic tilt and Trendelenberg gait. However, the child is able to run short distances, participate in sports, attend school, and has experienced no significant psychological issues (Figure 110.6) [58].

Although not yet reported in the academic literature, a surgical team in Valencia, Spain confirmed by press conference that they performed bilateral above-knee leg transplants in July 2011 [59]. It is too early in this patient's course to comment on functional recovery.

Vascularized fibula transplantation

In 1988, a Japanese group transplanted a vascularized fibula from a living mother to her 2-year-old son in order to reconstruct a large

Figure 110.6. Lower extremity transplant. (A) Preoperative appearance of ischiopagus twins. Twin A is the donor with a lethal cardiac anomaly; Twin B is the recipient. (B) Postoperative appearance following twin separation and lower extremity transplant. (C) Transplanted leg is 6.5 cm shorter than native leg. Reproduced from [58] with permission from John Wiley and Sons.



(A) (B)

Figure 110.7. (A) Tongue allograft. (B) Postoperative appearance of tongue allograft. Reproduced from [62] Kermer C, Watzinger F, Oeckher M. Tongue transplantation: 10-month follow-up. Transplantation, 2008;85:654–655 with permission from Wolters Kluwer Health.

tibial defect due to congenital pseudoarthosis and neurofibromatosis after failed surgical procedures. The vascular pedicle included donor peroneal artery and veins; these were anastomosed to the recipient anterior tibial artery and veins. Immunosuppression included cyclosporine and a 2-week course of steroids. One day following transplant, technicium bone scan demonstrated moderate tracer uptake. However, 2 weeks following transplant, unintentional drop of cyclosporine levels below target range resulted in rejection. Repeated bone scan 3 weeks after transplant showed decreased tracer uptake, and arteriography at 3 months demonstrated vessel obstruction. Bony union was achieved only at the distal end. In order to achieve proximal union, a short vascularized fibula autograft was performed 1 year after transplant [60].

Uterus transplantation

Uterus transplantation has been suggested as possible treatment for women with either a congenital absence or following surgical removal of the uterus. There is one reported case of human uterus allotransplantation, performed in Saudi Arabia in 2000. The 26-year-old female, 6 years following hysterectomy for postpartum hemorrhage, received an orthotopically transplanted uterus from a 46-year-old unrelated living donor. Immunosuppression regimen included cyclosporine, azathioprine, and steroids. One episode of acute rejection at 9 days was reported and treated with antithymocyte globulin. The suspicion of rejection was initiated by systemic symptoms such as vaginal discharge, abdominal pain, fever, and malaise. Doppler ultrasound revealed myometrial edema. Flow cytometric analysis of peripheral blood also revealed a reversed CD4/CD8 ratio. No biopsies were taken to confirm histologic rejection or its resolution. Initial functional success was evidenced by endometrial proliferation in response to hormonal therapy and two episodes of withdrawal bleeding. However, hysterectomy of the transplanted uterus was necessary after 99 days due to acute vascular thrombosis resulting in uterine infarct. The vascular thrombosis was attributed to lack of uterine support resulting in kinking of the vascular grafts [61]. However, an antibody-mediated response cannot be excluded as a cause of graft loss. At the time this chapter is written, there are no other reports of human uterine transplantation.

Tongue transplantation

A tongue transplant was performed in 2003 in Vienna, Austria, to a 42-year-old recipient who had lost the ability to speak, eat, or swallow due to squamous cell carcinoma of the tongue. Radical en bloc resection of the tumor immediately preceded transplantation. Induction immunosuppression with antithymocyte globulin and steroids, followed by maintenance with tacrolimus mycophenolate mofetil, and steroids was administered. No perioperative complications or rejection episodes were reported. At 10 months, the patient was able to speak, swallow saliva and small amounts of pulp-consistency foods, and the tongue was partially sensate. Tongue motility was never achieved. One year after transplant, tumor recurrence was identified in the patient's neck and the patient died at 13 months post-transplant from a presumed pulmonary embolism (Figure 110.7) [46,62].

Fasciocutaneous radial forearm transplantation for hemi-face reconstruction

In 2008 an Egyptian team transplanted a vascularized fasciocutaneous radial forearm allograft from a living mother to her child, who was afflicted by xeroderma pigmentosa (XP). XP is a disease characterized by defects in DNA repair and results in numerous skin cancers upon exposure to ultraviolet light. Patients typically die before 20 years of age. In this unique case, surgeons performed this VCA as a prophylactic measure to replace the patient's own skin with donor skin that does not carry the XP defect. Full thickness excision of the recipient's hemi-face native skin was performed and replaced by an allografted fasciocutaneous radial forearm allograft. Immunosuppression included steroids, mycophenolate mofetil, and cyclosporine. Initial healing was uneventful. An isolated area of skin discoloration was noted on the allografted skin 6 weeks after transplant, but this resolved without intervention. However, after missing three consecutive follow-up appointments and presenting with below-target immunosuppression levels 5 months after transplantation, the allograft experienced irreversible rejection and required complete removal. The wound was then left to heal by secondary intention. Of note, no cancers developed on the transplanted skin, although new cancers continued to develop on the contralateral side that was not transplanted [63].

Summary

Throughout history, clinical transplantation has developed in close relation with research. VCA has made significant progress in the last decade; however, similar to other transplants, VCA has its challenges and considerations, some of which are common across allograft types, and others that are unique to VCA. Although these transplants are technically feasible, many challenges remain: defining the best candidates; balancing the burden of immunosuppression with a QoL transplant; defining the appropriate timing of the transplant; achieving the timely reporting of outcomes to the scientific community; and defining the immunological specificities that, although beyond the scope of this chapter, must be recognized and kept in the forefront of this investigational field.

From their inception, VCA programs should target an interdisciplinary approach. Currently, groups from at least 17 countries have performed these complex transplants. At this time, there are fewer than ten patients alive with VCAs longer than 10 years since transplant. Patient non-compliance with immunosuppression or prophylaxis regimens has been reported in hand, face, larynx, and knee transplant recipients and has been implicated in one hand allograft loss and the death of one face transplant recipient [14,32]. The natural history of these transplants, even in conditions of optimal immunosuppression and rehabilitation, is unknown. The profile of chronic rejection and antibody-mediated processes in VCA is undefined. Critical areas of investigation as the field develops include: cortical plasticity, the impact of ischemia-reperfusion injury on function, potential VCA applications for congenital and pediatric defects, tissue donor acceptance and public education, and regulatory issues. Today, transplantation continues to be a unique setting for the study of immunology and transplant-related applications to improve patient care. As a partner in transplantation, VCA should advance with a focus on ensuring the best outcomes for VCA recipients, both present and future.

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SECTION 8

Pediatric Transplant

CHAPTER 111

An Introduction to Pediatric Organ Transplantation

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The chapters in this section focus exclusively on pediatric transplantation. Although many aspects of transplantation of children bear close parallels to transplantation in adults, there are many ways in which pediatric transplantation is unique. The current section is designed to focus on these differences and to complement and not replace earlier chapters in this text.

The assembly of a section specifically dedicated to pediatric practice has its rationale in recognizing that the care of children typically involves a distinct set of practitioners, and similarly, that pediatric transplantation must be clearly distinguished from adult transplantation not only on technical grounds, but also with regards to specific differences in immunobiology, pathology, pharmacology, psychology and protective immunity that manifest in unique clinical challenges. Investigators and practitioners need to recognize these issues as a primary condition of caring for pediatric recipients of transplanted organs.

In the first section, Urschel and West provide a detailed description of the maturation of the immune system and its relevance to organ transplantation across the age groups. Although it is often stated that the immune system is quite mature in humans at birth, any neonatologist or pediatrician caring for young infants knows this is not entirely correct. While its antigen receptor repertoire is certainly established at birth, it is now clear that immune function develops with increasing immune experience. A clear account is provided of the protean ways in which all aspects of the immune system differ between birth and adult life, with differences in both quantity and quality of all components of the immune system. These findings help explain age-related differences in alloimmune responses, with clear patterns of lower rates of acute rejection and chronic graft dysfunction in patients transplanted in the earliest stages of life. These findings are perhaps most noticeable in neonatal and early infant heart recipients, since transplantation of other organs is rare in this very young age group. This can be considered a period of relative "immunologic privilege", although the precise window for this immunologic advantage is not entirely clear. By contrast, these young transplant recipients have high rates of infection, and children have the highest rates of lymphoproliferative disorders.

There follows organ-specific chapters focusing on kidney, liver, heart, lung and intestinal transplantation. These chapters remind us of the prominent role that pediatric transplantation has played in the history of organ transplantation. Pediatric transplantation evolved in parallel with adult transplantation, not as a result of the success of the latter. Few are aware that the first pediatric kidney transplant was performed in Paris on Christmas Eve, 1952 [1]. The graft worked immediately, but was lost, presumably due to rejection, on the twenty-first day leading to the death of the teenage patient. None-the-less, feasibility of the procedure was demonstrated. The first pediatric heart transplant was planned (1966) prior to the pioneering first human heart transplant by Christian Barnard in December 1967. The donor deteriorated and the transplant was abandoned. A new recipient was identified late in 1967 and the transplant was performed only three days after Barnard's first procedure. This second ever orthotopic heart transplant in a human occurred in a three-week old infant with lethal congenital heart disease. The donor was an anencephalic newborn who had been transported to the recipient center [2]. The organ beat for approximately six hours. Infants and young children also feature prominently in the history of liver transplantation, including the first recipient operated upon by Starzl in March 1963. Unfortunately, the recipient procedure could not be completed and the "three year old bled to death as we worked desperately to stop the hemorrhage" [3]. Although most of the earliest transplants were not successful, these observations affirm the place of children and their families as pioneers in the field of solid organ transplantation and much was learned from these earliest experiences.

The organ specific chapters on pediatric transplantation draw attention to the manner in which pediatric transplantation differs from transplantation of adults. The most notable difference perhaps relates to the markedly different spectrum of causes of end-stage organ failure in children. This requires specialized pretransplant evaluation with teams who are focused on the care of the child with end-stage organ failure. Some of these differences mandate specialized surgical techniques, for example for children with congenital urinary tract anomalies, or congenital heart disease. These differences can make the transplant procedures technically very challenging, requiring a surgical team with extensive experience in reconstruction of these congenital anomalies. Failure of adequate reconstruction of congenital anomalies frequently leads to early graft loss.

Post-transplant management strategies are also influenced by age and pretransplant diagnosis. Immune maturity may influence immunosuppressive strategies, and patient size and anatomic variations may impact ability to perform post-transplant biopsies of the allograft. Corticosteroid avoidance has been pioneered in pediatric populations, a strategy now being studied in many adult transplant

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programs. Prior infectious agent exposure (notably human herpes viruses) informs us of infection risk, and risk of lymphoproliferative disorders, and influences strategies for post-transplant viral prophylaxis and detection.

Following the organ-specific chapters, we focus on several issues of critical importance to the pediatric transplant recipient and their families. The goal of organ transplantation is to prolong recipient survival as long as possible, return the recipient to optimal state of health, to enhance quality of life, and to return the child to normal, age appropriate, activities. Many barriers exist to achieving these goals. Medications have many side effects, and growth and puberty may be delayed for many recipients. The impact of corticosteroids, and the use of therapeutic growth hormone, on somatic growth have now been extensively studied in pediatric organ recipients. Cognitive development may also be impaired and psychiatric disorders are common. Non-adherence to immunosuppressive medications may be the most challenging problem facing the pediatric transplant team members and barriers to achieving adherence are now the focus of a number of studies. Studies of novel interventions to enhance adherence are also described. This section concludes with discussion of a topic of great importance to both pediatric and adult transplant team members, that is "transition of care". This is a vulnerable period for all recipients associated with increased risk of graft rejection and loss. Specific recommendations for creation of an effective transition program are made.

In summary, this section focuses on pediatric aspects of organ transplantation. The chapters can be considered in isolation as a monograph on the unique aspects of pediatric transplantation, but are best read in concert with other sections of the book, which are all relevant to the field of transplantation in children. References to chapters in the more general sections of the text are made to highlight where common practice exists between adult and pediatric transplantation.

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CHAPTER 112

Developmental Immunity: From Birth to Adult

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Introduction

Ontogeny of the immune system occurs in distinct stages in exquisitely integrated interplay, giving rise to 'critical windows' in the continuum of immune development [1,2]. Antigen exposures or therapeutic interventions during these windows may have long-term or even permanent consequences both unfavourable and favourable (Table 112.1). In contrast to other organ systems that are closer to being fully developed in their anatomy and functionality by the end of gestation, the immune system is incompletely formed and is subject to major developmental changes throughout childhood and youth. Immunologic immaturity at the time of birth is related both to general lack of antigen contact and to inherent processes that undergo continued maturation long after birth. Understanding the various aspects of immune development and their influence on optimal decision-making in pediatric transplant medicine requires integration of knowledge gathered from various fields of medical research and clinical practice including infectious diseases, autoimmunity, immunodeficiencies and endocrinology. Not the least, information from animal models and other experimental work over more than half a century contributes to the understanding of the complex changes that occur during immune maturation. In this chapter we will compile current concepts of developmental immunology in the context of their relevance to transplant medicine. It will complement the more general discussion on physiological immune function found in Chapter 2.

Natural development of the immune system with childhood maturation

When considering neonatal immune responses it is important to acknowledge that despite abundant information on development derived from elegant experimental systems, particularly murine models, the complex in vivo interplay in the intact human neonate is clearly less amenable to precise analysis. Nonetheless, since experiments carried out by Peter Medawar and colleagues in the 1950s [3,4], the neonatal mouse has served as an ideal model for the investigation of immunologic development as a species that is both readily available for laboratory investigation and easy to manipulate, as well as very immature at the time of birth. The event of birth, of course, does not imply any particular degree of immunologic maturation but rather marks the moment of departure of the individual from the intrauterine environment. The degree of immunologic development reached at birth, as well as the rate of post-natal maturation, varies with the species; the maturity of the neonatal mouse is far less advanced than that reached by the human at the time of birth (developmental stages between mice and humans are roughly approximated in Table 112.2) [5].

Innocent and naïve: The immune system of the neonate and infant

While the strict definition of a neonate includes children aged from 1 to 28 days after birth, changes of the immune system are obviously fluid and proceed with wide individual variability. This is particularly relevant given that birth and survival of a newborn currently may occur as early as 23 weeks gestational age, thus 17 weeks before the new human being would be expected to be prepared for life outside the protective maternal environment. Together with deficits in the developmental stage of other organs, the child born prematurely faces additional challenges associated with immune immaturity, thereby increasing the risk of infection. However, all newborns are vulnerable to severe systemic infections caused by agents that are not particularly harmful later in life. Elements that have been reported to contribute to less than fully effective immunity in neonates include quantitative and qualitative factors in both the adaptive and innate compartments that have been described in murine models as well as in humans. These are summarized in Table 112.3 and discussed in detail in the following sections.

Innate immune responses of the newborn and infant

As described in Chapter 7, the innate immune system includes a variety of cells and enzymes that respond to structures indicating the presence of pathogenic or non-human organisms, or altered human cells, and can be summarized as a non-specific first-line defense. As the name suggests, innate immune responses are operative at birth, likely beginning early in gestation, and are not dependent on exposure to specific antigens or to the development of immune memory. However the term 'innate' also implies a misconception that no maturation occurs or is required; in truth, immaturity in innate immunity is illustrated in various clinical and experimental observations. As one example, about 2 in 1000 mature newborns experience early onset sepsis; in ~41% the causative agent is Group B *Streptococcus*, a bacterium to which the neonate has limited resistance, acquired from otherwise harmless colonization of the maternal birth channel [6]. Neonatal phagocytic cells show a reduced response compared to cells from adults when stimulated with various pathogen-associated and damage-associated

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Table 112.1. Major stages of human development

1 Preconceptual: that is, oocyte and spermatozoa development, particularly important for oocyte development as susceptibility may be prolonged for many years before fertilization
2 Gestational: embroynic to mid-gestation, late fetal (organogenesis, cell migration, growth education)
3 Perinatal: early postnatal (lactation)
4 Infancy: early childhood — infectious exposures
5 Middle childhood — thymic involution
6 Adolescence — hormonal influences
7 "Maturity"
8 Senescence

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Table 112.2. Comparison of T cell development stages between mouse and human

Function	Mouse	Human
Length of gestation	21 days	40 weeks
Lymphohematopoietic cells colonize primordial thymus	11–12 days	week 9
Morphologic division of thymus into cortex and medulla	13–14 days	weeks 11–14
Expression of γδTCR then αβTCR	14–16 days	weeks 11–13
Proliferative response demonstrable in MLR	16–18 days	week 12 (thymus) week 19 (spleen) (weak until week 23)
Mitogen responsiveness	day 18 (thymus) (to some mitogens only after birth)	weeks 13–14 (thymus) weeks 16–18 (spleen, peripheral blood)
Cytotoxic response demonstrable (CML)	Weak until postnatal day 7	Beginning from about weeks 20–23 (thymus)

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molecular patterns (PAMPs and DAMPs) through pattern recognition receptors (PRRs) including toll-like receptors (TLRs) [7,8]. Dendritic and monocytic antigen-presenting cells of neonates have been found to have reduced expression of a number of cell surface structures important to effective responses, including class II major histocompatibility complex (MHC) [9,10] as well as costimulatory molecules such as CD40, CD80 and CD86 [11]. During bacterial infection and sepsis, proliferation and release of polymorphonuclear neutrophils from the bone marrow of neonates exhaust faster than later in life [12] leading to neutropenia during sepsis with associated higher mortality [13,14]. The capability of neutrophils of newborns to migrate spontaneously and to follow chemotaxis factors is also reduced [15], particularly in the context of sepsis [16]. The complement factor C9, a crucial component of the membrane attack complex in the response to bacterial infections, has been reported to be reduced in quantity in neonates [17]. Possibly associated with the lack of well-developed adaptive immunity and consequently specific antibodies, neonates show no detectable activation of complement through the classic pathway during bacterial infections, but an overwhelming response through the alternative pathway, identified through increased plasma levels of C3a and C5a [18].

This summarizes only some of the most relevant amongst currently recognized and studied elements that limit innate immune responses of the newborn and infant. The impact on allo-immune responses in transplantation remains mostly unknown although it is increasingly evident that the interplay between innate and adaptive immunity has been vastly underestimated. Recognition of the augmentation of antigen-specific adaptive immunity through innate immune responses, and thus the potential for increased long-term graft damage, is essential. This will not only provide better understanding of inter-individual differences in early and long-term graft acceptance but may also reveal possible therapeutic targets for intervention at the time of organ implantation that will ultimately improve graft survival.

Table 112.3. Immature components of the immune system of newborns and infants compared to older individuals

Innate immune system
- **Phagocytic cells**: low responsiveness of pattern recognition receptors
- **Dendritic cells and monocytes**: low expression of MHC II and costimulatory molecules
- **Polymorphonuclear neutrophil leukocytes**: small reservoir and impaired chemotaxis
- **Complement system**: low quantities of C9, predominance of alternative activation pathway
- **Natural killer cells**: numbers generally low at birth and functionally ineffective

Adaptive immune system
- Lymphopenia at birth, lymphocytosis in the first year
- **T cells**: phenotypical and functional naïveté; lack of memory; broad spectrum of newly generated non-replicated T cells; Th2 bias in specific immune response
- **Regulatory T cells**: high presence of naïve T regs, few activated T regs
- **B lymphocytes**: low numbers at birth, predominant lymphocyte in first year; lack of memory phenotypes and marginal zone B cells; predominance of naïve cells; increased proportion of transitional B cells
- **Antibodies**: maternal IgG at birth with nadir around six months; endogenous production of IgG, IgA, IgM starting at birth and reaching adult concentrations around ten years of age; impaired IgG2 production
- **Spleen and lymph nodes**: architecture (e.g. germinal centers) under development; absence of splenic marginal zone in the first 3–6 months

Developing T and B cell responses from the prenatal period to early childhood in animals and humans

The susceptibility of infants to various infectious agents is related not only to incompletely developed innate responses as described above, but also to lack of preexisting immune memory and to a paucity of lymphocytes and other adaptive immune components in peripheral lymphoid organs (Table 112.3). Deficiencies in lymphocytes (both T cells and B cells) have been described. Subsets of immune cells are differentially represented in quantity over the course of development, and may also have both phenotypic and functional differences compared to adult-derived cells (reviewed in [19,20]). T and B cells are both phenotypically and functionally naïve, typically lacking cells with memory phenotype and function. Natural killer (NK) cells are infrequent at birth and functionally ineffective. A relative lymphopenia has been reported, with peripheral T cell numbers lowest at birth and increasing thereafter [21,22], together with under-developed peripheral lymphoid organs containing few cells.

For decades, neonatal mice were reported to have T cells that are not only few in number but with defective function. However, more recent studies have reported the capability of neonatal T cells to mount effective responses in a variety of in vitro and carefully manipulated in vivo models [23–25]. Furthermore, murine neonatal helper T cell responses have been found to be not so much 'deficient' as skewed toward the Th2 lineage [26,27], thought to be caused, at least in part, by delayed maturation of an IL-12-producing dendritic cell subset [28]. It is not entirely clear that this is true in humans, as T helper responses in terms of both Th1 and Th2

function have been reported to be diminished in some cases but equally effective in others [29–31]. $CD4^+$ T cells of fetal origin are likely to persist in neonates, influencing early responses towards the Th2 bias that predominates in utero [32,33]. Furthermore, there are contradictory reports in the literature as to the effectiveness of neonatal $CD4^+$ T cell function and, as noted, wide diversity in T helper responses. Although $CD8^+$ T cells in neonates have been reported to develop mature cytolytic function in certain circumstances including congenitally acquired infections [34–36], their function in normal healthy neonates is nonetheless generally diminished compared to adults [37]. Additionally, the relative lymphopenia of neonatal life may give rise to homeostatic proliferation, well-studied in mice but not in humans, which may influence the emerging T cell repertoire. During early life, peripheral cell numbers increase as T cells continuously leave the thymus, resulting in higher proportions of recent thymic emigrants in neonatal spleen and lymph nodes compared to adults [38,39]. These cells are phenotypically and functionally distinct in neonates, with quantitative and qualitative differences compared to adults in terms of cytokine production and proliferative responses. This likely contributes to the diverse T helper responses observed in neonates. Recent studies of human cord blood-derived T cells using analysis of T-cell receptor (TCR) excision circles (TREC) as a marker of recent thymic emigrants have demonstrated higher numbers that proliferate in response to IL-7 than naïve $CD4^+$ cells in adults, suggesting that a functional distinction may also be present in humans [39,40].

The circulating B cell proportion is higher in newborns than later in childhood; however, neonatal B cells have an immature phenotype [41] and different isotype switching patterns compared to adult cells. These factors together with inadequate provision of 'help' from neonatal $CD4^+$ T cells for effective B cell function contribute to commonly noted deficiencies in neonatal antibody responses: later onset and shorter duration, low antibody levels and decreased antibody affinity and avidity. Marginal zone B cells are largely absent during the first months of life [42]; circulating plasma cells are rare. Lymphoid organogenesis is ongoing during early newborn life, with lymph node and splenic architecture still under development (germinal centres, marginal zone). Thus both 'T-dependent' and 'T-independent' antibody responses are less effective than later in life [43]. T-independent responses in particular are noted to be markedly impaired [44]; a number of factors have been reported to contribute to this as described below.

Inadequate capacity for antigen presentation further contributes to limited immune responsiveness during the neonatal period. Dendritic cells are few in number, with some reports describing poor function and low expression of costimulatory molecules. In clinical organ transplantation, recapitulation of this 'immature' phenotype in older individuals is sought through immunosuppressive strategies using pharmacologic or biologic blockade of costimulation pathways.

Regulatory cell populations are increasingly recognized as central participants in many aspects of immunity, and may also contribute to an enhanced state of 'suppression' during infancy that plays a role in prevention of the emergence of autoimmunity. $CD4^+$ regulatory T cells have been reported to be present in higher quantities in young children than in older individuals, however their functional abilities remain incompletely explored. Neonates mainly have regulatory T cells with a naïve phenotype, which is considered to be beneficial for tolerance to both self and, in experimental models, non-self antigens [45,46]. With regard to recently described regulatory B cells [47], evidence is emerging suggesting also a higher prevalence in infants [48] than in [49] older individuals, but the impact remains to be clarified. Additionally, the role of inhibitory pathways such as PD-1 [50] in transplant immunity in children remains to be explored.

Interaction between innate and adaptive immune system in early childhood

Elements of innate and adaptive immunity interact co-activate and modulate each other on many levels. In the context of transplantation the 'T-independent' response to polysaccharide structures is of major importance in regards to the immune response to non-self ABO blood group epitopes. Similar to other polysaccharides, ABO antigens have generally been thought not to be presented through the MHCII complex resulting in classic T-mediated B cell activation, but rather activate B cells through interaction with innate immune elements. If an antigen is complexed with complement fragment C3d, activation of its corresponding B cell receptor (BCR) requires a 100–1000 fold lower antigen concentration than without complement. This effect is mediated through binding of C3d to its receptor CD21, part of the B cell co-receptor complex that mediates its effect on the BCR through CD19 [51]. The complement-mediated co-signal plays a crucial role in the development of tolerance to self-antigens [52,53] and possibly to alloantigens. The impaired function of this interaction in infants is reflected clinically in a higher incidence and severity of infections with polysaccharide encapsulated bacteria [44]. Analyses of peripheral blood from children after ABO-incompatible and ABO-compatible heart transplantation (described in detail below) did not show a clear age dependency of C3d levels; however, recipients of ABO-incompatible heart transplants as infants were found to have significantly less C3d in the plasma than ABO-compatible counterparts [54]. While the presence of CD21 on B cells of neonates and infants was not found to differ significantly compared to later in life [54], the effect induced through complement binding appears to be impaired, be it through an altered signalling capacity [55] or due to mechanisms further downstream. A role for complement in the long-term accommodation of ABO-incompatible kidney grafts in adults has also been previously described [56].

Passive immunity through maternal immunoglobulins

Starting as early as 13 weeks gestational age, immunoglobulin G (IgG) starts to transfer through the placenta from the maternal into the fetal bloodstream [57,58]. This process is hugely enhanced after 32 weeks gestation and leads to a nearly complete reflection of the maternal serology in the fetus by about 36 weeks. A full-term newborn actually exceeds the maternal concentration of specific IgG by 20–30% [57]. This transfer occurs in an active manner as high molecular weight structures are prevented from passing the placental barrier by diffusion. Maternal IgG is bound to receptors for the crystallizing moiety of IgG (Fcγ) on the fetal side of the placenta ('neonatal Fcγ Receptor', FcRn) [59]. These MHC class I-like receptors are pH-sensitive, having 100 $\times$ higher affinity for Fcγ at a pH of 6.0 compared to the physiological human pH of 7.4 faced in the maternal blood. The current concept assumes that the maternal IgG is captured by fluid phase endocytosis in endosomes with low pH but protected from degeneration by lysosomal enzymes. The endosomes are actively transcytosed through the cells and fuse with the membrane facing the fetal blood vessels, where the IgG is released from the FcRn now again facing the physiologically higher pH in the fetal blood [60].

Maternal IgG provides the newborn with a certain degree of protection against infectious agents previously encountered by the maternal adaptive immune system or via immunization. The presence of IgG in the mother indicates previous contact with the antigen; strength of maternal antibodies partly reflects the time interval since this encounter. In the setting of infections that can be transmitted through transplacental transfer to the child, positive maternal serology indicates an increased risk of infection in the child compared to seronegative mothers, simply by being a surrogate of previous infection of the mother [61]. However, higher maternal antibody levels against certain pathogens have been found to decrease the risk of infection with these agents, confirming the passive protection provided to the child. For example, the risk of neonatal sepsis with Group B *Streptococcus* was found to be inversely correlated with the antibody titre detected against these bacteria in maternal and cord blood [62]. Nonetheless, passively transfused IgG can only provide partial protection, especially against intracellular pathogens, and the transfer of various IgG subclasses is unequal, with subclass IgG2 having the lowest transfer [63]. Analysis of commonly present antibodies shows that the proportion of placental transfer varies depending on the nature of the antigen. Antibodies against proteins such as tetanus toxoid were represented in higher proportions (median 165%) in the infant compared to maternal plasma, while anti-polysaccharide antibodies (e.g. against *Haemophilus influenzae* and *Escherichia coli* lipopolysaccharides) were slightly lower than in the mother [57]. This observation is of major importance for infant transplantation because the antigens defining the blood groups of the ABO system are surface polysaccharides and IgG2 is the predominant antibody subclass produced in response to polysaccharide antigens [64]. One might speculate that this contributes to protection of the fetus from hemolysis induced by maternal antibodies in constellations of a blood group O mother pregnant with an A or B fetus, or a blood group AB child being carried by a mother of any other blood group. The specific implications of transplacental immunoglobulin transfer are discussed further on.

Breastfeeding

In contrast to various other animals, humans do not express the FcRn in the intestine and hence seem not to have active absorption of undigested immunoglobulins from the gut lumen [65]. Consequently, healthy infant IgG levels undergo a physiological drop, reaching a low at approximately 6 months of age with a subsequent slow but steady increase by early adolescence [66] with median values in the normal adult range by the age of about 9 years. Plasma IgG (and IgM) levels of infants are not different in breastfed versus formula-fed children [67]. IgM and IgA are not transferred through the placenta nor via intestinal absorption but are produced by the child, showing a steady increase from birth until adult values are reached by 17 to 18 years of age [66]. However, based on selective enrichment of IgA-expressing B cells in the mammary acini, human breast milk contains high concentrations of maternal IgA (secretion of up to 5000 mg/day) in the colostrum and moderate concentrations of IgG and IgM (up to 70 mg/day in the first week, decreasing to 10 mg/day from post-partum weeks 3–4) [68,69]. Despite the lack of intestinal absorption, this provides protection to the newborn and infant via intra-luminal binding and neutralization of antigens and toxins [70]. Interestingly plasma IgA levels were found to be higher in breast-fed compared to formula-fed children, possibly due to adaption or conservation of the child's own IgA [67].

Elements of the maternal immune system are also transferred into the child's gastrointestinal tract through leukocytes. While macrophages form the largest proportion of up to 85% of transferred leukocytes [71], with the obvious function of engulfing and inactivating harmful substances [72], the presence of mainly memory phenotype T and B cells indicates that a fully functional adaptive immune response of maternal cells may occur within the child's intestinal tract [68]. Additional immunologically active substrates within breast milk include lactoferrin, reported to impair bacterial adhesion capacity to the mucosal surfaces [73], and lysozyme, which contributes to intraluminal lysis of bacteria [74].

A variety of primary non-immunologic components of breast milk play an immunological role within the developing infant immune system such as non-digestible oligosaccharides that compete with structures of the mucosal surface for bacterial adhesion receptors, reducing the likelihood of the bacteria to bind to and invade the infant circulation [75]. Immunomodulating effects of intra-luminal immune components of maternal origin are assumed given the lower incidence of atopic disorders in breast-fed infants [76] and lower likelihood of necrotizing enterocolitis associated with breastfeeding in premature infants [77]. It has been proposed that this imparts a possible 'priming' effect on the immature immune system, facilitating the discrimination between 'benign' foreign substances tolerated as nutritional or otherwise essential elements of ingestion and potentially harmful substances targeted and destroyed by the immune system. Mechanisms include reduction of physical interactions with pathogenic substances, modification of the immunological milieu by secretion of cytokines and interactive cellular 'cross-talk' of intra-luminal maternal lymphocytes [68].

Despite the vastly uniform opinion amongst experts that various specific and non-specific immune components in breast milk provide the child with some degree of protection towards harmful substances [78], there are very few controlled clinical trials proving this assumption. The published trials were mostly performed in developing countries reflecting different threats to newborns than faced in 'developed' countries by infants awaiting organ transplantation. Several Cochrane reviews have concluded that the summation of available scientific data strongly supports breastfeeding as the best option for newborns, particularly in developing countries, leading to recent WHO guidelines recommending exclusive breastfeeding up to 6 months of age whenever possible [78].

The impact of breastfeeding on transplantation options and diagnostics in the newborn are considered further on.

Toddler and school age: gaining maturity and experience

In contrast to infants and the elderly, in whom susceptibility to infections is noted to be higher and outcomes generally worse due to immunologic naïveté and senescence, respectively, the middle childhood years typically represent a period of vigorous immune responses. A number of infectious diseases are reported to have lower mortality rates and milder disease course during the ages of approximately 4 to 13 years of age when compared even to healthy young adults [79,80]. Notable examples of this robust immunity are observed with regards to decreased susceptibility to influenza and tuberculosis infection in children compared to all other age groups, and decreased severity of disease manifestations when viruses such as varicella, measles, mumps and Epstein-Barr virus (EBV) are first encountered as children. This is partly due to the acquisition of memory B cells that can persist for many years and

can be rapidly activated to produce protective antibodies, and also by antibody levels maintained in serum for long periods of time by terminally differentiated long-lived plasma cells [81–86]. Memory $CD4^+$ and $CD8^+$ T cells also contribute to protective immunity against pathogens due in part to the speed with which their effector functions develop. In addition to immune memory and protective antibodies, it has been suggested that regulation of the balance between protective versus pathogenic aspects of immune responses may be different in children compared to older individuals, ultimately favouring tissue repair and regeneration after pathogen elimination over ongoing or extensive destruction [87].

In the setting of transplantation, the impact of these normal developmental processes on clinical outcomes has not been extensively studied from the perspective of their evolution in children transplanted as infants. However, for children initially undergoing (heart) transplantation during the middle childhood years, it can be noted that long-term outcomes are not as favourable as in those patients undergoing transplantation during the first year of life [105].

Adolescence

As in many other aspects of life adolescence is a critical period of human development. The highest rate of graft losses in organ transplantation, independent of time post-transplant and other risk stratification, is found in the age group between 15 and 25 years, a consistent observation independent of the organ transplanted [88–90]. The inferior outcomes for patients of this age are likely due to the summative impact of various factors. They are reviewed here, and treated in depth in Chapter 120.

Behavioural impact

First and foremost amongst factors contributing to worse transplant outcomes in adolescents are the huge mental and psychosocial changes occurring during this developmental stage. Children become adults and struggle with transition of their roles and responsibilities. This includes an increasing wish to take control over their lives while mostly not yet possessing the tools to perform challenging tasks appropriately and reliably such as the medical regimen associated with post-transplant care. Authority figures are questioned and challenged including parents and medical staff; aggressive, risk-taking and self-destructive tendencies are paired with immature coping mechanisms and increased emotional sensitivity. Obviously adherence to medical treatment and associated restrictions in everyday life are frequently poor in this age group [90,91]. The need for daily immunosuppressive medications and recommended limitations regarding recreational substance use are often in direct opposition to the individual perception and aspiration not to be different from the peer group. Inconsistency in immune suppressive medications can therefore partially explain the higher rate of acute rejection, higher frequency of development of donor-specific human leukocyte antigen (HLA) antibodies and graft failure observed during adolescence [92]. However, limiting consideration solely to these explanations ignores major metabolic and immunologic changes associated with puberty.

Impact of hormonal and metabolic changes on the immune system

Neuroendocrine factors, sex hormones and steroids as well as growth hormones have a major impact on various aspects of the immune system. During adolescence all these hormones undergo massive changes in quantities and patterns of secretion. Differences of the immune system between the sexes have been clinically recognized and scientifically investigated for decades; nonetheless, many aspects have yet to be explained and understood fully. Female humans, similar to many other animals, manifest a higher prevalence of autoimmune disorders than males [93]. Further correlations have been identified of variations in the activity of autoimmune disorders with the quantitative differences in sex hormones during the menstrual cycle and during pregnancy. Similarly, recurrent symptomatic flares of persistent infections such as herpes simplex have been found to be associated with the menstrual cycle and associated alterations of immune components and cytokines/chemokines [94]. Some disorders such as multiple sclerosis [95] and rheumatoid arthritis [96] have been found to exhibit significantly decreased activity during pregnancy. In contrast, for other disorders such as systemic lupus erythematosus, both exacerbation and reduction of inflammatory activity have been observed, and hormonal changes associated with the end of pregnancy have been found to be a trigger for disease flares [97]. This effect is not limited to hormonal influences but also involves exposure to the HLA molecules of the fetus, with the degree of disparity possibly contributing to modulation of immune responses [98]. Corticosteroids, whether generated by the adrenal glands or taken as medication, enormously affect both innate and adaptive immune elements. Steroids are thus used therapeutically to modulate immune responses in a variety of autoimmune and allergic disorders, as well as to reduce the allo-immune response post-transplant. Androgens and estrogens intrinsically have glucocorticoid effects; similar impact on the immune system was shown when high doses of sex hormones were administered [99]. High doses of testosterone were further found to cause release of tumor necrosis factor alpha (TNF-α) and apoptosis of $CD4^+CD8^+$ 'double positive' immature thymocytes [100].

Involution of the thymus was found to correlate strongly with increased secretion of sex steroids and their regulatory hormones [101], and to be reversible by surgical gonadectomy or chemical castration [102]. Additionally, it has been reported that many cells of the innate and adaptive immune system are impacted by growth hormones [103] and sex hormones [104], however the physiological function and clinical relevance remain to be determined. These hormones reach lifetime peaks during adolescence; the effects of this 'chaos' of hormonal interactions may cause changes of the developing human far beyond the isolated effect of each endocrine messenger. This indicates that the impact on immune maturation, immune responsiveness as well as tolerance and other therapeutic consequences is currently underestimated and warrants further investigation.

Implications of immune maturation on transplantation and vice versa

'Permissive' environment of early childhood

When organ transplantation is performed in early life, at a time of dramatic immunologic change, two major factors inherent in the process have an impact that remains poorly understood and incompletely explored: implantation of a large and persistent source of foreign antigens and introduction of chemical immunosuppression neither designed for nor tested in a rapidly changing developmental framework. In the setting of heart (and lung) transplantation, this is further complicated by the potential

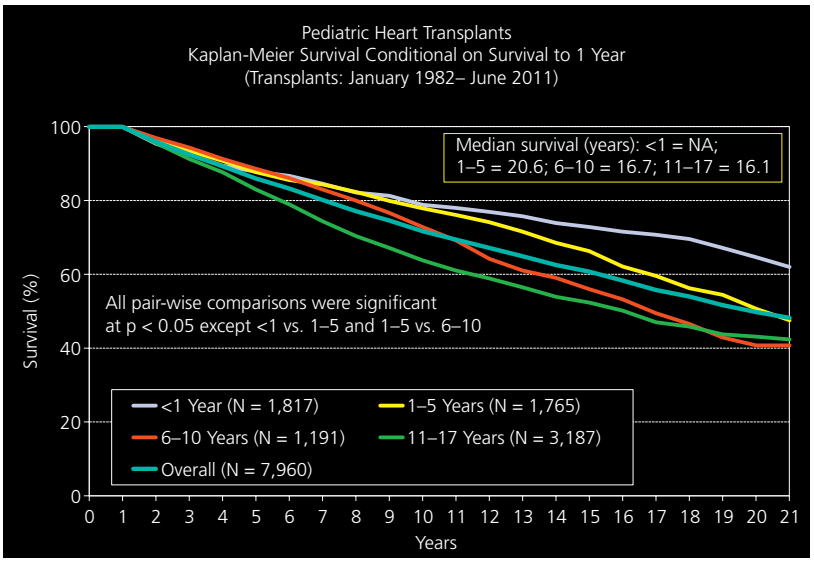

Figure 112.1. Conditional actuarial survival of pediatric heart transplant recipients surviving the first year post-transplant, stratified by age at transplant.
Source: International Society for Heart and Lung Transplantation, accessible from http://www.ishlt.org/downloadables/slides/2013/heart_pediatric.pptx

effects of neonatal thymectomy on immune function. The immunologic consequences of clinical organ transplantation are not limited to increased infectious complications (i.e. a higher susceptibility to infectious pathogens across a wider spectrum compared to later in life and a different course of post-transplant infections with common viruses). In contradistinction may also include provision of an advantageous environment in terms of transplant outcomes. The developing immune system is susceptible to tolerance induction and carries a degree of plasticity, diminishing over time, that renders it somewhat malleable, capable under the influence of chronic immunosuppression of 'adaptation' to the presence of a transplanted organ. Thus, the immature state offers unique opportunities to the very young patient such as safe blood group (ABO)-incompatible organ transplantation (see below), whereas later, the neurohormonal changes of adolescence paired with psychological and behavioural issues give rise to remarkable and complex challenges for the clinical teams taking care of transplanted children.

Superior long-term outcomes

It is well documented in the registry of the International Society for Heart and Lung Transplantation (ISHLT) [105] that children under one year of age at the time of heart transplantation have the best long-term survival compared to every other age group despite the higher early mortality found in multiple collaborative studies [106,107]. Infants undergoing heart transplantation are often in a more critical clinical state than older children before transplantation, including approximately 12% needing mechanical circulatory support in the form of extracorporeal membrane oxygenation (ECMO), and an increasing number being transplanted from ventricular assist devices (VADs). Despite the anticipated higher early post-transplant mortality, the actuarial survival curves for infant transplant recipients cross those of older children and adults, leading to a significantly better graft 'half-life', currently 19.7 years. The superior survival becomes particularly obvious when considering the conditional survival of children who have survived the first year after transplantation (Figure 112.1), which currently has not yet reached the 50% graft survival benchmark over more than 21 years of documentation [105].

After pediatric liver transplantation, nearly 20% of children achieve a state of operational tolerance, defined as normal graft function and absence of clinically relevant rejection without maintenance immunosuppressive drug therapy [108,109], a much higher proportion than reported in adults thus far. The same 'immunologic privilege' may also apply to very young kidney and lung recipients, but the low frequency of these transplants in very young children makes it harder to demonstrate any advantage of immunologic immaturity.

In transplant recipients, the role of regulatory T cells in the control of anti-donor immune responses is unclear. The effects of induction therapy and chronic immunosuppression on development and function of regulatory T cells and, in pediatric thoracic transplantation the additional impact of thymectomy, have yet to be studied in depth. T cell subsets and the T cell repertoire are clearly affected by neonatal thymectomy, which is typically performed with sternotomy procedures [110,111]. Preliminary data suggest that regulatory T cells in peripheral blood are indeed altered in these patients, with their numbers being significantly lower in heart transplant recipients compared both to thymectomized non-transplant patients (who do not receive T cell depletion and maintenance immunosuppression) and to healthy age-matched controls [112,113]. Moreover, the proportion of naïve versus effector regulatory T cells appears to be lower in transplant patients compared to healthy controls [114]. It remains to be elucidated whether this alteration in the diversity of regulatory T cell subsets under the persistent presence of allo-antigens together with immunosuppression promotes the development of stable donor-reactive regulatory T cells and contributes to better graft survival.

In summary, infants manifest particular maturational differences in components of the immune system that increase their susceptibility to various infections compared to older individuals. Development of effective vaccines for infants is aimed at overcoming these

immaturities to increase immune responses at an earlier age. It may be that these same developmental differences contribute to superior outcomes observed in infant transplant recipients and could be exploited still further to overcome remaining obstacles. Although documented only rarely in the literature [115], there is a common perception amongst pediatric transplant physicians that younger children generally require less intensive immune suppression after transplantation than older individuals. Many centers do not use induction therapy in infants, wean steroids earlier in young children (or avoid them altogether), and have lower target levels for calcineurin inhibitors in children transplanted at the younger childhood ages than required for older children. A recent multi-center study led by our group found de novo donor-specific HLA antibodies to occur only in children transplanted at older ages [116]. Moreover, de novo anti-class II HLA antibodies assessed by sensitive flow-based screening did not develop in any patients transplanted under the age of six years. Graft vasculopathy, representing chronic rejection, occurs least frequently in newborn and infant transplant recipients even as they age, while the incidence increases in the group of 1–10 year olds, and in teenage recipients is equal to that observed in adults [105].

Clearly this effect cannot be explained solely by immature immunity and is confounded by the lower frequency of additional risk factors in young children compared to adults including hypertension, non-favourable plasma lipid profiles and absence of associated disorders including extra-cardiac atherosclerosis, diabetes mellitus and chronic kidney failure [117]. Nonetheless there is little doubt that the developing immune system of young transplant patients provides them with a better chance of persistent graft acceptance than patients undergoing transplantation at any time later in life.

Acceptance of ABO-incompatible grafts

Beyond infancy, implantation of an ABO-incompatible graft in an unconditioned recipient carries a high risk of hyperacute antibody-mediated rejection due to high levels of pre-formed isohemagglutinins. In adult kidney transplantation, the barrier of ABO-incompatibility between donor and recipient has been successfully surmounted when preexisting antibodies to the donor blood group can be reduced at the time of transplant by a variety of means [118–123], thereby expanding the potential donor pool. For infants needing heart transplants, ABO-incompatible transplantation was initially contemplated as a reasonably safe option due to their natural lack of the immune mediators of hyperacute rejection, together with their inordinately high risk of death while awaiting an ABO-compatible donor [124]. In the decade since our original report of the first cohort of ten intentional ABO-incompatible heart transplants, it has been demonstrated that ABO-incompatible heart transplantation in young children carries no higher risk of mortality or morbidity than ABO-compatible transplantation and is an effective strategy for expanding the available donor pool for infants [125–127]. Indeed, pediatric cardiac transplant centres in a number of countries now routinely list very young patients for transplant without requiring donor blood group compatibility [128,129], although the limits of safety remain to be determined conclusively [130]. It is notable that in contrast to Canada and the UK, UNOS regulations continue to impair realization of the full benefits of this strategy in the US. [130–132]. Based on organ allocation rules developed decades ago for adult kidney transplantation, donor organs must first be allocated to ABO-identical then ABO-compatible recipients before being considered for ABO-incompatible recipients, despite this policy having no scientific rationale or relevance for infant heart transplantation.

In adult recipients of ABO-incompatible kidney grafts, it was noted decades ago that when antibodies to the donor blood group eventually re-accumulated, the graft was often resistant to antibody-mediated injury. Platt and colleagues described this phenomenon as 'accommodation' [56,133]; mechanisms of graft accommodation in the setting of both HLA and ABO antibodies are still under active investigation and are considered in detail in Chapters 4, 6, and 36. In contrast, young recipients of ABO-incompatible heart grafts, generally lacking abundant isohemagglutinins before transplant, typically remain deficient over time in the development of antibody production to the donor blood group while producing antibodies to other non-self antigens (Figure 112.2), a pattern more consistent with B cell tolerance than graft accommodation [134–138]. Moreover, when challenged with various specific and non-specific stimuli in vitro, cultures of peripheral blood cells from ABO-incompatible transplant recipients did not produce

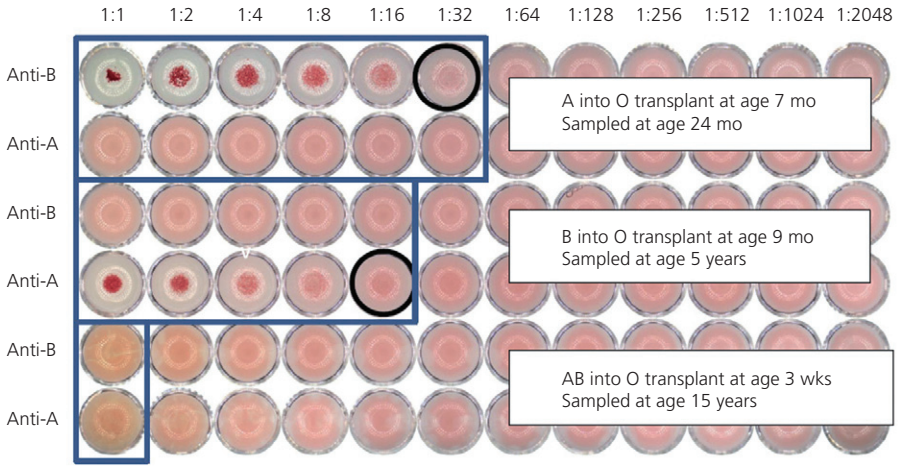

Figure 112.2. Examples of isohemagglutinin titres following ABO-incompatible heart transplantation assessed by microplate agglutination assay. Reproduced from [138] West LJ. (2011). ABO-incompatible hearts for infant transplantation. Curr Opin Organ Transplant 16:548–554 with permission of Wolters Kluwer Health.

antibodies against the donor blood group, suggesting that chronic exposure of maturing B cells to graft A/B antigens in this setting during early life results in their elimination in a process analogous to the development of self-tolerance [139].

When considering ABO antibodies and their potential importance in the setting of ABO-incompatible transplantation, it is crucial to understand the inherent limitations of the methods currently used for clinical measurement of isohemagglutinins [140]. Agglutination of reagent erythrocytes by antibodies present in serially diluted serum samples is read and reported as 'antibody titre'. It is widely known that this method has many limitations, including subjective assessment of degree of agglutination as well as lack of standardized methods from centre to centre [141]. Another potential confounder to interpretation of ABO antibody titres is that expression of ABO carbohydrate structure sub-types varies between reagent erythrocytes used for agglutination testing and tissue expression in organ transplants. Erythrocytes have been reported to bear all six carbohydrate chain sub-types of the A and B antigens while tissues generally do not [142,143]. This gives rise to the obvious conclusion that antibodies detected solely by agglutination of erythrocytes bearing A or B antigens that are not present in tissues may be clinically irrelevant in the setting of ABO-incompatible organ transplantation. Thus information from the ABO agglutination assay must be interpreted cautiously. While lack of agglutination may reliably indicate *absence* of ABO antibodies, agglutination of A or B erythrocytes may indicates presence of antibodies that may not be donor specific in regard to the organ graft.

Impact of transplacental immunity and breastfeeding on transplantation

Infectious disease diagnostics in the newborn and infant

Examination of the serum from newborns reflects the serologic memory of the mother and therefore cannot be used as a reliable diagnostic marker of infant infection. However, for infections that can be transmitted vertically from mother to child such as toxoplasmosis and cytomegalovirus (CMV) this situation leads to a diagnostic challenge [144,145]. One parameter that allows distinction between passively acquired maternal antibodies and infection of the child is the presence of IgM isotype antibodies in the child's serum. IgM pentamers do not undergo transplacental transfer due to their large molecule size and are not subject to FcRn-mediated active transcytosis. Therefore, unless the child received plasma infusion, IgM isotype antibodies in the infant blood will have been produced by the child's immune system and confirm infection of the child. However, IgM antibodies represent the first-line response of the adaptive immune system that subsequently switches to the IgG isotype and are found only for the first few weeks post-infection. Consequently, absence of IgM antibodies in presence of IgG in the infant's serum does not rule out infection earlier during pregnancy with an already faded IgM response. Direct testing for the infection may be helpful but does also not provide 100% sensitivity. In some cases of suspected infection the only reliable procedure is to treat the child as infected until the parallel fading of total and antigen-specific IgG confirms absence of infection [146].

In the context of neonatal and infant transplantation, infections of particular concern include CMV, EBV, toxoplasmosis and Herpes simplex (HSV), with the first two viruses having a major impact on long-term outcomes in pediatric transplantation. In the presence of positive IgG serology for CMV, cultures for virus detection from urine and a throat swab provide a sufficiently high negative predictive value to allow labelling a child as non-infected. For EBV, testing for virus capsid antigen (VCA)-specific antibodies is helpful to indicate recent infection; presence of IgM suggests infection of the infant, while high IgG titers not correlating with the maternal serology will be suggestive [147]. Nucleic acid testing polymerase chain reaction (PCR) has a very high specificity for infection if positive [148].

Since maternal antibodies from breast milk are not absorbed, breastfeeding should generally not alter infectious disease serology testing for infections acquired during the post-partum period. However, the significant presence of maternal leukocytes in the breast milk carries a risk of transmission of maternal infections to the child, especially in the setting of impaired host defence due to post-transplant immunosuppression. Therefore caution with breastfeeding is indicated whenever the mother develops clinical symptoms of an infection. Serious infectious diseases in the mother such as human immunodeficiency virus (HIV) that carry a risk of vertical transmission through breast milk should prohibit breastfeeding, as well as newly acquired or clinically re-activated infections with specific risk for immunosuppressed patients such as CMV or EBV [149]. A unique situation arises if the child of a seropositive CMV mother is deemed CMV-negative based on negative cultures and is transplanted with an organ from a CMV-negative donor. Given the low but existing risk of infection through breast milk in this case, post-transplant breastfeeding would be discouraged.

HLA and ABO diagnostics in the infant and newborn

It has been reported that up to 60% of healthy adults without previous surgeries or blood transfusions may have HLA antibodies when screened with highly sensitive techniques [150]. As with other IgG isotype antibodies, those directed against HLA epitopes are subject to transplacental transfer and can lead to passive presence of HLA antibodies in the newborn [57] that may persist up to 18 months of age with declining intensity [151,152]. Therefore, assessment of neonatal sensitization can be difficult and may only be confirmed by documenting the course and natural decline of the maternal antibodies relative to the infant's own immune response. Even a newborn is capable of generating a specific antibody response towards foreign HLA molecules if, for example, early cardiac surgery is required involving transfusion of thrombocytes and other blood products, cardiopulmonary bypass and especially implantation of human tissue allografts ('homografts') to reconstruct vessels. Indeed, infant recipients of vascular tissue allografts typically mount a robust and broad antibody response due to continuous exposure to the foreign tissue antigens in a setting in which immunosuppression is not used [153–155]. For those subsequently requiring transplantation, this clearly confers a high immununologic risk. Our group previously reported that simple intraoperative immersion of the homograft in glutaraldehye solution effectively prevents sensitization while not impairing homograft integrity [156]. Infant HLA sensitization is now rarely encountered at our centre, an enormous advantage for cardiac surgery patients who later require transplantation.

Differentiation between active HLA sensitization and passive antibody acquisition is crucial for planning peri- and post-operative clinical management. With passive antibody acquisition, antibody removal at the time of transplant should be sufficient to protect the child from antibody-mediated rejection, whereas actual sensitization with presence of immune memory may require aggressive

desensitization before proceeding with transplant, including antibody removal, B cell depleting (rituximab) and plasma cell reducing (bortezomib) treatments and immune modulation with IVIG. These patients may be excluded from transplantation if a suitable donor cannot be identified against whose HLA the patient does not have specific antibodies; if crossing the HLA-incompatibility, they face high risk of aggressive early antibody-mediated injury. The long-term burden of anti-HLA antibodies in a child with genuine sensitization and production of (donor-specific) antibodies and immune memory is much higher than in cases of transient passive antibody acquisition. These transplant recipients are at high risk of later re-activation of their immune response leading to antibody-mediated graft injury. This may not be limited to acute antibody-mediated rejection but may also include accelerated chronic rejection processes such as rapid graft vasculopathy in heart transplantation.

The differences in placental transfer of IgG subclasses and specificities may partly explain our observation that passively acquired anti-A and anti-B antibodies in neonates considered for ABO-incompatible heart transplantation are typically found less frequently and at lower titres than other antibodies, for example antibodies against infectious agents such as CMV or EBV. Also, in our own experience and observations from other groups, maternal blood group-specific antibodies tend to decline rapidly or disappear within a few weeks in infants whereas other maternal serologic markers persist regularly until 9 to 12 months of age and sometimes up to 18 months.

As detailed earlier in this chapter, maternal IgG or IgM antibodies are not absorbed through the intact intestinal barrier, hence the presence of maternal HLA or ABO antibodies should not preclude breastfeeding for infants awaiting transplant or post-transplant.

Transfer into adulthood: the recovering immune system

Children represent only a small proportion of organ transplant recipients; nonetheless, knowledge of mechanisms and elements of immaturity that contribute to superior graft acceptance in young children compared to adults has a major impact on transplant medicine for all ages. In preparation for hematopoietic cell transplantation, the recipient's own lymphoid system is depleted through irradiation and leukocyte-depleting agents. One could postulate that this represents a virtual 'reset' of the immune system leading to a relatively more forgiving immunologic environment of the graft toward host allo-antigens. Analysis of the recovering immune system in this setting shows that regeneration of the various components follows similar stages as observed in the developing immune system of the newborn [157–159]. However, overall reconstitution depends on multiple additional factors that are different in the adult compared to the infant or young child [160]. This includes some aspects discussed above such as absence of the thymus, differential representation of sexual hormones and growth hormones, and possibly differences in the grafted tissue itself after completion of growth and development and structural changes associated with multiple cell cycle replications. Even current multipronged approaches to eradicate the immune system in preparation for hematopoietic cell transplantation with a combination of 'lethal' irradiation and additional therapies do not deplete every immune cell [161]. In particular, cells that are in a resting or hibernating stage may persist and be reactivated during recovery. Thus the recovering adult immune system is not likely to achieve the same level of naïveté, or the potential to adapt to non-self antigens, as the evolving immune system of the very young. Indeed, attempts to induce immune tolerance in adults to transplanted organs using lymphocyte depletion strategies, or with partial bone marrow depletion and reduced dose irradiation, have met with limited though improving success, as described in Chapter 76. Therefore, for development of targeted therapeutic approaches in adults and older children, precise identification and characterization of immature pathways and interactions become even more important. In this regard, a possible key role for B cells is emerging. Following bone marrow depletion, newly produced B cells undergo similar developmental stages as during prenatal life and early infancy. In particular there is a high occurrence of the different stages of transitional B cells. These are characterized by surface expression of CD5 and CD10 and, in the first stage, low density CD21 [162]. During maturation, associated with 'Bruton tyrosine kinase'-dependent genetic rearrangement, transitional B cells increasingly express CD21 as well as CD23, CD44, Bcl-2, BAFF-R and IgD [162]. In a murine islet transplant model, this immature cell type was successfully induced by blocking B cell maturation signals or their receptors [163,164] and led to humoral allograft tolerance [165]. Similar findings were observed in humans when analyzing reconstitution of the B cell compartment in the first successful clinical trial of operational tolerance in kidney transplant recipients, in which long-term survival and function of HLA-mismatched grafts was achieved by partial bone marrow depletion followed by transfer of stem cells from the kidney donor, described in Chapter 76 [166]. It was noted that transitional B cells and plasma levels of the B cell activating factor (BAFF) were higher in patients in whom tolerance induction was successful [167].

Whether the state of donor-specific tolerance observed in infants receiving ABO-incompatible heart transplants (described earlier in this section) will develop in adults through depletion of B cells with monoclonal antibodies such as rituximab remains subject to speculation. In larger cohorts of adult recipients of ABO-incompatible kidney grafts, the majority showed eventual re-accumulation to some degree of antibodies to the donor blood group post-transplant, albeit at lower titres than pre-transplant and typically without clinical rejection. However, a group was observed with persistent absence of antibodies to the donor blood group, while a different minority was found to develop unmanageably high titres and graft loss [120,122]. Thus, only a minority of adults showed reconstitution after B cell depletion mimicking the primary immaturity of infants, while the majority followed a different pattern.

Summary

In conclusion, understanding the immature and developing immune system will not only help personalize and adapt clinical transplantation to the specific needs of the individual along the continuum of human development, but will also continue to open windows into the delineation of mechanisms of self-tolerance and allo-tolerance. Exploiting naturally occurring aspects of immaturity to the benefit of graft and recipient survival must be a major part of transplantation research.

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CHAPTER 113

Pediatric Kidney Transplantation

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Introduction

Kidney transplantation is in children, as in adults, the preferred treatment for most causes of end stage renal disease, particularly in children over 10 kgs. However, the indications bringing children to require transplantation differ from those of adults, as do the co-morbidities. Additionally, the pediatric population offers unique technical (particularly urological) challenges related to congenital anomalies, social support concerns and issues of non-adherence, and nuances of immune, physical and emotional maturity that clearly distinguishes the practice of pediatric transplantation from adult transplantation. This chapter will provide an overview of pediatric kidney transplantation with particular attention directed toward those aspects of the patient population served that define the field. This chapter complements the information on adult kidney transplant conduct and patient management found in numerous chapters elsewhere in this title.

The role of transplantation in children

Chronic dialysis and renal transplantation are both excellent treatments for end-stage renal disease (ESRD). The majority of adults with ESRD are receiving dialysis rather than undergoing renal transplantation. The number of adults seeking kidney transplantation is rising but the number performed has been limited by the lack of appropriate donors [1]. There is a survival advantage of transplantation for virtually all candidates. Renal transplantation was recognized as the better form of treatment for children with ESRD almost three decades ago [2] and has repeatedly shown to provide a survival benefit for children [3,4].

Both peritoneal dialysis, delivered as continuous ambulatory or cyclic peritoneal dialysis (CAPD or CCPD), and hemodialysis provide a worse quality of life and unsatisfactory growth rate as well as ongoing complications. Data from the dialysis component of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registry [5] show that the overall height deficit of −1.8 standard deviation (SD) became more negative reaching a value of −2.16 SD at 24 months after initiation of dialysis. Additionally, children do not tolerate being "dependent" on the modality and maintenance dialysis which induces loss of self-esteem and emotional maladjustment in them [6]. Cognitive achievement testing may diminish with prolonged time on dialysis [7]. In contrast, the mobility and freedom from dietary restrictions afforded by a functioning renal transplant enable children to live nearly normal lives. Although renal transplantation has not lived up to the promise of normal growth for all children, dramatic short-term improvements in height can be seen in many and final adult height is improving after transplantation [8–12]. Importantly, successful transplantation permits the child to attend school and to develop normally, and school function testing improves dramatically following transplantation [13,14]. Young children have the best long-term outcomes of all age groups of transplant recipients, verifying the utility of transplantation in this age group [15,16]. For all of these reasons, successful renal transplantation remains the primary goal of programs that care for children with ESRD [3,17]. Pediatric recipients of kidney transplants have the highest percentages of living donors; and, they receive substantial preference on the deceased donor (DD) transplant waiting list [18], leading to potentially short waiting times. Thus, pediatric patients with ESRD should not have substantial delays in undergoing renal transplantation after they develop ESRD, although the goal of rapid progression to kidney transplantation has not been universally achieved [19].

The incidence and frequency of pediatric renal transplantation

Between 2008–2012, there were about 16 500 kidney transplants performed in the US annually and about 800 of these were in children younger than 18 years [19] (Figure 113.1), suggesting that pediatric patients comprise about 5% of all transplant recipients. Although the number of pediatric transplants performed each year has generally varied by no more then 10%, the donor origin has undergone substantial changes. The Scientific Registry of Transplant Recipients (SRTR) data show that living kidney donation has expanded substantially and the number of living donors (LDs) exceeded the number of DDs for the first time in 2001 [20]. However, the number of LDs rose slightly until 2004 and has fallen since that time; while the number of DDs has remained stable [21]. Living donation now accounts for 36% of all kidney transplants in the US. In 1987, only 40% of all transplants performed in children were from a LD source; by 1991, the figure had risen to 53%, and until 2005 LDs were the predominant source of donors for pediatric kidney transplantation. A change in DD allocation that provided substantial preference to waiting children as soon as they were activated on the DD list led to a surge in DD transplants and a current predominance in DDs for children awaiting kidney transplantation (Figure 113.1). Although this change has undoubtedly led to more rapid transplantation for these children, there may be a decrease in mean graft survival rates related to this shift [22].

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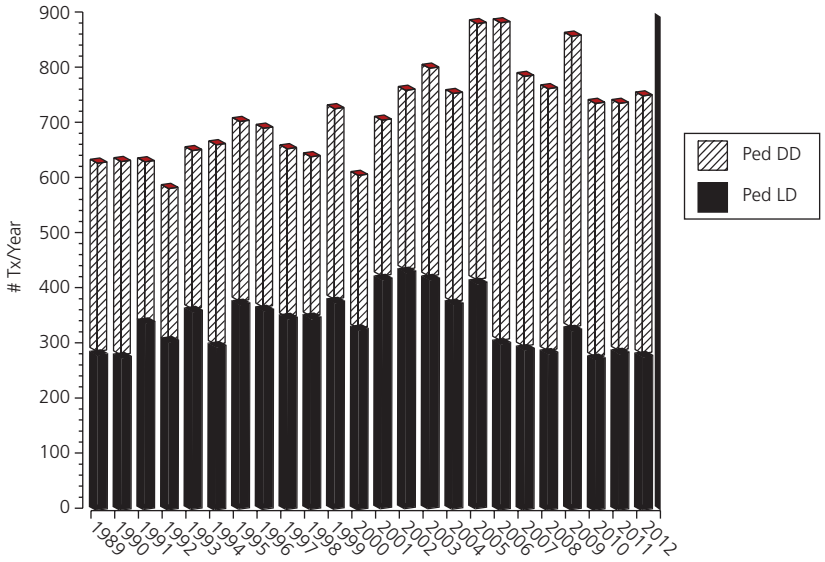

Figure 113.1. Number of living donor (LD) and deceased donor (DD) kidney transplants in children younger than 18 years of age by year between 1989 and 2010 (adapted from UNOS data: www.optn.org).

Parents comprise the majority of LDs. Mothers comprise the majority of parent donors; fathers account for 46%. Since there are more boys than girls who receive kidney transplants, it should not be surprising that fathers donate to sons 64% of the time and mothers to sons 60%. There is no outcome advantage to either parent, with the possible exception that infants less than one year of age seem to have fewer rejections if the mother is the donor [23,24]. Due to the fact that children most often have siblings who are too young to donate (less than 18 years), there are few transplants between siblings. NAPRTCS has identified150 grafts were from donors less than 21 years of age. A review of the NAPRTCS registry identified only 12 living donors under 18 years of age, of which 11 were transplants between siblings and one was from parent to an infant. It is quite clear that most programs are very reluctant to use minor donors [25,26]. However, a review of United Network for Organ Sharing (UNOS) data revealed that from approximately 40 000 LDs in the US between 1987 and 2000, 60 were from donors less than 18 years of age [27]. Twenty-four of the recipients were children and 36 were adults; only seven of the transplants were between identical twins. In recent years there has been a substantial interest in living-unrelated donation in adult transplant literature since the outcome of the grafts has been shown to be better than that of DD kidneys [28]. NAPRTCS identified 123 instances of living-unrelated donation between 1987 and 2001. In a preliminary analysis of the first 38 living-unrelated recipients, 23 (61%) were male, 30 (79%) were Caucasian, eight were younger than six years old, and 20 were older than 12 years [29]. This was the primary transplant for 29 of the 38 recipients. Of the 38 donors, 22 were non-biologic parents, and a family friend was the donor in ten of the cases. In 2008, UNOS documented 293 pediatric LD kidney transplants: 189 (68%) were from parents, 24 from siblings and 30 from other biologically related. Thus 83% of LD kidney transplants in children came from biologically related donors.

The majority of DD kidneys for children are recovered from adult donors. In the 1980s there was a tendency to preferentially place kidneys recovered from infants into infant recipients, with disastrous consequences for patient and graft survival [30]. As a result of widespread dissemination of these data [31,32], there has been a marked change in the practice. From 1987 through 1990, the percentage of DDs older than ten years ranged from 59–68%. From 1991 through 1994, these percentages ranged from 78–88% and has continued to rise to greater than 90%. Prior to 1991, children younger then two years of age comprised 3.2% of DDs. In 1991, no pediatric recipient received a kidney from a DD less than two years of age; and in 1995 and 1996, there were no such kidneys utilized in children [33]. The early change in allocation of kidneys from young donors led to improvement in graft survival [30]. More recently there has been improvement in outcomes of kidney transplantation using young donors [17,34,35], but many programs transplant grafts from very young donors into older recipients [36,37].

Patient selection and indications for transplantation

Etiology of ESRD in children

Generally ESRD in children is due to congenital or inherited diseases. In reviewing 10 632 index transplants in the NAPRTCS database, the most common congenital diagnoses are obstructive uropathy and aplastic/hypoplastic/dysplastic kidneys, each representing about 16% of the patients [3] (Table 113.1). Among glomerular disorders, focal segmental glomerulosclerosis (FSGS) is the most common with 1246 children receiving a renal transplant for FSGS between 1987 and 2011. The primary diagnosis also varies with the race of the recipient. Overall in the NAPRTCS registry, Caucasian children account for 64% of all recipients; however, Caucasian children account for less than 50% of the children transplanted for FSGS. The data regarding the role of FSGS in leading to ESRD can be better appreciated by observations from the dialysis section of the registry, in which the two most common diagnoses are FSGS and aplastic/dysplastic kidneys at 14% each. Of 733 children with FSGS on dialysis, Caucasian children account for only 34%, with African-American and Hispanic children accounting for 62% of the patients. Twenty-four percent of African-American children on dialysis and 30% of those >12 years old have FSGS. The information regarding primary diagnosis is critical in predicting graft survival as well as recurrence of the original disease, as discussed below.

Table 113.1. Pediatric kidney transplant gender and race distribution by primary diagnosis

	N	%	% Male	% White	% Biopsied
Total	10632	100	59	64	56
Diagnosis					
Aplasic/hypoplastic/dysplastic kidney	1681	15.8	62	67	30
Obstructive uropathy	1630	16.5	85	67	30
Focal segmental glomerulosclerosis	1246	12.6	58	48	94
Reflux nephropathy	549	5.5	44	78	35
Chronic glomerulonephritis	340	3.4	44	50	75
Polycystic disease	323	3.2	51	78	52
Medullary cystic disease	287	2.9	50	87	66
Congenital nephrotic syndrome	277	2.8	52	68	87
Hemolytic uremic syndrome	273	2.7	57	82	52
Prune belly	268	2.7	98	63	38
Familial nephritis	241	2.4	81	60	72
Cystinosis	221	2.2	53	90	45
Membranoproliferative glommerulonehritis — Type I	187	1.8	45	60	96
Pyelo/Interstitial nephritis	186	1.8	47	74	77
Idiopathic crescentic glomerulonephritis	181	1.8	34	56	95
SLE nephritis	159	1.6	17	27	95
Renal infarct	140	1.4	48	82	37
Berger's (IgA) nephritis	135	1.3	54	72	94
Henoch-Schonlein nephritis	113	1.1	40	75	85
Membranoproliferative glommerulonehritis — Type II	85	0.8	49	78	96
Wegener's granulomatosis	66	0.6	44	68	91
Wilms' tumor	56	0.5	55	75	93
Drash syndrome	55	0.5	56	70	91
Oxalosis	55	0.5	51	92	75
Membranous nephropathy	47	0.4	64	51	94
Other systemic immunologic disease	34	0.3	12	61	94
Sickle cell nephropathy	13	0.1	56	0	75
Diabetic glomerulonephritis	11	0.1	36	36	64
Other	1110	11.2	53	63	64
Unknown	663	6.7	53	34	34

Adapted from Smith et al. [3].

Indications for renal transplantation in children

There has been a substantial change in long-term renal allograft outcome for children during the past decade [3,38–40]. Previously, young children were thought to have poor short- and long- term graft survival related to several factors, most prominently a proposed heightened immune response, especially in infants [41,42]. The most recent comprehensive registry reviews have clearly demonstrated a dramatic reversal in outcomes with marked improvements in patient and kidney graft survival for infants and young children [16,21,38,39,43]. Indeed, several analyses have identified these very young recipients as now having the best long-term survivals of all age groups [16,23,38,44] (Figure 113.2). Thus, children of all ages are excellent transplant candidates. By the time the child has reached chronic kidney disease (CKD) stage 4–5 planning for kidney transplantation and pretransplant preparation should have begun. There are very few contraindications to kidney transplantation in children. Perhaps the only two are another otherwise-fatal condition with a short projected survival, such as metastatic Wilms' tumor; and, severe neurological compromise. About 75% of children are treated with a course of chronic dialysis prior to renal transplantation, but unless there is a need for specific pretransplant preparation, there is no advantage to pretransplant dialysis. Preemptive renal transplantation, indeed, has a survival advantage [45–49].

Recipient age at transplantation

Kidney transplantation prior to six months of age, or in a recipient who weighs less than 6 kg is exceptional. Since 1987, UNOS has recorded 111 transplants performed in children younger than 12 months compared to about 3500 performed in children 1–5 years of age. Of these, seven transplants were performed in children between 3–5 months, 22 were performed in children between 6–8 months, and 63 were performed in children between 9–11 months of age. Only 31 infants have been reported since 2000. Since infants and adolescents have different risk factors for both patient and graft survival, children frequently have been grouped into five age categories: 0–1, 2–5, 6–12, 13–17 and 18–21 years of age. In 1987, 119 children 1–5 years old received kidney transplants whereas in 2012 the same age group received 187 transplants. Early on, excellent results were obtained is very young patients in some individual centers [44,50] but the concept of a heightened immune response in young recipients suggested that they were a high-risk group [51–53]. The unique problems associated with transplantation in young recipients may have been related to infections, technical issues and differences in pharmacokinetics [23,54–58] rather than their immune response.

There has been a substantial change in long-term renal allograft outcome for children during the past decade [38–40]. Previously, young children were thought to have poor short- and long- term graft survival related to several factors, most prominently a proposed heightened immune response, especially in infants [41,42]. The most recent comprehensive registry reviews have clearly demonstrated a dramatic reversal in outcomes. Improvements in surgical technique [59–63], donor selection [64], immunosuppression practices [65–69], and the enhanced experience of specialized pediatric transplant teams [70], as well as the development of

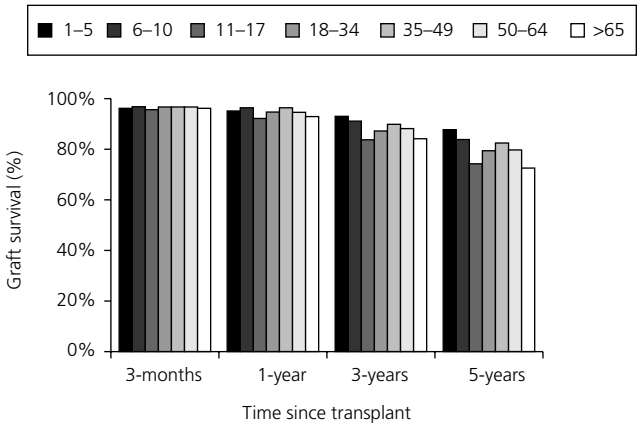

Figure 113.2. Living donor kidney transplant survival by recipient age at the time of transplantation. Reprinted from Magee JC, Krishnan SM, Benfield MR, et al. Pediatric transplantation in the United States, 1997-2006. American Journal of Transplantation. 2008;8(4 Pt 2):935–945.

multi-center research consortia have all led to marked improvements in patient and kidney graft survival for infants and young children [38,39,43]. Indeed, several analyses have identified these very young recipients as now having the best long-term survivals of all age groups [16,23,38,44,71]. In fact, young recipients of adult-sized kidneys who have immediate graft function have been reported as having the longest projected graft half-lives, exceeding even those of adult recipients of 2-haplotype matched living donor (LD) transplants [72].

Pediatric recipients under age 11 who receive LD kidney transplants have three-year graft survival rates that are as good or better than older age groups (93% for those aged 0–5 years, and 92% for those aged 6–10 years) [16]. The results of young recipients of DD kidney transplants are similar to those seen in adults, with recipients aged 1–5 years and those aged 6–10 years having three-year graft survival rates of 82% [16]. Unfortunately, this excellent outcome is not seen in adolescents [73,74] whose three-year graft survivals for both LD (85%) and DD (76%) grafts are worse than all other age groups (Figure 113.2).

Management of the transplant list

Recipient preparation

Before a child can undergo renal transplantation, the problems caused bychronic renal insufficiency (CRI) must be addressed and repaired if possible. In those cases where ESRD is due to urologic abnormalities, corrective reconstructive surgery should be undertaken, especially to the lower urinary tract, prior to transplantation. Two of the major consequences of CRI are anemia and growth retardation both of which should be addressed prior to transplantation. A recent report of final adult height in pediatric renal transplant recipients suggests that the current improvement in final adult height post-transplanttransplantation is more related to improving height deficits prior to transplant than to any net gains achieved after transplantation [10,12,75]. Uremia also leads to wasting and malnutrition in the child, and this can compromise the success of the procedure. For example, prophylactic native nephrectomy and reversal of protein wasting and malnutrition improves the outcome of transplantation in children with congenital nephrotic syndrome [76–78]. Careful preparation is particularly important in children undergoing pre-emptive transplants. Since live-viral vaccines are generally not indicated or effective in chronically immunosuppressed patients, children should receive all appropriate vaccines pretransplantation. There are various reports concerning the efficacy of immunization protocols for children pretransplantation [79–82].

Urological preparation

Children with urologic causes of ESRD require a thorough urologic evaluation prior to transplantation and they frequently require pre-transplant reconstructive urological surgery [83–86]. In a NAPRTCS report, 1878 of 7651 (25%) pediatric transplant recipients were identified as having lower urinary tract abnormalities [43]. For all such patients, a history of voiding pattern prior to development of renal failure is most helpful. Preliminary investigations consist of measurement of urinary flow rate and ultrasound estimation of the post-transplant micturition urine volume. Urinary flow rate should be at least 15 ml per second [87], and the residual volume should be less than 30 ml. Further investigations would consist of urethrocystoscopy in patients suspected of a urethral stricture, and a voiding cystometrogram is essential for complete assessment of bladder function [88]. This provides information about bladder capacity, pressure rise, and the efficiency of voiding. Still more information can be obtained by combining the urodynamic studies with radioisotope imaging. Routine voiding cystourethrogram is not indicated in older patients with no symptoms related to the urinary tract [89].

A bladder with a very small capacity may not be adequate for a functioning transplant. Occasionally a small capacity bladder may be seen in patients with prolonged oligoanuria. However if the bladder is distensible and the bladder wall compliant, such a bladder may be used safely for kidney transplantation. Other criteria for a useable bladder are an end-filling pressure less than 30 cm of H_2O, and a good flow rate. In patients with a poor flow rate, if urethral and bladder outlet obstruction are ruled out, the problem may be due to detrusor malfunction [87]. When a bladder fails to empty completely, infection and obstruction are potential complications that may shorten graft survival. Intermittent, clean, self-catheterization, which is widely used in urologic practice, can be safely used post-transplanttransplantation in patients where the primary abnormality is inefficient and uncoordinated detrusor function. Most pediatric patients have a urinary bladder that will adapt to the new kidney. Although the bladder may not appear to have the capacity, especially in patients on long-term dialysis prior to transplantation, it will most often distend with usage [90]. However in patients with a truly low capacity or high pressure, bladder augmentation may be necessary prior to transplantation [91–94].

The goal of modern reconstructive pediatric urology is to have a competent low-pressure urinary reservoir which can be emptied by voiding or at least by intermittent catheterization. Augmentation cystoplasty consists of adding bowel or gastric wall to the bladder, whereas substitution cystoplasty is performed when most of the bladder is excised and replaced with bowel. Gastric remnants have been popular for augmentation, however they do tend to cause excessive loss of acid in the urine, leading to discomfort and metabolic alkalosis. Early attempts to reconstruct bladders with bioengineered material are ongoing. There are promising reports of "bio-engineered" bladder material, although these have not yet been tried in transplant recipients [95–97]. Urologic reconstruction, including augmentation cystoplasty, typically occurs prior to

transplantation [85,86,93,94,98–102]; although some programs have reported successful reconstruction after transplantation also [84]. In those patients in whom augmentation has been performed, long-term antibiotic therapy and intermittent catheterization may have to be carried out to prevent urine stasis and infection. In general, the incidence of urinary tract infection and other complications is higher in these recipients, their course is generally no worse than pediatric recipients without urologic abnormalities [94]. If native kidneys in children with ESRD are causing hypertension, chronic infections or excess losses of protein, urine or other substances, there should be consideration for nephrectomy prior to or at the time of transplantation [103]. About 25% of children have native nephrectomies prior to index transplants [104].

Donor preparation

Donor selection

The selection of the appropriate donor is an integral part of the transplantation procedure and may be a limiting factor in the long-term outcome of kidney transplantation for any individual child. The use of living donors has generally been more common in pediatric kidney transplantation than in adults [3,16,32,33,38,43,104,105]. On average, graft survival can be twice as long when a LD is used compared to a DD [22,38,104,105]. There are limitations to the use of living donors however, such as donor suitability, blood group incompatibility and age; and, thus, not every child may have a suitable living donor. Moreover, there is concern about using living donors when there is a substantial risk of early graft failure or recurrent disease [106,107]. When DDs are used for children, careful attention should be paid to utilizing low-risk donors since the mortality risk for children after kidney transplantation is low and children are expected to require the grafts for long periods of time [18,64,108–110].

Living donor

The first successful kidney transplants utilized living donors and the use of living donors has been a mainstay of pediatric kidney transplantation since that time. Importantly, as the donor shortage has become more acute, considerations about donor safety have also become apparent [111]. Recently, the transplant community has examined this issue and has reached consensus on the appropriateness of the use of living donors, their evaluation process and the appropriate follow-up and care of those donors [1,112]. The donor should never be put at physical, emotional or psychological risk for the benefit of the recipient. Included in this principle was the agreement that children less than 18 years of age should not be considered as living donors, although that has not been universally observed [27,113]. The LD pool has been increased by the use of genetically unrelated donors, which initially included spouses, but has expanded to include other genetically unrelated family members, friends or even anonymous non-directed ("good Samaritan") donors. Importantly, the outcomes of these unrelated donors appear to be comparable to related donors [114–121]. There have even been proposals that non-directed living donors be compensated for the donation, leading to a commercial system to expand the donor pool [122,123]. Although commercial systems using paid donors have existed in many parts of the world, most countries have declared such practices illegal and the broad transplant community has condemned them [124,125]. The use of non-directed living donors have also played an important role in expanding the donor pool through the practice of donor exchange. These programs have allowed living donors to donate to an unrelated recipient in exchange for that recipient's incompatible donor to donate to the former's family member [126–128]. These programs have included simple two-recipient exchanges, multiple domino-style exchanges and exchanges with the DD waiting list [129–139]. In depth treatment of the ethics of living donation can be found in Chapter 138, and specific coverage of Pair Exchange donation is found in Chapter 27.

The work up for a donor proceeds as described in detail in Chapter 23. In general, all living donors are evaluated for ABO blood type, human leukocyte antigen (HLA) histocompatibility typing and some type of cross-matching with potential transplant recipients. Parents predominate as living kidney donors for children, accounting for about 80%. Unrelated donors, siblings and occasional non-directed donors make up the balance [105]. Thus, there are few 2-haplotype donors for pediatric kidney transplantation, with most donors being 1-haplotype HLA matched, or less. Although there may be some disadvantage to the lack of any HLA-B matching [105], overall the lack of histocompatibility matching for pediatric recipients, especially infants, does not seem to compromise long-term outcomes [23,72].

As noted above, donors are carefully evaluated in order to assure that they are not being paid, coerced or that they might be injured in any way as the result of the donation procedure. The components of an appropriate donor evaluation have been published [140,141]. Much of the evaluation is performed to assure donor safety, but also it is designed to assure that the donor does not transmit infectious or other diseases to the recipient and that the donor organ will provide sufficient function for the recipient. In general, donor candidates are excluded if they are too young (<18 years of age), too old (as individuals age, they lose glomerular filtration rate (GFR) and the potential for cancer, cardiovascular and other diseases increases) or have medical problems that may be exacerbated by the donation surgery or living with only one kidney [112]. Conditions such as hypertension, diabetes and a history of metastatic cancer typically exclude donors. Also, chronic infections such as HIV, or hepatitis B or C are exclusionary because of the likelihood of transmission. Although cytomegalovirus (CMV), human papillomavirus (HPV) and Epstein Barr Virus (EBV) may also be transmitted, these are typically not exclusionary, since they can be treated in the recipient.

Deceased donor

The majority of kidney transplants currently come from DDs. While living donors were more common for children in the past, changes in allocation policy in the US has led to a predominance of DD transplants for children since 2006 (Figure 113.1) [4,16,18]. The consequences of this change in donor selection will not be known for years; but, on average, the long-term outcomes of LD kidney transplants in children are superior to those obtained from DDs (Figure 113.3) [3,16,18,104,105], indicating that the short term benefit of having rapid access to the DD list may be counterbalanced by shortened graft survival.

For many years there was a tendency to utilize kidneys recovered from infants and young children for transplantation into young recipients [30]. Early studies showed that 25% of all DD transplants performed in children were recovered from children under ten years [41]. A special study of the NAPRTCS [64], demonstrated that the preferential placement of small kidneys into infants and very young children had disastrous consequences for graft survival and this was subsequently confirmed by a larger study [142]. As a result,

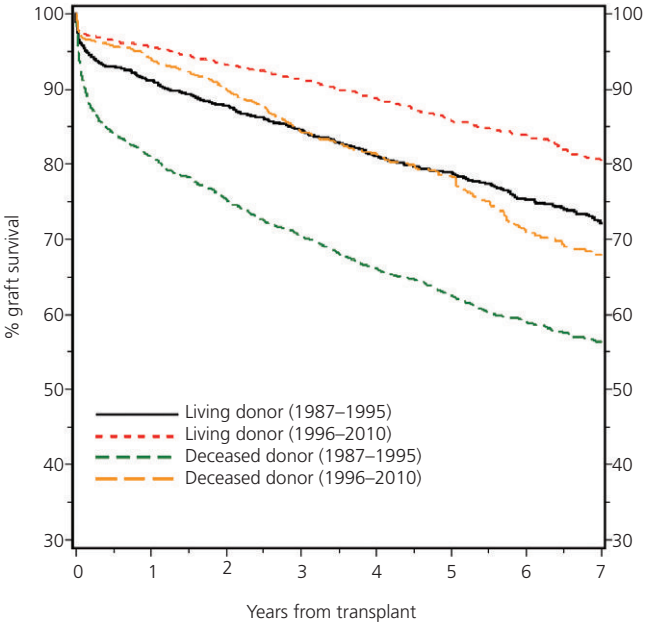

Figure 113.3. Seven-year graft survival of living and deceased donor pediatric kidney transplants from two eras. Reproduced from NAPRTCS 2010 Annual Report: www.naprtcs.org. https://web.emmes.com/study/ped/annlrept/2010_Report.pdf. [December 19, 2013].

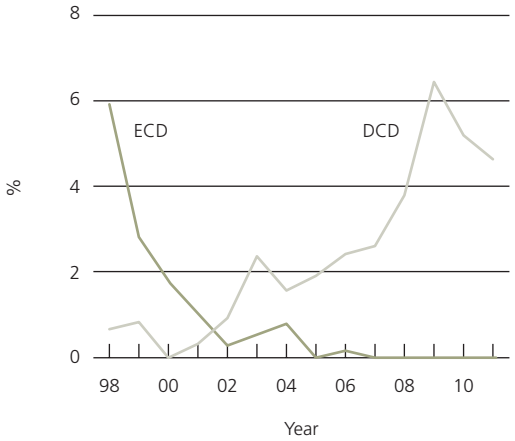

Figure 113.4. Frequency of extended criteria donors (ECD) and donors after cardiac death (DCD) as source of deceased donors for pediatric kidney transplantation 1998–2010. SRTR 2012 Annual Report: http://www.srtr.org/ [accessed December 19, 2013].

DD allocation policies for pediatric recipient were changed resulting in improved outcome [30]. More recent information shows that this practice has now undergone substantial change [43]. Since 1991, less than 1% of DD kidneys for pediatric recipients have come from donors younger than two years old. And, during the same time, the percentage of kidneys recovered from donors over the age of ten years increased from 78% to 88%. Importantly, the kidneys from young donors have not been discarded, but have been utilized for transplantation via the en bloc technique into adult recipients [36,142,143]. Single centers have reported better success with young donors, utilizing carefully controlled protocols [34,35]. Advanced donor age is also associated with diminished long-term graft survival [144,145] and likely should be avoided for young recipients [64,146,147].

The influence of matching on transplant outcome used to be substantial, but it has diminished significantly since the introduction of improved immunosuppression [148–151]. Pediatric DD kidney transplant outcomes are influenced very little by donor histocompatibility matching [18,104,105]. ABO blood type compatibility and cross matching are still necessary, however. In general, transplant candidates who have preexisting antibodies or who develop antibodies after transplantation are at higher risk of early rejection and should have enhanced immunosuppression protocols [152–156].

Deceased donors are typically evaluated to assure that the graft will provide sufficient function for the recipient and that the donor does not transmit infectious or metastatic cancer diseases to the recipient. In the past, the standard for determining the donor eligibility rested on the determination of brain death. However in efforts to expand the donor supply, recent advances have been made in recovering organs from donors whose death has been determined on the basis of cardiac death, so-called donation after cardiac death (DCD). Children are receiving these grafts at an increasing frequency (Figure 113.4). Although there is a substantially higher incidence of delayed graft function when such donors are used, the short term outcomes of kidney transplants from DCD donors is equivalent to that from brain-dead donors [118,128,157,158] but there is some concern about late graft loss [159].

Most countries with national organ allocation systems have provided some sort of preference for pediatric deceased-donor transplant candidates. In the US, a recent change of the Organ Procurement and Transplant Network (OPTN) allocation system gives children under18 years of age at the time of listing, preference over most other candidates, resulting in much shorter waiting times than adult candidates [18]. The use of elderly or extended criteria donors (ECD) has continuously been associated with poor outcomes [119,128,145,160]. Children rarely receive organs from elderly DDs or from extended criteria donors (Figure 113.4) [104].

The transplant procedure

Technical issues in transplantation

The operative technique differs based on the weight of the child. For small children, less than 15 kg, the transplant is frequently done through a midline incision and the larger vessels are utilized for anastomosis with the donor kidney [90]. After reflection of the cecum and the right colon, the anterior wall of the aorta and the inferior vena cava are exposed and dissected [161]. The aorta is mobilized from above the inferior mesenteric artery to the external iliac artery on the right side. After ligating and dividing the lumbar branches, the iliac arteries and the inferior mesenteric are encircled. Next the inferior vena cava is mobilized from the left renal vein to the iliac veins. After ligating the lumbar veins the iliac veins are encircled. The donor renal vein is anastomosed to the recipient vena cava in an end-to-side technique [162]. The donor renal artery is then anastomosed to the recipient aorta in an end-to-side fashion. Careful attention needs to be paid to the recipient hemodynamic response upon clamping and unclamping of the major vessels, and it is desirable to maintain a central venous pressure of 15–18 cm H_2O prior to unclamping [90,161].

The filling of the transplanted kidney may be slow due to the fact that a large adult kidney will take up a significant portion of the normal pediatric blood volume. Hemodynamic studies suggest

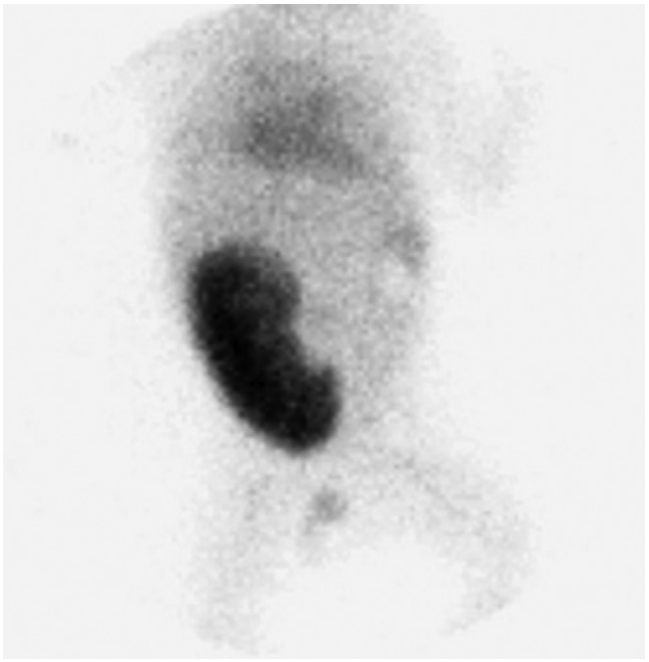

Figure 113.5. ^{99M}Tc-MAG3 radionuclide renal scan in a nine-month-old infant who received a living donor (LD) renal transplant from his father. The graft is intraperitoneal and occupies most of the right side of the peritoneal space. Note the relative sizes of the graft and the heart. Reprinted from [315] Harmon WE. Pediatric kidney transplantation (Chapter 75). In: Avner ED, Harmon WE, Niaudet P, et al., editors. Pediatric Nephrology, 6th ed. Springer; 2009: Oxford University, UK: 1867–1901.

that the cardiac output of infants must double in order to perfuse the adult donor kidney adequately [163]. Thus volume replacement is critical (Figure 113.5). The ureteral anastomosis is done by implanting the donor's ureter into the recipient's bladder using either a Ledbetter-Politano procedure or a modification of it to assure non-refluxing anastomosis. However, most surgeons now prefer a non-refluxing extravesical rather than transvesical approach for ureteroneocystostomy (see Chapter 55) because it is faster, a separate cystotomy is not required and less ureteral length is necessary, thus assuring a distal ureteral blood supply [164–166].

The transplantation technique utilized in children with a body weight greater than 15–20 kg is similar to that employed in adults described in Chapter 55. Unlike the transperitoneal approach necessary in younger children, this transplant is extraperitoneal, with the renal vein anastomosed to the common iliac or the external iliac vein [161]. The arterial anastomosis can be to either the common iliac or internal iliac artery. The ureterovesicular anastomosis is done utilizing the techniques described above.

The use of a laparoscopic technique to remove the LDkidney has become very popular recently, because it tends to shorten the hospitalization and rehabilitation time for the donors. The procedure does require substantially longer time in the operating room and the rate of delayed graft function is slightly higher. Although most programs indicate that the procedure is safe, a review of 10 000 living organ donations identified three deaths or serious complications; all three followed laparoscopic procedures [167]. Although some studies suggest more complications in the pediatric recipients [168,169], most reports demonstrate good outcomes when the donor has a laparoscopic nephrectomy [170–175].

Evaluation of graft dysfunction

At the completion of the vascular anastomosis and release of the vascular clamps immediate function of the transplanted kidney is demonstrated by the production of urine. Various causes however, may prevent initial function, and evaluation of immediate non-function and the differential diagnosis of this condition is a critical component of the transplant physician's role.

Delayed graft function (DGF)

A well-functioning kidney graft should lead to normal renal function within two–three days. The lack of attainment of normal renal function, as demonstrated by a fall of the serum creatinine to normal levels, is termed delayed graft function (DGF). There is no consensus concerning the definition of DGF [176]. In some settings, DGF is used only to distinguish recipients who require dialysis after transplantation, which is a stringent but commonly-used definition. Acute tubular necrosis (ATN) represents the most frequent cause of immediate graft non-function. The risk of early ATN is related to factors such as prior transplants, prolonged cold ischemia, absence of prophylactic antibody therapy and the use of more than five pretransplant blood transfusions. If recovery of graft function is delayed, however, a transplant biopsy may be necessary since other diagnostic tests cannot distinguish between ATN and rejection or sudden recurrence of primary disease [177–179]. Importantly, early acute rejection can mimic ATN or coexist with it [180]. The presence of ATN does not auger well for the transplant, particularly for recipients of DD grafts since graft failure and death are more common among patients with ATN [181,182]. DGF is an independent risk factor for graft loss and death [183–185]. Importantly, although the incidence of DGF is increased when a DCD donor is used, the detrimental affect of DGF on long-term outcomes in this setting does not appear to be as severe as when it occurs after living donation or after transplantation from brain-dead DDs [157,158,176].

Graft thrombosis

Graft thrombosis is an almost unique complication of pediatric transplantation. Although usually a major cause of immediate graft non-function, it can be seen later on in the course, and has been recorded to occur as late as 15 days post-transplanttransplant following initial engraftment and function. Graft thrombosis has been the third most common cause of graft failure in pediatric renal transplantation, accounting for about 10% of graft losses [3,43]. It is irreversible in most cases and necessitates removal of the graft. Graft thrombosis should be suspected in cases where there has been immediate function followed by the development of oligoanuria. The diagnosis is established by a radionucleide scan using diethylenetriamine pentaacetic acid (DTPA) or MAG3 [186], which reveals a photopenic defect with no uptake by the transplant kidney (Figure 113.6).

Since the outcome of graft thrombosis is uniformly dismal, numerous studies have been conducted in an attempt to understand and anticipate this complication. The etiology of graft thrombosis is multifactorial, but it is more commonly seen in young recipients [110]. In a special study of 2060 LD and 2334 DD kidneys [187], the NAPRTCS has shown that a history of prior transplantation increases the risk, whereas increasing recipient age has a protective effect for LD kidneys. The prophylactic use of

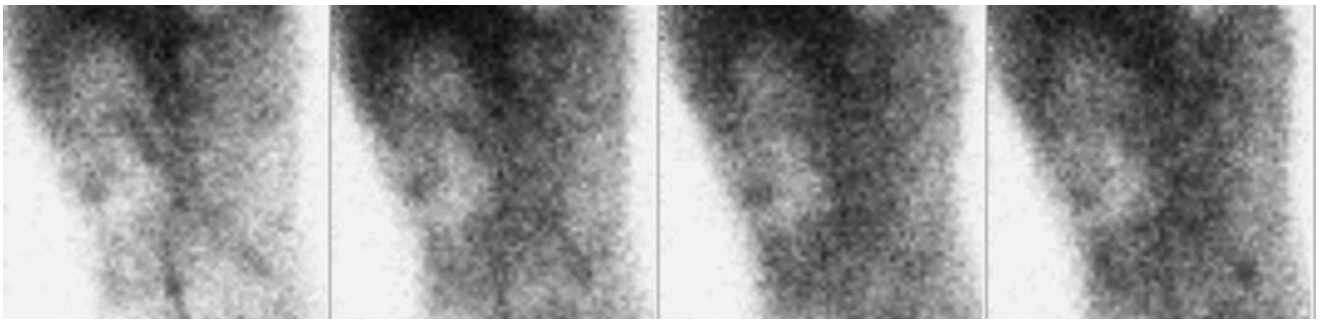

Figure 113.6. ^{99M}Tc-MAG3 radionuclide renal scan in a six year old girl with focal segmental glomerulosclerosis (FSGS) who received a living donor (LD) renal transplant, performed 16 hours postoperatively. Note the photopenic area in the right abdomen, indicating thrombosis of the graft with no perfusion. Reprinted from [315] Harmon WE. Pediatric kidney transplantation (Chapter 75). In: Avner ED, Harmon WE, Niaudet P, et al., editors. Pediatric Nephrology, 6th ed. Springer; 2009: Oxford University, UK: 1867–1901.

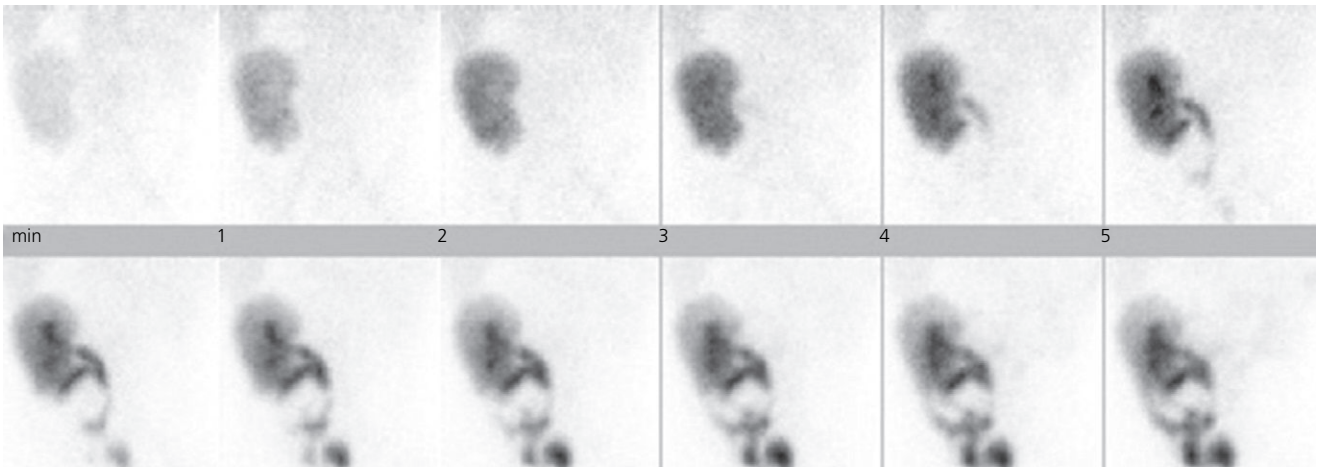

Figure 113.7. ^{99M}Tc-MAG3 radionuclide renal scan in an eight year old girl who received a deceased donor renal transplant, performed 12 hours postoperatively. Note the good perfusion of the graft and the rapid concentration and excretion from the kidney. Tracer, however, rapidly accumulates in the right lower quadrant, outside of the bladder. Investigation demonstrated a traumatic bladder rupture. Harmon WE. Pediatric kidney transplantation (Chapter 75). In: Avner ED, Harmon WE, Niaudet P, et al., editors. Pediatric Nephrology, 6th ed. Springer; 2009: Oxford University, UK: 1867–1901.

antilymphocyte antibody also decreases the risk and this may be particularly true for the monoclonal IL2r antibodies [188]. For DD kidneys, a cold ischemia time longer than 24 hours increases the risk of thrombosis. The use of antibody induction therapy, the use of donors greater than five years of age, and increasing recipient age were factors that decreased the risk of thrombosis. A heightened thrombotic state has also been implicated [186,189,190]. One study showed that centers that performed fewer infant transplants had higher rates of graft thrombosis [70]. Other studies suggested that pretransplant use of peritoneal dialysis increased the risk of thrombosis [191,192], but a later study disputed that relationship [46]. Some centers routinely administer anticoagulants to pediatric recipients at high risk of graft thrombosis, but no clinical studies of their effectiveness have been performed and its use is not without complications [193].

Obstruction, urinary leak, urologic complications

An uncommon but correctable cause of immediate graft dysfunction is obstruction of the urinary flow, which presents as decreasing urine output and the development of hydronephrosis. An ultrasound or radionuclide scan with a furosemide washout enables the clinician to establish this diagnosis. Obstruction can be due to kinking of the ureter, to edema or blockage of the implantation site of the ureter or to development of a lymphocele. A more ominous cause of immediate non-function is the rare case of urinary leak due to disintegration of the distal ureter or rupture of the bladder. This condition is extremely painful due to the extravasation of urine into the pelvis or peritoneal cavity and is established by radionucleide scan (Figure 113.7). The appearance of the tracer in the peritoneal cavity or in the scrotal, vulvar, or inguinal area clinches the diagnosis and immediate surgical correction is necessary.

Immunosuppression strategies

Immunosuppressive strategies are consistent with those described for adults throughout this text. One-half of pediatric kidney transplant recipients receive antibody-based induction therapy following kidney transplantation, with about half of these receiving lymphocyte-depleting agents [3,194–198]. At day 30 post-transplanttransplant, majority of children receive "triple" maintenance immunosuppression with tacrolimus, mycophenolate mofetil and steroids [3,108], but an increasing percentage are no longer receiving chronic steroids. As shown in the NAPRTCS annual report, the "typical" immunosuppression protocols have changed frequently over the past two decades (Figure 113.8). However, it

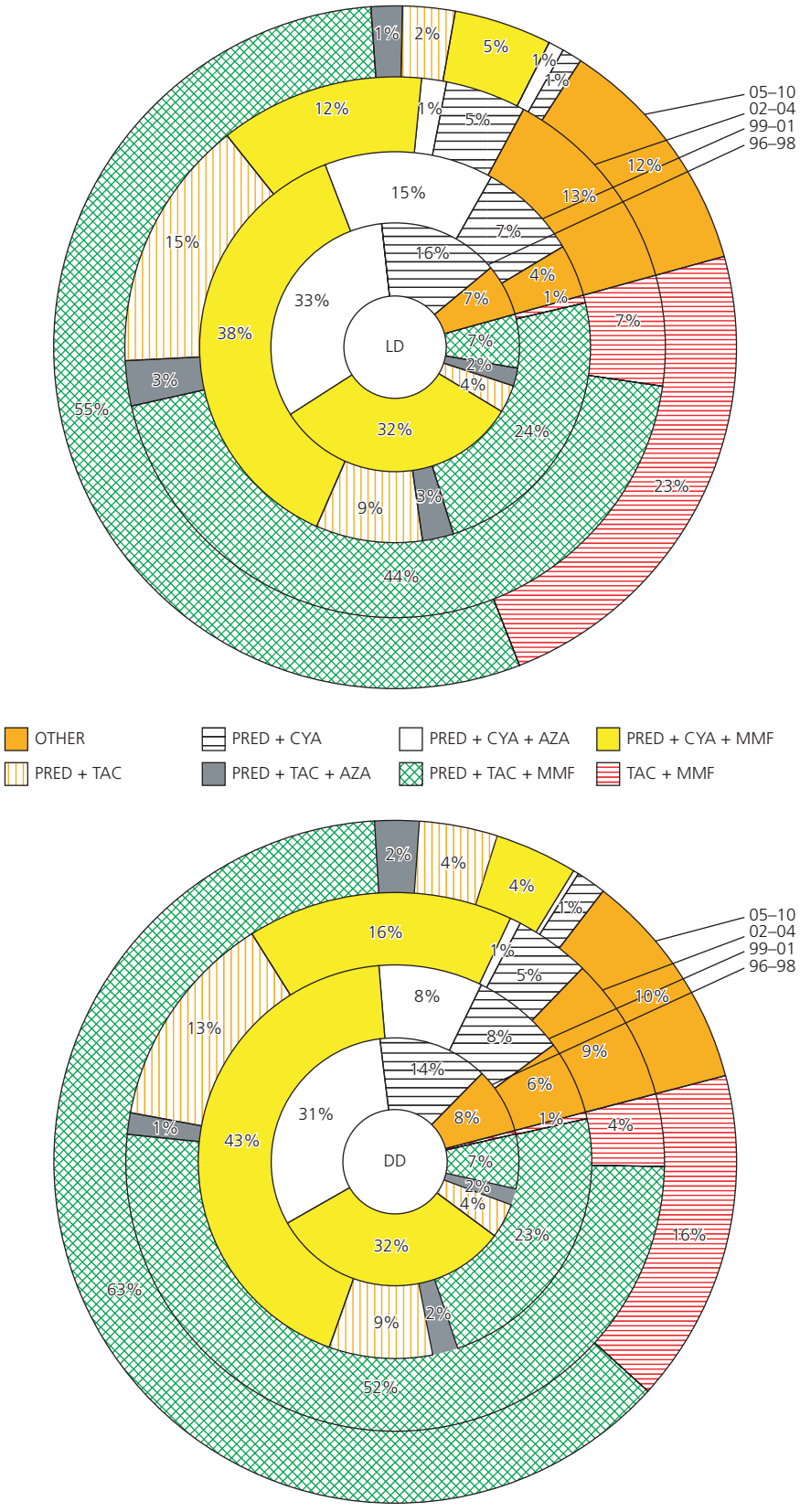

Figure 113.8. Maintenance immunosuppression at Day 30 posttransplant in pediatric living donor (LD) and deceased donor (DD) renal transplant recipients by three-year cohorts:1997–2010; adapted from NAPRTCS 2010 Annual Report: www.naprtcs.org. https://web.emmes.com/study/ped/annlrept/2010_Report.pdf. [December 19, 2013].

also appears as if children who begin treatment under one combination stay on that regimen. Several recent multi-center research efforts have been directed to attempt to decrease the number of types of chronic immunosuppression, with corticosteroids and calcineurin inhibitors being the most common medications targeted for removal [199]. Several single center [175,199–201] and multicenter trials [202–204] have reported success in avoiding or withdrawing corticosteroids, although some of these have had substantial side effects [202,205,206]. There have also been reports of avoidance of calcineurin inhibitors in children designed to prevent nephrotoxicity. At least one of these trials resulted in excellent long-term graft function, but had a high rate of early acute rejections [207]. A subsequent trial with enhanced antibody induction may have better short-term results [195].

Post-transplant transplant management

Hyperacute rejection

Hyperacute rejection is the result of specific recurrent antidonor antibodies against HLA, ABO or other antigens [208]. Irreversible rapid destruction of the graft occurs. Histologically there is glomerular thrombosis, fibrinoid necrosis, and polymorphonuclear leukocyte infiltration. In the early years of transplantation, when the HLA matching techniques were not well developed, hyperacute rejection was more common. In most centers, it occurs very rarely. The latest data from the NAPRTCS shows only19 cases over the last 24 years [3]. The only treatment is surgical removal of the allograft.

Acute rejection

Information regarding the incidence and outcome of acute rejection in pediatric renal transplantation is difficult to standardize since there are multiple different definitions. A common definition of a rejection episode is based upon the circumstance of a patient having been treated with anti-rejection therapy although biopsy confirmation is becoming more common. In a recent review of pediatric kidney transplantation, the biopsy confirmation rate was 95% and the one-year cumulative acute rejection rate was reported to be 15% [3]. A remarkable decrease in the incidence of acute rejection has occurred over the past two decades (Table 113.2). Risk factors for DD transplants include the absence of prophylactic T cell antibody therapy, donor age less than five years, black race, and no Dr matches. Risk factors for LD transplants are the absence of T-cell antibody and one or two Dr mismatches, black race and ATN. There are conflicting data about whether infants and small children have a "heightened" immune response and an increased incidence of acute rejection episodes. Indirect evidence suggested a more vigorous immune response especially in infants [42]. Also, data from the UNOS registry demonstrated a higher rate of acute rejections in young children after both LD and DD transplantation, although adolescents were noted to have a higher rate of late acute rejections [23]. On the other hand, data from surveillance transplant biopsies suggest equivalent rejection responses in all groups [52]. Data from one large pediatric transplant program demonstrated that infants have a lower rate of acute rejection than older children [54]. A recent SRTR report demonstrated that infants and young children now have the best outcomes of all age groups [15]. Thus, either the proposed heightened immune response has been overcome by improved immunosuppression or the cause previously poor outcome was related to other factors.

Table 113.2. 12-month probability (%) of first rejection, by transplant year

Transplant year	Living donor		Deceased donor	
	%	SE	%	SE
1987–1990	54.2	1.7	68.9	1.5
1991–1994	44.9	1.5	60.3	1.6
1995–1998	33.0	1.4	40.5	1.7
1999–2002	21.9	1.3	26.6	1.8
2003–2007	12.8	1.3	17.1	1.5
2007–2010	8.6	1.8	16.6	2.1

Adapted from Smith et al. [3].

Diagnosis of acute rejection

Rejection is suspected when there is decreasing urinary outflow and a rising serum creatinine. In the past, classical signs of acute rejection included fever and graft tenderness. Under calcineurin inhibitors and prophylactic antibody therapy however, these signs are rarely seen; thus early evidence of graft dysfunction even without other signs, should initiate concern. The differential diagnosis consists of ureteral obstruction, renal vascular compromise from stenosis, urinary leak and an infectious process. When rejection is suspected, a urinalysis and urine culture should be performed to assess the possibility of infection. The urinalysis is also helpful if it suggests intra-graft inflammation or immune response as evidenced by proteinuria and the presence of leukocytes and other cells in the sediment. An ultrasound is performed to rule out anatomical obstruction. Obstruction can be the result of perirenal fluid collection, a large lymphocele, hematoma, or rarely, an abscess. The ultrasound can also provide information about intragraft blood flow and pressure [177]. A radionuclide renal scan, using a tracer such as MAG3, is a very helpful tool in establishing some diagnoses (Figures 113.6 and 113.7) [209]. Rejection is suggested by rapid uptake of the tracer by the kidney but a delayed excretion. Unfortunately radionucleide scans cannot distinguish among various causes of intragraft dysfunction, such as rejection, cyclosporine toxicity and ATN. Thus, a definitive diagnosis of rejection requires a transplant biopsy.

Pediatric renal transplant biopsy

The renal transplant biopsy procedure is very easy and safe when conscious sedation and ultrasound guidance are utilized. Data evaluating over 150 pediatric renal transplant biopsies, including some in intraperitoneal kidneys and many performed during the first week post-transplanttransplantation, have demonstrated a very low risk [178]. A major factor in reducing post-transplantbiopsy bleeding is the use of a automated biopsy "guns" using a small (18 gauge) rather than the standard (15 gauge) needle. Biopsies should be performed in pediatric renal transplant recipients whenever the diagnosis of rejection is in doubt.

Treatment of acute rejection

Standard treatment of an episode of acute rejection is intravenous methylprednisolone in a single daily dose of 10–20 mg/kg (maximum dose: 0.5 Gm), for three consecutive days. Most Grade I and II rejections will respond to steroid therapy. Steroid resistant rejection episodes are treated with T cell antibody, such as the polyclonal antithymocyte globulin Thymoglobulin. Thymoglobulin is given in a dose of 1.5–2 mg/Kg /dose for a total of 10–14 days. It may be advisable to monitor CD3+ cells during treatment and restrict the frequency of dosing only to days when the count is

greater than 20 cells/mm^3 [210]. All antibodies have several side effects. Precaution against the potential anaphylactic reaction related to polyclonal antibodies consists of using 500 mg of methylprednisolone with the infusion of the antibody and administration of an antihistamine, such as diphenhydramine (Benadryl), one-half hour prior to drug administration.

Chronic allograft nephropathy

The gradation from acute to chronic rejection is gradual; however many biopsies may show features of both, and some characteristic vascular changes of chronic rejection may be seen as early as ten days post-transplant-transplant [211]. The clinical picture is that of gradually declining renal function together with varying degrees of proteinuria and hypertension [212]. The clinical condition may be referred to as transplant glomerulopathy, chronic rejection, chronic allograft dysfunction (CAD), chronic allograft nephropathy (CAN) or interstitial fibrosis and tubular atrophy (IFTA) [213]. The succession of names reflects lack of clarity of the etiology, clinical course, or treatment of this disorder. Nonetheless, this process, which will be referred to as CAN in this chapter, is the leading cause of graft loss following kidney transplantation in children.

An ongoing controversy exists as to whether the changes seen in chronic rejection are immune mediated, secondary responses to infection, ischemic in nature, or non-immunological injury due to hyperfiltration [214–217]. Data in children have shown clearly that acute rejection is a predictor of chronic rejection [40]. In a study of 1699 LDs and 1795 DD recipients NAPRTCS noted acute rejection was a relative risk (RR) factor for chronic rejection (RR = 3.1), and multiple acute rejections increased the RR to 4.3. Late acute rejections are also clinical correlates of chronic rejection [218]. Even if acute rejection is the most critical element in the genesis of chronic rejection, other immune mechanisms may mediate its progression, such as antibodies directed against the donor, MICA, endothelial cells and B lymphoblasts [156,219]. Gene expression profiles in graft biopsies of patients with established CAN demonstrate upregulation of profibrotic and growth factors [220].

Recurrent kidney disease

As described in depth in Chapter 77, some diseases will recur in a transplanted kidney, and the recurrent disease may lead to loss of the graft, as it had done to the native kidneys previously. Recurrence of the original disease is the cause of 7% of all pediatric kidney graft losses [3]. The five-year graft survival for children with FSGS is 71% and for children with glomerulonephritis it is 77%; in contrast to all other causes of ESRD in which five-year graft survival is greater than 83%. In the DD group, five-year graft survival rates for children with FSGS, glomerulonephritis and congenital nephrotic syndrome are below 64%; hemolytic uremic syndrome (HUS) and familial nephritis have rates of 66%; and all other causes have a rate of 70%. Several publications have reviewed the course of recurrent disease in pediatric kidney transplantation [106,221,222]. In some cases, recurrence of some features of the disease without affecting graft survival up to recurrence of the full disease with substantial reduction in graft survival. Unfortunately, there has been very little change in frequency of recurrent disease in pediatric grafts, despite substantial changes in immunosuppression during the past two decades [3,223].

Focal segmental glomerulosclerosis is the most common cause of steroid resistant nephrotic syndrome leading to ESRD and is the most common acquired cause of ESRD in children. Reports of recurrence of FSGS vary from 15–50% and about 50% of the recurrences lead to graft loss [221–223]. The genetic diseases do not seem to recur in a graft. Risk factors for recurrence include early onset of nephrotic syndrome, rapid progression to ESRD, resistance to treatment, white or Asian race, recurrence in a previous transplant, and possible presence of a circulating "glomerular permeability factor" [179,223]. Recurrence can occur immediately after transplantation and result in massive proteinuria, acute tubular necrosis and even graft failure related to small vessel thrombosis [179]. In general, children with active nephrotic syndrome are not candidates for pre-emptive transplant because of the heavy proteinuria and consequent risk of graft thrombosis and delayed diagnosis of recurrence [221]. Many programs will perform native nephrectomy and will maintain the children on chronic dialysis for some period of time, certainly to improve nutritional status and to normalize the serum albumin. There is no benefit to LD transplantation in children with recurrent FSGS: although graft loss due to rejection is lower in recipients of LD transplants, graft loss due to recurrence is higher, leading to equivalent graft survivals in living and DD transplants [179,224]. Plasmapheresis is often used prophylactically prior to transplantation or immediately after it to attempt to prevent or treat recurrence of FSGS [225–228] and some programs report complete remission in up to 60% treated in that manner. Although no specific immunosuppression protocol has demonstrated clear efficacy in treating or preventing recurrent FSGS, there is some evidence that high dose cyclosporine may be effective in doing so [227,228]. Whether the high dose is needed to counteract the effect of high serum levels of low-density lipoprotein which binds free cyclosporine, or whether the beneficial effects are due to direct action on podocytes is not clear [106,229]. Rituximab has also been used to treat recurrent FSGS in children, with mixed results [230–233].

Hemolytic uremic syndrome in children is most commonly caused by enteropathic bacteria and the disease typically does not cause ESRD or recurrence in a kidney transplant [234]. On the other hand, children with atypical or "non-Shiga toxin-associated" HUS have a much higher incidence of progression to ESRD and recurrence of the disease after transplantation [107,235,236]. Although calcineurin inhibitors have been associated with de novo HUS in a few kidney transplants, their use in recurrent disease seems to have no effect [106]. In patients with factors H, I or B mutations, the recurrence rate appears to be high but recent reports of prophylactic or rescue treatment using a monoclonal antibody that binds to complement factor protein C5, eculizumab, are very promising [237–239].

Both forms of MPGN can recur in transplants, with variable frequency from 30–60% [106]. Type II seems to be more severe, and neither form seems to be treatable after recurrence [240].

Primary oxalosis recurs almost immediately and universally after kidney transplantation and was once considered a contraindication to kidney transplantation. However, treatment with intensive pre and post-transplant dialysis to lower the body burden of oxalate and the use of combined kidney-liver transplantation has led to substantially better outcomes [241–246]. If liver transplantation is being considered, however, careful consideration must be paid to determining whether the child has a variant that might be responsive to lifelong treatment with pyridoxine rather than liver transplantation [106]. Methylmalonic acidaemia may be partially ameliorated by kidney transplantation, but full treatment may require liver transplantation in select recipients [106]. ESRD is typically the earliest organ failure in children with cystinosis and often accounted for the bulk of deaths from this disorder. However, the

use of kidney transplantation and cystine-depleting therapy with cysteamine has extended their life expectancy to the fifth decade [247–250]. Although cystine may accumulate in the interstitium of renal grafts, it does not cause graft failure. However, the unremitting accumulation of cystine results in substantial non-renal morbidity and mortality [249,251].

Outcomes and risk factors

In order to obtain a proper population mix representing gender, age, and racial diversity, multi-center registry results such as SRTR and NAPRTCS annual reports have been used to accurately report kidney graft outcomes [3,16,21].

NAPRTCS has recorded a total of 11 603 kidney transplants in 10 632 pediatric recipients between 1987 and 2010. Of the 2920 graft failures, about 9% were deaths with a functioning graft, 13% were due to acute rejection and 36% were due to chronic rejection. Vascular thrombosis remains a major cause of failure, and 13.5% were attributed to primary non-function, vascular thrombosis, or miscellaneous technical causes. These data show that such problems occur in about 4% of all pediatric transplants [70,110,187,189, 191,192,252]. Recurrence of original disease as a cause of graft failure accounted for 7% of graft failures. The specific diseases include: focal segmental glomerulosclerosis accounted for 44% of these graft losses, membranoproliferative glomerulonephritis Type II 9%, HUS 9%, oxalosis 5%, chronic glomerulonephritis 4%, others 28%.

Overall ten-year LD graft survival curves by recipient age are shown in Figure 113.2 and seven-year graft survival rates by donor source and era are shown in Figure 113.3. Expected graft survival for index transplants performed in the last decade at one, three and five years for LD kidneys is 97, 92, and 84% respectively, and for DD kidneys it is 95, 84, 78% [3,21]. There has been a continuous improvement in short and mid-term graft survival rates, mostly due to marked improvements in early graft survival rates. This may be related to the decreased frequency of acute rejection rates and the decreased incidence of acute rejection as a cause of graft loss. It is notable, however, that as shown in Figure 113.3, the slope of the graft survival curves have not changed significantly over the past two decades. These important trends in improved graft survival in pediatric LD and DD renal transplant outcome have been reported frequently over the past decade [3,15,21,32,105].

Table 113.3 shows relative hazards for graft failure for selected transplant characteristics for both living and deceased kidneys.

Table 113.3. Relative hazard analysis for graft failure in pediatric kidney transplantation

	Living donor		Deceased donor	
	RH	*P* Value	RH	*P* Value
Recipient age (>2)	1.23	NS	0.67	0.0040
Prior transplant	1.50	<0.0001	1.45	<0.0001
Induction antibody	0.84	0.0050	0.92	NS
>5 Lifetime transfusions	1.22	0.0160	1.25	0.0006
No HLA-B mismatches	1.32	0.0183	1.15	0.0170
No HLA-DR mismatches	0.82	NS	1.13	0.0333
Black race	1.94	<0.0001	1.58	<0.0001
Prior dialysis	1.16	0.0375	1.23	0.0326
Cold storage time >24hours	–	–	1.15	0.0200
Transplantyear (1987–2010)	0.95	<0.0001	0.94	<0.0001

Adapted from Smith et al. [3].

Relative risks of graft failure are derived using Cox proportional hazards regression models. For recipients of LD grafts, the most influential prognostic variables of graft survival are race (African American vs. non-African American), prior transplant, lack of induction antibody treatment and transplant year. For DD recipients, the important prognostic factors include: African-American race, prior transplant, age older than two years and transplant year. A history of prior dialysis, lack of HLA-B and -DR matches and prolonged cold-storage time may be slight relative risks. Male recipients seem to have improved risk compared to females for both LD and DD transplants. Also for both LD and DD transplants, a history of more than five lifetime blood transfusions seems to be associated with worse graft survival rates, but the significance of this finding in the modern era is not clear. Also, the interpretation of the use of induction antibody treatment is hampered by selection factors that motivate its usage; the size and direction of these biases cannot be quantified and the evaluation of this factor cannot be considered definitive. Importantly, the improvement in graft survival rates in very young recipients is strongest in the LD recipients and the overall improvement in this age group may be related to the high percent of living donors used for them.

Growth following transplantation

A major distinguishing feature of pediatric from adult recipients is the need for children to grow. The growth failure commonly observed in children at the time of transplantation is multi-factorial; however the most important cause is the reduced response to endogenous growth hormone [253], related to several mechanisms. For several years it has been suggested that a functioning transplant would enable the child to achieve catch-up growth [9]. Unfortunately, long term data from registry studies has shown a more disappointing outcome.

NAPRTCS data shows that the mean height deficit at the time of transplantation is −1.75. Males (−1.78) and younger recipients have greater height deficits at the time of transplantation [43]. The deficit has been improving steadily, with a mean deficit of −2.43 in 1987 improving to −1.23 in 2009 [3]. Younger children generally show early catch-up growth [3,9] with complete inversion of Z-score up to 0.60 at two years for those <5 years of age at transplant. Older children may grow at a normal rate, but rarely show catch-up growth. Final adult height for children with ESRD is improving, but all of the improvements seem to be related to the gains achieved during treatment for CKD rather than after transplantation [10,12]. The final adult height of children transplanted more recently is much better than those transplanted years ago. The Z-score for children transplanted in 1987–1991 who have reached their terminal height was −1.93 as compared to −1.08 for those in the 1997–2001 cohort.

Adherence to chronic immunosuppression treatment

Non-adherence is often cited as a cause of long term graft loss in pediatric renal transplant recipients, especially adolescents [254]. This is covered in depth in Chapter 120. A major reason for non-adherence is thought to be the alteration in appearance that accompanies immunosuppressive medications, including the cushingoid facies and growth retardation related to long-term daily corticosteroid administration and the hirsuitism and gingival hypertrophy associated with cyclosporine. However, the true incidence of non-adherence is unknown. Non-adherence rates of 22% [255], 43% [256] and as high as 64% in adolescents [257] have been reported.

Some factors, such as young age, adolescence, poor socio-economic status, and family stress have been associated with increased levels of non-adherence [255,257–259]. Importantly, however, healthcare workers are not able to identify a significant proportion of non-adherent patients [260]. Treatments such as educational programs [256] and family-based therapy [261] have been proposed, but these types of programs have not been universally successful in changing motivation [254]. An alternative proposal for improving non-adherence would be to change the type or frequency of immunosuppressive medications so that the recipients do not have to adhere to rigid schedules; but these proposals are currently only hypotheses [175,262].

Hospitalization

The re-hospitalization rate has fallen substantially between 2000 and 2010. Living donor recipients have decreased from 49 to 39% with the stay has decreased from eight to six days. For DD recipients, the rate has fallen from 57 to 42% and from ten to six days [3]. The most common reason for hospitalization used to be for treatment of rejection. However, a recent analysis supports that treatment of viral and bacterial infections are the next most common reasons for hospitalization [3,263]. Treatment for hypertension is the cause for hospitalization in the first six months in 5–8% of recipients, and falls to ~1% five years after transplantation [33].

Post-transplant transplant lymphoproliferative disease and malignancy (PTLD)

Although PTLD has been reported as a complication of pediatric organ transplantation for many years [264], the number of published reports seem to be increasing [265]. It is not clear whether this indicates that the incidence of this potentially lethal complication of immunosuppression is increasing or if it is just more readily recognized. If the incidence is increasing, it may be the unfortunate consequence of "improved" immunosuppression [266]. In a review of UNOS data age <18 years, Caucasian race, and male gender are significant risk factors [267]. Current incidence appears to be 1–2% of all pediatric renal transplants with some reports being much higher [206].

This complication is specifically covered in Chapter 96. PTLD often presents within lymph nodes, but it can be extra-nodal, frequently occurring within the gastrointestinal tract [268], proximate to or within the graft [269], or distant from it [270]. Presentation of PTLD within the central nervous system is often devastating and rapidly fatal. PTLD is generally thought to emanate from an EBV infection [268,271,272]. Thus, the pretransplant EBV status of the donor and recipient may be an important determinant of the disease and may explain why the disease is more common in children than in adults [273,274]. In several reports, the incidence rate of PTLD for EBV-seronegative recipients was many times higher than for EBV-seropositive recipients [275–277] and in others, the source was the donor in most of the cases [278]. However, EBV seropositive recipients may be at risk for later onset and more lethal disease [279]. Concomitant primary infection with CMV may increase the risk of PTLD five-fold [275]. The intensity of immunosuppression may also predispose the child to PTLD [277,280]. Treatment with anti-lymphocyte antibodies, such as OKT3, as either induction or anti-rejection therapy, may increase the risk of developing PTLD substantially [275,276,279,281]. Although it has been reported following both cyclosporine and tacrolimus treatment, programs that have used both drugs have suggested that the incidence was higher in tacrolimus-treated recipients [266,273,282]. However, a recent registry report suggests that neither mycophenolate mofetil (MMF) nor tacrolimus were independent risk factors for PTLD; rather, the intensity of immunosuppression was most important [267].

The diagnosis of PTLD has generally been made on the basis of characteristic pathologic findings and the diagnosis cannot be made without biopsy material. Advances in detection of EBV DNA [283–287] and in the outgrowth of transformed lymphocytes [288–290] have permitted early detection of patients at high risk to develop PTLD. Surveillance of blood and prospective adjustment of immunosuppression has been proposed, but there are no universally-accepted standards in this area [291]. Similar tests have been used also to guide treatment [283] but their absolute value for this function is not established.

The mainstay of treatment of PTLD is the reduction or discontinuation of immunosuppression [278,292,293]. Of interest, in many of these cases, the graft is not rejected despite the marked lowering or discontinuation of immunosuppressive medications. Interferon-α and intravenous γ globulin [294,295], ganciclovir [296,297], and even chemotherapy have been suggested, but their efficacy has been variable. Prophylaxis of high-risk patients may be useful [298]. Treatement with the monoclonal antibody Rituximab has shown promising results [299–303].

Other infections

Immunosuppression renders both adult and pediatric recipients susceptible to numerous viral and bacterial infections. These are covered as they relate temporally to the transplant procedure in Chapters 92–94. Infections account for the majority of complications post-transplanttransplantation in children and are the principal cause of morbidity. Prophylactic therapy against the more common infections seen in the context of a renal transplantation is employed by most centers.

Cytomegalovirus infection in immunocompromised allograft recipients may cause serious or even fatal disease, especially when the recipient is sero-negative and the donor is positive. Prophylaxis with oral valganciclovir for 3–12 months post-transplanttransplantation is routinely practiced in pediatric kidney transplant programs [297,304,305]. Similarly, prophylaxis against pneumocystis jirovecii pneumonia using trimethoprim-sulfa, pentamidine, dapsone or atovaquone.

Varicella can be very serious or even fatal in immunocompromised individuals [306,307]. Children should receive varicella vaccine prior to transplantation [308,309] and should be repeated as necessary to assure sero-conversion prior to chronic immunosuppression [81]. The use of varicella vaccine post-transplanttransplantation has been reported in only a small series [309], but it is likely safe, although not uniformly successful. BK virus nephropathy is an increasingly recognized complication of renal transplantation and its incidence in children is similar to what is seen in adults [310–313]. As is done with EBV, the typical approach is preventative, with careful screening for viremia and selective reduction of immunosuppression when it occurs. Some patients may benefit from treatment with leflunomide [314].

Mortality

Mortality rates for children receiving kidney transplants is remarkably low. Patient survival has improved for both LD and DD recipients (Figure 113.9) [3] and current five-year patient survival is now 97% and 96% respectively. Survival for infants, especially those

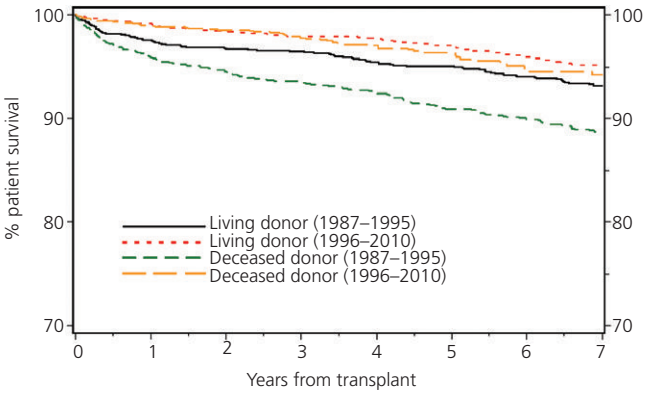

Figure 113.9. Seven-year actuarial patient survival in children from living donor (LD) and deceased donor (DD) renal transplantation from two eras; adapted from NAPRTCS 2010 Annual Report: www.naprtcs.org. https://web.emmes.com/study/ped/annlrept/2010_Report.pdf. [December 19, 2013].

receiving DD grafts, was not as good as older children, but the current rates are now comparable. The major cause of death for children is infection, followed by cardiopulmonary problems, malignancy and dialysis-related problems. About half of the deaths occurred with a functioning graft [3].

Summary

In general, pediatric transplantation has improved through the implementation of therapies designed for adults and then adapted post-transplant hoc to the specific needs of children. Surgical techniques relate to the size of the child and the congenital anomalies that often are found in this population. Outcomes are outstanding and significantly better than those achieved through dialysis, although this remains suboptimal in adolescents. Immune management is similar to adults transplant recipients, although this is largely a product of the development pathways for drugs, with the small population of children commanding limited attention from pharmaceutical companies. Future opportunities in the field include specific immunosuppressive approaches that specifically consider the dynamic, developing immune system in children (see Chapter 112), and begin providing therapies uniquely responsive to the needs of children.

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CHAPTER 114

Pediatric Liver Transplantation

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Introduction

Although the basic principles of evaluation and selection for liver transplantation are the same for children as for adults with acute or chronic liver disease, clearly the candidate populations are divergent. Pediatric recipients are distinct from adult recipients not only with respect to age but also with respect to the spectrum of liver diseases, increased penetration of technical variant grafts, decreased risk of disease recurrence, protracted exposure to immunosuppression medications, and remarkable potential for life years gained [1].

Deceased donor organ allocation

Allocation of deceased donor (DD) livers to pediatric candidates is based on the regulatory framework of the Final Rule established in 2000 and implemented by the US Department of Health and Human Services in 2002. The pediatric end-stage liver disease (PELD) and Mayo end-stage liver disease (MELD) scoring systems use objective measures to estimate disease severity and medical urgency. Ethical considerations involved in the allocation of organs can be found in Chapter 137, and national allocation policies are considered in Chapter 128. The PELD score is calculated from age, serum albumin concentration, total serum bilirubin concentration, international normalized ratio (INR) and the presence or absence of growth failure [2] while the MELD score is calculated from total serum bilirubin concentration, INR, and serum creatinine [3]. Organs are allocated to children under age 12 years based on the PELD score and to adolescents aged 12 to 18 years based on the MELD score. Providers may request additional exception PELD/MELD points from a regional review board if the calculated PELD/MELD score is believed to inadequately reflect the severity of the patient's disease [4–6].

Indications for liver transplantation for pediatric patients

The indication data depicted here are retrieved from the Studies of Pediatric Liver Transplantation (SPLIT) Registry and represent over 2000 pediatric liver transplants done in North America (Table 114.1). Biliary atresia is the most common underlying condition for liver transplantation, accounting for approximately 37% of the cases. Acute liver failure, retransplantation, and primary liver tumors including hepatoblastoma account for 13.5, 10, and 9% of transplants, respectively. Approximately 21.5% of the pediatric liver transplants are performed for inherited gene defects that be categorized into (1) disorders of cholestasis (4.5%); (2) disorders of metabolism refractory to medical therapy, without cirrhosis (6%) and (3) disorders of metabolism that lead to structural liver injury with cirrhosis (11%). The remaining cases comprise a heterogeneous group of chronic conditions including cryptogenic cirrhosis, autoimmune hepatitis, sclerosing cholangitis and mitochondrial hepatopathy. Disease recurrence is rare, occurring only in patients with autoimmune disease and in a subset of recipients with inherited disorders of cholestasis [7]. As a result, liver disease distribution differs distinctly between children and adult as the latter is dominated by hepatitis C cirrhosis, alcoholic and non-alcoholic steatohepatitis, and hepatocellular carcinoma.

Biliary atresia

For children with biliary atresia, there is no doubt that liver transplantation improves survival. Left untreated, all patients with biliary atresia die by two years of age; more than 70% ultimately require liver transplantation [8–10]. Transplantation offers the only option for patients with failed portoenterostomy. With improved immunosuppression and operative techniques, adjusted five year post-transplant patient survival for biliary atresia is 92.3% after living donor (LD) liver transplantation and 85.8% after DD liver transplantation (2009 OPTN/SRTR Annual Report 1999–2008. HHS/HRSA/HSB/DOT; UNOS; Arbor Research Collaborative for Health). The decision is more complex for older patients who develop complications of portal hypertension after successful portoenterostomy in whom survival benefit is less well-defined [9,11–14].

Acute liver failure

Decision-making relative to liver transplantation for children with acute liver failure (ALF) is complicated as the disease is characterized by rapid clinical changes. Moreover, the increased risk of early and late post-transplant mortality compromises survival benefit [15–17]. Children with indeterminate ALF, compared to those with an established cause of ALF, are 2.5 fold more likely to require liver transplantation. For the latter, which include children with acute acetaminophen toxicity, herpes simplex, and hemophagocytic lymphohistiocytosis [16,18–21], targeted treatment alternatives can be life-saving without transplantation. Existing acute liver failure scoring systems including the Kings College Hospital Criteria (KCH) [22], the Clichy score [23,24], MELD/PELD [24–26] fail to

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 114.1. Indications for pediatric liver transplantation. Derived from the SPLIT Registry (Patients with first transplant between June 1, 2005 and May 31, 2009)

Primary Diagnosis	%Primary Transplant
Biliary atresia	41.2%
Alagille syndrome	3.1%
Acute liver failure	14.5%
Liver tumor	5.2%
Metabolic liver disease without cirrhosis	11.2%
Other	15.8%

Table 114.2. Indications for liver transplantation for children with metabolic liver disease. Derived from the SPLIT Registry (Patients with first transplant between June 1, 2005 and May 31, 2009)

Primary Diagnosis	N
α1-Antitrypsin deficiency	71
Wilson's disease	28
Tyrosinemia	24
Primary hyperoxaluria	6
Cystic fibrosis	37
Urea cycle defects	58
Crigler-Najjar	15
Glycogen storage disease	18
Neonatal hemachromatosis	13
Inborn error in bile acid metabolism	2

reliably distinguish between those who will spontaneously survive and those who require transplantation [16,21]. The pressing clinical questions faced when managing children with ALF are:

1 Does the child have a condition that is medically treatable?
2 What is the risk of deterioration or improvement for each successive day that the child remains alive with his/her native liver?
3 Is transplantation necessary for patient survival?
4 Is full recovery possible without transplantation?
5 Are associated morbidities recoverable or irreversible?

Liver tumors

Hepatoblastoma is the most common primary liver tumor in children, accounting for approximately 70% of transplants for hepatic malignancy and 5% of all pediatric liver transplants performed. Primary curative resection yields outstanding results with >90% event-free survival. Unfortunately, only one third present with resectable disease. For the remainder, neoadjuvant chemotherapy is administered. For children deemed unresectable even after chemotherapy, liver transplantation is required for long-term survival [27–29]. The main predictors of clinical outcome are the presence or absence of metastatic disease, histology, and tumor resectability as determined by PRETEXT staging [27,30–32]. Current US allocation policy stipulates assignment of status 1B at listing.

Metabolic disease

Transplantation corrects the expression of mutated proteins by the liver and any co-existing structural damage [33] (Table 114.2). For the sub-group of metabolic diseases with structural liver damage, the decision to proceed with transplantation is based on survival benefit and medical urgency as determined by PELD/MELD score. However, for the subgroup without structural liver disease, the goal of liver transplantation is not only to correct the gene defect but to prevent damage to extra hepatic organ(s) occurring consequent to the gene defect. Urgency therefore reflects the risk of irreversible damage to the central nervous system or the kidneys, rather than the risk of death. Transplant decision-making must balance the risk of transplantation against the likelihood that transplant will prevent extrahepatic disease progression and requires a multi-disciplinary approach inclusive of relevant specialists.

Retransplantation

The main indications for retransplantation are primary non-function, hepatic artery thrombosis, chronic rejection, and late vascular or biliary complications. Primary non-function and early hepatic artery thrombosis are medically urgent, usually occurring within days after transplantation. Patient and graft survival after retransplantation are lower than for primary transplant [34]. Donor age less than one year, use of a technical variant graft, and INR at time of retransplantation are independent predictors for survival; INR likely acts as a surrogate marker of liver failure.

Transplant evaluation

Evaluation for liver transplantation should be initiated when a child is determined to have irreversible, potentially life-threatening liver disease. The timing of evaluation depends on the child's clinical status along with an understanding of the primary disease process. For instance, an asymptomatic six-month-old child with biliary atresia and a serum bilirubin of 8 mg/dl will require liver transplantation by age 24 months to ensure survival. In contrast, a six-month-old child with Alagille Syndrome and the same laboratory profile in the absence of cardiac disease will not likely require transplantation. The evaluation process seeks to: (1) estimate the survival benefit offered by transplantation [35]; (2) identify progressive and irreversible non-hepatic disease that may negate the benefit of transplantation; (3) determine if psychosocial support is sufficient to optimize outcome.

The impact of liver transplant on quality of life remains a critical part of the evaluation and discussion with the patient and family. Nevertheless, given limited data on quality of life over time after liver transplantation and the DD organ shortage, quantity of life supercedes quality of life [4,36]. An interdisciplinary transplant team comprised of surgeons, hepatologists, coordinators, psychologist, social worker, financial counselor, and any other relevant consultants should participate in the assessment of any candidate for liver transplantation. General considerations for the preoperative evaluation of transplant candidates can be found in Chapter 29.

Survival benefit

On many occasions, there is insufficient data to make evidence-based decisions on the presence and magnitude of the survival benefit offered by liver transplantation for children. As with the adult population, short-term survival benefit is strongly driven by liver disease severity. For children with a PELD score of 17–27, liver transplantation yields survival benefit after one month. In contrast, for patients with PELD scores of 7–16, survival benefit does not begin to accrue until ten months after transplantation [37].

Is there irreversible and progressive non-hepatic disease that compromises transplantation outcome? Liver transplantation is generally contraindicated by uncorrectable multi-system failure, extra-hepatic malignancy, active infection outside the hepatobiliary system or advanced and/or irreversible cardiopulmonary or central nervous system disease. There are however exceptions. A single, surgically resectable hepatoblastoma metastasis does not preclude

transplantation [27,28]. Often, the distinction regarding irreversible, extra-hepatic disease may be ambiguous. Arguably, liver transplantation should not be considered if futility is likely. However, the definition of futility is elusive as it depends on a complex interaction of factors including primary diagnosis, transplant center skill, and the availability and quality of organs. Moreover, the definition of futility for children may differ from adult, considering the greater potential for life years gained and the lower burden of medical co-morbidities? Are we, as a society, willing to accept more risk for children given the potential for greater survival benefit return? Analysis of SRTR data has delineated survival benefit during the first year after liver transplantation from time of listing for children [38]. Using the PELD score as an estimate of disease severity, a futility threshold could not be identified. Given the lack of a defined threshold, an effective interdisciplinary team to promote good decision-making is critical for cases in which a substantial concern for futility has been raised.

Is there psychosocial support sufficient to optimize outcome? Psychosocial evaluation does not focus solely on suitability of the candidate but on identifying and mitigating psychosocial risk which might diminish post-transplant outcomes [39]. A structured assessment should aim to determine if: (1) there is evidence of child abuse; (2) the child or parent is depressed or has evidence of post-traumatic stress; (3) the family can support the child through the transplantation process. None of these factors should be considered contraindications to transplantation but challenges to be addressed by the liver transplant team.

Pretransplant care

The pretransplant period is a time of significant risk. Mortality risk on the waiting list is highest for children with acute liver failure and for those who are less than one year old [21,37] (Table 114.3). The outcome of transplantation reflects an interaction of multiple processes: (1) the primary disease; (2) pretransplant care; (3) organ allocation; (4) donor/graft quality; and (5) operative and peritransplant care [40]; also covered in Chapters 20 and 38.

Care during the pretransplant period impacts early patient and graft survival. In order for a pediatric candidate to be medically and psychosocially optimized at the time of transplantation, the transplant team must develop processes to:(1) address patient and family education; (2) maximize access to a suitable organ, living or deceased; (3) optimize nutritional status; (4) prevent infection; and (5) prevent and/or treat complications of portal hypertension.

Hepatopulmonary syndrome

Hepatopulomonary syndrome (HPS) is defined by the presence of severe liver disease, hypoxemia on room air, intrapulmonary vascular dilatation [41,42] in the absence of underlying primary cardiopulmonary disease. HPS is a risk factor for poor prognosis in patients with cirrhosis [43]. However, HPS is reversible after liver transplantation, particularly if transplant occurs prior to the development of severe hypoxemia [44,45]. While data in children are limited, liver transplantation has been shown to improve survival for adults with HPS [45]. As a result, current allocation policy recognizes HPS as an exceptional indication for transplantation. Adults who satisfy predetermined criteria receive a MELD score equivalent to a 15% mortality risk at three months. MELD score then increases by a 10% mortality equivalent every three months. No standard criteria have been established for children with HPS.

Table 114.3. Prevalence of specific symptoms as the primary indication for pediatric liver transplantation. Derived from the SPLIT Registry (Patients with first transplant between June 1, 2005 and May 31, 2009)

Primary Indication for Transplant	N	%
Failure to thrive	255	26.6
Ascites	308	32.1
Spontaneous bacterial peritonitis	31	3.2
Hepatic encephalopathy	130	13.6
Portal hypertensive bleeding	80	8.3
Intractable pruritus	99	10.3
Other	56	5.8
Total	959	100.0

Variceal hemorrhage

The efficacy of primary and secondary prophylaxis for variceal bleeding is established for adult patients but unknown for children. Management of acute variceal bleeding should parallel that for adult patients, beginning with hemodynamic stabilization, diagnostic and therapeutic endoscopy followed by a defined for plan secondary prophylaxis.

Malnutrition

Maintenance of adequate nutrition is a challenge given the effect of chronic liver disease on intake and endocrine function, the latter including diminished response to growth hormone [46]. Based on the data from the SPLIT Registry, 40% of transplant candidates less than two years old are more than two standard deviations below norms for weight and height at evaluation, and even more are greater than two standard deviations below norms at time of transplant. It is likely that an even larger proportion suffer milder degrees of nutritional deficiency. Optimizing nutritional status for children with moderate or high liver disease severity (MELD/PELD score >15) requires thorough, serial assessments on a monthly basis. In-depth diet history, evaluation of anthropometrics and growth, review of medication and vitamin/mineral supplementation, determination of energy, protein, macronutrient and/or micronutrient intake, and fluid requirements should be reviewed. Nutritionally replete children on the waiting list suffer fewer infections that may delay or complicate transplantation. After transplantation, a favorable nutritional status is associated with fewer infections, improved wound healing, and decreased risk of intractable ascites [12,47].

Ascites and spontaneous bacterial peritonitis

Ascites management should be directed towards maintaining a patient's intravascular volume status, serum sodium greater than 130 meq/l, and adequate nutritional and respiratory status. A sudden, marked increase in ascites may reflect portal vein thrombosis that should be assessed using Doppler ultrasonography. Guiding principles and management strategies for adults are outlined in several reviews but are also applicable to children [48,49].

Spontaneous bacterial peritonitis (SBP) is defined as bacterial infection of ascites not due to specific intra-abdominal pathology. Registry data indicate that SBP occurs in 10% pediatric patients awaiting liver transplantation. Fever, worsening encephalopathy, abdominal pain, and essentially any clinical change in a patient with ascites should be investigated with diagnostic paracentesis. SBP is most frequently diagnosed by increased neutrophils in the ascitic fluid (>250 cells/mm^3), with or without a positive culture. Broad-spectrum anti-microbial therapy should be initiated on the basis of a positive cell count. Cefotaxime has been the most widely studied antibiotic, and covers the three most common isolates: *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae* [48,49].

Repeat paracentesis is not needed unless symptoms persist or response to treatment is atypical. Studies in pediatric patients are limited. Even so, primary and secondary prophylaxis for children with cirrhosis and ascites is likely to improve outcomes and, as such, is often prescribed.

Transplant procedure and surgical techniques

Historically, children have been instrumental in the initial establishment of and subsequent innovation in liver transplantation. Both the first attempted liver transplant in 1963 [50] and the first three successful liver transplants in 1967 involved young children [51]. Later, during the late 80s as the field of liver transplantation burgeoned, it became clear that waitlist mortality rates of 25–46% (compared to 8% for adults) secondary to the insufficient supply of size-matched organs demanded innovation [52–54]. The liver's segmental anatomy inspired surgeons to transplant partial grafts of a size suitable for the specific candidate [55,56]. Initially, whole livers underwent size reduction into a right lobe, left lobe, or left lateral segment graft through formal resection on the back table to accomplish transplantation of one child with outcomes comparable to whole liver transplantation [53,57,58]. Shortly thereafter, innovation progressed to divide whole livers into two transplantable grafts accomplishing transplantation of either two children or one adult and one child [58–60] (Figure 114.1). Initially, complication rates for split grafts were higher than whole or reduced grafts, reflecting the increased surgical complexity of dividing the critical structures optimally for the two recipients [59]. These difficulties were quickly overcome, paving the way for a final innovation, transplantation of a partial graft procured from a LD [61,62]. With the rapid development and dissemination of a deep menu of options to achieve transplantation for children, that waitlist mortality dropped precipitously [54,63], thereby maturing the field of pediatric liver transplantation. Technical details of the whole liver transplant procedure can be found in Chapter 56 and of the segmental and live donor liver transplant procedures in Chapter 57. The prevalence increased steadily from two transplants in 1989 (0.4%) to 120 transplants (20.4%) in 2000 (Figure 114.2). Coincident with a major change in allocation policy for deceased donor organs in February 2002, the trend reversed such that living donors currently accounts for 9–12% of all pediatric liver transplants. Figure 114.3 shows that the frequency of living donor transplantation varies by candidate age. Perhaps not surprisingly, living donor transplants consistently account for the largest percentage of transplants for infants (<1 year) followed by toddlers (1–5 years). However, interestingly, older children (6–17 years) comprise a larger percentage of LD transplant recipients in the past few years, perhaps a consequence of the maturation of adult to adult LD liver transplantation.

Early post-transplant surgical complications

Vascular complications

The substantial technical challenge of pediatric liver transplantation is reflected by the observed rates of surgical complications in the early post-transplant period. Hepatic artery thrombosis (HAT)

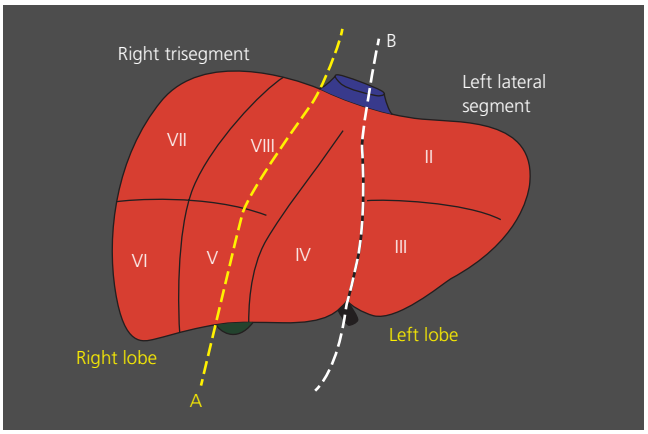

Figure 114.1. Splitting a deceased donor liver. For transplantation of one child and one adult, the liver is typically divided into two grafts as indicated by the white dashed line "B": (A) left lateral segment (Couinaud's segments II and III) accounting for approximately 20–25% of the liver mass, and (B) right trisegment graft (Couinaud's segments I, IV–VIII) accounting for approximately 75–80% of the liver mass. The left lateral segment typically comes with the left hepatic vein, the left portal vein, the celiac axis with Carrel aortic patch, and the left hepatic duct. The right trisegment graft typically comes with the inferior vena cava, the main portal vein, the right hepatic artery, and the common bile duct. An alternative splitting approach is to divide the liver into the right lobe (Couinaud's segments V–VIII) and the left lobe (Couinaud's segments I–IV) as indicated by the yellow dashed line "A".
Reproduced from Renz JF, Yersiz H, Reichert PR, Hisatake GM, Farmer DG, Emond JC et al. Split-liver transplantation: a review. Am J Transplant 2003;3(11):1323–1335, with permission from John Wiley and Sons.

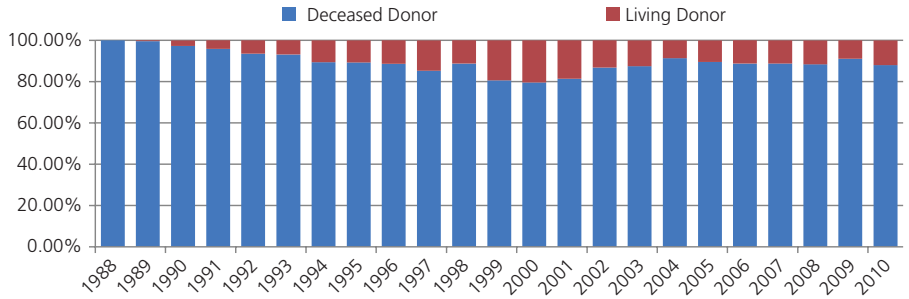

Figure 114.2. Annual proportion of deceased versus living donor liver transplants for children (<18 years). Since the first pediatric living donor liver transplant in 1989, their proportion increased steadily through the 1990s. However, coincident with implementation of the MELD/PELD liver allocation policy in February 2001, the national volume of living donor liver transplantation decreased and currently accounts for approximately 10% of pediatric liver transplants.
Based on OPTN data as of January 12, 2012.

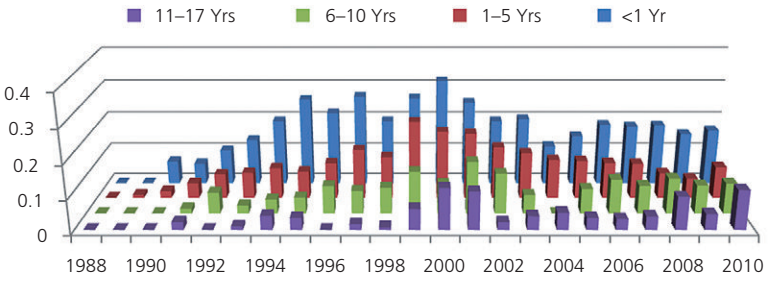

Figure 114.3. Proportion of living donor transplants according to recipient age. The proportion of living donor transplants varies significantly with donor age and year of transplantation. Although living donor transplants have predominantly served infants and toddlers (0–5 years of age), there appears to have been a relative increase in the percentage of pediatric liver transplants. Based on OPTN data as of January 12, 2012.

looms as a serious, potentially life threatening complication after pediatric liver transplantation. Although HAT can be clinically silent, it can also lead to acute allograft failure with massive hepatic necrosis or subacute problems with abscess formation or ischemic biliary complications of leak or stricture. Historically, thrombosis rates reached as high as 38% but, more recently, many centers have reported rates of less than 10% [64–70]. Similar to portal vein complications, demographic factors that likely translate into small recipient and/or donor vessel size such as age less than three years, weight less than 15 kgs, use of a whole graft, and multiple/arteries requiring reconstruction and/or separate anastomoses have been implicated risk factors [67,68,70–72].

Substantial thrombosis rates led to exploration of alternative surgical techniques and additional medical therapies. Early on, in the setting of DD whole or partial liver transplantation, aortic revascularization either directly or with an interposition graft and the use of an interrupted suture technique was tauted as an advance [67,68,70,73]. However, with the emergence of LD transplantation, adaptation of microvascular surgical techniques with high magnification (greater than 10×) afforded by an operative microscope led to dramatic reduction of hepatic artery thrombosis rates [66,74–77]. More recently, several reports suggest that high power loupe magnification (3.5 to 6×) may be sufficient [64,65,78]. Medical maneuvers to mitigate risk of HAT center on anti-coagulation strategies. Aspirin, heparin, and/or low molecular weight dextran are frequently employed but are of unproven efficacy [65,71,79,80].

Considering the frequency of HAT and the knowledge that successful intervention depends upon early diagnosis, many pediatric transplant centers perform daily or even twice daily Doppler ultrasounds for one or two weeks after liver transplantation [65]. Suspicious findings are typically confirmed by angiography although some centers recommend proceeding immediately to exploration [72,81,82]. In the operating room, thrombectomy with or without thrombolysis is performed with reassessment of the original anastomosis and revision as necessary. Full anti-coagulation may also be instituted.

Patient and graft outcomes of HAT are mixed. Many reports indicate that HAT does not impact post-transplant patient survival [69,72,81]. Graft survival, however, is clearly compromised as 60–75% of recipients with HAT require retransplantation [69,82,83]. Urgent revascularization has a success rate of 30–50% [69,82]. However, even after successful revascularization, many children with HAT suffer biliary complications [69,72,84].

Portal vein complications, most often stenosis or thrombosis, are the second most common vascular complications after pediatric liver transplantation. Reported frequencies range from 4 to 16% although higher rates, upwards of 35%, were observed in early series [85–94]. Potential risk factors for portal vein complications include recipient demographic variables, anatomic and functional characteristics, as well as anastomotic technique. Demographic characteristics including age less than or equal to one year and weight less than 10 kgs have been variably found to be associated with portal vein complications as they are associated with a diminutive portal vein diameter [86,87,92,94]. Moreover, a subgroup of children with biliary atresia has co-existent structural abnormalities that complicate portal venous reconstruction. These abnormalities include visceral heterotaxy (left isomerism), splenic anomalies (usually polysplenia), preduodenal portal vein, portal vein hypoplasia, absent portal vein, absent inferior vena cava, or intestinal malrotation [95–99]. Finally, unfavorable functional characteristics including low portal flow or non-hepatopetal (fluctuant, absent, or hepatofugal) flow has also been correlated with increased post-transplant portal venous complications [86,87,94].

The most commonly used approach is an end to end anastomosis of the donor to recipient portal vein. However, if the recipient portal vein is unsuitable, more complex strategies must be employed. One option is complete resection with end-to-end anastomosis to the confluence of the splenic and mesenteric veins or end-to-side anastomosis to the superior mesenteric vein. A second option is portoplasty or reconstruction of the native segment typically with a venous patch. On balance, the use of a conduit or interposition graft appears to be associated with higher rate of portal venous complications [87,90,91].

Early portal vein complications have been treated with surgery for thrombectomy and revision of the portal anastomosis, interventional radiology with clot fragmentation/lysis and stenting, or a combination procedure [97,100–102]. The literature, dominated by small single center case series, generally indicates good long-term patency.

The third type of vascular complication after pediatric liver transplantation is venous outflow obstruction. As in adults, the frequency of this complication is lower when the allograft is implanted using a caval replacement rather than a piggyback technique. Therefore, particularly early in the days of partial liver transplantation for children, rates of outflow obstruction ranged between 17–28% [62,103]. Rates are more modest in later series, ranging between 4.5–10.5% [103–106]. It is widely believed that a simple end-to-end anastomotic approach is a risk factor for graft torsion that can obstruct outflow resulting in allograft dysfunction with fluid retention and ascites accumulation or even acute allograft failure. Several have proposed alternative anastomotic geometries with an emphasis on creating a wide and short outflow tract. Options include a triangulated anastomosis using the confluence of the native hepatic veins further opened onto the the inferior vena cava or a site fashioned directly onto the inferior vena cava [62,103,107,108].

Clinically, compromised venous outflow can present dramatically early after transplantation with acute allograft failure. More

commonly, presentation is dominated by the development of ascites and/or peripheral edema, with or without biochemical evidence of allograft dysfunction. Diagnosis is often suggested by Doppler ultrasonography showing hepatic venous waveforms without the typical phasicity and confirmed by transjugular venography. Surgical intervention is typically reserved for acute presentations in the immediate peritransplant period. Otherwise, venous outflow obstruction is addressed with percutaneous endovascular approaches to resolve the pressure gradient [103,104,109,110]. Balloon dilatation is first attempted, with high rates of both initial success and recurrence. Persistence of a gradient or recurrence strongly merits consideration of stent deployment. Long-term outcomes are generally excellent with clinical resolution and graft preservation.

Biliary complications

The typically small bile ducts of both the allograft and the recipient have led to the dominance of Roux en Y hepaticojejunostomy over choledochocholedochostomy as the preferred anastomosis strategy for pediatric liver transplantation. Unlike vascular complications which occur with higher frequency in children over adults, biliary complications occur at a similar frequency ranging between 10–33% [111–116]. Complications are usually either leak or stricture. Biliary leaks typically present early after transplantation. Although most commonly anastomotic, leaks can also emanate from the cut edge of partial grafts. Biliary strictures can present either early or late after transplantation. Similarly, the most common are anastomotic strictures as diffuse intrahepatic strictures are rare. Risk factors for biliary complications after pediatric liver transplantation include hepatic artery thrombosis resulting in biliary ischemia, multiple ducts, and ABO incompatible transplantation [112–114,116]. Graft type — whole grafts or partial grafts from deceased or living donors — has not been consistently identified as correlated with biliary complications. More recently, a few reports have explored choledochocholedochostomy with an external stent as an anastomotic strategy [117–120]. Conversion to Roux en Y was frequently necessary for complications, particularly with infant recipients.

Although non-invasive approaches such as ultrasonography or biliary scintigraphy can suggest the presence of a biliary complication, percutaneous transhepatic cholangiography is required for definitive diagnosis. If a diagnosis is made, it is critical to assess the hepatic artery prior to treating the biliary complication. In the immediate peritransplant timeframe, surgical intervention is generally undertaken. However, later after transplantation, percutaneous and, occasionally, endoscopic approaches are more frequently exercised [121–125]. Although biliary complications certainly result in morbidity, they typically do not compromise graft or patient survival.

Early post-transplant management considerations

Immunosuppression strategies

In the early days of transplantation, when the immunosuppression armamentarium comprised solely of corticosteroids and azathioprine, and even after the emergence of cyclosporine, induction immunosuppression was commonly utilized in both adult and pediatric liver transplantation. With the gradual development and increasing penetration of more potent immunosuppression agents such as tacrolimus followed by mycophenolate, induction was essentially abandoned. However, as the toxicity profile of corticosteroids and calcineurin inhibitors in children has been better delineated, there has been a resurgence of interest in induction immunosuppression. Rather than using induction to augment overall immunosuppression and reduce rates of acute rejection, induction immunosuppression is now used to facilitate minimization or even complete avoidance of corticosteroids and/or reduction of exposure to calcineurin inhibitors while maintaining stable rates of acute rejection, graft, and patient survival [117,126–132]. Currently, induction is administered to 31% of pediatric liver transplant recipients. The dominant choice for induction has been anti-interleukin-2 (IL-2) receptor antibodies, over anti-thymocyte globulin [133]. Historically, two anti-IL2 receptor antibodies have been available, basiliximab and daclizumab, but the latter is no longer available. In general, the IL-2 receptor antibodies have had an excellent safety profile with no increases in hemodynamic, metabolic, nephrologic, neurologic, infectious or malignant adverse events including post-transplant lymphoproliferative disease [127–130,132].

Maintenance immunosuppression regimens have evolved tremendously over the past two decades, and are covered in detail in Chapter 66. Although it is widely recognized that the calcineurin inhibitor, cyclosporine, brought transplantation into the modern era, it has long been displaced by tacrolimus as the dominant maintenance immunosuppression agent. In randomized control trials, tacrolimus proved superior to cyclosporine with significantly lower rates of acute and chronic rejection albeit no improvement in patient or graft survival [134–136]. The toxicity profile of tacrolimus compared to cyclosporine was mixed. Tacrolimus was associated with significantly higher rates of diabetes and post-transplant lymphoproliferative disease (PTLD) but significantly lower rates of hirsutism and gingival hyperplasia [137–141]. Over time, the penetration of tacrolimus has steadily increased such that currently, greater than 90% of pediatric liver transplant recipients are discharged from the transplant hospitalization on tacrolimus [142].

Similar to tacrolimus and cyclosporine, mycophenolate mofetil has largely replaced azathioprine as the anti-metabolite of choice for maintenance immunosuppression. The first report of incorporating mycophenolate mofetil into a de novo maintenance immunosuppression regimen with microemulsion cyclosporine and corticosteroids appeared in 1999 [143]. When compared to a historical cohort treated with azathioprine, oil-based gel-encapsulated cyclosporine, and prednisone with anti-T-cell antibody induction therapy, the authors concluded that the new regimen was safe, well-tolerated, and eliminated the need for intravenous induction immunosuppression. Utilization of mycophenolate increased as reports of its use to improve renal function through reduction of calcineurin inhibitor exposure [144–150] and as rescue therapy for refractory rejection emerged [148,151–153]. Mycophenolate is now prescribed as maintenance immunosuppression for approximately 40% of de novo pediatric liver transplant recipients.

With the widespread use of tacrolimus, with or without mycophenolate, there has been substantial interest in exploring corticosteroid minimization or avoidance strategies. The first trial compared a prospective cohort of 20 recipients treated with basiliximab induction and tacrolimus with a historical cohort of 20 recipients treated with corticosteroids and tacrolimus [131]. Recipients in the steroid free regimen enjoyed superior one year rejection free survival and exhibited significant linear growth. Subsequently, additional studies have confirmed that basiliximab supported complete steroid elimination with a significantly lower incidence of acute

rejection [129,132], overall infection [132] or viral infection [129]. Moreover, steroid elimination reduced anti-hypertensive use and improved catch-up linear growth [129]. Currently, in the US, approximately 20% of children undergo liver transplantation with complete steroid avoidance. Although the majority of children still receive corticosteroids at the time of transplantation, there has been a strong trend of minimization through discontinuation, particularly in the interest of optimizing (catch-up) growth [154–160]. One year after transplantation, approximately 40% of pediatric liver transplant recipients remain on corticosteroids.

Diagnosis and management of acute cellular rejection

The diagnostic and therapeutic algorithms for early acute cellular rejection (within the first six months) for pediatric liver transplant recipients parallel those for adult liver transplant recipients, covered in Chapter 70. In light of the increased technical difficulty and higher rate of surgical complications for pediatric liver transplantation, ultrasound and Doppler examination is an important first step in the assessment of allograft dysfunction that is typically manifest by elevated liver tests. If there is no evidence of hepatic arterial, portal venous, or biliary complications, then liver biopsy should be obtained. The most common approach to liver biopsy is percutaneous, with ultrasound guidance. A transjugular approach performed by an interventional radiologist has gained popularity as any associated bleeding is typically contained within the intravascular space [161,162].

The histological diagnosis and grading of acute rejection are according to the Banff Classification [163] that is presented in detail in Chapter 82. In the immediate peritransplant timeframe, the primary differential diagnoses for allograft dysfunction are acute cellular rejection, ischemia/reperfusion or preservation injury or pericholangitis suggesting biliary stricture. Currently, the incidence of early acute cellular rejection approximates 40–45% with the vast majority of episodes occurring within six weeks of transplant (Figure 114.4) [164]. Analysis of the SPLIT database showed that

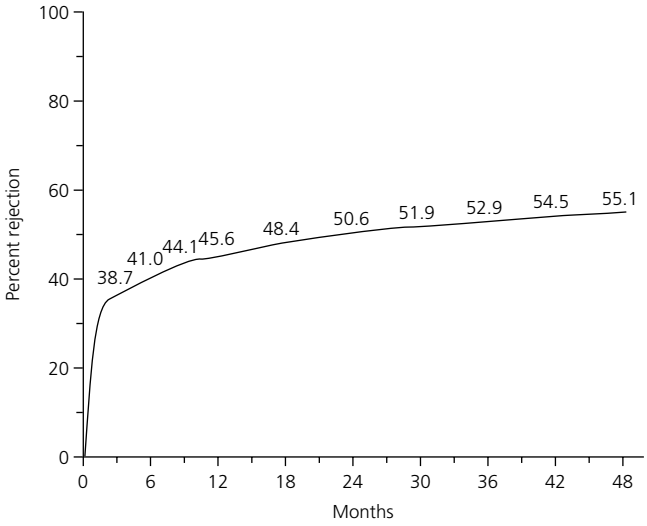

Figure 114.4. Probability of rejection according to time after transplantation. Kaplan-Meier plot showing the probably of rejection over time in 2291 primary pediatric liver transplant recipients registered in the SPLIT database between 1995 and 2006. Reproduced from [164] Shepherd et al. with permission from John Wiley and Sons.

independent factors that negatively associated with acute rejection included age at transplant of 0–5 months compared to all other age categories, cholestatic, metabolic, or other etiology of liver disease compared to biliary atresia, ABO incompatible compared to ABO identical transplantation, tacrolimus compared to cyclosporine primary immunosuppression, and transplantation after 2002 [164].

Early acute cellular rejection after pediatric transplantation is most commonly treated with intravenous bolus corticosteroids. Follow-up biopsy may be necessary to assess treatment efficacy, particularly if there is suspicion of incomplete response. An episode of acute cellular rejection that does not resolve with corticosteroid bolus is termed steroid refractory rejection and often requires more intense therapy with a monoclonal or polyclonal antibody. In addition to the immunosuppression bolus administered to treat acute rejection, augmentation of maintenance immunosuppression is often considered. Options include intensification (dose increase), conversion [137] (change from less to more potent agent, typically in the same class), or addition (adding an agent) [153,165–168]. In special circumstances, these options, in and of themselves, may represent viable treatment approaches [169]. While it is always desirable to choose the least intense option that will be effective in reversing the rejection process, inadequate treatment risks potential evolution to chronic rejection, a more serious and difficult condition to treat and reverse.

Management of infection risk after transplantation

While improvements in surgical technique, immunosuppression medications, and general medical care have undoubtedly coalesced to revolutionize pediatric liver transplantation, advances in the prevention, detection, and treatment of infections also deserves substantial credit for dramatically improving post-transplant patient survival. The achievements in controlling infection are particularly notable when considered within the context of increasingly potent and efficacious immunosuppression regimens that heighten patients' vulnerability. Pediatric liver transplant recipients are susceptible to infection as the majority enters transplantation as infants and toddlers, lacking immunity to many pathogens. Moreover, chronic liver disease and its frequent companion, malnutrition, weaken cellular immune defense mechanisms and predisposes to specific infections such as spontaneous bacterial peritonitis and biliary sepsis [170–172]. Finally, at the time of transplantation, immunosuppression is administered. Therefore, it is not surprising that, in spite of substantial progress, infectious complications continue to compromise both quality and quantity of life for children after liver transplantation [17,164,173–175].

Profile of infection according to time after transplantation

It is widely accepted that uncontrolled infection is a contraindication to transplantation. Although some children come to transplantation without any particular infectious issues, many may have had recent infection, current infection controlled by treatment, and/or additional vulnerabilities such as indwelling catheters/drains. Like every patient who undergoes major surgery, children undergoing liver transplantation are prone to infections involving the surgical site(s) particularly if there are unfavorable anatomical or technical factors and infections related to indwelling devices such as foley catheters, vascular catheters, and/or endotracheal tubes. Pathogens most frequently encountered within the first month of transplantation are bacteria, both gram positive and gram negative, and candida species [164,175–178]. While anti-microbials and anti-fungals are

important therapies, durable resolution requires correction of any underlying anatomic or technical problem, if present. Otherwise infection will recur and potentially involve resistant organisms reflecting previous treatment with anti-infective agents. Data from SPLIT indicates that 38% of pediatric liver transplant recipients experience a culture proven bacterial or fungal infection during the first month after transplantation [164].

Between one and six months after transplantation, assuming resolution of anatomical and technical issues that may have been present, opportunistic infections reflecting the effect of immunosuppression emerge, along with (seasonal) community acquired infection [174,175]. Routine prophylaxis against candida and pneumocystis has had their intended effect. Although viral infection or reactivation has been the dominant problem, these have receded with the advent of effective prophylactic and/or preemptive treatment as discussed further on in this chapter. As additional time passes after transplantation, the burden of infection generally diminishes, particularly for those with good allograft function on standard maintenance immunosuppression. Exceptions are children who have required aggressive treatment for rejection, those with some sort of chronic, often viral, infection, and those whose allograft function is not optimal.

Important viral infections: Cytomegalovirus

Cytomegalovirus (CMV) is a DNA virus in the herpes family that is a well-known pathogen for immunosuppressed liver transplant recipients. A spectrum of clinical scenarios can result, ranging from an asymptomatic infection to a self-limited febrile illness to a serious tissue-invasive disease of the lungs, the liver, the gastrointestinal tract, or, very rarely, the central nervous system [174,175,178–181]. In the setting of documented viral replication, infection refers to asymptomatic cases, while disease refers to symptomatic cases. The main risk factor for CMV infection and/or disease, the lack of previous infection and therefore the lack of immunity, affects the majority of pediatric liver transplant recipients who are predominantly infants and toddlers at the time of transplantation. This vulnerability is accentuated by exposure to the graft and blood products from donors with previous history of infection. Primary infection is widely considered to be more virulent than reactivation infection. The second most important risk factor is receipt of a potent antibody preparation such as rabbit thymoglobulin or OKT3 [178,180,182,183]. Among pediatric liver transplant recipients, these medications are typically prescribed for steroid refractory rejection and therefore, affect only a small minority of patients.

Recognition of the potential for morbidity and mortality posed by CMV has led to two dominant strategies for patient management beginning at the time of transplantation when immunosuppression is initiated. Many centers prescribe anti-viral medications at a prophylactic dose to all transplant recipients, often with different duration based on risk profile [184–189]. Ganciclovir is often administered intravenously during the transplant hospitalization with conversion to oral therapy to complete a predetermined course. This approach is arguably the simplest to implement but does expose all patients to anti-viral medications for months with attendant side effects. Moreover, data has shown that infection/disease often emerges after prophylaxis is discontinued [181], leading programs to extend the course thereby further increasing toxicity. The alternative approach is preemptive therapy whereby serial determinations of CMV DNA copy-numbers are obtained and treatment is initiated when the titer exceeds a predetermined threshold [185–187]. While this approach minimizes drug exposure, it is essentially individualized care and, as such, is more resource intensive. As a result, many transplant centers have designed various hybrid strategies. Possibilities include risk stratification with prophylactic treatment for high risk recipients and preemptive treatment for low risk recipients or prophylaxis for a time period followed by preemptive monitoring.

The diagnosis of CMV infection and disease has also evolved substantially over the past several decades. Today, detection and quantification of circulating CMV DNA is the mainstay, having replaced assays for pp65 antigenemia and viral cultures of blood, urine, or other secretions [190–192]. Positive viral DNA titers must be interpreted in the context of the patient's clinical condition. CMV DNA may be detected in the absence of or prior to any evidence of clinical disease. Depending on the titer, treatment may be initiated or follow-up testing performed. Moreover, CMV DNA may not be detected in the presence of tissue-invasive disease most commonly of the gastrointestinal tract. Tissue biopsy should be obtained for histological examination as dictated by clinical suspicion.

The primary therapeutic agent for CMV infection and disease is intravenous ganciclovir, a nucleoside analog that has myelodepression as its primary toxicity. Oral ganciclovir or valganciclovir, a prodrug with improved bioavailability, are typically prescribed for prophylactic or preemptive treatment although use of the latter as a therapeutic for established disease is being explored [181,182,184,186–189,193]. Alternative medications include CMV hyperimmune globulin as adjunctive therapy and foscarnet, or cidofovir as second line drugs with less favorable side effect profile [189]. Finally, consideration should be given towards immunosuppression reduction for serious CMV disease.

Important viral infections

Epstein-Barr virus, including post-transplant lymphoproliferative disease

Epstein Barr virus (EBV) is a lymphotrophic herpes virus. As the etiologic agent of a wide spectrum of disease ranging from infection to malignancy, EBV causes substantial morbidity and mortality after pediatric liver transplantation [178,194–198]. A detailed consideration of PTLD is found in Chapter 96. Like CMV, primary infection produces more serious clinical syndromes than reactivation infection. Infants and toddlers are particularly vulnerable, as they typically undergo transplantation in a naïve state without previous exposure or immunity. Primary infection typically occurs in the peritransplant period, transmitted through the graft itself and/or transfused blood products although the routine incorporation of leukocyte depletion has diminished the importance of the latter. Acute infection can be entirely asymptomatic or result in a mild, non-specific viral illness evidenced after the fact by seroconversion. Alternatively, acute infection can produce a more serious viral syndrome with a variety of generalized symptoms including fever, malaise, anorexia, and myalgia. There can also be signs or symptoms of organ inflammation including pharyngitis, lymphadenopathy, splenomegaly, hepatitis, pneumonitis, and gastroenteritis. The inflammation signals EBV-driven proliferation of lymphocytes [predominantly (>85%) B cells] and is thereby termed post-transplant lymphoproliferative disease (PTLD). In addition to an EBV-naïve immune state, the intensity of overall immunosuppression is well recognized as the other dominant risk factor for the development of PTLD [195,199–203].

There are significant parallels between the management of CMV and EBV risk, infection, and disease, particularly since the same

anti-viral agents are thought to be effective for both viruses [184]. Some centers administer prophylaxis, either to all recipients or to high-risk recipients — those who are EBV naïve receiving an organ from a donor with previous infection. Others use a preemptive strategy, following peripheral blood EBV titers. Because the development of PTLD has been associated with variably defined "high" EBV DNA copy-numbers, immunosuppression reduction is often practiced to augment anti-viral therapy. Several reports suggest that this strategy has reduced the incidence of PTLD [204–213]. The modern incidence of PTLD ranges has decreased to <5% while historically, incidence upwards of 20% had been reported [199,200,207,214–216].

Thorough clinical, laboratory, and radiographic assessment should be carried out to delineate extent and burden of disease. Definitive diagnosis of PTLD requires biopsy of involved tissue(s). The pathology is classified according to the criteria of the World Health Organization. In children, most are EBV driven and of polymorphic histology. Complete details of the spectrum of pathology, clinical findings and range of therapies are described in Chapter 96. As for other organs, treatment of biopsy proven PTLD has historically followed a sequential approach, ascending a hierarchy of therapeutic modalities. At the base of the pyramid is immunosuppression reduction, which is often sufficient for patients with "early lesions" and polymorphic disease, and even for some children with monomorphic disease. Some centers have advocated complete cessation of immunosuppression [217]. Surgical or radiation therapy may also be a front-line approach for symptomatic relief or localized disease. The next therapeutic in the modern armamentarium is rituximab, a monoclonal antibody against a cell surface antigen expressed by all B cells that is present in the majority of PTLD cases, CD20. Since the first reported use for PTLD in children in 2002, there have been several subsequent reports that have solidified its role for PTLD with positive CD20 staining [218–221]. At the top of the pyramid is the standard chemotherapy regimen that is administered to non-transplant patients diagnosed with lymphoma. Many reports have shown that these regimens result in prohibitive rates of morbidity and mortality, motivating the design of less intensive, and therefore, less toxic regimens that have yielded improved toxicity profiles while maintaining adequate efficacy [222,223].

The outcomes of pediatric liver transplant recipients with PTLD illustrate the delicate balance that must be achieved to emerge with a healthy recipient and a healthy allograft. Primary causes of mortality after the diagnosis of PTLD is either the PTLD itself or toxicities related to its treatment (infection/sepsis/multi-system organ failure or severe allograft dysfunction). The latter is the dominant etiology of graft loss, typically resulting from immunosuppression reduction that precipitates acute and subsequently chronic rejection [207,209,217]. Reported mortality rates have varied widely over the past one to two decades, from 12% to as high as 60% [195,199,215–217,224,225].

Late post-transplant challenges

Late mortality and graft loss are infrequent among pediatric liver transplant recipients. Late mortality is predominantly attributable to complications of immunosuppression such as infection (sepsis or multi-system organ failure) or malignancy (PTLD or hepatoblastoma recurrence) [17,226]. However, late graft loss is often attributable to inadequate immunosuppression resulting in chronic rejection or technical complications such as hepatic artery thrombosis and/or biliary obstruction. Common predictors of late death and late graft loss are transplantation for tumor and greater than five admissions during the first post-transplant year. Additional independent predictors for late death are transplantation for fulminant hepatic failure, weight deficit at transplantation, and hepatic artery thrombosis. Additional independent predictors for late graft loss are early reoperation and steroid resistant acute cellular rejection [17]. Children face substantial risk of morbidity from non-immune complications of immunosuppression given their longer potential life span and greater cumulative exposure. Current immunosuppression medications increase risk for diabetes, hyperlipidemia, hypertension, obesity, and the metabolic syndrome. In addition to these critically important medical outcomes, chronic immunosuppression likely compromises growth and functional outcomes including health related quality of life, issues considered in greater depth in Chapter 118. Presently, only one third of 10 year survivors have stable allograft function defined by normal liver tests while on immunosuppressive monotherapy, with normal growth and absence of complications of immunosuppressive medications [227].

Late surgical complications

Vascular and biliary complications can develop or be diagnosed late (more than one year) after transplantation, resulting in considerable morbidity and potential graft loss [17,226]. The true prevalence of late hepatic artery thrombosis is unknown as the condition can be clinically silent. If the diagnosis is made incidentally in the setting of preserved liver function and the absence of any symptoms, then intervention may not be either necessary or advisable. Late hepatic artery thrombosis can, however, be the cause of refractory biliary complications and thereby necessitate retransplantation. Late biliary complications occur in up to 13% of pediatric liver transplant recipients and are dominated by strictures [111,116,228]. Common presenting signs and symptoms are elevated liver tests, particularly in a fluctuating pattern, or a frank episode of cholangitis. Patency and adequacy of hepatic arterial flow must be assessed and considered when planning therapy. Percutaneous or endoscopic intervention is typically the first-line approach to treat late strictures, particularly if hepatic artery thrombosis is present, to maximally preserve collateral circulation [111,115,123]. Retransplantation may be necessary for recurrent biliary sepsis and/or secondary biliary cirrhosis. Approximately 15–20% of late graft loss has been attributed to biliary complications [17,226,229,230].

Late venous complications occur less frequently than biliary complications [85,89,231]. Presenting symptoms of portal vein stenosis or thrombosis typically reflect the development of portal hypertension and include gastrointestinal bleeding, ascites, and/or splenomegaly [85]. Outflow obstruction most often presents with ascites, with or without peripheral edema, First line therapy for a portal or hepatic vein stricture is balloon dilatation. Initial success is high but recurrence is common, frequently necessitating repeat dilatation or stent deployment [89,104,105,110,231]. Portal vein thrombosis necessitates standard management of the complications related to portal hypertension as they develop.

Late allograft dysfunction

Allograft rejection

Late allograft rejection, defined as acute cellular rejection occurring more than 90 days after transplantation, is not common, occurring in about 10% of pediatric transplant recipients between 3–12 months after liver transplant and less frequently thereafter (Figure 114.4) [164]. Allograft rejection is usually associated with a change

in immunosuppression; either a scheduled change according to the center's management protocol or non-adherence to treatment regimen [232–234]. Non-adherence is particularly relevant in pediatric transplantation and is covered in-depth in Chapter 120. The latter may be detected by excessive variation of calcineurin inhibitor blood levels [235,236]. Clinically, acute rejection is usually asymptomatic, often suspected as a result of elevated liver tests compared to baseline and evaluated by biopsy. Allograft biopsy is the gold standard to diagnose and grade acute and/or chronic rejection. Biopsies should be interpreted according to the Banff global assessment criteria [163] (see Chapter 82). If the biopsy does not demonstrate rejection, other causes of liver dysfunction should be thoroughly considered.

The treatment strategy for acute rejection should take into account the clinical and histological severity, any objective evidence of non-adherence, and potential to precipitate new or exacerbate preexisting co-morbidities such as renal dysfunction or diabetes. Mild or moderate acute rejection (rejection activity index less than six) can be treated with intensification (dose increase, most frequently, of a calcineurin inhibitor), conversion to a more potent agent, addition of a new medication, or some combination of the above. If liver tests do not stabilize or improve within two weeks, corticosteroids can be given. Moderate to severe acute rejection can be treated with bolus corticosteroids, either intravenously, orally, or both. If liver tests normalize or return to baseline, then rejection is considered resolved. Failure to see improvement in liver tests may require a repeat liver biopsy to determine whether the rejection process is ongoing and therefore, resistant to corticosteroids, necessitating administration of potent antibody preparations such as rabbit thymoglobulin or OKT3. Chronic rejection is uncommon, occurring in less than 2% of pediatric liver transplant recipients [17]. Even so, chronic rejection accounts for approximately 40% of late graft loss [17]. Chronic rejection occurs less frequently in transplant recipients who receive primary immunosuppression with tacrolimus compared to cyclosporine-based regimens [136,237]. Diagnosis is based on liver histology according to the Banff criteria [163,238]. Enhanced immunosuppression is indicated for children with chronic rejection, particularly if diagnosed at an early stage when the process might still be reversible. However, with the exception of early studies of conversion from cyclosporine to tacrolimus [137,239], reliable treatment strategies other than retransplantation are not well-defined.

Chronic hepatitis and fibrosis

Chronic inflammation and fibrosis are prevalent among pediatric liver transplant recipients more than five years after transplantation even in the face of normal aminotransferases [240–243]. Fibrosis is of variable severity occurring in as many as 75% of the population by ten years after transplant. The pathobiology of chronic hepatitis and/or fibrosis is not defined and may reflect late complications of technical variant allografts, atypical rejection, chronic viral hepatitis or de novo autoimmune hepatitis. For patients with clinical, serologic, and histological features of de novo autoimmune hepatitis, treatment with azathioprine, corticosteroids, and/or sirolimus may be effective [244,245].

Non-immune complications of immunosuppressive agents

Post-transplant renal disease

Estimates of chronic renal dysfunction in pediatric liver transplant recipients range from 24% to >70% [246–248]. Recipients with inborn errors of metabolism, Alagille syndrome, congenital hepatic fibrosis, and those exposed to nephrotoxic agents (chemotherapeutic agents, aminoglycosides) prior to transplantation are at increased risk of chronic renal dysfunction [246,247,249]. Indirect evidence also suggests that acute kidney injury in the peri-transplant timeframe contributes to development of chronic renal dysfunction [250,251]. Strategies to preserve renal function during this critical period include close monitoring of serum creatinine, maintenance of adequate intravascular volume, avoidance of nephrotoxic medications and early recognition and mitigation of acute kidney injury [252]. Immunosuppressive medication treatment strategies can decrease risk of renal dysfunction especially for patients with preexisting renal disease and/or those who have decreased glomerular filtration rate (GFR) at transplant. Observational and controlled studies in adults and children with decreased GFR more than one year after transplantation have demonstrated improved renal function in association with lowering the calcineurin inhibitor dose by 25–50% and adding mycophenolate mofetil [147].

Diabetes

De novo post-transplant diabetes occurs in 10–15% of pediatric liver transplant recipients and is therefore less common than in adults [253–255]. The mean duration of post-transplant diabetes is 75 days, suggesting that it may be due to the high doses of corticosteroids often administered in the peri-transplant timeframe [253]. Risk factors include Hispanic ethnicity, adolescent age at transplantation, cystic fibrosis etiology of liver disease, corticosteroid and tacrolimus administration [141,254]. As with adult liver transplant recipients, minimization or early discontinuation of corticosteroids may improve glycemic control, particularly in children with diabetes prior to transplantation or those at increased risk.

De novo malignancy

Increased risk of cancer over time has been convincingly demonstrated in adult solid organ transplant recipients but the data in children is less substantial [226,256–258]. Anecdotal experience suggests that skin cancer is rare during the first 10–15 years post-transplant among pediatric transplant recipients [259,260]. In a Swedish study that linked all solid organ transplant pediatric recipients between 1970 and 2007 (n = 536) to the National Cancer Registry, two cases of non-melanoma skin cancers were identified [261]. However, given the longer life expectancy of children, strategies towards prevention of skin cancers are critical. Other cancers have been reported but the relative risk compared to the general population has not been determined. Risk factors, screening guidelines, and optimal treatment have not been established for pediatric transplant recipients.

Health-related quality of life

Health-related quality of life (HRQOL) refers to those aspects of QOL that are directly related to health and are potentially affected by the health care system. There are five aspects of health that comprise HRQOL as defined by the World Health Organization: (1) physical health; (2) mental health; (3) social functioning; (4) role functioning; and (5) general health perception [262–264]. A large, cross-sectional, multi-center study of pediatric liver transplant recipients in North America demonstrated that all aspects of HRQOL including school function were decreased in pediatric liver transplant recipients compared to population norms but that the largest effect size was in school functioning [265]. A more detailed study showed that the mean intelligence quotient for liver transplant recipients at least two years after transplantation was decreased

compared to the normal population [266] (see Chapter 119). In the same cohort, learning disability and reduced executive function were also more common among liver transplant recipients than in the normal population [265]. These observations have significant implications for education strategy, management of non-adherence, and transition to adult care.

Transition to adult care

The transition of a child transplant recipient to an adult-oriented care model is a complicated issue covered in depth in Chapter 121. For successful transition to the adult care system, the adolescent or young adult must understand his/her cause of organ failure and need for transplantation. The patient should recognize behaviors which promote good outcomes including adherence to medical regimens and avoidance of risk taking behavior and have accumulated knowledge of the health care system [233,267–271]. Medical adherence is considered a critical factor in the transition process and may decline after the transfer to adult care. To ensure a safe transfer of care, a strategy for transition of responsibility from the parent to the adolescent or young adult should begin several years before the actual transfer of care.

Summary

Pediatric liver transplantation has evolved into the preferred treatment for children with end stage liver disease, and in doing so has developed many practices that are distinct from adult liver transplantation. These include technical, pharmacological, and social nuances that must be understood for the clinician to engage in this highly complicated but immensely rewarding practice.

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CHAPTER 115

Pediatric Heart Transplantation

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Introduction

Pediatric heart transplantation has been an integral part of heart transplantation in general since the earliest days of the field. During its development, pediatric heart transplantation has led to substantial insights important not only to the technical aspects of cardiac replacement, but also to the understanding of immune development and tolerance. Today, cardiac transplantation is an established option for children with anatomical or functional heart disease. This chapter will cover the development of heart transplantation in children, and provide an overview of the indications, preparations, conduct, and follow-up for this procedure, highlighting pediatric-specific issues to be contrasted with similar topics for adult transplantation covered elsewhere in this textbook.

Historical notes

On December 3, 1967, the first cardiac allotransplantation procedure was performed by Christian Barnard at the Groote Schuur Hospital in Cape Town, South Africa [1]. The first pediatric heart transplant followed only three days later, in the early morning hours of December 6. The procedure was performed by Kantrowitz and colleagues at the Maimonides Medical Center in Brooklyn, New York [2]. A previous attempt at infant heart transplantation had been abandoned by the same team the prior summer (June 1966). The new recipient was felt to have tricuspid atresia, although in fact was later found to have severe Ebstein's malformation with functional pulmonary atresia. A systemic-to-pulmonary shunt had been performed on the third day of life, but the infant developed severe heart failure and pulmonary edema. On November 24th, it was decided to offer the family the option of orthotopic heart transplantation using the heart from an ancephalic infant when it became available. A consent form for the operation was signed by the parents over a week before Barnard's first transplant in South Africa. Both donor and recipient were blood group A, and the "irradiated hamster test disclosed no evidence of major incompatibility". In the early hours of December 6, an ancephalic child was identified as a potentially suitable donor and both infants were brought to the operating room where external cooling was begun by immersion in iced water (Figure 115.1). Donor cardiac activity ceased at a temperature of 27 °C and the transplant proceeded (this was prior to agreed upon criteria for brain death). The implantation essentially used the technique of Lower and Shumway (Figure 115.2), though the procedure was performed under circulatory arrest. Initially the infant appeared to do well and was moving all limbs spontaneously. However, after several hours metabolic and respiratory acidosis ensued and after approximately six hours cardiac arrest occurred. Attempts at resuscitation failed. Autopsy revealed a good surgical result with appropriate hemostasis from the suture lines. However, the aortopulmonary shunt had been incompletely closed. These first heart transplants in South Africa and New York were performed following many years of laboratory experimentation with heterotopic and orthotopic transplantation in small and large animals. The race to be the first may well have been part of the driving force behind the timing of these first transplants, though there is also evidence to suggest that these pioneering surgeons believed that successful thoracic transplantation was feasible in both children and adults by the end of the 1960s.

Following Barnard and Kantrowitz, a large number of surgeons attempted heart transplants in 1968. By the end of that year some 102 procedures were recorded from 17 countries. Mean survival for these early transplants was only 29 days [3]. Over the next decade enthusiasm for human heart transplantation declined worldwide, as it became apparent that the therapeutic armamentarium for controlling allograft rejection was inadequate for achieving allograft and patient survival [4]. Surgeons at a small number of centers, most notably Stanford University, persevered with attempts at heart transplantation in selected adults. The Stanford group also started to transplant a few adolescents, performing seven procedures between 1974 and 1980. Although only corticosteroids and azathioprine were used for immunosuppression, four of these patients survived greater than ten years from transplantation [5]. In addition, donor organ availability improved as the concept of brain death became accepted [6,7].

The favorable impact of the introduction of cyclosporine on survival of adult heart transplant recipients was immediately apparent [8,9] and led to renewed interest in pediatric heart transplantation in Stanford, at the University of Pittsburgh (1982) and subsequently at a number of other centers around the world. The earliest recipients were older children and adolescents, but it was not long before infants and younger children were also considered to be suitable recipients. Bailey and colleagues, as a result of extensive work in the animal laboratory, suggested that the neonate might make an excellent solid organ recipient [10,11]. Their first attempt at neonatal transplantation was a baboon-to-human xenotransplant in a 2.2 kg 12-day-old infant with aortic and mitral atresia [12]. The infant only survived for 20 days but the surgical feasibility of newborn

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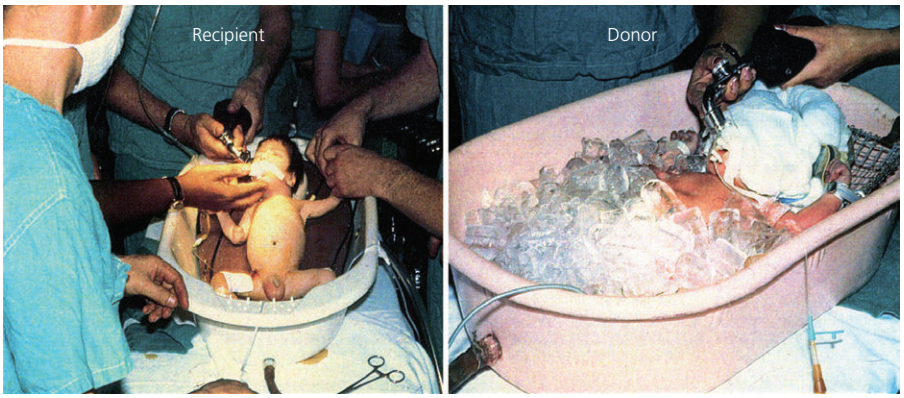

Figure 115.1. The first pediatric heart transplant was performed on December 6th, 1967. Hypothermia was induced by placing both the ancephalic donor (right panel) and the recipient (left panel) in baths of iced water. When donor cardiac activity ceased, the transplant procedure commenced.

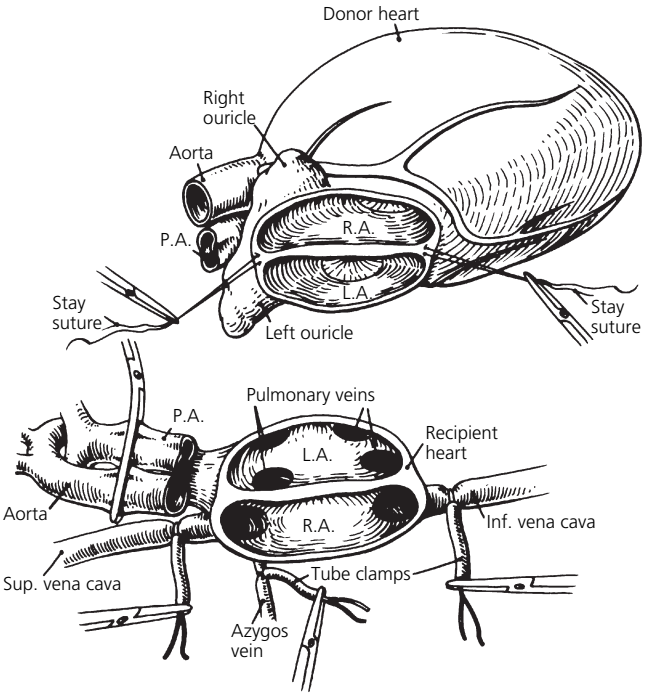

Figure 115.2. Surgical technique of the first pediatric heart transplant. The drawing was made shortly after the procedure. Reproduced from [87] Fine R, Webber SA, Harmon W, et al. (2007). Pediatric Solid Organ Transplantation, Second Edition, with permission from John Wiley and Sons.

transplantation for hypoplastic left heart syndrome (HLHS) had been demonstrated. Orthotopic newborn heart transplantation was introduced at Loma Linda, in November 1985 [13]. The first recipient was a four day old infant with HLHS, a condition generally considered to be lethal at that time, despite the recent introduction of a palliative operation by Norwood [14]. During the 1990s, transplantation for HLHS became the fastest growing area of pediatric heart transplantation. More recently, improved results of staged surgical palliation for HLHS and the long wait times for donor organs for neonatal candidates have led many centers to abandon transplantation as the primary procedure of choice for infants with this condition.

Many other challenges have been overcome, and milestones achieved, in the field of pediatric heart transplantation over the last 15 years. Early results with transplantation for previously palliated complex congenital heart disease were disappointing [15]. However, with improved patient selection and increased surgical experience, results similar to transplantation for cardiomyopathy can now be achieved in experienced centers [16].

The shortage of donor organs remains a critical issue, and death on the waiting list remains highest in the infant heart candidate population, especially for children receiving mechanical circulatory support with ECMO [17]. The use of ABO incompatible transplants has recently been advocated by the Toronto group for neonatal and infant candidates, since antibodies to the major blood group antigens are not produced until later in infancy (discussed in more depth in Chapter 112) [18]. Such a strategy may improve survival for select infants with high wait-list mortality [19]. No comprehensive solution to the donor shortage will occur unless xenotransplantation can be shown to be safe, efficacious and socially acceptable.

Indications for transplantation and candidate evaluation

Indications for transplantation in childhood

Transplantation of the heart is generally considered indicated when expected survival is less than two years, and/or when there is unacceptable quality of life. Cardiomyopathy (predominantly dilated forms) and complex congenital heart defects remain the primary indications, and together account for approximately 90% of transplantations undertaken in children [20]. Transplant activity has remained approximately constant over the last decade reflecting the limited number of donor organs. Diagnoses leading to transplantation are age dependent, with congenital heart defects accounting for over half of transplants in the infant age group, and cardiomyopathy accounting for almost two-thirds among adolescents [20]. Of note, the proportion undergoing transplantation in infancy and for a diagnosis of congenital heart disease (CHD) are significantly higher in North America than the rest of the world [20].

The appropriate indications for heart transplantation in childhood were summarized in a 1999 report from the Pediatric Committee of the American Society of Transplantation [21]. More recently, a consensus group of the American Heart Association has addressed the same topic [22]. In general, there is broad consensus in the pediatric cardiology community as to when transplantation is indicated. Perhaps the most controversial indication for heart

transplantation is HLHS and related pathologies in the newborn. Survival rates in excess of 80% at one year may be achieved in experienced centers with either Norwood reconstruction or primary transplantation for this condition. Median waiting times for newborn heart transplant candidates are approximately two months in the US (and longer in some countries), resulting in very high costs of care prior to transplantation, significant pretransplant morbidities, and a wait-list mortality as high as 30% [23]. In light of these observations, most centers have moved away from transplantation, and towards staged reconstruction, for neonates with HLHS. This strategy increases availability of organs for other infants with cardiac disease unsuitable for surgical palliation.

Relative and/or absolute contraindications for heart transplantation include chronic infection with either hepatitis B or C, or human immunodeficiency virus (HIV), prior non-adherence with medical therapy, recent or current treatment of malignancy with inadequate follow-up to ensure likely cure, active acute viral, fungal or bacterial infections, excessive and fixed pulmonary vascular resistance (above 10 IU), inadequate intraparenchymal pulmonary vascular bed, diffuse pulmonary vein stenosis, and major extracardiac disease felt to be non-reversible with heart transplantation (e.g. severe systemic myopathy). Inevitably, some centers consider specific contraindications absolute, whereas others may feel they are relative. Decision-making is based on consensus discussion among all team members and with consultation with the family and referring physicians.

Evaluation of the candidate

The evaluation process broadly follows the principles laid out for evaluation of the adult potential cardiac candidate (see Chapter 30). The evaluation includes the assessment of expected survival without transplantation, current quality of life, the potential for alternate surgical or medical therapies, as well as the inherent risks of the transplant surgery itself.

Anatomic and hemodynamic considerations

The most complex anatomy may be successfully transplanted provided the lung vasculature is adequately developed and pulmonary vascular resistance is acceptable. Anatomic points of most interest to the surgeon include abnormalities of cardiac and visceral situs (especially anomalies of the systemic and pulmonary venous return), as well as the size and anatomy of the main and branch pulmonary arteries, including the presence of stenoses, distortions and non-confluence. Intracardiac anatomy is less important since the bulk of the cardiac mass will be explanted. Abnormalities in the relation of the great arteries usually pose few problems. Attention must also be given to the relationship of key structures such as right ventricular-pulmonary artery conduits or giant right atria (after Fontan procedure) to the posterior aspect of the sternum. Computed tomography and/or magnetic resonance imaging are well suited to delineating cardiac anatomy, but cardiac catheterization is usually indicated pretransplantation to assess pulmonary vascular resistance. Excessive fixed resistance may result in acute donor right ventricular failure and an inability to wean the patient from cardiopulmonary bypass.

In general, children with indexed pulmonary vascular resistance (PVRI) $\leq$9 IU are considered low risk for acute donor right heart failure and early death. PVRI in excess of 9 IU is considered as high risk [24] and should be a contraindication to isolated heart transplantation unless there is a major fall with pulmonary vasodilator therapy. In borderline cases, restudy of hemodynamics after several days of inotropic and vasodilator therapy may be indicated, as pronounced falls in PVRI are occasionally seen. It should be noted however, that rapid fall in pulmonary resistance can lead to acute elevation in left atrial pressure in patients with very poor left ventricular function, even precipitating pulmonary edema. The role of ventricular assist device (VAD) therapy as a strategy to unload the left ventricle and reduce excessive PVRI to prepare children for orthotopic transplantation is unknown at this time but recent case reports suggest that short-term VAD support can lead to significant fall in PVRI in selected cases with subsequent successful transplantation.

Laboratory investigations

Blood typing is necessary to assure ABO compatibility with the transplanted organ, though infants and young children with absent or low anti-A and anti-B isohemagglutinin titers may be safely transplanted across traditional ABO barriers. This strategy was introduced by West and colleagues in Toronto [18,19] and is based on the principle that isohemagglutinins against blood group antigens do not normally develop until the latter part of infancy. Transplantation across blood group barriers prior to the development of naturally occurring anti-A and anti-B isohemagglutinins appears to result in outcomes comparable to ABO compatible transplants [25,26]. Furthermore, most of the transplanted infants did not form antibodies against donor blood group antigens during long-term follow-up [27], possibly due to the development of B cell tolerance [28]. Even when antibodies do develop, outcomes have still been reported to be excellent [27]. This strategy has important implications for pretransplant care, since use of blood products pre and post-transplant must be carefully planned to avoid transfusion of blood products containing inappropriate anti-A and anti-B antibodies. Acceptable products should be summarized in collaboration with the blood bank and posted at the child's bedside. These restrictions will apply indefinitely and information on acceptable blood products must be provided to the family prior to hospital discharge.

Evaluation for the presence of preformed anti-HLA antibodies ("sensitization") was traditionally performed using panel reactive antibody (PRA) testing. Today, almost all centers use solid phase assays, in particular the Luminex platform with or without cell based (PRA) assays. The solid phase platforms are highly sensitive and data (unpublished) from the Clinical Trials in Organ Transplantation in Children (CTOT-C) show that approximately half of all pediatric heart candidates have preformed anti-HLA antibodies. Many are at low titer and the significance of this observation (even when donor specific) remains to be determined. Most centers perform recipient molecular HLA typing as part of the evaluation process. This is essential if "virtual" cross-matching is to be performed in sensitized candidates (covered in more depth in Chapter 89).

Infectious disease evaluation includes serologic testing for cytomegalovirus (CMV), Epstein–Barr virus (EBV), varicella, herpes simplex virus, T. gondii, HIV, measles and hepatitis viruses A, B, C and D. Serologic status for these agents may guide prophylaxis as well as the diagnostic evaluation of post-transplantation fever. Very rarely, it may lead to exclusion of candidacy of the child for transplantation. The infectious disease evaluation should also include review of immunization history. Those candidates in whom transplantation is not likely to be imminent should undergo an update of appropriate immunizations on a regular basis. In addition, re-evaluation of antibodies against infectious agents for which

the patient was naïve at the time of the transplant evaluation should be repeated at the time of transplantation since this will influence CMV prophylaxis and assessment of risk for PTLD post-transplantation.

Consultations

A multidisciplinary team that includes the transplant cardiologist and surgeon, social worker, transplant coordinator, and infectious disease experts evaluates each candidate. A screening psychiatric/psychological examination of the child and their family is also very beneficial. The primary purpose of this evaluation is to identify patients and families at high risk for poor psychosocial outcome while waiting for transplantation and after transplantation. Evaluation of past history of non-adherence to medical therapy is critical. Additional consultations may be required from specialist services such as hematology-oncology (when there is past history of malignancy), child development, genetics, neurology and feeding/nutritional specialists. Patients with Fontan circulation require evaluation of the liver for evidence of cirrhosis and may require formal hepatology consultation.

Wait-list management and outcomes

Patients and families are interested in the chances of survival once a decision has been made to proceed with listing for transplantation. Despite this, emphasis is rarely given to pre-transplant mortality, risk factors for wait-list mortality and the optimal timing of transplantation. Premature transplantation results in exposure of the recipient to the hazards of transplantation and long-term immunosuppression. Excessive delay may result in death without transplantation or the development of co-morbidities that may increase operative risk. These co-morbidities include progressive end-organ dysfunction (especially renal), malnutrition associated with advanced heart failure, and progressive rise in pulmonary vascular resistance.

Data from the US Scientific Registry of Transplant Recipients (SRTR) reveal that children in all age groups have substantially shorter waiting times for heart transplants than do adults, but they have a greater risk of death while waiting (www.ustransplant.org). The highest death rate is among infants less than one year of age, and this rate is dramatically higher when the underlying diagnosis is congenital heart disease. The use of ABO incompatible heart transplants may decrease wait-list mortality in infant heart transplant candidates [19].

Several analyzes of the Pediatric Heart Transplant Study (a multi-institutional research database focusing on outcomes following listing for transplantation and after transplantation in over 40 centers world-wide since 1993), have focused on understanding risk factors for survival after listing for transplantation, and for defining the optimal timing of transplantation. Children awaiting transplant at the lowest urgency status (UNOS Status 2 in the US) have a very low risk of sudden death while waiting if the underlying etiology is dilated cardiomyopathy. This contrasts with the high risk of sudden death in adults with ischemic etiology on the transplant waiting list. These data suggest that routine use of automatic implantable cardioverter defibrillators (AICD) in all children who are awaiting transplant is not indicated, though certain subpopulations may benefit [29]. Despite these observations, one study has suggested a survival advantage with transplantation for patients listed as Status 2 out to at least four years [30].

Detailed analyses of outcomes for children with cardiomyopathy after listing have been performed using the Pediatric Heart Transplant Study (PHTS) database [31–33]. For dilated cardiomyopathy, the commonest indication for transplantation in children, competing outcomes show that waitlist mortality by one year is 10% with 74% achieving transplantation and 16% remaining alive waiting [31]. Risk factors for mortality while wait-listed included a history of mechanical ventilation, extracorporeal membrane oxygenation (ECMO) and arrhythmias.

By contrast, patients with congenital heart disease fair less well on the waiting list, especially those who are Status 1A and those who are listed in infancy. Wait list mortality for certain diagnostic subgroups is as high as 30% in infancy [23], perhaps reflecting the inability to support certain groups of patients to transplantation with current medical and surgical therapies. Although there has been a dramatic increase in the use of VAD support in children over the last 15 years, outcomes are far inferior for patients with CHD when compared to cardiomyopathies [34]. Almost all children are supported by paracorporeal pumps, and although the introduction of the Berlin Heart [35,36] in children has led to successful bridge to transplant for children less than 30 kg (the approximate limit of use of adult devices), the outcomes for infants are still disappointing [35,36]. None-the-less, the alternative mechanical support used most often in infants is ECMO, and this usage is associated with both very high pretransplant mortality and also high post-transplant mortality [17,36]. Because the median wait time in most centers exceeds the time that most children can be supported on ECMO, the latter is being increasing used as a bridge to VAD support when urgent mechanical support is required [35]. However, for most infants with CHD, anatomical considerations severely limit the successful applicability of this technology [34].

Donor evaluation, surgical techniques and early post-transplant management

This section focuses on evaluation of the donor for pediatric heart transplantation, surgical techniques (with focus on modification for patients with CHD) and early post-transplant management during the intensive care period/initial hospitalization. The technical aspects of the procedure described here are focused on specific issues common in the pediatric population and should be considered in concert with the more general technical aspects of heart transplantation presented in Chapter 58.

Evaluation of the cardiac donor for the pediatric recipient

Evaluation of the donor heart begins with a careful review of the history. This includes donor age and gender, body size, cause of death, presence of any chest trauma, need for cardiopulmonary resuscitation, length of resuscitation and evaluation of the hemodynamic status of the donor (including blood pressure, heart rate and central venous pressure if available). The amount of inotropic support, and trends in usage over time, are also noted. A history of cardiopulmonary resuscitation is not, in itself, a contraindication to cardiac donation for pediatric recipients [37,38]. It must be recognized that brain death results in dramatic physiological disturbances in the donor. These include temperature instability with hypothermia, circulatory volume changes (most commonly depletion) and neuro-endocrine dysfunction. There is depletion of circulating thyroxine, cortisol, insulin, glucagon and antidiuretic hormone (ADH).

To rule out structural abnormalities and to evaluate cardiac function, a complete echocardiogram should be performed. Most centers avoid the use of donor hearts whose systolic function is more than mildly impaired after treatment with inotropic agents or thyroid hormone (e.g. shortening fraction less than 26%, ejection fraction less than 50%). Some degree of atrioventricular valvular regurgitation is common after brain death and mild degrees do not constitute a contraindication to organ donation. Pericardial effusion may be indicative of myocardial contusion. A twelve-lead electrocardiogram should be performed. Mild non-specific ST and T wave changes are commonly present, and usually reflect central nervous system effects, electrolyte disturbances or hypothermia. These do not contraindicate organ donation. Interpretation of cardiac enzymes may be difficult in the setting of generalized trauma. It has been suggested that elevation in cardiac troponin I levels in donor serum is a useful predictor for acute graft failure after infant heart transplantation [39]. However, a recent analysis using the SRTR database concluded that troponin I elevation before procurement is not associated with increased graft failure or hospital length of stay among pediatric heart recipients [40].

Evaluation of adult donors for coronary artery disease by selective coronary arteriography is commonplace in adult transplantation. Use of older donors (e.g. above 35 years of age) for pediatric recipients is associated with high risk of post-transplant coronary disease and poor long-term survival [41]. Such donors are generally avoided for pediatric candidates and thus coronary arteriography is rarely requested on the donor for pediatric recipients.

Size matching is a critical issue in the selection of potential donors. Most centers avoid under-sizing the donor below 75–80% of recipient weight. Below this, cardiac output of the donor may be insufficient to meet the needs of the recipient. None-the-less, a recent report from the SRTR database suggests that donor-recipient mismatching as low as 60% of recipient size is not associated with worse graft outcomes [42]. This retrospective analysis was not able to look at the complex interaction of donor size, cold ischemic time and preprocurement donor organ function. Therefore, it cannot be concluded that severely undersized organs can be safely used on a routine basis.

Use of oversized donors is common in children, and indeed is a necessity for infant candidates since there are so few donors available that are well matched for recipient size. Most candidates with cardiomyopathy will have marked cardiomegaly, leaving ample room within the chest for an oversized donor heart. Use of donor:recipient weight ratios of 2.5 : 1 is common in pediatric practice, and ratios of 3 : 1 and greater have been successfully used [43], especially in newborn and infant candidates. Marked over-sizing often results in delayed sternal closure and in infant recipients, donor: recipient weight ratios of greater than two have been associated with a more prolonged ventilator course and increased risk of primary graft failure [44]. Oversized donor hearts may also give rise to a postoperative syndrome characterized by high output state associated with systemic hypertension, raised intracranial pressure and even mental status changes. Although widely recommended, it is unclear if over-sizing of donors may improve outcome when there is significant preoperative pulmonary hypertension in the recipient. Most authorities would agree that significant undersizing should be avoided in the presence of recipient pulmonary hypertension.

All donors should be screened for CMV, EBV, HIV 1 and 2, human lymphotropic viruses 1 and 2 (HTLV-1, HTLV-2), and hepatitis viruses A, B and C. Donors are also screened for syphilis and for antibodies to *Toxoplasma gondii*. Presence of antibodies to CMV, EBV or *T gondii* do not constitute contraindication to transplantation but helps guide post-transplantation therapy and surveillance. Evidence of donor retroviral infection (HIV or HTLV) is considered an absolute contraindication to heart transplantation. The presence of donor hepatitis B surface antigen is also usually considered an absolute contraindication to heart donation. The usage of hepatitis C positive donors remains controversial.

Surgical techniques of graft implantation

Detailed review of surgical techniques is outside the scope of this text and has recently been reviewed [45]. In 1960, Lower and Shumway enumerated the basic surgical principles of orthotopic heart transplantation, initially in dogs, and several years later in humans [46]. The pioneering efforts led to the adoption of the *biatrial anastomosis* for cardiac transplantation. This technique has been applied to thousands of patients, of all ages, with excellent results.

Despite the great success of the biatrial technique, this is nonetheless a non-anatomic technique that results in large atrial cavities. The resulting abnormal atrial geometry is thought to contribute to tricuspid valve dysfunction. Sinus node dysfunction, from surgical trauma or ischemia, has also been described. For these reasons, many centers now perform *bicaval anastomosis* in children of all ages undergoing orthotopic heart transplantation. Some specific forms of congenital heart disease lend themselves particularly well to the bicaval technique. For example, patients who have previously undergone Mustard or Senning operations, and those with a Glenn anastomosis. The bicaval technique may be associated with superior caval vein stenosis, especially in infants [47]. This can generally be successfully treated with interventional catheter techniques beyond the first few weeks after transplantation when wound healing is complete [47].

Transplantation for congenital heart disease is often performed after multiple palliative procedures, and may present formidable surgical challenges. The anatomic substrate can be broadly classified as abnormalities of the systemic venous return, abnormalities of the pulmonary venous return, and abnormalities of the great vessels, including HLHS. Surgical modifications of the two basic techniques, atrial and bicaval anastomoses, are required for transplantation of these anatomic variants. In general, abnormalities of great arterial positions pose few challenges. Abnormalities of systemic venous return are among the most challenging anatomic variants to transplant. Fashioning unobstructed connections in patients undergoing heart transplantation with left sided cavae (for example in dextrocardia with situs inversus or in heterotaxy syndromes), requires special consideration and planning. A variety of techniques have been devised to overcome these difficult anatomic arrangements (Figure 115.3) [16,45]. Of key importance is procurement of generous sections of donor caval veins including the innominate vein. When there are bilateral superior caval veins without a connecting vein, the donor innominate vein can be used with an end-to-end anastomosis to the recipient left cava. When there is recipient complete mirror image arrangement (situs inversus with dextrocardia), resection of the interatrial septum may be performed with rerouting of superior venae cava and inferior venae cava flow to the right side using atrial flaps as tunnels.

Often times, prior cardiac operations have involved the pulmonary arteries in the form of systemic-to-pulmonary shunts, cavopulmonary anastomoses, or other procedures. In all cases as much donor pulmonary artery as possible should be harvested.

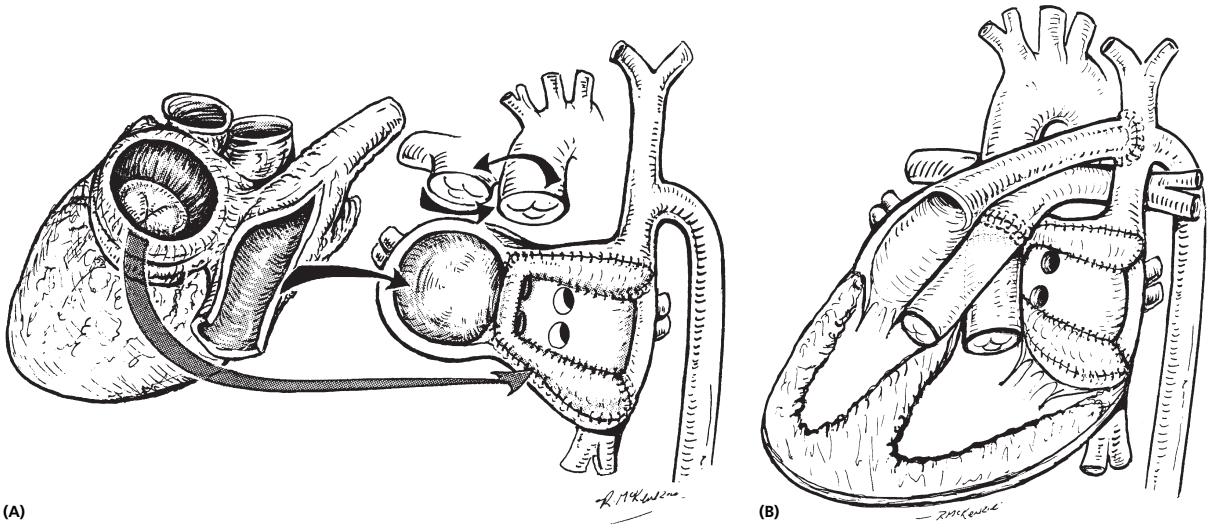

Figure 115.3. Transplantation for previously repaired transposition of the great vessels with mirror image arrangement ("situs inversus") following prior mustard (atrial baffling) procedure. (A) The mustard baffle was preserved to direct left-sided systemic venous return to the donor right atrium. (B) Because of stenosis of the superior caval baffle, the donor superior caval vein was anastomosed to the recipient superior caval vein proximal to the baffle.

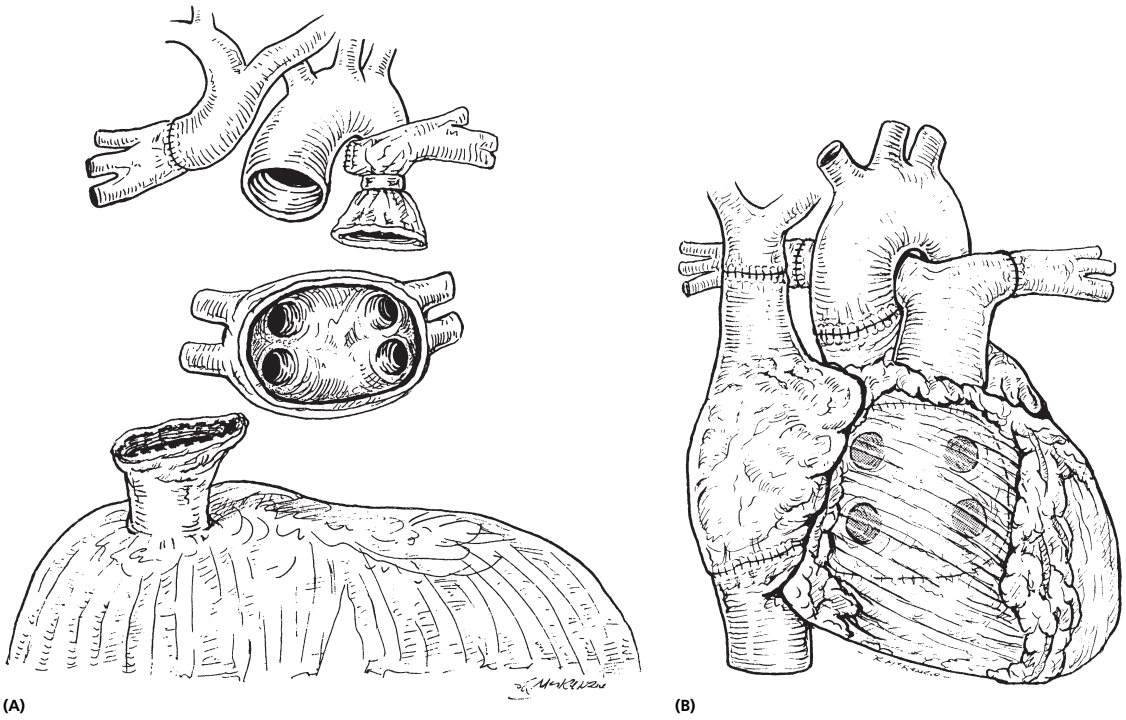

Figure 115.4. Bicaval heart transplantation in a child with discontinuous pulmonary arteries following prior right classical Glenn shunt and pulmonary artery banding. (A) After recipient cardiectomy, inferior cavo-atrial cuff and left atrial button were prepared and the classic Glenn shunt was taken down. (B) The donor organ was procured with both branch pulmonary arteries and an extended segment of the superior caval vein. Bipulmonary and bicaval anastomoses were performed.

Most commonly, as is the case in bidirectional Glenn or Fontan operations, the cavopulmonary anastomosis is taken down, the pulmonary arteries are patched, and bicaval anastomoses are performed. Patch material may take the form of bovine pericardium, homograft, or donor or recipient discard. Repair is best accomplished after recipient cardiectomy and before organ implantation.

In the case of discontinuous pulmonary arteries, the donor organ should be procured with the pulmonary bifurcation and pulmonary arteries intact (Figure 115.4). Direct left and right pulmonary artery anastomoses are then performed. Alternatively, excess donor aorta can be used to connect the right and left pulmonary arteries prior to implantation. Transplantation to a single lung is feasible when there

is unilateral absence or severe hypoplasia of a pulmonary artery [48]. This should only be performed when the dominant pulmonary artery is well developed with very low pulmonary vascular resistance. Otherwise the risk of acute donor right heart failure is high.

Few centers currently perform primary transplantation for HLHS. Because of the extensive arch hypoplasia, the donor surgeon must procure the entire transverse and proximal descending aorta en bloc with the organ. Surgical reconstruction of the arch is performed under deep hypothermic circulatory arrest.

Postoperative management and early complications

Many of the fundamental principles of early postoperative management after heart transplantation are similar to those for pediatric patients undergoing other procedures with cardiopulmonary bypass. This section focuses on aspects of care that are specific to the transplant recipient.

Cardiovascular considerations

Low cardiac output

Abnormalities in cardiac function are inevitable due to the obligatory hypoxic/ischemic insult that the donor heart endures. Recovery of systolic function is usually rapid. Abnormalities in diastolic function, however, may persist for many weeks. Most heart transplant recipients will benefit from low dose inotropic support in the immediate postoperative period, though often this is only required for 2–3 days. The choice of inotrope will reflect both physician preference and hemodynamic factors such as heart rate, pulmonary vascular resistance and blood pressure. Low dose dobutamine and isoproterenol are common choices. The latter is sometimes recommended because of its combined properties of chronotropy, inotropy and pulmonary vasodilatation. The addition of a combined vasodilator/inotropic agent such as milrinone is logical when there is low cardiac output and evidence of high systemic vascular resistance. In infants with markedly oversized donors, the simplest way to improve cardiac function is to leave the chest open at the end of the transplant procedure. Occasionally, there is failure to wean from cardiopulmonary bypass, or early postoperative graft failure in the first 24 hours. This primary graft failure is a serious complication associated with high mortality. The term primary graft failure is often reserved for the finding of acute left ventricular or biventricular failure not due to high pulmonary vascular resistance. Poor donor selection, very prolonged ischemic time, poor preservation technique and hyperacute rejection should all be considered. When primary graft failure occurs (not due to hyperacute rejection), recovery is frequently possible if the circulation can be supported. This is usually achieved with extracorporeal membrane oxygenation (ECMO) [49]. Retransplantation for early graft failure is generally associated with very poor outcomes [50], and many consider this a contraindication to retransplantation [51].

Systemic hypertension

In contrast to the non-transplant cardiac surgical patient systemic hypertension is common. Many factors contribute including vigorous function of an oversized donor organ and use of high dose corticosteroids. It is not unusual to observe quite severe systolic hypertension within 24 hours of a successful transplant procedure. If good ventricular function is confirmed by echocardiogram, rapid wean of inotropic support is performed. Where systemic resistance appears high, intravenous vasodilators are a logical choice. In the case of a vigorous oversized organ, some have advocated beta blockade.

Pulmonary hypertension

The importance of pulmonary vascular resistance as a risk factor for acute donor right ventricular failure is discussed above. If there is a concern about elevated pulmonary vascular resistance, on the basis of preoperative evaluation, additional precautions should be taken. Nitric oxide is begun in the operating room and is used to wean from cardiopulmonary bypass. Acidosis must be avoided and high levels of inspired oxygen are provided. Hyperventilation is performed and generous sedation is provided in the early postoperative period. The right heart may require significant inotropic support, and sometimes epinephrine may be required in addition to milrinone and dobutamine, despite potential negative effects on pulmonary vascular resistance. If right ventricular dysfunction persists with poor cardiac output despite this level of support, then mechanical assistance should be provided. If pulmonary vascular resistance is moderately elevated pretransplantation, (or if there is an acute elevation in resistance following bypass in a child with previously low resistance), then 24–48 hours of support will often enable the right ventricle to recover enough to support the circulation, despite elevated pulmonary pressures. If acute donor right heart failure reflects poor candidate selection (e.g. indexed pulmonary resistance greater than 9 IU after vasodilator challenge), then recovery of right heart function is unlikely.

Cardiac rate and rhythm

Postoperative tachy- and bradyarrhythmias have been observed in children following heart transplantation. The commonest rhythm abnormality (other than sinus tachycardia) is sinus node dysfunction leading to sinus bradycardia, with or without an atrial or junctional escape rhythm. The denervated sinus node responds appropriately to exogenous chronotropic agents and isoproterenol is useful in this respect. A simpler approach is atrial pacing, and all transplant recipients should have temporary pacing wires placed in the operating room. Sinus node dysfunction reflects ischemic and/or traumatic injury, but usually recovers in a few days. Ventricular ectopy and non-sustained ventricular tachycardia are also quite common in the first week or two after transplantation. These presumably relate to the obligatory ischemia-reperfusion injury, and rarely require treatment. The fresh cardiac allograft has limited ability to increase stroke volume, and therefore establishing an adequate heart rate is important for maintaining cardiac output. Atrial pacing is most commonly used to control the heart rate.

Respiratory support

The principals of respiratory support do not differ from those of other pediatric open heart procedures. Early extubation should be the goal. The patient who has required prolonged preoperative mechanical ventilation will usually need more prolonged ventilatory support postoperatively as retraining of respiratory muscles will be required. Infants with long-standing cardiomegaly will often have significant tracheobronchomalacia and persistent or recurrent pulmonary atelectasis is not unusual, especially of the left lower lobe.

Renal function

The combination of chronic heart failure, cardiopulmonary bypass and use of cyclosporine or tacrolimus all contribute to postoperative renal dysfunction. This is exacerbated if there is low cardiac output state postoperatively. Oliguria is common. Fortunately,

acute renal failure is rare in children and dialysis is seldom required. Persisting oliguria is managed with loop diuretics and low dose dopamine (e.g. 3–5 mcg/kg/minute). Low output is managed with inotropic agents as discussed above. Administration of a continuous furosemide infusion (up to 6 mg/kg/day) may be helpful. These maneuvers are usually successful in stimulating an adequate urine output (>1 ml/kg/hour). When urine output remains low, it may be necessary to withhold calcineurin inhibitors (tacrolimus or cyclosporine) for several days. This can be facilitated by the use of intravenous induction agents as part of the early immunosuppressive regimen (see further in this chapter).

Gastrointestinal considerations

Gastrointestinal complications are quite common early after pediatric heart transplantation [52]. All patients should receive intravenous, and subsequently oral, H_2 antagonists to decrease the risk of stress ulcers in the early post-operative period. These are usually continued until corticosteroids have been weaned to low doses or discontinued. The nasogastric tube is removed as soon as the patient is extubated and able to take oral feeds and medications. Attention is paid to providing optimal calories without use of excessive volumes since most patients will tend to retain fluid in the early postoperative period. Pancreatitis is not uncommon following transplantation and should be sought when there is abdominal pain or unexplained feeding intolerance. Immunosuppressive regimens that avoid the use of azathioprine and corticosteroids may reduce this complication. Symptoms of gastrointestinal perforation may be subtle in small children on immunosuppressive medications, especially if corticosteroids are being used. Many children with chronic heart failure have gastroesophageal reflux disease. This should be aggressively managed, but with knowledge that there are many drug interactions between immunosuppressant medications and drugs used for gastroesophageal reflux disease including antacids, antihistamines and prokinetic agents.

Infectious precautions

Infections are a leading cause of death and morbidity in the first year following pediatric heart transplantation [20]. Most severe infections occur during the initial hospitalization. During the first week after transplantation, invasive lines and drains are removed as soon as possible. A short course of antibiotics (e.g. 72 hours) is given as prophylaxis against mediastinal and wound infection. Usually a first generation cephalosporin will suffice. Broader staphylococcal coverage (i.e. vancomycin) is given if the patient has had a prolonged ICU stay and has long-standing lines in place. Such lines are usually replaced in the operating room. Patients colonized with MRSA are also covered with vancomycin. Oral nystatin is started in the intensive care unit, along with ganciclovir, if recipient or donor are seropositive for CMV. Patients at high risk for yeast infections (e.g. patients on pretransplant ECMO) are frequently given prophylaxis with fluconazole. However, it should be noted that all "azole" antifungals have a profound effect on calcineurin inhibitor metabolism (via the cytochrome P450 system). A marked reduction in tacrolimus or cyclosporine dosing (50–90% reduction) is required during concomitant use of an azole antifungal agent. Initiation of prophylaxis against *Pneumocystis jiroveci* can follow nearer to the time of hospital discharge.

Immunosuppression and early acute rejection

High dose intravenous methylprednisolone (e.g. 15–20 mg/kg) is given in the operating room. A tapering course of corticosteroids is often given over the next 1–2 weeks, with the majority of centers discharging patients on maintenance corticosteroid therapy [2]. Some centers attempt to wean patients off steroids if the rejection course has been benign [53]. However, there is increasing use of steroid avoidance immunosuppressive regimens in pediatric practice [54]. This likely requires induction therapy to be successful. Cyclosporine or tacrolimus is commenced generally within 24–48 hours of surgery once good urine output has been established. Both agents can be given intravenously or enterally. If anti-T cell induction therapy is used (most commonly polyclonal rabbit antithymocyte globulin; less often with an interleukin-2 receptor antagonist), then there is less urgency to introduce a calcineurin inhibitor (CNI) in the immediate (first 1–2 days) post-transplant period. Cyclosporine or tacrolimus can then be commenced by the oral route rather than intravenously. Delay in commencement of these agents for several days (under coverage of induction therapy) may be particularly useful when urine output is low or renal function is deteriorating. Of interest, use of induction therapy has progressively increased over the last decade in children reaching 70% in 2010 [20]. This trend has not been mirrored in the adult population. This may reflect the desire to avoid corticosteroids in low immunologic risk cases, as well as delay the need for invasive endomyocardial biopsy (EMB) in pediatric recipients.

Careful daily assessment is performed for signs of rejection, though severe rejection before 7–10 days is rare (except in the sensitized patient). Rejection is generally delayed with use of induction therapy. Infants and young children experience less acute rejection than adolescents. Pallor, increasing tachycardia, abdominal pain, gallop rhythm and oliguria all are suggestive of severe rejection. Ideally, rejection is identified by echocardiography and/or surveillance biopsy before such signs develop. The electrocardiogram may show reduced precordial voltages. The tempo of rejection can be quite abrupt in the early post-transplant period and any deterioration in the patient's condition after initial recovery from surgery must be taken very seriously. If there is unequivocal evidence of new graft dysfuction, empiric treatment (usually consisting of bolus intravenous corticosteroids), or immediate EMB, should be performed. Biopsy generally shows lymphocytic infiltrates (predominantly T cells) with varying degrees of edema and myocyte damage. Endomyocardial biopsies are graded according to an internationally agreed classification system developed by the ISHLT [55].

Post-transplant management and long-term complications of transplantation

The long-term management of pediatric heart recipients is aimed at maximizing long-term survival, minimizing complications of immunosuppressive therapy, and improving quality of life. A detailed discussion of all complications of heart transplantation that occur beyond the immediate postoperative period is outside the scope of this chapter and are covered elsewhere in this text. This section focuses on how specific complications impact the pediatric cardiac transplant recipient and the strategies that are employed to prevent and manage these complications.

Maintenance immunosuppressive therapy

Immunosuppressive therapy aims to prevent or minimize any damaging immune response of the host to the donor organ, while avoiding complications of therapeutic immunosuppression. Immunological complications of transplantation fall into two main

groups: (1) allograft rejection and graft dysfunction (both acute and chronic) reflecting inadequate or ineffective immunosuppression and manifestations of non-specific immunosuppression, including infections and malignancy. (2) non-immune side effects of immunosuppressive therapy (i.e. tissue and organ toxicities) are an important cause of morbidity, and occasionally mortality, after heart transplantation in children.

As discussed previously, most children currently receive some form of induction therapy in the perioperative period, most commonly with rabbit anti-thymocyte globulin. As with adults, almost all children receive a CNI with an anti-metabolite/anti-proliferative agent. Presently, the combination of tacrolimus and mycophenolate mofetil is the most commonly used combination of agents, with approximately 66% use of tacrolimus and 70% use of mycophenolate mofetil (MMF) or mycophenolic acid (MPA) at one year after transplantation [20]. Use of MMF/MPA has fallen to approximately half of patients by five years [20], perhaps reflecting the relatively high intolerance of MMF/MPA in children [56]. Use of corticosteroids is highly variable, though internationally, 64% are still receiving corticosteroids at one year after transplantation falling to 39% at five years [20]. However, as discussed previously, many centers have experience with complete steroid avoidance beyond the first few days after transplantation in non-sensitized children after heart transplantation [54]. This offers many obvious advantages to pediatric recipients. Further work is needed to identify which patients are most suitable for steroid avoidance regimens.

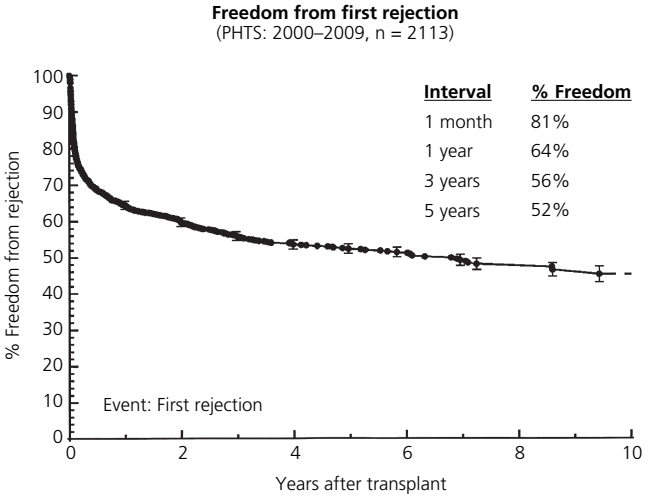

Figure 115.5. Freedom from first acute rejection event in patients transplanted within the Pediatric Heart Transplant Study (PHTS) database from January 2000 to December 2009. Published with permission of PHTS.

Acute rejection

Patients remain at risk for acute rejection indefinitely, though it is clear that the hazard declines over time. There is no evidence that heart transplant recipients become truly tolerant to their allograft. The importance of acute rejection episodes becomes evident when causes of death after heart transplant are examined. Data from the ISHLT show that acute rejection is the commonest cause of death between 30 days and three years after heart transplantation [20]. During this time period, 20% of deaths are identified as being due to acute rejection with another 31% attributed to "graft failure". The peak hazard, or instantaneous risk, for first rejection is between one and two months after transplantation. By one year after transplantation, approximately 64% of pediatric heart recipients are free of acute rejection [23] (Figure 115.5). Late acute rejection episodes (occurring beyond the first year after transplantation) appear to carry a particularly poor long-term prognosis especially if associated with graft dysfunction [57,58]. When there is any degree of systolic dysfunction with acute rejection, rapid deterioration is common, even when the patient appears well and free of heart failure at presentation. Thus, it is prudent to admit all patients with acute graft failure to the intensive care unit for initiation of therapy. If systolic failure is more than mild, intravenous milrinone should be initiated and the patient should be monitored for arrhythmias. Unless graft failure is known to be due to coronary artery disease, treatment for acute rejection/graft dysfunction should be initiated with intravenous methylprednisolone 10–15 mg/kg (maximum 1 g) daily for 3–5 days. Unless the patient is unstable, it is optimal to obtain an EMB since acute graft dysfunction maybe associated with "acellular rejection" (which carries a worse prognosis) or with clear histological evidence of antibody mediated rejection (AMR). Additional therapies may then be required, including urgent plasmapharesis. It should be emphasized that treatment of severe acute rejection should not be delayed while waiting for EMB to be performed or results to be obtained. Acute rejection with hemodynamic compromise can rapidly lead to graft failure. Unless there are specific contraindications, such patients should receive full hemodynamic support, including use of mechanical support since the condition is generally reversible in nature. ECMO is most commonly used in this setting [59]. Retransplantation should be avoided in the setting of active acute rejection since outcomes are poor [51].

Three recent analyses from the PHTS have investigated the presence of era effects for acute rejection [58,60,61]. There have been significant reductions in acute rejection events from 1993 to the current era both during the first year after transplantation [60], as well as reduction in first and recurrent episodes of late rejection (occurring more than one year after transplantation) [57,58]. This is important since late rejection episodes have been shown to be associated with high risk for subsequent development of moderate to severe graft vasculopathy, death and need for retransplantation [57,58]. Of particular note, the incidence of acute rejection with severe hemodynamic compromise has not changed over time within the PHTS, despite the overall fall in frequency of all acute rejection events [61]. Furthermore, survival has not improved for acute rejection associated with severe hemodynamic compromise with similar survival across eras at one and five years after the rejection event (63% at one year and 49% at five years) [61].

Risk factors for acute rejection have also been extensively investigated with the PHTS. Risk of first rejection is associated with earlier era of transplantation, older age at transplantation and positive donor-specific cross-match [60]. Of note, race was not a risk factor in the recent era for first acute rejection, but has remained a risk factor for late rejection episodes and for episodes of rejection with severe hemodynamic compromise [58,61].

Antibody mediated rejection is also observed after pediatric heart transplantation, as for adults. Most episodes occur in patients transplanted across a positive donor-specific cytotoxicity cross-match [62,63]. Sensitization is common in the pediatric age group

due to the high frequency of prior surgical procedures, including previous use of aortic and pulmonary homografts for repair of congenital heart defects. These cryopreserved allografts have a profound sensitizing effect [64]. Some children have such broad sensitization that wait-list times are very long, and wait-list mortality high, due to inability to find a compatible donor with acceptable HLA antigens [65]. Pretransplant desensitization regimens do not appear effective in this population. In some cases ("100% PRA"), it is apparent that an acceptable donor will never be found. Some centers have attempted to transplant these patients knowing that the donor-specific cross-match will be positive. Typical perioperative regimens include intraoperative plasma exchange, short-term post-transplant plasmapharesis, T cell depleting antibody induction therapy, triple drug immunosuppression (tacrolimus, MMF, steroids) and serial infusions of intravenous immunoglobulin. A trial is under way to evaluate the success of such a strategy [66]. The role of rituximab, bortezomib and other experimental agents in this setting is unknown. All these agents have also been used for the treatment of established AMR in pediatric heart recipients [67], though no prospective, randomized trials have been performed.

Chronic rejection or cardiac allograft vasculopathy

The terms chronic rejection and cardiac allograft vasculopathy (CAV) are generally used synonymously. Coronary disease subsequent to transplantation is an accelerated vasculopathy that is the leading cause of death among late survivors of pediatric heart transplantation [20,41]. It accounts for at least 25% of deaths in the period beyond three years after transplantation [20]. Details of CAV pathophysiology are found elsewhere in this text. Suffice it to say that pediatric recipients are not immune from this complication, though freedom from CAV appears superior to adults at any given time point after transplantation, especially in young children [41]. Intriguing data have recently been published showing that persistence of viral genome of various viruses (especially adenovirus) detected in the myocardium of heart biopsy samples by polymerase chain reaction predicts the development of coronary disease and late graft loss in children [68]. Use of older donors, late acute rejection episodes, and older recipient age are all risk factors for the development of post-transplant CAD in children [41]. The role of cytomegalovirus serostatus and infection in CAV in children is not entirely clear and is less well studied than in adults [69].

Symptoms of ischemia are often absent, though some children will experience episodes of abdominal pain and/or chest pain, despite operative denervation of the heart [70]. Syncope and sudden death are also common presentations of CAV in children. In the current era, the diagnosis is most often made during surveillance selective coronary angiography. Intravascular ultrasound has much greater sensitivity for this diagnosis, though experience in children is much more limited than adults [71,72]. Assessment of coronary flow reserve is also feasible in children, but has rarely been utilized due to the technical challenges involved and uncertainly about interpretation of the findings [73].

Unfortunately, no curative treatment exists for established coronary arterial disease. Diastolic dysfunction tends to develop early and may be observed even when there is little evidence of epicardial coronary artery narrowing [74]. This may be a reflection of diffuse small vessel disease in many patients. Once overt systolic failure ensues, survival is poor and consideration should be given to retransplantation. Outcomes for late retransplantation (beyond six months from primary transplant) are similar to those for primary transplantation [50]. Children with ischemic-induced syncope should receive automatic implantable cardioverter-defibrillators (AICD) if they are to be discharged from hospital while awaiting retransplantation. Beta blockers may be given for their anti-ischemic benefits if heart failure is not advanced and beta agonists are not required. Stenting and bypass grafting may play a limited role in bridging to retransplantation, but are limited by the diffuse nature of CAV. Graft loss was reported in 52% of children within one year of a percutaneous revascularization procedure, emphasizing the limited role of these interventions [75].

Infections

An increased prevalence of all forms of infection is seen compared to the general population of children. Most infections are caused by pathogens that also cause infection in the non-immunocompromised host. Common examples include respiratory viruses, *Streptococcus pneumoniae*, and varicella virus. All infections that occur in non-immunocompromised patients can cause greater disease severity in the recipient of a transplanted heart. Of particular note in this respect are infections due to cytomegalovirus (CMV) and Epstein-Barr virus (EBV), which only rarely cause severe disease in the immunocompetent host. More rarely, opportunistic infections are seen such as that due to *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*) [76]. Although most infections are well tolerated, infection is second only to graft failure as the leading cause of death in the first 30 days after transplantation, and remains an important cause of death during the remainder of the first post-transplant year [20].

Broad spectrum antibiotic coverage is required in any septic appearing child after heart transplantation until an organism has been identified. *Streptococcus pneumoniae* infections occur with increased frequency and choice of antibiotics must include coverage of this agent. When there is clinical and radiographic evidence of pneumonia and deteriorating clinical status, there should be a low threshold for performing bronchoalveolar lavage in order to obtain deep cultures for viruses, fungi and bacteria. *Pneumocystis jiroveci* should be ruled out when there is hypoxia and characteristic chest X-ray changes [76]. Respiratory viral pathogens (e.g. RSV, influenza, parainfluenza, adenovirus) should be sought when there is evidence of severe respiratory infection in a heart transplant recipient. While viral respiratory disorders tend to be well tolerated in older children and later out from transplantation, acquisition of one of these respiratory viruses in the first few weeks after transplant can occasionally cause devastating disease, especially in infants. Invasive fungal infections make up approximately 7% of serious post-transplant infections in children, and are most commonly due to *Candida* and *Aspergillis* spp [77]. These are associated with incremental numbers of invasive pretransplant procedures (e.g. ECMO, prior surgery, VAD) and are associated with high mortality, predominantly within the first six months after transplantation [77].

Primary CMV infection is less problematic in heart transplant patients than in lung transplant recipients, but can still cause serious morbidity and occasionally fatality. In heart recipients, pneumonitis is quite rare, whereas it is a common site of disease in lung and heart-lung recipients who develop primary infection post-transplantation. By contrast, gastroenteritis, hepatitis and bone

marrow suppression are relatively common findings. Diagnosis is facilitated by evaluation of peripheral blood by PCR or antigenemia (pp65) testing. Diagnosis of CMV *disease* remains a tissue diagnosis. When the diagnosis is made early, treatment with intravenous ganciclovir and/or oral valganciclovir is usually very effective. Prophylaxis strategies generally do not differ from adults (see Chapter 94).

Infection from EBV in the immunocompromised pediatric recipient can be asymptomatic, or may cause a non-specific viral syndrome, mononucleosis, fulminant "viral sepsis" or a post-transplant lymphoproliferative disorder (PTLD). The strongest risk factor for the development of PTLD is the development of primary EBV infection post-transplantation, though children who are seropositive for EBV at the time of transplant are not completely protected from this complication. Two analyses of the PHTS database provides the most comprehensive analysis of PTLD in the pediatric heart population [78,79]. PTLD was diagnosed in 147 of 3170 primary heart transplants between 1993 and 2009 among 35 institutions, with probability of freedom from PTLD being 90% at ten years after transplantation. Rebound rejection during therapy for PTLD remains a major concern, and graft failure (in contrast to PTLD disease progression) accounts for approximately half of all deaths after a diagnosis of PTLD is made [78]. Further details of the pathobiology, epidemiology, treatment and prognosis of PTLD are given in Chapter 96.

Non-immune complications

In addition to the consequences of over or under-immunosuppression, transplant recipients experience a wide array of non-immune toxicities of immunosuppressive therapies. Toxicities of immunosuppressive agents in children have been previously reviewed [80,81]. In contrast to adults, growth failure is a unique problem in children and contributes to the desire for steroid minimization and avoidance regimens in many pediatric transplant centers. One complication worthy of particular attention is that of progressive renal dysfunction due to calcineurin inhibitor renal toxicity. This is becoming increasingly problematic as larger numbers of children survive long-term after heart transplantation. Some have already developed end-stage renal failure requiring renal transplantation [82]. It should be noted that estimates, and direct measures, of creatinine clearance overestimate glomerular filtration rate (GFR) in this population and that the severity of renal disease is generally greater than perceived [82]. In a recent analysis of renal dysfunction within the PHTS, late renal dysfunction was found to be associated with earlier era of transplant, black race, rejection with hemodynamic compromise in the first year and lowest quartile estimated GFR at one year post-transplantation [83]. Another recent multi-institutional study in children was unable to confirm earlier reports that common genetic variations might influence post-transplant renal function under CNI-based immunosuppression [84]. Of note, clinical trials of CNI avoidance from the time of transplantation have not been performed in the pediatric heart transplant population.

Post-transplant survival and risk factors

Data from the registries of the ISHLT [20], the SRTR and the Pediatric Heart Transplant Study [23] all demonstrate important trends in post-transplant survival over the last two decades. Importantly, there have been significant improvements in outcome

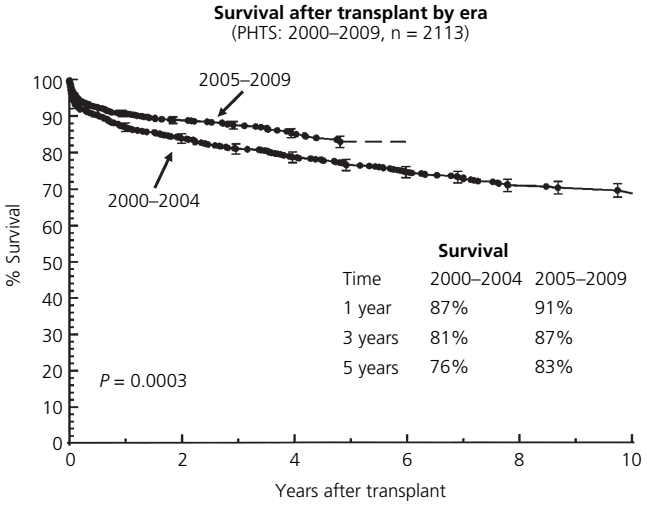

Figure 115.6. Overall survival in patients transplanted within the Pediatric Heart Transplant Study (PHTS) database from January 2000 to December 2009 stratified by era. Published with permission of PHTS.

in recent years; the improved survival being most evident in the infant age group and in smaller volume centers. Of note, there has been significant improvement in outcome even between the first and second halves of the first decade of the new millennium (Figure 115.6). Most of the improvement appears to be due to reduction in early mortality. One-year survival is now approximately 90% in many centers, with only a relatively small drop over the next 1–4 years. The PHTS and the ISHLT databases continue to show a slightly higher perioperative and early mortality for infant recipients, but interestingly, these youngest recipients have a patient half-life that now approaches 20 years, and the conditional half life for infants surviving to one year is superior to all other age groups [20]. By contrast, patient half-life for adolescents is only 11.9 years [20]. It is likely that this reflects a lower incidence of post-transplant coronary artery disease in very young recipients and a degree of immune privilege. The higher graft loss and mortality rates in older children likely reflects, at least in part, inferior adherence in this age group. Use of induction therapy, choice of initial CNI (cyclosporine or tacrolimus) and use tacrolimus versus cyclosporine at one year post-transplant have not been associated with differences in survival after pediatric heart transplantation [20].

The results of transplantation for congenital heart disease still lag behind those of transplantation for cardiomyopathy; this difference is due to higher perioperative mortality. This difference is most notable in infant recipients and least noticeable in adolescents. ISHLT reports a 12% superior survival at ten years for infants with cardiomyopathy compared to CHD; the difference is entirely due to increased early mortality. The difference did not reach statistical significance for the adolescent age group.

Importantly, there remains evidence of reduced survival among black pediatric recipients compared to other racial groups [85,86]. This difference is primarily due to increased graft loss beyond the first year after transplantation. Lower socioeconomic status has also been associated with increased graft loss beyond the first year [86].

In the most recent analysis of the ISHLT registry, risk factors at transplant for death at one year included the following: ECMO,

retransplantation, congenital diagnosis, dialysis, mechanical ventilation, prior sternotomy, donor age, pretransplant creatinine level and total ischemic time [20]. Donor cause of death was also found to be a risk factor with cerebrovascular events having higher relative risk for one-year mortality compared to head trauma, and anoxia being protective compared to head trauma. The significance of these latter observations is unclear, since a recent analysis of the PHTS database (3122 donors) did not reveal traditional donor risk factors to influence post-transplant outcomes including donor cause of death, donor CPR and high donor inotropic requirements (prior to procurement) [37]. In infants less than six months of age, no donor-related factors influenced post-transplant survival. Longer ischemic time and greater donor-recipient age difference did influence survival in recipients over ten years of age.

Summary

Excellent outcomes are now being achieved after pediatric heart transplantation, with five-year survival now exceeding 80%. The improved outcomes appear to be limited primarily to the early postoperative period, and this improvement is most apparent among infants and those with congenital heart disease. Infants surviving the first year have the best long-term outcomes with conditional graft half-lives in excess of 20 years. Pretransplant wait-list mortality also appears to have improved and likely reflects multiple factors including better medical care for critically sick candidates, extension of VAD support as a bridge to transplantation in children, and perhaps changes in allocation policy. Despite these advances, heart transplantation remains palliative and all transplant recipients are at risk for the adverse effects of non-specific immunosuppression, including infections, lymphoproliferative disorders and non-lymphoid malignancies. In addition, current immunosuppressive agents have narrow therapeutic windows and exhibit a wide array of organ toxicities. This poses special challenges for the young patient who must endure life-long immunosuppression. New immunosuppressive regimens have lowered the rates of acute rejection (but not rejection with severe hemodynamic compromise) but appear to have had relatively little impact on the incidence of CAV, the principal cause of late graft loss.

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CHAPTER 116

Pediatric Lung Transplantation

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Introduction

In 1963 Dr. Hardy performed the first successful human lung transplant into a convicted felon serving a life sentence. Immunosuppression consisted of azathioprine, prednisone and radiation therapy. The patient died 18 days later from renal insufficiency. Despite an ABO mismatch, no rejection was found on autopsy. Dr. Cooley and his team performed the first human heart-lung transplant on a two-month-old infant with complete atrioventricular canal defect and pulmonary hypertension in 1968. The patient survived for only 14 hours [1]. Outcomes such as this relegated lung transplantation mostly to the laboratory until surgical techniques, preservation solutions and immunosuppressive regimens improved or became available. Reports of successful heart-lung and lung transplantation in adults in the early 1980s [2,3], allowed for the expansion and application of this procedure to the pediatric population. Between 1986 and June 2010, 1664 lung and 653 heart-lung transplantations, in patients less than 18 years old, had been reported to the Registry for the International Society for Heart and Lung Transplantation (ISHLT) [4].

The decision to transplant a child presents unique challenges. For all the experience gained in the field of adult lung transplantation, the indications, approaches, complications, immunosuppressant pharmacokinetics, and monitoring are often very dissimilar for children. Therefore, lung transplantation in the pediatric population must be treated as a distinct entity and studied as such. This chapter will attempt to highlight some of the unique features of pediatric lung transplantation. Chapters 31, 45, 59, 72, 80, 84, and 106 cover general and adult-specific aspects of lung transplantation. Special considerations in children relate to size and growth of the child, complications of airway anastomoses, increased exposure to infections in daycare or school settings, non-adherence to immunosuppression, transitioning to adult healthcare providers, and obstacles related to monitoring the allograft.

Patient selection and indications for transplantation

Lung transplantation should be considered in selected children with end-stage or progressive lung disease or pulmonary vascular disease for which there is no other therapy. The diagnoses that commonly bring a child to a lung transplant center for evaluation are often different than those in adults (Chapter 31). Chronic obstructive pulmonary disease (COPD), emphysema, and idiopathic pulmonary fibrosis are the three most common adult pretransplant diagnoses. Indications for lung transplantation in children have undergone considerable change in the last two decades as experience with this procedure even in infants has grown [5,6]. The most common diagnoses for which children are transplanted are listed in Figure 116.1 according to the age in years at time of transplantation. In children younger than one year, the most common indications are pulmonary hypertension, usually associated with congenital heart disease, other pulmonary vascular diseases, primarily pulmonary vein stenosis, and rarely alveolar capillary dysplasia. Disorders of surfactant metabolism include surfactant protein B (SPB) and C (SPC) deficiencies, ATP binding cassette A3 (ABCA3) transporter and NKX2.1 mutations [6–9]. Less common indications include interstitial lung disease, bronchopulmonary dysplasia and pulmonary hypoplasia [10]. In the 1–5 years age group, disorders leading to pulmonary hypertension remain a common indication. In patients 6–11 years of age, cystic fibrosis (CF) becomes the most common indication. The relative percentage of children with primary pulmonary hypertension coming to lung transplant has diminished significantly during the past decade, largely because of the introduction of effective medical therapies including prostaglandins (epoprostenol), phosphodiesterase-5 inhibitors (e.g. sildenafil) and endothelin receptor antagonists (e.g. bosentan) [4]. In adolescents, CF accounts for almost three quarters of the population that receives a lung transplant. In other words, it is more likely that pediatric lung transplant candidates, in contrast to their adult counterparts, will present with septic lung disease and continue to harbor organisms in their upper airways after lung transplant.

Historically, timing of referral for lung transplant has been predicated on matching predictions of mortality with the anticipated waiting time for donor lungs. Before committing a child to lung transplant, given imperfect disease specific criteria, most pediatric centers carefully consider multiple factors beyond lung function including trajectory of the underlying disease process, growth and nutrition status, frequency of hospitalizations, psychosocial factors, and potential for improvement in overall quality of life (QOL).

Absolute contraindications encompass situations that would clearly put the child at risk for a poor outcome after transplant, are similar between pediatric and adult patients and include systemic disease such as malignancy or multi-organ failure. Relative

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

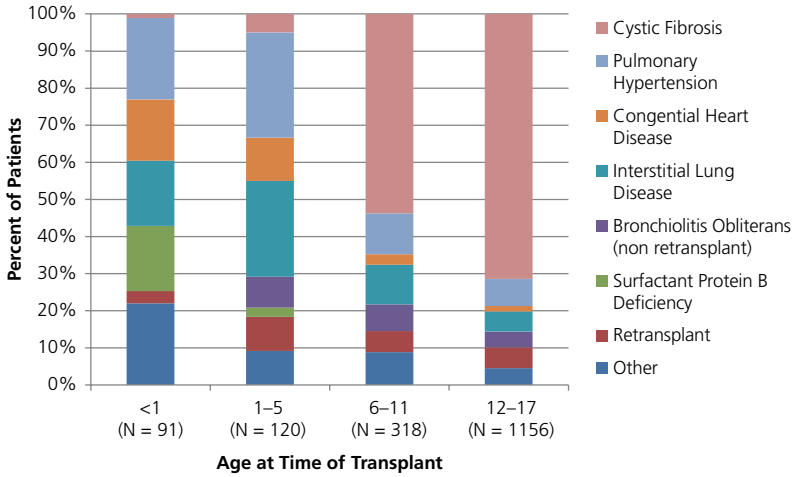

Figure 116.1. Distribution of pediatric lung transplants by age and indication. Data adapted from [4] with permission.

contraindications increase the risk of transplant but are weighed on a case-specific basis. In general contraindications are not static and can change over time with the emergence of new information, therapies or techniques. Contraindications are also center-specific based on that center's individual experience. For example, many centers will not consider patients requiring veno-arterial extracorporeal support (VA-ECMO).

One challenging concern that all pediatric transplant programs must address at times is a history of documented non-adherence to medical management. This is more problematic for pediatric programs than adult centers because the responsibility for providing the child their medications or for bringing them to clinic appointments belongs to the parents and not to the minor. A detailed discussion of this can be found in Chapter 120. In these situations the referring center and the transplant center must share the responsibility of working with the family to identify and address any barriers to care. This may take the formulation of a contract between the referring physician and family that outlines the need for strict adherence to a treatment program over a three to six month period prior to listing for transplant. In general, concerns about non-adherence become an absolute contraindication at some transplant centers when it is refractory to intervention. Although in some centers this may serve as an impetus to involve legal authorities to place the child in foster care, many centers will view temporary placement in a foster home as an absolute contraindication to transplant.

The transplant evaluation itself consists of a number of laboratory and radiographic studies as well as consultation with multiple services (Table 116.1). The evaluation is multi-disciplinary in nature, involving both medical and psychosocial components.

Table 116.1. Recommended evaluation for pediatric lung and heart-lung candidates

Bloodwork:
Blood type (ABO)
Complete blood count
Coagulation studies (PT, INR, PTT)
Complete biochemistries including electrolytes and renal and liver function tests
Lipid profile
Serologies including CMV, EBV, HIV, hepatitis B and C, measles, varicella, herpes simplex, *Toxoplasma gondii*
Anti-HLA antibody screen
Arterial blood gas
Autoimmune screen (ANA, ANCA, rheumatoid factor, quantitative immunoglobulins)
Thyroid profile
Pulmonary function testing:
Six minute walk test
Sputum or deep throat culture and susceptibility testing
Tuberculin testing
Electrocardiogram
Cardiac catheterization (in select patients)
Imaging:
Chest radiograph
Chest CT
Sinus CT (in patients with CF or immunodeficiency)
Echocardiogram
Bone densitometry
Consultations with:
Cardiothoracic surgery
Cardiology (in select patients)
Infectious diseases
Social services
Psychology
Nutrition
Physical therapy
Child life

Disease specific evaluation

Cystic fibrosis

Cystic fibrosis (CF) is a highly variable disease in which end stage lung disease is the most common cause of death. Improvements in care have led to better median survival, but there are still children with CF who progress to respiratory insufficiency. Liou et al. used a proportional hazards model to determine if lung transplantation provided a survival benefit to children with CF and concluded that it did not [11]. Their model however had several flaws [12]. It was derived using data obtained at the time of listing and not at the time of transplant. Their study was done prior to 2005 at a time when lungs were allocated in the US based on time spent on the waiting list and not by the Lung Allocation System (see further in this chapter). Patients were typically listed two and three years prior to their actual transplant to allow them to survive to transplant. Clinicians were astutely taking into account that the date of listing was not going to be the date of transplant, but rather acknowledged that if patients were going to survive to transplant, they would need to accrue time on the list. This flaw, in and of itself, impacts the calculated survival benefit

of the procedure. Other studies [13] have found a benefit and lung transplantation remains the only treatment option available to patients with CF and end stage lung disease with the potential to extend life [14].

Specific indications

Early studies in CF led to recommendations for referral for lung transplantation once the forced expired volume in one second (FEV_1) declined below 30% predicted [15]. Although more recent studies have attempted to add to these criteria [16], none have improved significantly on the ability to predict waiting list mortality. Even in the best model, the positive predictive value is less than 50% [17].

Specific contraindications

Individual transplant centers have specific microbiologic contraindications to transplantation; most commonly chronic infection with *Burkholderia cenocepacia*. Case reports suggest that outcomes for patients chronically infected with *Mycobacterium abscessus* may be worse [18], but other investigators have demonstrated successful outcomes in these patients [19]. The presence of severe liver disease may be a contraindication in some centers, but may be an indication for combined lung-liver transplantation at other centers. Pleurodesis is not an absolute contraindication to transplant, but may prolong the ischemic time because of excessive intra-operative bleeding.

Surfactant processing abnormalities

Patients with SPB deficiency typically present with respiratory failure shortly after birth. Children with SPC deficiency, ABCA3 transporter mutation, or NK2.1x mutation can have variable presentations [20]. Lung biopsy may show diffuse alveolar type II cell hyperplasia, alveolar septal thickening, and alveolar proteinosis. Diagnosis is confirmed by genetic analysis.

Specific indications

Once a decision to pursue transplant has been made, patients with SPB deficiency should be transferred as soon as a possible to the transplant center, as many of these patients ultimately require high frequency oscillator ventilation or ECMO. For the other surfactant processing abnormalities lung transplantation is indicated for refractory respiratory failure or progressive respiratory insufficiency unresponsive to medical interventions.

Specific contraindications

As noted above in many transplant centers VA-ECMO dependence is an absolute contraindication to transplantation. Significant cerebral hemorrhage or other severe neurologic injury associated with a significant likelihood of developmental delay is also a contraindication.

Pulmonary vascular disease

There are multiple potential etiologies for pulmonary vascular disease in children including idiopathic pulmonary hypertension (IPH), congenital heart disease and pulmonary vein anomalies. These entities must be carefully distinguished to allow for appropriate management. Therapeutic advances have dramatically improved outcomes for children with IPH and they should only come to transplant when medical therapy has failed. Determining when to proceed with transplantation for patients with Eisenmenger syndrome continues to be challenging. Many of these patients can live for years, if not decades after diagnosis.

Specific indications

A decreased cardiac index of less than 2 L/min/m^2, elevated pulmonary vascular resistance, right atrial pressures of greater than 7.4 mmHg, and right ventricular end diastolic pressure of more than 10.4 mmHg portend poor survival and are indications for lung transplantation. Other factors that may correlate with survival include von Willebrand factor levels of more than 240%, elevated uric acid levels, and plasma levels of brain natriuretic peptide (BNP) greater than 180 pg/mL.

In patients with Eisenmenger syndrome and progressive disease, including severe hypoxia, syncope, or a very limited QOL, heart-lung transplantation must be considered. Patients with simple cardiac lesions may be candidates for cardiac repair at the time of bilateral lung transplantation [21,22].

As in patients with SPB deficiency, once the decision to transplant is made patients with alveolar capillary dysplasia (ACD), pulmonary veno-occlusive disease or pulmonary vein stenosis should be referred as soon as possible as these entities are poorly responsive to medical or surgical interventions.

Specific contraindications

In this population, the presence of extensive aorto-pulmonary collateral circulation (often seen in pulmonary atresia with diminutive or absent central pulmonary arteries), coupled with multiple prior thoracotomies has been associated with poor outcome [23,24]. In addition, patients who have undergone multiple thoracic surgeries or who have had implantation of homograft valves or vessels have in increased likelihood of developing allosensitization and subsequent risk of antibody medicated allograft injury. These risk factors are a relative contraindication in many centers.

Management on the waiting list

In the US listing practices for children >12 years of age and adults are driven by the "Lung Allocation System" (LAS) adopted in 2005 by the Organ Procurement and Transplantation Network (OPTN) [25] (see Chapter 31). The LAS allocates donor lungs based on models of waiting list mortality and post-transplant survival, in order to maximize the one-year transplant survival benefit. Factors in the models include diagnosis, age, height/weight, need for supplemental oxygen, pulmonary arterial pressures, six minute walk distance, and lung function. Waiting time and waiting list mortality have decreased in the US coincident with the LAS adoption [26]. However, an effect of the double contribution of the waiting list mortality to the LAS (LAS = post-transplant survival – 2 * waiting list survival, normalized to a 0–100 scale), has been an increased number of sicker patients receiving transplant and an overall poorer survival of those patients transplanted with high LAS scores (primarily those requiring mechanical ventilation) [27]. An important aspect of the LAS is the potential for serially collected data from patients listed for lung transplant to be used for refinement of the underlying models that generate the priority score; a proposal for LAS updates was released for public comment in early 2012. Although sufficient data has not been available to create a LAS type system for children younger than 12 years of age, since 2010 lung allocation for this group has been based on two urgency tiers and broader geographic sharing (Table 116.2) [28].

The goal of preoperative management is to maintain a state of health that will maximize the patient's likelihood of a successful post-transplant outcome and to address deficiencies discovered during the evaluation. Therefore many of the interventions during this time period are the same no matter the underlying diagnosis.

Table 116.2. Priority status for children under 12 years of age awaiting lung transplantation in the US

Respiratory failure, defined as:
• requiring continuous mechanical ventilation; ***or***
• requiring supplemental oxygen delivered by any means to achieve FiO_2 greater than 50% in order to maintain oxygen saturation levels greater than 90%; **or,**
• having an arterial or capillary PCO_2 greater than 50 mmHg, or a venous PCO_2 greater than 56 mmHg.
Pulmonary hypertension, defined as:
• having pulmonary vein stenosis involving three or more vessels; ***or***
• exhibiting any of the following, in spite of medical therapy: suprasystemic PA pressure on cardiac catheterization or by echocardiogram estimate, cardiac index less than 2 L/min/M^2, syncope, or hemoptysis.

Strategies will include: maximizing the child's nutritional status; allowing the child to participate in physical and occupational therapy and therefore minimizing muscle relaxation; minimizing infectious exposures; providing appropriate vaccinations; and if there have been issues in regards to adherence, working with the family to establish routines that will benefit them later with the complex post-transplant regimen.

Other specifics of preoperative management will vary by disease state. In patients with CF, continuation of aggressive airway clearance, nutritional supplementation, antimicrobial therapy and physical rehabilitation is vital. In patients with pulmonary vascular disorders, depending on the specific etiology, use of pulmonary vasodilator therapy, diuretics, anticoagulation, supplemental oxygen, and/or atrial septostomy may prolong life. In children with interstitial lung disease steroids may be of some benefit.

It is worth noting that although mechanical ventilation is a risk factor for a poor outcome in adult lung transplant recipients that does not seem to be the case for infants and toddlers. Graft survival at one and three years in mechanically ventilated children under three-years-old was not different compared to nonventilated patients [29].

In patients who remain difficult to manage despite mechanical ventilation, the Interventional Lung Assist device (Novalung® iLA), a pumpless venovenous membrane ventilator, has been shown to be an efficient remover of carbon dioxide and may be a useful bridge to transplant. Additional detail regarding artificial lung devices can be found in Chapter 50 [30]. The device has been used successfully in a child as young as two years of age [31]. At our center we have also utilized the Maquet® Quadrox-iD membrane oxygenator in an attempt to provide adequate gas exchange while avoiding muscle relaxation. These devices carry similar risks for anticoagulant related complications to ECMO; therefore their role in long term support remains to be clearly defined.

Transplant procedure and surgical techniques

Potential donor lungs for children are evaluated in a similar fashion to adult patients. In contrast to adult lung transplantation (chapter 59), most procedures in children are performed while on cardiopulmonary bypass. The surgical approach still uses the bilateral anterolateral trans sternal ("clamshell") incision providing optimal access to both pleural spaces and visualization. Most procedures involve bilateral sequential lung transplantation with end-to-end rather than telescoping bronchial-to-bronchial anastomoses to reduce the likelihood of stenosis [32]. To provide optimal blood supply to the anastomosis and reduce the risk of extension of infection from the airway to adjacent vascular structures, the anastomosis is generally covered with pericardial or peribronchial lymphatic tissue from either the donor or recipient [32,33]. In patients with CF, vigorous washing of the recipient trachea and bronchial stumps with an antibiotic solution prior to implantation is used to reduce the likelihood of transmission of airway organisms to the donor allograft.

Heart-lung transplantation is a relatively rare event in pediatrics [4]. Although in the past, pulmonary hypertension with significant right ventricular dysfunction was considered an indication for heart-lung transplant, resolution of even marked right ventricular hypertrophy and dysfunction can be seen with successful bilateral lung transplantation [34,35]. When a congenital heart defect requiring repair is part of the clinical picture, intracardiac repair may take place at the time of, or after, the bilateral pneumonectomies and prior to implantation of the donor lungs, obviating the need for heart-lung transplantation [22]. Because of the high prevalence of septic lung disease in pediatrics, and concerns about lung growth, single lung transplantation is used rarely among children [4].

Living donor lung transplant has virtually disappeared in the US since the introduction of the LAS. Nonetheless, the procedure, where a right lower lobe from one healthy donor and a left lower lobe from another (generally family members) are implanted in the recipient [36] is still used in Japan where access to donor organs suitable for children has been restricted. Other surgical techniques used in children include donor downsizing using linear stapling devices or lobectomy and lobar transplant [37]. These are typically used to increase the scope of organs available for transplant in infants and small children.

Post-transplant management

The post-transplant management in pediatrics differs from the adult regimen in a few important ways that we will highlight. Specifics regarding adult management can be found in Chapter 80. Certain post-transplant management issues are identical to both pediatric and adult lung transplant recipients such as the approaches to primary graft dysfunction or rejection and these will only be briefly mentioned or not be explored in further detail here.

Immunosuppression

Pediatric transplant physicians must consider not only the efficacy of the immunosuppressive regimen, but also how immunosuppression affects normal growth or causes cosmetic side effects that may promote non-adherence to the medical regimen. Almost 80% of pediatric lung transplant recipients are on tacrolimus and 65% are on MMF at the end of the first year [4]. Because lung transplant recipients have a higher risk for rejection episodes than other solid organ transplant recipients, more intense immunosuppression regimens have been developed. For example, the initial targets for trough levels for tacrolimus are typically in a range of 10–20 ng/mL. Initial dosing for prednisone is typically 0.5–1.0 mg/kg/d with the goal of 0.25–0.5 mg/kg/d by three to four months after transplant. Few patients are weaned from prednisone by five years post-transplant [4]. Induction immunotherapy is used by the majority of pediatric lung transplant centers; recent registry data showed that over 60% of patients received either a polyclonal agent (anti-lymphocyte or anti-thymocyte globulin) or an IL-2 receptor antagonist (i.e. basiliximab) [4].

The use of steroids in children deserves special mention because of the potential for adverse impact on a child's linear growth. There

are data in other pediatric solid organ recipients that steroid avoidance strategies can lead to successful immunologic outcomes but no systematic study in pediatric lung transplant recipients [38]. Further study is warranted in this area.

Antimicrobial regimen

Most patients receive prophylactic intravenous antibiotics before and after lung transplantation to cover common airway pathogens as well as the organisms known to colonize their airways. The antibiotic regimen may be modified based on donor cultures. Patients without CF generally receive a single antibiotic with broad Gram positive and Gram negative coverage. In recipients with CF, their pretransplant sputum flora cultures help guide therapy; typically, such antimicrobial therapy is chosen to cover Gram negative organisms such as *Pseudomonas aeruginosa* and occasionally *Achromobacter* spp. Vancomycin is often included to cover Methicillin resistant *Staphylococcus aureus* (MRSA), which has recently become more prevalent in children with CF and advanced lung disease. Most centers now use voriconazole or anidulafungin prophylaxis for patients in whom *Aspergillus fumigatus* has been isolated prior to transplant. In some circumstances, aerosolized amphotericin, oral itraconazole or oral voriconazole has also been used [39]. Prophylaxis against *Pneumocystis jiroveci* is begun shortly after transplant with three times weekly trimethoprim/sulfamethaxozole (TMP/SMX), (alternatively nebulized pentamidine, oral atovaquone or dapsone). Oral nystatin is given as prophylaxis for candidal disease.

Although ganciclovir prophylaxis has significantly reduced the incidence of CMV pneumonitis in lung transplant recipients, CMV remains a serious potential risk for mortality [40,41]. The approach to CMV prophylaxis in the pediatric lung transplant recipient varies considerably across transplant centers. In general when both recipient and donor are CMV seronegative, no prophylaxis is given [42]. When the donor or recipient is seropositive for CMV, 4–12 weeks of ganciclovir or valganciclovir is administered. Recently some centers have extended the duration of prophylaxis (with IV ganciclovir or oral valganciclovir) to six months or longer, based on studies suggesting benefit [43,44]. Although based on adult experience some pediatric centers use CMV hyperimmune globulin (CMVIg) in addition to ganciclovir [45], the benefit of CMVIg remains unclear [46].

Monitoring of the allograft

Measurement of lung function in children is more challenging, particularly in children less than six years of age because spirometry is generally not available/reliable in that age group. For older children, as in adults, spirometry is essential for the clinical diagnosis of bronchiolitis obliterans syndrome (BOS). For infants, using thoraco-abdominal compression techniques, pulmonary function testing can identify the presence of airflow obstruction, but requires specialized equipment and experience and cannot be done once the child's length is greater than 90 cms [47,48]. In addition, because they require sedation, infant pulmonary function testing cannot be performed as frequently as conventional spirometry. Moreover, criteria for BOS using infant pulmonary function testing are not included in the most recent BOS definition [49]. Some pediatric transplant centers therefore routinely perform high resolution CT scanning of the chest along with ventilation/perfusion scans to aid in the early identification of air trapping indicative of BO.

Impulse oscillometry (IOS) is a validated technique to assess the presence of airflow obstruction in children as young as two years old [50,51]. The technique requires that the subject breathe normally into a mouthpiece (or a special face mask for young children unable to close their lips around the mouthpiece) for a short period of time. It does not require active co-operation and probably deserves more intensive study in the post-transplant pre-school aged child.

Obtaining surveillance biopsies in infants and toddlers is also more challenging. Although smaller transbronchial biopsy forceps that will fit in the suction channel of pediatric bronchoscopes have been available for some time, they typically produce much smaller pieces of tissue. Therefore, obtaining sufficient tissue for the reliable diagnosis of rejection in infants is often quite challenging [52].

Outcomes and risk factors

Survival

Survival after pediatric lung transplantation for patients transplanted between January 1990 and June 2009 is comparable to adult survival (Figure 116.2). See Chapter 106 for additional discussion of lung transplant outcomes. Overall, pediatric recipients have a median survival of four and a half years, which is not statistically

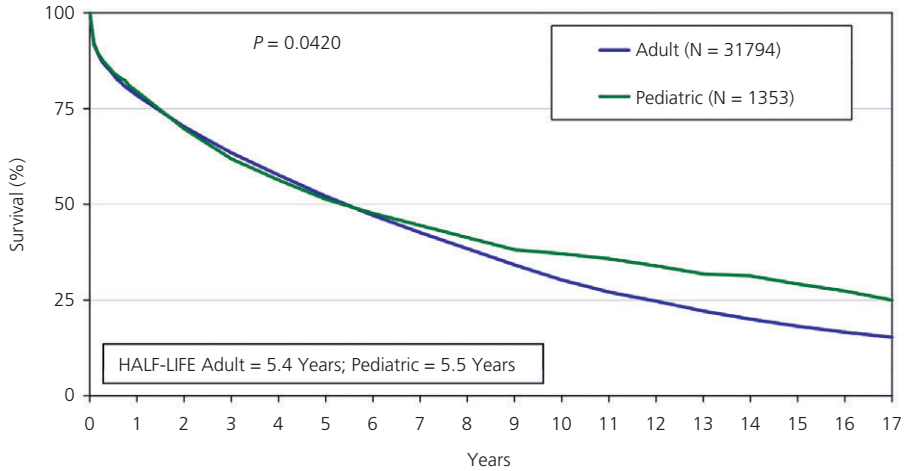

Figure 116.2. Comparison of adult and pediatric post-lung transplant survival. Reprinted from [4] Benden C, Aurora P, Edwards LB, Kucheryavaya AY, Christie JD, Dobbels F, et al. The Registry of the International Society for Heart and Lung Transplantation: Fourteenth Pediatric Lung and Heart-Lung Transplantation Report (2011). J Heart Lung Transplant. 2011 Oct;30(10):1123–1132. Copyright © 2011, with permission from Elsevier.

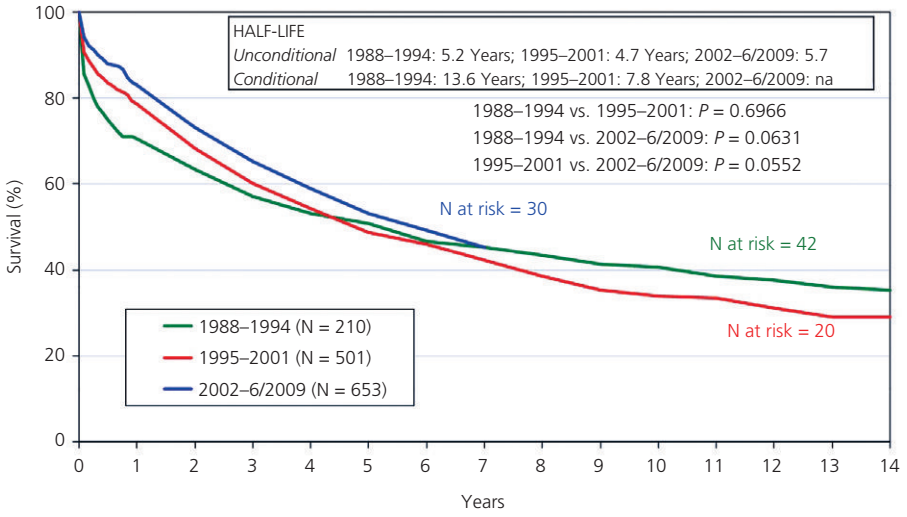

Figure 116.3. Comparison of pediatric lung transplant survival between different eras. Reprinted from [4] Benden C, Aurora P, Edwards LB, Kucheryavaya AY, Christie JD, Dobbels F, et al. The Registry of the International Society for Heart and Lung Transplantation: Fourteenth Pediatric Lung and Heart-Lung Transplantation Report (2011). J Heart Lung Transplant. 2011 Oct;30(10):1123–1132. Copyright © 2011, with permission from Elsevier.

different from the adult experience. One-year survival rates for pediatric recipients have improved significantly, from 67% in the era between 1988 and 1994 to 83% in the recent era (2002–2009). (Figure 116.3) There is, however, no significant improvement in five-year survival when comparing eras, suggesting that improvements in early mortality are primarily responsible for the era effect. Kaplan-Meier survival analysis also demonstrated significantly better survival in children transplanted between the ages of 1–11 years compared to those between 12–17 years (Figures 116.4a and 116.4b). In the most recent ISHLT report, the two most common causes of death within the first year post-transplant were infection and graft failure. By one to three years after transplant, bronchiolitis obliterans becomes the leading cause of death, accounting for nearly 40% of the mortality. Infection and graft failure remain an important cause of death throughout the follow-up period.

It is important to note that although outcomes remain poorer for retransplantation (overall 37% five year survival) than for primary transplantation, retransplantation has become a more frequent occurrence in the recent era [4,26], perhaps due in part to increased access provided by the LAS for children 12 years and older as well as reports of improved survival for selected recipients [53]. Appropriate selection of pediatric candidates for lung retransplantation will remain an important priority.

Complications

Airway complications

Dehiscence at either the vascular or bronchial anastomoses is a rare but emergent complication requiring reoperation. Most transplant centers assess the integrity of the airway anastomoses via flexible bronchoscopy during the first 24–48 hours after transplantation. Cultures from the lower airways are also obtained. The development of techniques to cover the anastomosis with vascularized tissue has significantly reduced the frequency of this complication [3,33,54]. Perhaps surprisingly, airway complications including fibrotic strictures, excessive granulation tissue or airway collapse at the site of the anastomosis occur at a frequency comparable to that seen in adult lung transplant recipients [55,56]. Stenosis of the airway lumen, when clinically significant, is treated via balloon dilatation by bronchoscopy. Most centers avoid placement of metallic expandable stents because of their limited ability to expand to the degree necessary to accommodate for maximal growth of the child's airway, requiring eventual removal. Unfortunately removal of these stents is fraught with potentially fatal complications [57]. In addition, the formation of granulation tissue may cause severe airway obstruction. At our institution, silicone rubber stents were used in 12 bronchial anastomoses and four were responsible for complications including dislodgement or obstruction [56]. Newer biodegradable stents have the potential to relieve the stenosis, but still allow for subsequent growth of the airway [58] and are deserving of further study.

Vascular complications

As the majority of pediatric lung transplant procedures are performed on cardiopulmonary bypass, bleeding, particularly in the pleural space or at the site of the vascular anastomoses, is a not infrequent problem, particularly in patients who have had prior thoracotomy [10]. Vascular anastomotic complications are often associated with hypotension and radiographic abnormalities. Thrombus formation in the pulmonary veins or left atrial anastomotic suture line may occur in the early postoperative period [59]. Perfusion scans and echocardiography should be used to assess the anastomoses.

Immunologic complications

The risk of developing acute cellular rejection and BOS appears to be lower in the infant population [29]. Otherwise the diagnostic and treatment approaches for immunologic complications are the same as in adult transplant recipients. Antibody mediated rejection is a more recently recognized immunologic complication of lung transplantation and seems to effect children and adults in a similar fashion. Although there is debate regarding the specific criteria for treatment, identification of circulating donor specific antibodies (identified using solid phase flow cytometry techniques), alveolar capillary complement (C4d) deposition, and capillaritis in the setting of allograft dysfunction is usually considered sufficient evidence [60]. Treatment of humoral rejection is also quite variable among pediatric centers. Most centers use some combination of steroids, plasmapheresis, intravenous immunoglobulin and B cell

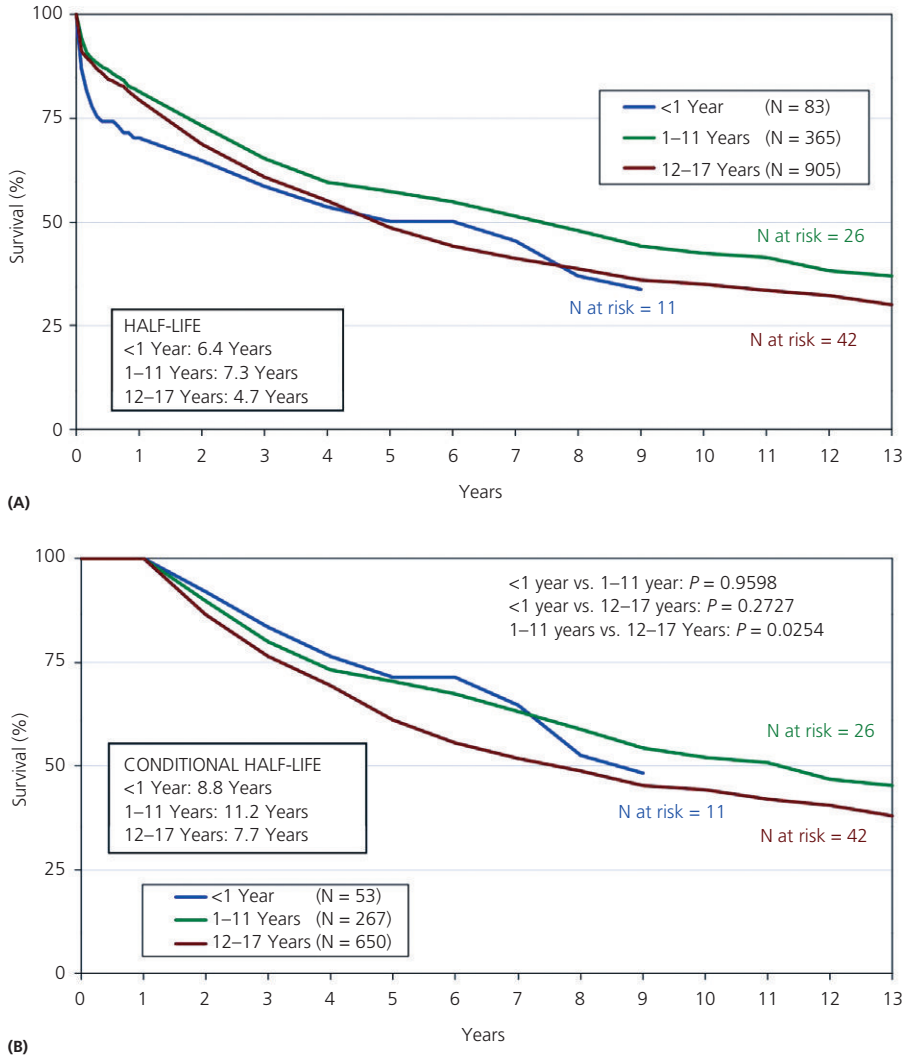

Figure 116.4. (A) Comparison of lung transplant survival between different age groups. (B) One year conditional survival between different age groups. Reprinted from [4] Benden C, Aurora P, Edwards LB, Kucheryavaya AY, Christie JD, Dobbels F, et al. The Registry of the International Society for Heart and Lung Transplantation: Fourteenth Pediatric Lung and Heart-Lung Transplantation Report (2011). J Heart Lung Transplant. 2011 Oct;30(10):1123–1132. Copyright © 2011, with permission from Elsevier.

reduction (cyclophosphamide or rituximab) [61]. Newer agents such as bortezomib, a proteosome inhibitor targeted at plasma cells or complement inhibitors such as eculizumab are increasingly being used [62,63].

An important complication of immunosuppression is the development of malignancy. The incidence of malignancy after lung transplantation in children is 5.9% and 13.1% at one and five years post-transplant, respectively; post-transplant lymphoproliferative disease (PTLD) is by far the most common malignancy [4]. In immunosuppressed pediatric patients, PTLD is typically an EBV driven, B cell lymphoproliferation with highly variable histology [64,65]. PTLD is more common in lung transplant recipients (than other solid organ recipients), in patients with CF, and in children as compared to adults [66]. These findings are probably explained by the intensity of immunosuppression in lung transplant recipients and the fact that many pediatric patients are EBV seronegative at the time of transplantation [65]. PTLD often presents with an unanticipated finding of a single or multiple round or ovoid pulmonary nodules on chest imaging without other symptoms [67]. The incidence of extrapulmonary PTLD increases after the first post-transplant year. Sites include the GI tract, the skin, and other lymphatic tissue including lymph nodes and the nasopharynx [68,69]. Most centers monitor quantitative measurements of EBV by PCR, a sensitive and somewhat specific marker for PTLD [70]. Positron emission tomography (PET) can be a sensitive and specific test [71]. Histologic diagnosis of PTLD is critical for prognostic purposes as CD20 positive tumors may be more amenable to antibody therapy and monomorphic PTLD has a worse prognosis [72].

Although some adult centers respond to the presence of elevated EBV PCR by reducing immunosuppression [73], a recent study suggests caution with this approach in children [74]. Although it can be successful in some patients, reduced immunosuppression carries a significant risk for the development of acute rejection. In many cases additional therapy is needed to induce remission. Many centers now follow the Children's Oncology Group protocol ANHL 0221 (ritixumab, an anti-CD20 monoclonal antibody shown to be effective in non-Hodgkin's B cell lymphoma, low dose cyclophosphamide and prednisone), which has shown promise as an approach to therapy for PTLD in pediatric solid organ transplant recipients [75].

Community acquired respiratory viruses

Children tend to have greater exposures to respiratory viral infections because of daycare, school, or the presence of siblings. These infections can be particularly problematic post-transplant. Adenovirus [76] and paramyxoviruses including parainfluenza and respiratory syncytial virus (RSV) can cause significant lung injury or mortality [77,78]. Many centers treat these viruses aggressively with cidofovir and ribavirin respectively [78–80]. Respiratory viral infections are also associated with the later development of BOS [81].

Transplant benefit, functional outcome and quality of life

Although QOL and survival in adult lung transplant recipients was better than candidates who remained on the waiting list [82–84], extrapolation of these findings to children and adolescents must be approached cautiously. Although registry report data indicate that more than 80% of lung transplant survivors report no activity limitations at one, three and five years post-transplant [4], there are limited well-controlled studies of QOL in pediatric lung transplantation. One small study showed a 24% improvement in quality of well-being in thoracic transplant recipients [85]. In another study, a group of 47 thoracic organ recipients (six lung transplant recipients) reported lower health status compared to a normal population but had similar health status as children with asthma, juvenile rheumatoid arthritis and intractable epilepsy [86]. It is important to note that assessments of QOL may be affected by the child's baseline capabilities prior to transplant and their expectations after transplant [85]. Moreover, consideration of the child's development level must also be included in QOL studies. It will be important to include systematic assessment and developmental impact of transplant in future longitudinal studies of pediatric QOL lung transplant recipients.

Lung growth

Successful lung transplantation in infants and young children raised concerns whether the lung allograft would grow with the child [87]. Allogeneic lung transplantation in animal models is associated with an increase in lung volumes and airway size with age [88]. In humans, spirometry and lung volume measurements have been reported to be normal in infants [89] and older children [24]. However, these measurements may reflect increased alveolar volume rather than increase in alveolar number and thus surface area for gas exchange. Ro and co-workers reported serial CT scan data supporting growth of intrathoracic airways over time [90]. However, a single center study of the diffusing capacity of carbon monoxide (DLCO) among pediatric recipients of cadaveric and living donor transplants suggested that no growth occurred [91]. Although DLCO provides a better estimate of gas exchange surface area, it is not easily measurable in infants. Further study is needed to clarify the extent of lung growth in infant and toddler lung transplant recipients.

Child development

Predictions of neurocognitive outcome are difficult to make. Infants possess some degree of neurocognitive plasticity that serves them well in the face of altered environmental interactions, circulatory compromise, infection, and abnormal nutrition and growth states. Infants who receive solid organ transplants are at risk for the development of learning disabilities, visual and spatial deficits, and motor delays [92]. However, transplantation in children in chronic renal failure actually allowed for significant improvement in development to occur. Measurements of head circumference, motor skills, and cognitive functioning all showed improvement post-transplant, with most children normal or near normal [92]. It is vital that transplant programs caring for infants have in place a protocol that assesses a child's neurodevelopment so that interventions may be implemented early. This is an area deserving of further study to better define the pertinent risk factors associated with developmental delay.

Adolescence

Adolescent transplant recipients deserve special mention. Adolescence is an emotionally fragile stage of life in which individuals struggle with self-identity, cognitive development, and emerging autonomy. Teenage transplant recipients have the added potential for risk-taking behavior which may manifest itself as non-adherence with medical therapy. At a time when they feel well, the importance of the immunosuppression regimen may be underestimated and may result in a poorer clinical outcome in terms of an increased incidence of late acute rejection, graft failure, and mortality [93,94]. There is little doubt that the adolescent transplant population requires an even more intense effort than other populations to establish a strong relationship with the transplant team in order to provide regular education, encouragement, and open communication. Because adolescents require such an intensive effort from the team, the authors recommend that programs considering offering lung transplants to adolescents include on their team dedicated pediatric practitioners with experience dealing with the unique and special needs of this population.

Eventually, however, the goal for all pediatric practitioners is to have our patients "outgrow" us. Unfortunately, rather than being viewed as a victory, oftentimes, the transition period is greeted with great apprehension by all involved. For the young person preparing to transition their care to an adult transplant provider, it is a time when they are expected to be fully responsible for managing their own healthcare yet they are moving to an adult healthcare and insurance system they have no experience navigating. For the parents, it is a time where they must relinquish control of managing every detail to their children. It is therefore no surprise that medical outcomes during this period can be poor [95]. Transition warrants a concerted effort to improve communication between the pediatric and adult centers and specifically between the providers [96,97]. How to best approach the process of transition needs to be an area of active study as this is a challenge that will only increase in scope as we become more successful in managing pediatric lung transplant recipients [98,99].

Summary

Pediatric lung transplantation presents unique challenges and numerous opportunities. It is clear that lung transplantation can be successfully performed in infants and children with end-stage pulmonary parenchymal and vascular disease providing some with prolonged survival and improved QOL. However, long-term outcomes for many fall far short of our expectations. Critical obstacles like donor graft shortage and effective approaches to bronchiolitis obliterans are shared with the adult lung transplant world. However, some issues encountered in the field of pediatrics distinguish themselves and warrant approaches specifically designed as such.

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CHAPTER 117

Pediatric Intestinal and Multivisceral Organ Transplantation

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Introduction

The origins of intestinal transplantation are closely associated with intestinal and liver diseases in children. It is these diseases that drove the early transplantation attempts. Several important milestones are worth noting. First, the foundations for liver inclusive intestinal type transplants were laid in the first successful pediatric liver transplant cases performed in 1967 by Thomas Starzl [1]. Concomitantly, there were several attempted pediatric intestinal type transplants without success [2]. With the clinical introduction of parenteral nutrition in the late 1960s [3], no other clinical intestinal cases were performed for nearly 20 years. Then in the mid-1980s, spurred by several reports of successful adult intestinal transplants [4–6], pediatric intestinal transplantation was re-attempted [7]. In fact, the longest survivor received her intestinal transplant in 1988 in Paris France [8]. Soon thereafter, case series were published [9] thus ushering in the modern era of pediatric intestinal transplantation.

Pediatric intestinal transplantation has changed dramatically over the ensuing two decades. Herein we will review some of the major advancements, surgical techniques, outcomes and complications of the procedure. General features of intestinal and multivisceral transplantation, and adult specific aspects are covered in Chapters 33, 42, 62, 86, and 109.

Indications and patient selection

The major indication for intestinal transplantation is irreversible intestinal failure: a clinical condition in which the fluid/electrolyte, caloric, macro- and micronutrient needs of a patient cannot be maintained by the gastrointestinal tract. Less common secondary indications are usually technical issues such as unresectable tumors involving the root of the mesentery or pan porto-mesenteric-splenic vein thrombosis not amendable to standard surgical therapies. Additional treatment of this topic is found in Chapter 33.

Intestinal failures are broadly grouped into three categories, which may have overlapping syndromes/diagnoses: surgical, functional, or mucosal (see Table 117.1).

Children who develop intestinal failure are first stabilized with parenteral nutrition management. In general, the initial goal is enteral adaptation thus avoiding the need for transplantation. The specific management of these patients at this stage of intestinal failure/rehabilitation is beyond the scope of this chapter and the reader is referred elsewhere [10,11]. Several prognostic factors are assessed in an effort to establish the likelihood of adaptation in contrast to the irreversibility of intestinal failure. Many of these factors (Table 117.2) have been shown in large studies to predict adaptation, although none are absolute [12].

Patients with a poor prognosis and/or those not demonstrating early evidence of enteral adaptation should be referred to a transplant center for assessment. Other medical and surgical means to assist with adaptation can be considered. However, the indication to proceed with transplantation is the presence of irreversible intestinal failure and one or more life-threatening parenteral nutrition related complications. These complications commonly include:

1 Loss of central venous access.
2 History of multiple central venous catheters.
3 History of multiple blood stream infections.
4 Presence of intestinal failure associated liver disease.
5 Chronic pain syndromes associated with intestinal failure.
6 Difficult fluid/electrolyte/nutrition management.

There is a subset of patients with an extremely poor prognosis for adaptation that consideration for transplantation should be given prior to the onset of the common parenteral nutrition related complications. This standard was set forth in a position paper published by the American Society of Transplantation in 2001 [13] and continues to be used today.

Wait-list issues

A major obstacle to successful pediatric intestinal transplantation has been wait list mortality. This was first exposed in a study of the UNOS database from 1993–2001 [14] where wait-list mortality rates for children listed for liver intestine or multivisceral grafts far exceeded that for child listed for isolated liver grafts. In retrospect, these findings were not surprising as liver-intestine and multivisceral candidates are basically waiting for size-matched pediatric donors while candidates waiting for liver only grafts have several options including size-matched pediatric donors, adult cadaveric split grafts, or living donor grafts. In addition, it was retrospectively discovered that the end-stage liver disease and pediatric end-stage liver disease (MELD/PELD) scoring system did not accurately predict three month survival in patients with intestinal failure

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Table 117.1. Common pediatric causes in each category are:

Surgical	Functional	Mucosal
gastroschisis	pseudo-obstruction	microvillous inclusion disease
jejunoileal atresia	Hirschsprung's disease	tufting enteropathy
midgut volvulus	–	congenital neuroendocrinopathy
necrotizing enterocolitis	–	–

Table 117.2. Assessed prognostic factors to establish the likelihood of adaptation in contrast to the irreversibility of intestinal failure

Factor	Positive predictor	Negative predictor
Age	younger	older
Length of remnant bowel	long	short
Ileocecal valve	present	absent
Colon	present	absent
Enterostoma	absent	present
Time since onset	short	long
Underlying intestinal disease	absent	present
Small bowel segment	ileum	jejunum

associated liver disease. Therefore, significant changes in the US intestinal allocation system including the addition of 23 PELD/MELD points to children awaiting liver-intestine or mutivisceral grafts as well as the allocation of pediatric organs to pediatric recipients first over a wider geographical region. These changes have resulted in a dramatic decrease in wait-list mortality for these candidates [15].

Graft options and surgical techniques

The type of intestinal transplant offered to children is tailored to the underlying disease. In general, four types of intestinal transplants are considered – isolated intestine grafts, combined liver-intestine grafts, multivisceral grafts, and modified multivisceral grafts. Technical details of these operations are covered in Chapters 62 and 63.

Bear in mind, significant controversy exists over the exact nomenclature used [16]. The reader is referred to other related chapters in this text for further information. For the purposes of this chapter, the following definitions will be used:

1 Isolated intestinal transplant — transplantation of part or all of the donor jejunoileum into the recipient. This is reserved for patients with irreversible intestinal failure, early or no liver disease, and the absence of foregut (stomach, duodenum, pancreatic) disease. Arterial inflow is commonly established from either the infra-renal aorta or the superior mesenteric artery. Venous outflow is common performed into the mesenteric veins or the inferior vena cava.
2 Liver-Intestine transplant — transplantation of part or all of the donor liver, pancreas, and jejunoileum. Note, the donor pancreas is now commonly included in this organ complex as a technical modification to simplify both the donor and recipient procedure [17]. The recipient liver and remnant jejunoileum is removed. However, the recipient pancreaticoduodenal complex remains in situ. This transplant is reserved for patients with irreversible liver disease and irreversible intestinal failure in the absence of foregut disease. Arterial inflow is generally established with vascular conduits off the aorta. Venous outflow is via the transplanted liver into the suprahepatic inferior vena cava.
3 Multivisceral transplant — transplantation of part or all of the donor liver, pancreas, and jejunoileum with or without inclusion of the donor stomach. This transplant is reserved for patients with irreversible liver disease, intestinal failure, and foregut disease. The major difference between this type of transplant and the liver intestine type is that the recipient foregut is removed along with the liver and remnant jejunoileum. Otherwise vascular inflow and outflow are similar.
4 Modified multivisceral — transplantation of all or part of the pancreas and jejunoileum with or without the stomach. Importantly, the liver is not included in this graft. This option is reserved for patients with irreversible foregut disease and intestinal failure. As before, the recipient foregut is removed. This graft is arterialized from the aorta using vascular conduits with venous outflow via the portal vein.

Most of these graft types can be modified, again based on patient specific needs, to include the stomach, colon, and/or kidney(s).

Immunosuppression and rejection

One of the major advances in intestinal transplantation has been in immunosuppression. Conceptually, the intestine is the only organ transplanted that relies on itself to absorb critical medication to prevent its own immune mediated destruction. Therefore, the development of highly absorbable and potent immune suppressants and/or intravenous formulations of immunosuppressants have been crucial to the advancement of the field. Without question, the first breakthrough came with the discovery [18] and clinical introduction [19] of tacrolimus (Prograf®; Astellas Pharma; Deerfield, IL). Prior to the clinical use of this agent, successful intestinal transplantation was rare; afterward, it became commonplace as noted above.

The next major breakthrough in this field was the use of induction immunotherapy. Prior to its routine use, the short-term survival after intestinal transplantation was commonly reported at 25–50% [20]. In 1999–2000, most centers began using induction therapies with either an interleukin-2 receptor antagonist (daclizumab; Zenapax®; Roche Pharma, Nutley, NJ) or basiliximab; Simulect®; Novartis Pharma, East Hannover, NJ) or an antithymocyte product (rabbit antithymocyte gobulin/rATG; Thymoglobulin®; Genzyme Corp, Cambridge, MA). More recently, alemtuzumab (Millenium and Ilex Partners, LP, Cambridge, MA) has also been used for induction therapy with improved results. All agents led to a marked increase in short-term survival rates as high as 75–90% [21–24].

Outcomes

Survival

General outcomes following intestinal transplantation are discussed in detail in Chapter 109. To review outcomes after intestinal transplantation, there are several different data sources. The standard multicenter data set remains the Intestinal Transplant Registry [25]. This data set is believed to have captured nearly all intestinal type transplants performed worldwide since 1985. The other available registry data set is the United Network for Organ Sharing/Organ Procurement and Transplant Network (UNOS/OPTN) data which is reported through the Scientific Registry of Transplant Recipients (SRTR) [26]. As with all registry type data, there are limitations as to the number of data points collected per patient, the completeness of the data points entered, and the variability in

interpretation of entered data from the different centers. The other major source of outcome data is single center reports. Few reports are dedicated to children alone. The experience reviewed herein is contained within the Table 117.3 [20,21,22,24–31].

The UNOS/OPTN data set indicates that between 1988–2011, 2138 intestinal transplants had been performed in the US with 56% occurring in children [26]. At three and five years post-transplant, the patient survival was 51–79% and 33–76%, respectively while the corresponding graft survival was 44–64% and 30–69%, respectively. Examining the 2009 ITR report on pediatric intestinal transplantation indicates that, worldwide, 1151 patients received 1236 transplants [32]. Five-year survival improved with each era analyzed whereby after 2000, a 70% patient survival was reported. The most common causes of patient death were sepsis (46%) and multiorgan failure (35%).

The single center reports are shown in Table 117.3. In general, similar trends are seen. That is, patient and graft survival improve in the more recent time intervals analyzed with three year patient and graft survival between 56–72% and 53–92%. The most common causes of death and graft loss are infection, rejection, and post-transplant lymphoproliferative disorders (PTLD).

Acute rejection

Acute rejection is the most formidable obstacle to successful intestinal transplantation. Historically, rejection has occurred almost universally after intestinal transplantation and has been the leading cause of graft loss. Single center reports remain the optimal source for assessing rejection rates. Unfortunately, there is no unified system for reporting with rates reported by transplant era, immunosuppression group, total rejections per graft, and patients with and without rejection. From these reports, the lowest rejection rates are with alemtuzumab induction combined with sirolimus (Rapamune®, Wyeth Pharma, Philadephia, PA) maintenance therapies (36% and 17%, respectively).

One of the most detailed reports on this subject is from the University of Miami [33]. Two hundred and nine patients including 128 children underwent intestinal transplantation during the study interval. Thirty-two percent did not have a rejection episode while the rest had 290 episodes for an average of two episodes per patient. The median time to first rejection was 18 days. Statistical analysis showed that transplant era before 2001, lack of alemtuzumab induction, the lack of donor spleen inclusion, and a longer cold ischemia time all predicted rejection with the transplant era being the strongest predictor.

These data indicate that while rejection is still a major obstacle to successful intestinal transplantation, the rates and severity have improved over time. Rigorous endoscopic monitoring protocols have been implemented at most centers which helps detect rejection at early stages. Induction immunotherapy of any kind appears to help reduce the incidence of rejection. Noninvasive tests that predict rejection need to be developed to aid with this problem.

Cytomegaloviral disease

Tissue invasive cytomegaloviral disease (CMV) disease is a problem after intestinal transplantation. This should not be a surprise as this virus is trophic to the gastrointestinal tract and thrives in immunoincompetent patients. Reports from earlier in 2000, indicates rates as high as 26% [34]. However, more recent reports indicate reduction in this infection to 11–24% see Table 117.3. A recent review at the University of California, Los Angeles, indicates an overall rate of CMV disease down to 5% [35]. Progress has been made due to the institution of prophylactic and preemptive therapeutic protocols targeted against this virus. The UCLA protocol includes weekly monitoring of blood for viral DNA as well as prophylactic treatment with intravenous ganciclovir [36].

Post-transplant lymphoproliferative disorders

Similar to the CMV virus, the Epstein Barr Virus (EBV) with associated infection and malignant viral transformation is another major problem after intestinal transplantation. Again, this virus thrives in immuno-incompetent patients. The actual incidence of EBV infection is not clear in the literature. In contrast, the incidence of PTLD is clearly reported. However, not all PTLD are EBV-associated/driven and therefore there is a lack of uniformity in the literature. From single center reports, the historical incidence of PTLD (EBV related or not) is between 23–44% (see Table 117.3). In more recent studies, these rates have decreased to 11% from 24%. At UCLA, the incidence of PTLD is 8% [35]. The improvement in the rate of PTLD would appear to be largely related to the same pre-emptive and prophylactic strategies used against CMV, although causality is hard to determine. Detailed discussions of PTLD are found in Chapter 96.

Renal failure

The extent of the problem with kidney function after intestinal transplantation was first elucidated in a report from the OPTN/SRTR in which the incidence of chronic renal failure in 228 recipients was 21 $\pm$ 3.4% at five years [37]. This finding has spurred several important single center studies on the subject. In a study of 44 children after intestinal transplantation, Ueno and colleagues found that while the pretransplant estimated glomerular filtration rate (eGFR) was 138 $\pm$ 42 mL/min/m2, by 24 months post-transplant, the corresponding eGFR had dropped to 81% of baseline [38]. This decrease was correlated with high trough levels of tacrolimus in the first 12 postoperative months. At UCLA, an analysis of 45 children and 23 adults after intestinal transplantation was undertaken to assess postoperative renal function [39]. Multiple time points were assessed and renal function was reported as eGFR/normal GFR as reported in standardized tables. Prior to transplant, the eGFR was 83–91% of normal. After transplant, the eGFR ranged from 64 to 95% of normal. Several factors were found to predict an eGFR of $<$75% normal including a low pre-transplant eGFR, ICU status prior to transplant, long time period on TPN prior to transplant, high dose tacrolimus, and age $>$18 years. From these studies, we can conclude that renal dysfunction/failure is a major problem after intestinal transplantation and that high tacrolimus levels are an important contributor to this problem.

Nutrition

The ultimate goal of intestinal transplantation is enteral nutritional autonomy. Surprisingly, there are few studies on this subject. To address this issue, Venick and colleagues performed a detailed assessment of nutritional outcomes in 33 children after intestinal transplantation [40]. Enteral nutrition was initiated a median of eight days after transplantation and parenteral nutrition was discontinued a median of 31 days after transplant. Improvement was seen in the median Z scores for both height and weight at 48 months post-transplant when compared to pretransplant (Weight: -1 to 0; Height: -3 to -2.4). Similar improvements were noted in the serum albumin and prealbumin (albumin: 2.7 to 3.7 g/dL;

Table 117.3. Summary of experience with intestinal transplantation from large registry and single-center publications

Source/ reference	Year	N	Patient survival	Graft survival	Cause of death	Cause of graft loss	Rejection	Post-transplant lymphoproliferative disorder	Cytomegalovirus
ITR [25]	2011	1351C	72 MO: 50% 6+ 47% 2000+ 42% 1995+ 33% 1990+ 19% 1985+	72 MO: 40% 2006+ 45% 2000+ 35% 1995+ 20% 1990+ 0% 1985+	NR	NR	NR	NR	NR
ITR [20]	2009	1151C	60 MO: NR% 2005+ 70% 2000+ 68% 1995+ 58% 1990+ 65% 1985+	NR	SEPSIS 46% MOF 35%	NR	NR	NR	NR
UNOS/OPTN [26]	2011	1198C	72 MO: 51%–79% 60 MO: 33%–76%	72 MO: 44%-64% 60 MO: 30%-69%	NR	NR	NR	NR	NR
PITTSBURGH [24]	2011	213C	72 MO: 73%; 60 MO: 68%	72 MO: 66%; 60 MO 55%			82% none; 79% CPP/IL2RA; 68% RATG; 36% CAMPATH	23% CPP/IL2RA; 11% RATG	26% CPP/IL2RA; 13% RATG+ CAMPATH
PITTSBURGH [21]	2009	500T 184C	60 MO: 70%C 61%T	60MO: 50%T	NR	rejection 20%T; infection 11%T	90D rate: 50%–75%T	41/184 (22%)	16%T
PITTSBURGH [27]	2002	84C	72 MO: 59% 60 MO: 56%	72 MO: 53% 60 MO: 47%	13/39 (33%) infection 7/39 (18%) rejection	NR	345E/89G (377%) 73G/89G (82%)	15/34 (44%) ERA1; 12/51 (24%) ERA 2	22%
MIAMI [29]	2006	123C 141TX	72 MO: 32% 1994+ 38% 1998+ 60% 2001+ (IL2RA) 44% 2001+ (CAMPATH)	NR	20/67 (30%) rejection 22/67 (33%) infection	NR	191/141 (135%)	14/123 (11%)	NR
MIAMI [28]	2005	108C	60MO: 41%	NR	19/55 (35%) rejection 19/55 (35%) infection	NR	21/108 (19%) severe	NR	NR
UCLA [22]	2010	88T 63C	72 MO: 70% 60 MO: 65%	72 MO: 68% 60 MO: 64%	infection 58% neuro 16%	rejection 33% infection 10%	NR	NR	NR
PARIS [30]	2005	52C	72 MO: 72%	72 MO: 53%	technical 3/15 (20%); sepsis10/15 (67%); PTLD 2/15 (13%)	27/52 (52%); patient death;	25/52 (48%)T	12/52 (23%)	11/52 (21%)
MT SINAI MC [31]	2002	31T 18C	NR	12 MO: 58% vs. 92% RAPA	sepsis 4/6 (66%); PTLD 2/6 (33%)	NR	30D: 72% vs. 17% RAPA	NR	NR

Abbreviations: C, children; CMV, cytomegalovirus; COD, cause of death; COGL, cause of graft loss; CPP, cyclophosphamide; E, episodes; G, grafts; IL2RA, interleukin-2 receptor antagonist; ITR, intestinal transplant registry; MO, month; n, number; NR, not reported; PTLD, post-transplant lymphoproliferative disorder; RAPA, rapamycin; rATG, rabbit antithymocyte globulin; T, total patients; TX, transplants; UCLA, University of California, Los Angeles; MC,medical center; UNOS/OPTN, United Network for Organ Sharing/Organ Procurement and Transplant Network.

prealbumin: 15 to 25.1 mg/dL). However, micronutrient deficiencies were commonly seen especially with iron and zinc. Nevertheless, this study provided specific evidence over a prolonged postoperative time period that intestinal allografts can support nutritional autonomy.

Summary

Outcomes after intestinal transplantation have markedly improved over the past decade. The reasons behind this progress are no doubt multifactorial. However, advances in surgical technique, center specific experience, induction immunotherapy, and anti-infectious drugs have played a role. Successful intestinal transplantation appears to be associated with nutritional autonomy. However, major obstacles still remain. Rejection, viral infections, and deterioration in renal function are particularly relevant problems that need to be addressed. Despite these issues, patients undergoing intestinal transplantation now have outcomes nearly equal to those seen after other solid organ transplants.

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CHAPTER 118

Growth and Development after Organ Transplantation

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Introduction

The realization of a patient's genetic growth potential is one of the key objectives of organ transplantation in children and adolescents. In principle, the restoration of full organ function should permit catch-up growth and a normal onset and progress of puberty. However, the recovery of growth and development following organ transplantation is usually incomplete. To date, final adult height is below target in a major proportion of patients who received an allograft during childhood. This chapter will detail the factors affecting post-transplant growth and the current strategies to improve growth and development following solid organ transplantation. Chapters 119 and 121 will provide additional insights into the related topics of cognitive development, and developmental issues influencing the transition from pediatric to adult care.

Growth retardation and delayed pubertal development in chronic organ failure

Chronic organ failure in children leads to alterations of growth and maturation. Consequently, retarded growth and delayed puberty are common in pediatric solid-organ recipients at the time of grafting. Skeletal maturation and the onset of puberty is commonly delayed commensurate with the retardation of growth.

Registry and large single center surveys have provided information about the average growth deficit at time of transplantation in solid organ recipients. The natural history of growth in children end-stage renal disease (ESRD) is characterized by a gradual deviation from the normal growth channel by 0.2–0.5 standard deviation score (SDS) per year in infancy and mid-childhood, a marked deceleration of growth in the late prepubertal period and a pubertal growth spurt which is delayed by 1.5 to 2 years and is of subnormal size and duration, resulting in a 50% reduction of cumulative pubertal height gain compared to healthy children (see [1] for review). Fortunately, growth failure in chronic renal insufficiency and ESRD can be prevented and treated by aggressive nutritional management and recombinant human growth hormone (rhGH) therapy. The intense therapeutic attention to growth failure has resulted in improved growth patterns in the pretransplant phase. In the North American Pediatric Renal Transplant registry (NAPRTCS), mean standardized height at the time of first kidney transplantation has increased from −2.4 SDS in 1987 to −1.5 SDS in 1999, with only slight further improvement (0.25 SDS) between 1999 and 2009 [2] (Figure 118.1).

Since pediatric liver diseases most commonly occur in infancy, severe malnutrition and rapid growth failure are common sequelae of liver failure. Indeed, failure to thrive is considered one of the indications for elective liver transplantation in patients suffering from disorders such as biliary atresia or Alagille syndrome. The mean pretransplant height SDS of 569 patients enrolled into the SPLIT registry was −1.3 [3]. Single-center studies from UCLA [4], the University of Chicago [5], and the UK [6] comprising 236 294, and 107 pediatric recipients, respectively, reported mean baseline height SDS of −1.7, −1.6, and −1.2.

In children with end-stage liver disease, growth retardation is usually inversely correlated with age, supporting the notion that liver failure is more critical to the nutrition-dependent growth phase of infancy. The crucial role of nutrition is also underlined by the severe growth retardation observed in the majority of infants with short bowel syndrome requiring isolated small bowel or combined liver-small bowel transplantation [7]. In contrast, patients acquiring end-stage liver disease in the post-infantile period more commonly undergo rescue liver transplantation due to acute conditions such as fulminant hepatitis or intoxications, without preceding growth failure. A third group of patients with liver failure is comprised of syndromic disorders with hepatic involvement. In these children, chronic liver failure may be one of several factors contributing to severe growth retardation. A genetic disposition for small stature has been suggested in Alagille or Byler syndrome; intrauterine growth retardation occurs in 50% of patients affected with these disorders and normalization of liver function by transplantation does not appear to induce catch-up growth [8–10].

Growth is also suboptimal in patients with severe cardiac disease awaiting transplantation. Impaired tissue oxygenation can cause impaired intestinal motility and nutrient malabsorption, acidosis, increased energy expenditure and even ischemic injury to the hypothalamo-pituitary axis with resultant insufficiencies of one or more of the endocrine systems promoting growth and development [11]. The overall degree of growth failure appears to be moderate. In the most recent large single-center reports, median height SDS at time of transplantation was −0.7 in 46 prepubertal children from UCLA and −1.3 in 130 children from Toronto [12,13].

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

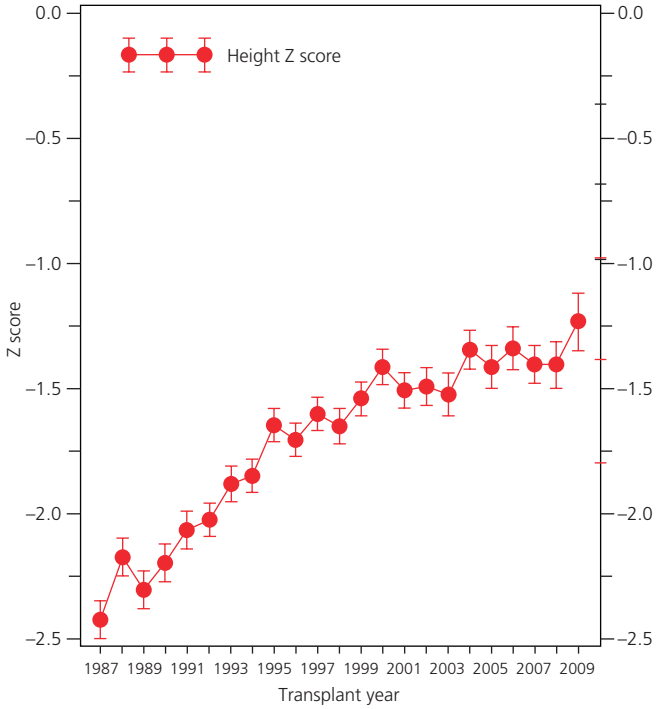

Figure 118.1. Improvement of average standardized height at the time of transplantation in North American children receiving kidney allografts between 1997 and 2009. Adapted from [2], with permission from North American Pediatric Renal Trials and Collaborative Studies.

Factors affecting post-transplant growth and development

Kidney transplantation

Post-transplant growth and development has been studied most systematically in renal allograft recipients. While a wide variety of growth patterns after kidney transplantation has been reported by individual centers, ranging from progressive growth failure to almost complete catch-up growth [14–28], most reports are in agreement about the main determinants of post-transplant growth. These are the age and the degree of stunting at time of transplantation [17,18,28–31], the level of allograft function and the modalities of glucocorticoid treatment.

The potential for post-transplant catch-up growth appears to be inversely related to the 'age at transplantation'. Excellent catch-up growth is usually observed in infants [17,29–32]. The critical age beyond which significant improvements in height SDS were no longer observed ranged from two to twelve years in different studies [17,27,29–31,33]. Pubertal growth seems to benefit least from renal transplantation. As in dialyzed patients, the onset of puberty and the pubertal growth spurt are delayed in renal allograft recipients by approximately two years. Although a distinct acceleration of height velocity occurs, total pubertal height gain is subnormal due to a shortened duration of the pubertal growth spurt [34]. An inverse relationship between pubertal peak height velocity and cumulative glucocorticoid intake has been observed [34,35].

In addition to the impact of age, the change in standardized height following renal transplantation is closely correlated to the 'height at time of grafting' [32,36]. Hence, the general principle of catch-up growth 'by demand', that is: the tendency of an organism to return to a predetermined growth channel after removal of growth-inhibitory conditions, holds also true for renal allograft recipients.

Post-transplant growth appears to be very sensitive to the 'function of the allograft'. Whereas native kidney function only affects linear growth when glomerular filtration rate (GFR) is consistently decreased to less than 30 mL/min/1.73 m^2, post-transplant growth velocity is significantly reduced at any GFR below 60 mL/min/1.73m [2,16,29]. Multivariate analyses have confirmed that the growth-suppressive effects of poor renal graft function is independent of glucocorticoid dosage [24,27].

In view of the profound growth-suppressive endocrine and metabolic effects of glucocorticoids (see further on), there is little doubt that this component of immunosuppressive medication plays a major role in post-transplant growth. Results from clinical trials using modified steroid administration, complete steroid withdrawal or even primary steroid avoidance protocols have provided some evidence that glucocorticoids affect catch-up growth in renal transplant recipients. On the other hand, surprisingly few studies found a consistent correlation between steroid dosage and the post-transplant change in standardized height [14,35]. The lack of a consistent correlation between glucocorticoid dose and post-transplant growth may be explained by a non-linear 'threshold' relationship, and by the large interindividual variability of glucocorticoid resorption and pharmacokinetics. Indeed, the area under the serum methyl-prednisolone concentration curve was superior to the administered glucocorticoid dose in predicting post-transplant growth rates [37].

Conflicting findings have been reported with respect to a possible growth inhibitory effect of mTOR inhibitors. On the cellular level, this class of immunosuppressive agents has profound antiproliferative properties by interfering with key anabolic signaling pathways [38]. In experimental studies rapamycin induced growth retardation via an antiangiogenic effect at the growth plate [39,40]. After a clinical case report first suggested growth failure induced by sirolimus [41], a retrospective analysis compared growth in 34 children switched from calcineurin inhibitors (CNI) to sirolimus due to CNI toxicity, acute or chronic rejection or malignancy, compared to 34 controls matched for age, gender, renal function and steroid dose [42]. Catch-up growth was observed over time in the children on CNI, but not in those receiving sirolimus. In a subset of prepubertal children with excellent GFR and very low steroid doses, the difference was even more marked with a mean two-year change in height SDS of −0.22 in the patients receiving sirolimus versus +0.77 in the CNI-treated controls. By contrast, a single-center study comparing 25 children receiving sirolimus, MMF and prednisone with 23 matched children treated with tacrolimus, MMF and prednisone from the time of transplantation did not find any adverse impact of sirolimus on growth rates during the first two post-transplant years, with unchanged mean height SDS in both groups [43].

After more than three decades of pediatric renal transplantation, several thousand patients worldwide have achieved 'final adult height'. This provides the opportunity to assess the ultimate completeness of post-transplant catch-up growth, and to analyze the eventual impact of the modulatory factors mentioned above on the ultimate endpoint of longitudinal growth. Final height has been assessed in single-center studies and registry reports [2,34,36,44,45]. Short adult height was found in 25–41% of all patients who developed end-stage renal disease and underwent kidney transplantation during childhood. Average final heights have substantially increased over time; the NAPRTCS Registry

reported mean SDS of −1.93, −1.51, −1.05, and −0.94 for patients achieving adult height in 1987–1991, 1992–1996, 1997–2001, and after 2002 respectively [2]. However, this increase appears to be entirely related to improved growth management prior to transplantation. In 237 children reported to the NAPRTCS registry who had received a graft at age ≤12 years, mean final height SDS was identical to standardized height at the time of transplantation, indicating a lack of catch-up growth in the pediatric renal transplant population as a whole [45]. As found for early post-transplant growth in the studies cited above, final height was inversely related to the age at transplantation; significant catch-up growth was limited to prepubertal patients. The most severely stunted patients exhibited the most marked post-transplant growth improvement, and renal graft function was an additional independent predictor of final height. In addition, an average prednisone dosage in excess of 0.15 mg/kg/day was independently associated with a retarded final height [45].

Liver transplantation

Post-transplant catch-up growth is observed in 40–50% of liver allograft recipients [5,8,46]. As in kidney transplantation, age and the degree of stunting at time of transplantation are important predictors of post-transplant catch-up growth [5,46], with young infants usually exhibiting most rapid and complete catch-up growth [4,5,46,47].

The 'primary hepatic diagnosis' might also impact post-transplant growth potential. McDiarmid et al. noted an increase in height SDS-score of +0.7 at five years post-transplant for recipients with biliary atresia compared to a decrease by −1.0 for those with fulminant hepatic failure, and −0.9 in those whose primary disease was a tumor [47]. Lack of catch-up growth was also noted in recipients with Alagille syndrome and familial cirrhosis [6,47]. This variation could either be due to persistent differences in post-transplant growth conditions or simply reflect differences of the age at which end-stage liver disease typically occurs.

Long-term growth outcome appears to be favourable in liver allograft recipients. McDiarmid et al. noted an improvement in standardized height from −1.7 SDS at baseline to −1.4 SDS at five years post-transplant in 236 children, Viner et al. an increase from −1.2 to −0.8 SDS at seven years in 107 recipients, and Fouquet et al. an increase from −1.4 to −0.5 SDS ten years post-transplant in 80 children with biliary atresia [4,6,47]. In 121 children followed in the North American SPLIT registry, mean height SDS ten years post-transplant was −0.5 [48]. Hence, the majority of pediatric liver allograft recipients reaches a height in the normal range.

Long-term growth performance of liver allograft recipients appears to be crucially determined by maintenance steroid administration (see further on in this chapter) and graft function [4,5,49,50]. In immunologically low-risk patients with good liver function who can be weaned off steroids entirely, nearly complete catch-up growth is common [4,51,52]. These findings were confirmed in a long-term growth analysis of 237 Japanese liver allograft recipients followed for 15 years [53]. Mean standardized height increased from −1.70 to −0.64 SDS in the first post-transplant year, followed by a plateau phase with slight further catch-up to −0.33 SDS at ten years post-transplant. Children transplanted before two years of age showed late growth deterioration beyond the tenth post-transplant year, which was mainly related to graft dysfunction. Mean height SDS at 15 years was −0.47. Long-term growth failure was strongly associated with steroid therapy; at ten years post-transplant, height was below the tenth percentile in 43% of patients still receiving steroids as compared to 11% in the steroid-free patients.

Heart transplantation

Varying growth patterns have been observed in cardiac allograft recipients. In patient cohorts on steroid-based immunosuppressive protocols, standardized height did not change or slightly decreased up to six years after transplantation [12,13,54–57].

Early propagation of steroid-free immunosuppressive protocols in heart transplantation has provided evidence that maintenance glucocorticoid therapy is the most important determinant of the long-term growth performance in pediatric cardiac allograft recipients.

A small group of heart transplant recipients in Newcastle, UK, followed for >1 year without maintenance corticosteroid therapy exhibited an increase in standardized height from a mean of −2.15 at baseline to −1.15 [58]. Similarly, in 77 infants transplanted at <6 months of age maintained on a steroid-free protocol catch-up growth was almost universally observed during the first post-transplant year [59]. Factors predicting subnormal height at five years post-transplant in patients with steroid-free immunosuppression included prolonged hospitalization during the first post-transplant year, frequent late rejection, and below-average parental stature.

Lung transplantation

Although more than 1500 pediatric lung transplants have been performed worldwide to date, the still limited long-term survival of children undergoing this procedure has precluded the analysis of post-transplant growth patterns [60]. It would be expected that these children have significant pre-existing growth retardation at time of transplantation due to the high incidence of malnutrition, infection and steroid treatment in this population. Catch-up growth after transplantation is unlikely since patients are still universally treated with steroid-based immunsuppressive protocols, and steroid withdrawal appears an option only in very few selected cases. A single paper in 1997 reported height data on 88 pediatric lung transplant recipients [61]. Mean patient height was '72% of normal' at the time of transplantation, and 64% at last observation.

Intestinal transplantation

Two reports describe the post-transplant growth performance in children undergoing intestinal, with or without liver, transplantation [7,62]. In a cohort of 46 children, 35 retained a functioning small-bowel graft for an average of three years without any need for additional parenteral nutrition [7]. Immunosuppression was maintained with tacrolimus and prednisone. Despite usually young age at transplantation (median 3.7 years), no catch-up growth was noted. Median height SDS was stable around −1.8 during the first two post-transplant years. The Paris group followed 31 children with a mean height SDS of −1.2 at transplantation for 2–15 (mean 7) years [62]. Height SDS remained stable in 25 children whereas growth velocity was impaired in five. Only a single child with severe pretransplant growth failure exhibited catch-up growth after transplantation.

Endocrine mechanisms of glucocorticoid-induced growth failure

In the postinfantile period, longitudinal growth is mainly driven by the hormones of the somatotropic and, during puberty, of the

gonadotropic hormone axis. These endocrine systems appear to be affected to a major degree by glucocorticoid immunosuppression in pediatric allograft recipients.

In children after renal transplantation, growth plates appear responsive to stimulation via the somatotropic axis as suggested by a positive correlation between growth hormone (GH) pulse amplitudes and height velocity [63]. However, pituitary GH secretion is variably reduced, most likely due to a stimulatory effect of chronic glucocorticoid therapy on hypothalamic somatostatin synthesis and release [64]. Hence, some children receiving post-transplant glucocorticoid medication appear to be partially GH deficient. During normal puberty, augmented pulsatile gonadotropin secretion stimulates sex hormone secretion. The increase of circulating sex steroid concentrations elicits the pubertal growth spurt, both by direct action on the growth plate and by stimulating pulsatile GH secretion. In kidney transplant recipients, the pubertal surge of gonadotropin secretion appears intact but, at least in boys, sex steroid levels rise insufficiently [64]. This is associated with a blunted augmentation of GH pulses and a subnormal pubertal height gain [20,64]. A recent report of Finnish patients transplanted at a mean age of five years reported more normal sex steroid patterns at puberty, suggesting that the use of low steroid doses and transplantation early in childhood may preserve the normal endocrine events during puberty [65].

In addition to suppressing GH secretion, experimental and clinical evidence suggests that glucocorticoid exposure may suppress GH action on the target organ level. Hepatic GH receptor expression is dose-dependently reduced by growth-suppressive doses of methylprednisolone treatment in rats [66,67] and circulating GH binding protein, reflecting hepatic GH receptor content, is low in pediatric kidney allograft recipients [20,68,69]. The steroid-induced alterations of GH secretion and action would imply reduced availability of IGF-1, the principal downstream mediator of GH. Indeed, reduced IGF-1 gene transcription has been demonstrated in glucocorticoid treated animals [67,70]. Surprisingly however, plasma IGF-I levels in glucocorticoid-treated renal allograft recipients are in the normal range [20,68,69]. This discrepancy may be explained by additional steroid-induced alterations of IGF-1 mRNA stability, post-trancriptional processing and secretion of IGF-1 peptide or increased plasma half-life by altered IGF-1 binding protein composition [70]. In contrast to the normal immunoreactive plasma IGF-1 concentrations, IGF 'bioactivity' was found significantly suppressed in patients on glucocorticoid treatment [68,71]. It has been suggested that glucocorticoids may induce the production of IGF inhibitors [71]. These putative IGF inhibitors appear to have a molecular weight of 12–20 kD, but have not been characterized any further to date.

In addition to these endocrine effects, glucocorticoids interfere with growth and endochondral ossification by multiple mechanisms on the level of the growth plate chondrocyte. They inhibit proliferation, suppress autocrine/paracrine IGF-1 synthesis, downregulate chondrocyte GH and IGF-1 receptor expression and interfere with the sulfation and mineralization of epiphyseal cartilage matrix [72,73].

Strategies to optimize post-transplant growth

Glucocorticoid treatment has profound effects on endocrine functions and growth performance in pediatric allograft recipients, but also on multiple other systems including body composition, lipid metabolism, bone mineralization and blood pressure regulation. The severe consequences of steroid-related side effects for long-term health and quality of life have stimulated the search for alternatives to daily methylprednisolone therapy in pediatric organ transplant recipients [74].

Modification of steroid therapy

Registry results and evidence from clinical trials suggest that improved longitudinal growth can be achieved without any risk for long-term graft function when the same total dose of corticosteroids is applied in an alternate-day rather than a daily fashion [22,27]. In the NAPRTCS registry, alternate-day steroid dosing (performed in 17% of all transplant recipients) predicted a positive change in height SDS during the first two post-transplant years independently of the absolute corticosteroid dose. In both studies, a gain in standardized height by 0.5 SD was observed with alternate day steroid dosing, contrasting to an unchanged height SDS observed on daily steroids. An analysis of long-term (five to seven years) growth performance in 30 young renal allograft recipients receiving alternate day steroids demonstrated nearly complete catch-up growth, from −1.9 to −0.4 SDS, in children aged two to five years at time of transplantation and with stable growth observed in children who received a graft at age <2 years (height SDS of −1.1 at time of transplant and at seven years post-transplantation) [32]. Due to its well established safety, alternate-day administration is the preferred mode of steroid therapy in many experienced centers beyond the first post-transplant year in allograft recipients with low immunological risk. In the 2010 Annual Report of the NAPRTCS registry, 34% of allograft recipients received alternate-day steroid treatment four years post-transplantation [2].

Steroid withdrawal

In the first two decades of pediatric 'kidney' transplantation, complete steroid withdrawal was problematic since steroid-free immunosuppressive protocols based on cyclosporin A (CsA)and azathioprine alone resulted in deterioration of renal function in at least 50% of children [21,75]. Nonetheless, several uncontrolled small scale studies suggested that if successful, steroid withdrawal may result in significant catch-up growth [76,77]. In the past 15 years the introduction of more powerful immunosuppressive agents, in particular the nearly complete replacement of CsA by tacrolimus and of azathioprine by mycophenolate-mofetil, has increased the therapeutic window for steroid withdrawal in kidney transplantation [78]. In the latest Annual Report of the North American Pediatric Renal Transplant Study (NAPRTCS) Registry the proportion of steroid-free patients three years post-transplant is 17% for cadaveric and 25% for living-related allograft recipients over the whole observation period 1996–2009 [2]. The fraction of patients receiving steroids 30 days after transplantation has steadily decreased from 95% in the 1990s to 49% in 2009.

Both late and early steroid withdrawal protocols have been evaluated in the past decade:

'Late steroid withdrawal' appears to be possible in at least 70% of pediatric allograft recipients receiving tacrolimus. In 52 children weaned off steroids six months post-transplant, mean height SDS had increased from −2.41 to −1.31 after three years [79]. Using a CsA and MMF based protocol, Höcker et al. successfully discontinued glucocorticoids in the second post-transplant year in 20 selected patients with major side effects of steroid therapy, stable graft function and not more than one preceding rejection episode [80]. After four years of follow-up, height SDS had increased from

−1.6 to −1.0, compared to an unchanged standardized height in a matched control group on continued daily methylprednisolone. Catch-up growth was limited to six prepubertal children who improved from −2.24 to −0.77 SDS, whereas pubertal patients had no significant benefit from steroid withdrawal with respect to growth. Other sequelae of steroid therapy such as obesity and hypertension were considerably improved.

The growth promoting efficacy of 'early steroid withdrawal' has been assessed in the randomized controlled TWIST trial, in which tacrolimus and MMF was administered to all 196 patients and randomization was made for either ongoing daily steroids or induction therapy with the monoclonal antibody daclizumab and steroid withdrawal on post-transplant day five. A significant improvement in standardized height was seen in the steroid withdrawal arm after six months (0.16 vs. 0.03 SDS, $P = 0.005$), along with significant improvements in metabolic and blood pressure control. Graft survival and rejection incidence did not differ at six months follow-up [82]. Extended outcome results of this trial have not been published to date. Several smaller studies exploring early steroid withdrawal with poly- or monoclonal antibody induction therapy with historical control groups obtained similar results during one to two-year follow-up periods, with excellent safety records and improved growth [82–85]. It seems, however, that the growth benefit of early steroid withdrawal may attenuate over time. In a prospective comparative trial evaluating the effect of early steroid withdrawal in 95 children on tacrolimus/MMF based protocols, mean height SDS improved from −2.4 at transplantation to −1.5 and −2.2 at one year, −1.3 and −1.9 at two years and −1.0 and −1.2 at five years in the steroid-treated and steroid-free patient groups respectively [85].

Steroids are still a cornerstone of immunosuppression in many pediatric 'liver' transplantation programs. Several uncontrolled [86] and one controlled study [87] evaluated the concept of steroid withdrawal in pediatric liver allograft recipients. In five uncontrolled studies attempting steroid withdrawal at 3–58 months post-transplant with concomitant CsA therapy, acute rejection episodes occurred in 7–27%, chronic rejection in 4–13% and graft loss or patient death in 3–13% of the recipients. In contrast, McDiarmid et al. [87], weaning steroids with very rigid inclusion criteria in 23 patients at a mean interval of three and a half years post-transplant, noted a 6% acute rejection rate and no resultant chronic rejection, graft loss or patient death. Unfortunately, no significant benefit of steroid withdrawal on linear growth compared to a control group on continued steroid therapy was observed. In three studies attempting steroid withdrawal within the first post-transplant year using concomitant tacrolimus immunosuppression, the incidence of acute rejection varied from 14 to 29% [86]. Reding et al. compared the impact of steroid withdrawal on linear growth in recipients receiving either CsA or tacrolimus [86]. Within 12 months of steroid discontinuation, standardized height slightly worsened with CsA (−0.2 to −0.4) compared to a modest improvement in the tacrolimus (−0.2 to +0.3) group. The observed difference could be due to the much shorter steroid exposure in the patients receiving tacrolimus (weaned on average 1.2 years post-transplant) compared to those treated with CsA weaned five to six years post-transplant.

In many pediatric 'heart' transplantation programs steroids are discontinued early in the post-transplant period; more than 50% of heart allograft recipients are on steroid-free maintenance immunosuppression 12 months post-transplant [88]. In an analysis of 25 infants who underwent heart transplantation at <3 months of age, growth performance was compared in 18 patients weaned off steroids seven months post-transplant versus eight patients continued on steroid therapy [89]. There was no difference in standardized height at follow-up between the eight patients maintained on low-dose steroids and the 18 who underwent steroid withdrawal. However, the overall post-transplant growth performance in this group of cardiac allograft recipients was rather poor. The mean standardized height worsened from −1.08 at transplant to −1.8 at four years post-transplant.

Steroid avoidance

With the increasing number of new potent and selective immunosuppressive agents, complete steroid avoidance has become a realistic option in pediatric organ transplantation. In addition to the advantage of preventing all side effects of glucocorticoid treatment from the beginning, animal data have suggested that the steroid avoidance strategy may even facilitate the induction of tolerance against allogenic tissue.

Steroid avoidance protocols have been pioneered in 'cardiac transplantation'. Almost universal catch-up growth has been observed in pediatric heart transplant recipients. In one large series, only 12% of recipients were below the fifth height percentile five years after transplantation [59].

In 'kidney transplantation', the Stanford group first reported favorable early results with entirely steroid-free immunosuppression in 50 children who received a combination of tacrolimus and MMF or sirolimus, complemented by IL-2 receptor blockade during the first 6 post-transplant months [90,91]. After a mean of 22 months follow-up, acute rejection had occurred in only 8% of the children, as compared to 32% in historical matched control patients on a steroid-based regimen. Growth was superior in the steroid-free group. This was most marked in children younger than five years, who gained 1.7 ± 0.8 SDS within the first 12 post-transplant months compared to an unchanged mean standardized height in the steroid-treated controls. This promising preliminary experience stimulated a three-year multicenter trial in which 130 children undergoing primary kidney transplantation were randomized to steroid-free or steroid-based immunosuppression, with concomitant tacrolimus, mycophenolate and standard dose daclizumab in the steroid-based and extended dose daclizumab in the steroid-free group [92].

Disappointingly, no significant difference for linear growth was observed at three years in those patients who still had growth potential at transplantation, with −0.92 ± 2.29 height SDS change in the steroid-free versus −0.96 ± 1.16 SDS in the steroid based study arm ($P = 0.732$). Only among children younger than five years those on steroid-free immunosuppression tended to display a superior change in height SDS, but this effect was lost at one and three years after transplantation. There were no differences in the rates of biopsy-proven acute rejection (16.7 vs. 17.1%; $P = 0.94$) or graft survival (95 vs. 90%) at 3 years follow-up. The steroid-free group showed lower systolic blood pressure and lower cholesterol levels.

Steroid-free immunosuppression has first been applied at the pediatric 'liver transplant' program in Brussels, Belgium. Twenty children receiving combined tacrolimus and basiliximab were compared with 20 matched historical controls treated with tacrolimus and steroids. The tacrolimus-basiliximab group exhibited higher rejection free survival rates and significantly better growth rates in the first year after transplantation. The slight growth retardation (−0.57 SDS) at time of transplantation was completely caught up

within six months post-transplant in the steroid-free patients, whereas growth recovered at a slower pace in the steroid-treated children [93]. After this positive initial experience, the same protocols were compared in two prospective trials including 72 and 84 pediatric liver allograft recipients respectively [94,95]. Both trials found similar patient and graft survival rates in the study arms but significantly longer rejection-free intervals, lower infection rates and reduced need for antihypertensive agents in the basiliximab-tacrolimus treated patients. Within three years, the steroid-free patients showed complete normalization of standardized height (mean −1.16 SDS at baseline, 0.21 SDS at three years) while the patients on maintenance steroids showed incomplete catch-up growth (−1.15 SDS at baseline, −0.42 at three years, $P < 0.001$) [95].

Growth hormone treatment

Chronic administration of rhGH has proven remarkably successful in the treatment of growth failure in children with chronic renal insufficiency, and resulted in a steady increase of height SDS at the time of transplantation in pediatric allograft recipients over the past 15 years. Hence, rhGH treatment has been considered as an alternative to modification or withdrawal of steroid treatment in growth retarded renal allograft recipients who do not exhibit post-transplant catch-up growth.

Experimental support for this concept has been provided by animal studies showing that the growth-depressing effects of glucocorticoid treatment are compensated by rhGH administration [96], mediated in part by stimulation of circulating or local IGF-I synthesis [97–99].

Numerous uncontrolled trials have suggested a growth-promoting effect of rhGH in children with renal allografts with up to three years of therapy [68,69,100–106], with a median annual standardized height increment of 0.5 SDS during the first two treatment years. Four randomized controlled trials confirmed the short term efficacy of rhGH in renal allograft recipients [107–110]; in the two larger trials one-year growth velocity increased to 7.7–9.0 cm/yr as compared to 4.2–4.6 cm/yr in the control groups. A long-term growth assessment in 513 pediatric post-transplant patients and 2263 untreated controls followed in the NAPRTCS registry suggested increased cumulative height gain and superior final height (−1.83 ± 0.14 vs. −2.63 ± 0.05 SDS) associated with rhGH treatment [33].

Successful growth stimulation was also achieved by rhGH treatment in seven liver allograft recipients on prednisolone. In the first treatment year, annual growth velocity increased from 3.9 to 8.2 cm, and median height SDS from −2.7 to −2.1 [111].

It has been argued that rhGH may not only antagonize the growth-inhibitory, but also the immunosuppressive effects of glucocorticoids. Two large controlled trials and the registry analysis of pediatric renal allograft recipients were powered to evaluate any changes in the risk of rejection and graft failure during rhGH treatment. While none of the studies found an increase in the overall incidence of rejection episodes or the rate of renal function decline, the prospective trials noted an increased risk of subsequent rejection episodes in those patients who had more than one rejection prior to treatment.

Summary

In conclusion, while large individual variation of growth and pubertal development exists in solid organ transplantation, many pediatric allograft recipients still grow up to become undersized adults. Major determinants of post-transplant growth and development are the age and the height deficit attained at time of transplantation, long-term graft function, the nature of the underlying disorder and the choice of maintenance immunosuppressive therapy. Complete withdrawal or even primary avoidance of steroids appears possible in the majority of children undergoing solid organ transplantation. However, the early growth-promoting efficacy and immunological safety of minimized steroid exposure awaits confirmation in long-term trials. Growth hormone treatment may be an alternative to steroid withdrawal in patients with high-immunological risk. The transduction of these approaches into routine clinical practice should allow improved catch-up growth and a normal final adult height in many, if not most, allograft recipients.

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CHAPTER 119

Cognitive Development and Functional Outcomes after Organ Transplantation

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Introduction

Solid organ transplantation is expected not only to prolong life and reverse the consequences of end-stage organ failure, but also to result in a health state that is desirable and a marked improvement from the pretransplant condition. In considering all aspects of health in children, physicians must examine not only physical outcomes but also psychosocial outcomes, including cognitive development and scholastic function. However, considerable gaps in this area of research exist in pediatric heart, kidney, liver and intestinal transplantation. In this chapter, we will review recent reports examining cognitive function in pediatric solid organ transplant recipients and discuss their findings in the context of present knowledge.

Assessment of cognitive development is complicated by the need to use age appropriate methods that investigate emerging skills. In assembling groups of children who have survived uncommon medical interventions, such as organ transplantation, the resulting samples are often small and include a heterogeneous mixture of patients with a wide array of ages. Thus, investigators find themselves testing these patients with different tools geared for different ages and attempting to collapse the results to form meaningful conclusions. To this point, many studies of cognitive development in children who have received solid organ transplantation have been limited to single center studies of relatively small samples. However, during the past few years some important studies have been published that help broaden our understanding of developmental outcomes and rehabilitation in these patients.

Cognitive function

Developmental outcomes can be measured in several ways dependent upon the age at testing. Measurement of infant development focuses on motor function, social and environmental interaction and language development and adaptive skills while older children can be tested for intelligence, academic achievement, behavior and more specific cognitive domains such as attention, executive function, language, visuospatial processing, learning and memory. Abnormalities that are observed later in childhood are more predictive of deficits that are likely to persist into adulthood. Many forms of chronic disease in childhood can have a negative impact on cognitive development. Children that experience solid organ failure during infancy may be at particular risk for cognitive delay since they experience the onset of their illness during a period of rapid neurologic maturation. The relative contributions of pretransplant versus post-transplant neurodevelopmental injury have not been carefully studied. Likewise, longitudinal studies of neurocognitive outcomes and developmental rehabilitation following solid organ transplant are limited.

Across the multiple organ recipient groups neurocognitive studies have included some of the same measures. One of the most commonly studied areas is intelligence, which is most frequently measured by intelligence quotient (IQ). IQ is most frequently measured with the Wechsler scales. Current versions for children are the Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-III) and the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV), which are standardized to a score of 100 with a standard deviation of 15 [1,2]. Developmental delay can be categorized as "mild to moderate", typically a "full scale intelligence quotient" (FSIQ) of 71–85, and "serious", patients with a FSIQ $\leq$ 70, which is two standard deviations below the normative population. Patients in the seriously delayed category have IQ scores typically indicative of mental retardation while those in the mildly to moderately delayed category have intellectual functioning that is in the borderline deficient to low average range [3]. Learning disability (LD) is also an outcome that has been frequently studied, but the definition of LD has evolved over time. In some studies, the operational definition of LD is a discrepancy of 15 points or more (one standard deviation) between intellectual ability as measured by IQ and academic achievement in reading and/or math computation [3]. Learning disability can also be measured by parental report of an established diagnosis, which is expected to be accurate, especially if the patients have been tested to support special education services within their school systems.

Kidney recipients

Chronic renal failure has been demonstrated to have a negative impact on neurocognitive development, which may be alleviated by renal transplantation. Intelligence levels in children following kidney transplant are usually in the normal to low normal range with one study reporting a mean FSIQ of 87 [4] (normative mean 100, standard deviation 15) and a more recent study reporting a median FSIQ of 97 in 27 recipients with only 22% having a FSIQ $<$ 85 [5]. It has been suggested that renal transplant recipients have lower verbal compared to non-verbal performance, shorter

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

attention spans and difficulty with memory, especially those who have had renal disease since birth [4]. In children who are older at the time of transplant, significant deficits have been observed in performance IQ, which measures intellectual ability that is less dependent upon one's fund of knowledge, vocabulary and basic knowledge and more dependent on fluid mental abilities [5]. Motor skills are also significantly delayed in renal transplant recipients as compared to norms with deficits observed in multiple areas of function including pure motor tasks $P < 0.001$, adaptive fine motor tasks $P = 0.01$, adaptive gross motor tasks $P < 0.001$, and static balance $P = 0.001$) [5]. Altered motor skills can play a pivotal role in peer relationships, and academic tasks such as drawing and handwriting.

Few longitudinal studies measuring cognitive function before and after renal transplantation have been conducted. A recent study assessed six children with chronic kidney disease (CKD) before and after transplantation and compared their outcomes to 28 children with moderate to severe CKD and 23 healthy controls [6]. The renal transplant recipients had on average a 12 point increase in FSIQ, even though they were tested relatively early in the first post-transplant year (mean six months post-transplant). Neither the CKD group nor healthy controls demonstrated this same trend.

Risk factor analysis for determinants of lower cognitive outcomes has been attempted in multiple groups of renal transplant recipients despite small sample sizes. Early onset of renal disease, longer duration on dialysis, pretransplant morbidity (e.g seizures) [4], and lower socio-economic status are believed to have a negative impact on developmental outcome [7–9]. Yet, other studies [5] have shown no relationship between medical factors and cognitive outcomes. However, it is generally accepted that avoiding prolonged dialysis may mitigate some of these deficits. Prolonged exposure of the developing brain to the complications of end stage renal disease including hypertension and uremia are hypothesized to have a negative effect on neuronal myelination and synaptic development. In fact, early transplantation has been shown to improve head circumference from a standard deviation score (SDS) of -1.4 to -0.9 ($P = 0.02$) and significantly improve gross motor, social and language development quotients ($P = 0.02$–0.0006) in infants one year post transplant [10].

Following renal transplantation, approximately 70% of patients receive routine classroom instruction while the remainder require some special educational support or have a history of serious neurological morbidity such as stroke [4,7]. There are a number of long-term studies that have reported final education levels that are lower than national averages [11,12]. While others have revealed education levels that were similar to the general population and their sibling controls [13].

Liver recipients

Even from the earliest experiences in pediatric liver transplantation clinicians have been particularly interested in neurocognitive outcomes in these recipients. Multiple studies have shown that children who have survived liver transplantation are more likely to have significantly lower intelligence than healthy children [14–18]. Onset of liver disease in infancy is thought to increase the risk of neurocognitive delay, which may persist even after successful liver transplantation. There are several unique aspects of the pediatric liver recipients that could theoretically place them at risk for cognitive deficits. Since approximately half of pediatric liver recipients are less than two years of age at transplant, it has been particularly important to study neurocognitive function in this infant population. Patients with chronic liver disease resulting in portal hypertension may develop clinical signs of chronic hepatic encephalopathy (HE). Serum levels of neuro-inhibitory substances, including ammonia may not only alter mental processes during periods of elevation, but may also contribute to altered function after successful transplantation by causing irreversible injury. Finally, calcineurin inhibitors may cause significant neurotoxicity and pediatric liver recipients appear to be at higher risk for this complication than other solid organ transplant recipients [19–21].

A series of studies in the early experience of liver transplantation were conducted at the University of Texas and examined patients with end stage liver disease and biliary atresia, finding that their IQ and developmental functioning ranged from borderline low to average. Early disease onset, liver function, and growth parameters (especially in younger children) were highlighted as important correlates of intellectual deficits. Younger patients (0.5–5 years) fared worse; overall cognitive functioning on the Bayley or Stanford-Binet fell in the borderline range with extremely low motor skills. Infants' "mental and motor" development were associated with growth parameters whereas developmental outcomes in older children were more closely related to liver function [22]. A small, recent study (n = 15; age 4–20 months) of infants with Biliary Atresia confirms these earlier findings, demonstrating significant delays particularly in very young patients [23]. In this study, all scales on the Mullen test were below norms, with the weakest performance apparent in "gross motor and expressive language skills". Modest associations were found with liver function, growth parameters, and age at Kasai procedure. Of note, nearly half the sample was primarily Spanish speaking and required an interpreter for testing, so the language results should be interpreted cautiously.

The University of Texas group also conducted two small studies examining neuropsychological outcomes after transplant by comparing pediatric liver transplant recipients to controls with cystic fibrosis (CF). Both studies reinforced and expanded on earlier findings in patients with end stage liver disease suggesting that deficits noted prior to transplantation were not reversible [24,25].

Historically, there have been very few studies successful in examining cognitive function before and after liver transplantation. The most important of which was a longitudinal study of infants with biliary atresia. While awaiting transplant, these patients had mental and motor developmental scores that are within the low average range [26]. Follow-up testing after transplantation revealed that scores dropped further in the immediate post-transplant period and only recovered to pretransplant levels at 12 months after the procedure. This observation suggests that infant recipients do not exhibit developmental "catch-up" until after the first post-transplant year. This study also demonstrated that infants with longer hospitalizations and those with growth impairment at transplant had more significant delays.

The most recent studies examining cognitive function in liver transplant recipients have mostly reinforced earlier findings. IQ has nearly universally fallen below published norms [15,27,28]. Pediatric LT patients clearly have a downward shift in IQ, with mean IQ scores typically in the mid-80s to low-90s and an increased prevalence (up to 27 vs. 2% expected) of scores falling below 70 [14,16,28,29]. Of note, the only multicenter study to examine IQ in pediatric LT patients, and the largest sample (n = 144; age 5–6), found the smallest proportion of patients with a FSIQ at or below 70 (4%), although FSIQ, verbal IQ (VIQ), and

performance IQ (PIQ) were all significantly below norms (FSIQ = 94.7 ± 13.5; VIQ = 95.0 ± 13.8; PIQ = 94.9 ± 13.5) and twice as many as expected (26 vs. 14% expected) had mild to moderate IQ delays (IQ = 71–85) [28]. Preliminary analysis of follow-up of this cohort suggests that IQ does not improve within a two-year interval.

Learning disability and school performance have also been examined in LT recipients. One study of long-term recipients, age three to nine years, revealed that almost 20% had an IQ of less than 70 and that 26% had a discrepancy between IQ and academic achievement thus meeting the definition of learning disability [30]. In that study, many of the children with low IQ and learning problems had not been identified by their school systems. A recent survey of school outcomes conducted by the studies of pediatric liver transplantation (SPLIT) network revealed that 40% of patients have required some type of special educational services, with 8% having been classified as learning disabled and 5% having a reported IQ of less than 70 [31]. Missed school days may also contribute to compromised school function. Data from the SPLIT network suggests that 30–40% of patients in long-term follow-up miss more than ten days of school per year, with teens having the highest rate of absences [31].

Heart and lung recipients

Studies performed in children with cyanotic heart disease consistently show that chronic cyanosis is associated with progressive cognitive impairment and that earlier correction of cyanotic heart disease leads to more favorable cognitive outcomes [32–34]. Other factors including circulatory arrest, cardiopulmonary bypass and embolic events have also been associated with lower than expected developmental outcomes [32–38]. Research findings examining developmental status of pediatric heart recipients, primarily infants, are detailed in a review by Chinnock et al. [39]. In general, reports describing developmental outcomes in children who have received cardiac transplantation suggest that the majority of these patients have intelligence in the low-normal range which is comparable to other children with surgically corrected congenital heart disease. However, a significant percent, perhaps as high as 40%, have intelligence scores in the low to borderline range (FSIQ < 85) and up to 10% may have significant neurological injury, frequently related to ischemic events which could preclude testing. Freier et al. in 2004, reported the longitudinal neurodevelopmental assessment, using the Bayley scales, of 39 patients transplanted as young infants (median age at transplant 53 days) [40]. Patients were tested four to six weeks after transplant and then every 6–12 months thereafter. The mean mental developmental index (mdi) of the group was within normal limits and the mean psychomotor developmental index (PDI) was in the mildly delayed range. Wray et al. in 2006 reported a longitudinal assessment of 34 older patients who received heart and lung transplants spanning a wide age range from 1.3–15.3 years [41]. Cognitive function and academic achievement were tested at 12 and 36 months following transplant. Mean scores of intelligence and academic achievement were within the normal range and appeared stable over the testing period with the exception of a drop in arithmetic scores for younger children. Several studies have examined risk factors for lower cognitive outcomes finding that circulatory arrest, birth head circumference, prolonged hospital stay, cardiopulmonary bypass, embolic events, waiting time to transplant and pretransplant diagnosis are all potentially associated with variability in developmental outcomes in this patient group [35–39,41]. Studies that have examined the impact of primary diagnosis suggest that patients transplanted for cardiomyopathy typically have higher scores than those with a history of cyanotic congenital heart disease. One such recent study included 18 recipients age 6–16.5 years and identified that mean FSIQ, VIQ and PIQ were all significantly lower than test norms with 26% scoring in the range of mental retardation (<70) [42]. When patients were grouped by history of neurological injury, such as abnormal neuroimaging results prior to transplant, it was clear that this high risk group was driving the mean difference, with all other patients scoring within the normal range. In this study, abnormalities on brain imaging were associated with the number of cardiac surgeries before transplant and with having congenital heart disease.

Another study of 21 heart recipients median age of two and half years (range 14 months to ten years) identified not only lower global intelligence, but also deficits in expressive language ($P = 0.01$, 46% >1 SD below norms), adaptive behavior ($P < 0.005$) and visual-motor ($P = 0.01$), and fine-motor skill ($P < 0.005$). Authors suggested that some aspects of adaptive behavior might have been influences by delays in the ability to use spoken language and poor coordination of motor skills [43].

Despite the encouraging results of studies examining intelligence in this patient group, the school performance of cardiac transplant recipients appears to be lower than healthy children and the prevalence of behavior problems may increase with time after transplantation [44]. Ikle et al. studied 26 children who had received cardiac transplants for hypoplastic left heart syndrome using the Vineland scales. The analysis found that many scored greater than 1 SD below the mean for measures of daily living skills (39%), socialization (29%), communication skills (48%), and overall adaptive behavior (52%) [45]. These findings suggest that interventions that target improvement in these skill areas might improve functional outcomes, including school performance in this population.

Lung transplant recipients have not been studied as extensively as cardiac recipients. A study that focused on a cohort of 18 infants and 11 toddlers who underwent lung transplantation found that 40–50% of infants and 20–30% of the toddlers had severe developmental delay. Of note, two of the infants with severe delay had significant hearing impairment. Patients were also tested relatively early in their postoperative course; more than 50% tested within the first year [46].

As mentioned above, Wray et al. [41] have reported mean scores for intelligence and academic achievement in the normal range. These same authors, studied a group of 99 patients, aged three to 17 years, consecutively listed for heart, lung or heart/lung transplantation to determine differences in cognitive delay between differing diagnostic groups. The study included 56 patients with congenital heart disease (CHD; 34% listed for heart/lung), ten patients with cardiomyopathy (CM), 15 patients with cystic fibrosis (CF; 100% awaiting lung) and nine with primary pulmonary hypertension (PPH; 100% awaiting lung). The mean scores for the group for all aspects of intelligence and academic achievement were significantly lower than the normative values. Ten percent (eight patients with CHD, one with CM) had an IQ of less then 70 and 23 % of the overall group had academic achievement that was more than two standard deviations below the norm. Underachievement or learning disability, defined as standardized scores for academic achievement that are more than one standard deviation below IQ,

was observed in 23% (22% in math and 14% reading). In general, the CM and PPH patients exhibited better performance than the CHD and CF groups. Bivariate analysis suggested associations between diagnosis, cumulative time spent in hospital since birth and age at assessment of IQ. However, in multivariate analysis, only time spent in the hospital accounted for a significant proportion of the variance (13%) in both IQ and academic achievement. These findings further highlight the impact of the duration of chronic disease on cognitive outcomes in children who will undergo transplantation. This effect may not be fully reversible following successful organ replacement.

Intestine recipients

Intestinal Transplant patients have been reported to have a high prevalence of cognitive delay [47–49] and patients may display worsening developmental delay in the first two months after transplant, similar to what has been reported in liver transplant recipients. Among pediatric patients with intestinal failure, the most studied group is premature infants with necrotizing enterocolitis (NEC). Several studies have found a higher prevalence of neurodevelopmental impairment (NDI) among those with NEC versus those without, even when groups have similar gestational age and birth weight. More serious NEC (Bell's Stage III) and NEC requiring surgery have been found to be associated with higher risk for NDI compared to NEC that can be managed medically or absence of NEC. However, it is unclear whether this association reflects severity of disease or factors related to surgery and complications (e.g., proinflammatory cytokines, transient blood pressure changes, more serious nutritional deficits, and more episodes of sepsis). Multivisceral transplant recipients are also more delayed than infants receiving liver only transplantation. These patients generally recover more slowly and have undergone a more extensive surgical procedure than patients receiving liver, heart or kidney transplantation. Some patients may have an ostomy, which necessitates repeat surgery for closure. Many continue to require parenteral nutrition while the transplanted intestine adapts to its new environment. Thus, intestinal recipients have a relatively larger burden of chronic disease than other solid organ recipients that may contribute to the slower developmental rehabilitation. Common risk factors experienced by infants with end stage liver disease compounded by chronic malnutrition and prolonged hospitalization and prematurity likely play an important role.

Cognitive function summary

The existing body of literature examining neurodevelopmental outcomes in pediatric solid organ transplant recipients is growing. The need to use different scales to measure cognitive ability in different age groups coupled with the small sample sizes available to study make it difficult to reach comprehensive conclusions. Children that receive heart transplantation may have better cognitive outcomes than expected considering the results of older studies of children with cyanotic congenital heart disease. The cognitive outcomes of both liver and kidney transplant recipients may also be improving, but many still require special educational resources. Infants requiring intestine or liver and intestine transplant are probably at the highest risk for cognitive delay, but longitudinal studies that affirm that these delays are not recoverable are lacking. In all cases neurologic co-morbidities increase the risk of delay. Despite recent advances, a significant proportion of both liver and heart recipients have serious developmental delay and/or neurological injury. Risk factors for developmental delay are still being examined, but strategies that limit the duration of unsupported organ failure and chronic malnutrition, especially during infancy, are likely to have a positive impact.

Solid organ transplant recipients display a wide range of developmental outcomes which include both patients with normal development and those with significant delays, see Table 119.1. While the exact mechanisms that contribute to these delays are not fully elucidated, factors such as long-term hospitalization, chronic malnutrition in the pretransplant period, and a history of surgical complications likely all play a role, see Figure 119.1. The primary care physician is an important partner in assisting in the early diagnosis of developmental delay and in facilitating appropriate evaluation and intervention within the patient's community. Early intervention with physical and occupational therapy can curtail physical disabilities. Prompt recognition of cognitive delay and abnormal school function can aid in securing special educational resources early in their academic experience. Again, setting expectations for the family and school system regarding cognitive outcomes is an important aspect of their anticipatory health care.

Functional outcomes

Children who survive organ transplantation are faced with multiple hurdles during the process of rehabilitation and, even when recovery from the transplant process is deemed complete, their health status often more closely resembles that of children with chronic diseases than that of their healthy peers. These patients struggle with co-morbidities that can include both physical and developmental deficits. These deficits become even more apparent when these patients, who were transplanted as children, grow into young adulthood and endeavor to live independently and find their place in society.

Assessment of functional outcomes includes measurement of health related quality of life (HRQOL) and specific measures of everyday living skills. Direct patient testing of physical skills and cognitive status can also be incorporated into functional outcomes assessments. Few pediatric solid organ recipients have obvious chronic physical disabilities, unless they have end stage graft failure or suffer a severe neurological injury during the transplant process. However, as detailed above, many have obvious cognitive deficits. Direct patient testing, such as neurocognitive testing or exercise/strength testing, allows investigators to gather specific information regarding functional skills that contribute to a patients' physical and psychosocial HRQOL. This information is essential to plan appropriate rehabilitation service and to assess the patient's ability to meet age appropriate expectations. Rehabilitation following transplant should focus on both physical and cognitive function and ongoing outcomes assessment should be sensitive to changes in both of these domains. Understanding deficits across this spectrum is vital to anticipatory guidance for education in intermediate follow-up during childhood, and also in job placement as these children mature to adulthood.

Health related QOL has been defined as an individual's subjective experience of their illness, and the impact that illness and its treatment has on the individual's functioning. Transplantation often leads to improved physical health. However, the impact of the medications, hospitalizations, clinic visits and invasive procedures, as well as the impact of living with the uncertainty of graft survival and long-term health status may all affect an individual's HRQOL.

Table 119.1. Intelligence testing in pediatric solid organ transplant patients

Publications	Sample size	Age range	Intelligence quotient (IQ)	Low or borderline IQ
Kidney				
Qvist et al [76]	33	7–12 yrs	Mean Verbal IQ 87.5 ± 13.4 Non-verbal IQ 87.5 ± 14.9	9% IQ < 70 42% IQ ≥ 70 < 90
Falger et al [5]	27	6–17 yrs	Median (range) FSIQ 97 (49–133) Verbal IQ 104 (50–146)	4% IQ < 70 19% IQ ≥ 70 < 85
Liver				
Adeback et al [14]	21	4–16 yrs	Mean Overall IQ 86.6 Verbal IQ 90.6 Non-verbal 84.5	15% FSIQ < 70 35% FSIQ ≥ 70 < 85
Sorensen et al [28]	134	5–7 yrs	Mean FSIQ 94.7 ± 13.5 Verbal IQ 95.0 ± 13.8 Performance IQ 94.9 ± 13.5	4% FSIQ ≤ 70 26% FSIQ > 70 ≤ 85
Heart/Lung				
Wray et al [41]	22 Heart and Heart-Lung	Mean age 7.88 ± 5.1 yrs	Mean Overall IQ 103.7 ± 13.3 (12 months post-transplant) Overall IQ 100.2 ± 12.9 (3 years post-transplant)	100% IQ ≥ 85
Haavisto et al [42]	18 Heart and Heart-Lung	6–16 yrs	Mean FSIQ 85.6 ± 18.8 Verbal IQ 89.8 ± 22.7 Performance IQ 82.2 ± 18.9	26% IQ < 70 16% IQ ≥ 70 < 85
Uzark et al [43]	21 Heart	1–10 yrs	Mean Overall IQ 86.7 Verbal IQ 88.4 Non-verbal IQ 87.5	43% Overall IQ < 85

All tests standardized to a score of 100 with a standard deviation of 15. Normative population expectations 14%, IQ > 70, ≤85, and 4% IQ < 70.

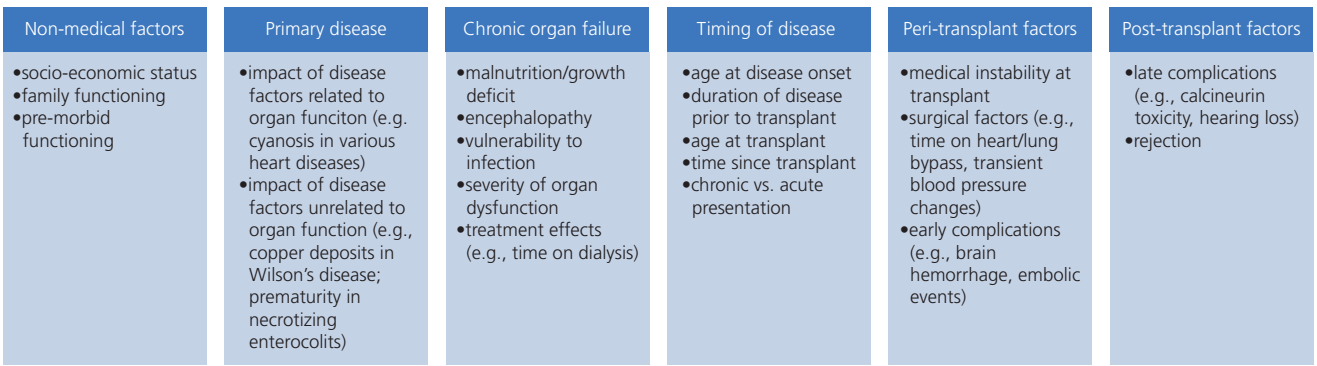

Figure 119.1. Factors impacting cognitive function in solid organ transplant recipients.

Health related QOL can be measured from either a generic or disease specific perspective. It can also be measured from the parent or child's perspective, and instruments must be developmentally sensitive, with age-appropriate versions. Assessment of HRQOL facilitates the evaluation of patient outcomes by providing a more thorough understanding of the child's and family's experience of solid organ transplant and its treatment.

Surveys of generic HRQOL include general questions such as: "Do you have trouble lifting something heavy?" or "Does your child have a low energy level?" — questions that are relevant for any child regardless of their health status. Assessing HRQOL from this generic perspective allows us to compare transplant recipients to both healthy children and to those with other chronic diseases. However, these questions may not tap into some of the real issues for children living with a transplant since many of these issues are unique. Disease specific HRQOL measurements help us understand these more subtle changes in health status over time and are likely more sensitive to changes in treatment and graft function. Most studies of HRQOL following solid organ transplantation have utilized only generic instruments, but now as new disease specific tools are available [50], data regarding disease specific HRQOL is emerging.

To measure HRQOL in young children parents must serve as proxies to report their child's status. However, older children, age five and above, may be able to accurately report their own status. Correlation between parent and child report in adolescents is reasonable, except for domains that are related to mostly internalized symptoms, such as pain or anxiety. Parent reports for school function and peer relationships are frequently well correlated, since these are issues older children frequently discuss with their family. Assessment of both parents' and the child's perspective are likely complimentary and inclusion of both respondents creates a fuller representation of the child's outcomes. Likewise, generic and disease specific measurements provide complementary information and inclusion of both in study designs is optimal.

We will briefly review functional outcomes assessment individually across all the solid organ groups, but it is important to note that these recipients experience very similar issues. Focus group discussion and individual interviews with recipients and their parents consistently identify medication and treatment related issues as highly important [51,52]. Measurements of disease specific HRQOL have identified treatment anxiety, recurring pain and discomfort, and concerns related to medication side-effects as significant issues across age groups and from both the child and parent perspective [50]. Immunosuppressive management strategies are evolving to reduce medication related physical side-effects such as weight gain and short stature. Evaluations of how these aspects of HRQOL change with differing treatment regimens are yet to be conducted. Likewise, analysis of predictors of lower HRQOL have been limited and have not consistently identified intervention targets. However, it is clear that demographic variables such as markers of socio-economic status are key determinants.

Kidney recipients

In general, physical and psychosocial HRQOL are considered to be good for pediatric kidney recipients and far better than that of patients remaining on dialysis. Pediatric kidney transplant recipients experience a higher level of HRQOL as compared to patients who continue hemodialysis or peritoneal dialysis with moderate effect size [53–56]. However, several studies comparing kidney transplant recipients to healthy controls reveal lower levels of HRQOL [53,56–58]. Patients report distress regarding their physical appearance and physical symptoms, difficulty with peer and family interactions, and school disruption as significant concerns [59]. Primary caregivers report a significant negative impact on their emotional state and limitations on family activities due to their child's health [58,59]. Significant discordance exists between child and parental reports of HRQOL with parents tending to report lower physical and psychosocial function [53,58]. Although the large majority of pediatric kidney transplant recipients who have transitioned into adulthood appear to be satisfied with their QOL and report successful interpersonal relationships [59–61] up to 83% report they suffer from anxiety, depression or both [13]. They are more likely to be unemployed and less likely to live independently when compared to the general population [11–13]. An interesting correlation has also been noted between final adult height and measures of functional outcomes including educational level, paid activity, marital life, and independent housing [11].

Liver recipients

Children that have survived liver transplantation have weathered multiple physical and psychological insults which have the potential to impact HRQOL in both short-term and long-term follow-up. Initial studies reporting HRQOL in children following liver transplantation suggested that survivors have few physical restrictions and general QOL that is good [62–64]. However, in subsequent studies using validated instruments it became clear that pediatric liver transplant recipients have a level of HRQOL that is significantly lower than healthy children and more on par with pediatric chronic disease populations [65–67]. In one of the few studies exploring changes in HRQOL before and after pediatric LT, Cole et al. [68] performed a longitudinal assessment of children under age five years which demonstrated significant improvement in all domains of function with the largest change observed in the first six months. A multi-center study that examined HRQOL at one time point two years after transplant included 102 children of two age groups; younger ($n = 7$) and older ($n = 35$) than five years [65]. Results differed by age, with parents reporting that older children had HRQOL that was significantly lower than both a normative group and younger recipients, age two up to five years. These younger patients experienced both physical and psychosocial HRQOL that was similar to a control group that was collected in parallel. Univariate analysis among the sub-scales identified demographic, but not clinical variables as significant predictors of HRQOL across all age groups.

The SPLIT Functional Outcomes Group (FOG) recently conducted a large cross-sectional analysis of generic HRQOL in 873 (363 self-report) pediatric liver transplant recipients between the ages of 2–18 years with a median interval from transplant to survey of 3.1 years [69]. Outcomes were compared to a sample of healthy children randomly matched by age group, gender, and race/ethnicity. The physical and psychosocial function of the liver transplant recipients compared favorably with children with other chronic pediatric illnesses, see Figure 119.2, but were not equal to the healthy sample. The Total Scale Score and subscales of the PedsQL™ 4.0 Generic Core Scales were all significantly lower than those of healthy children with effect sizes in the small (emotional functioning) to moderate range (school functioning). In considering survey questions that assessed school function, questions that related to missing school had the largest impact on score in this domain.

Demographic as well as medical variables may predict levels of HRQOL in this population [67,68]. The SPLIT/FOG cross-sectional data set was analyzed to examine the impact of age at testing on parent report of HRQOL. Results suggest that age at testing may indeed have an important impact on HRQOL with younger children having the highest scores and younger adolescents having the lowest scores. In fact, the impact of age at testing appears to be more significant than interval from transplant. Initial results from multivariant analysis examining the impact of various factors on parent reported HRQOL in the SPLIT/FOG study identified single parent household, length of initial hospitalization after transplant, older age, history of seizures, lower height z score at transplant and days hospitalized in recent follow-up as negative predictors.

The relationship between the patient's HRQOL and family dynamics bears further consideration. Studies that have included assessment of the impact of the child's health state on the parents have shown a considerable negative influence on parental emotional state and family life [14,58,65]. However, when family function was formally measured at two years post-transplant, family function was found to be equal to that reported by a reference population [65]. These preliminary results suggest that services that support the parents' ability to cope with their child's health

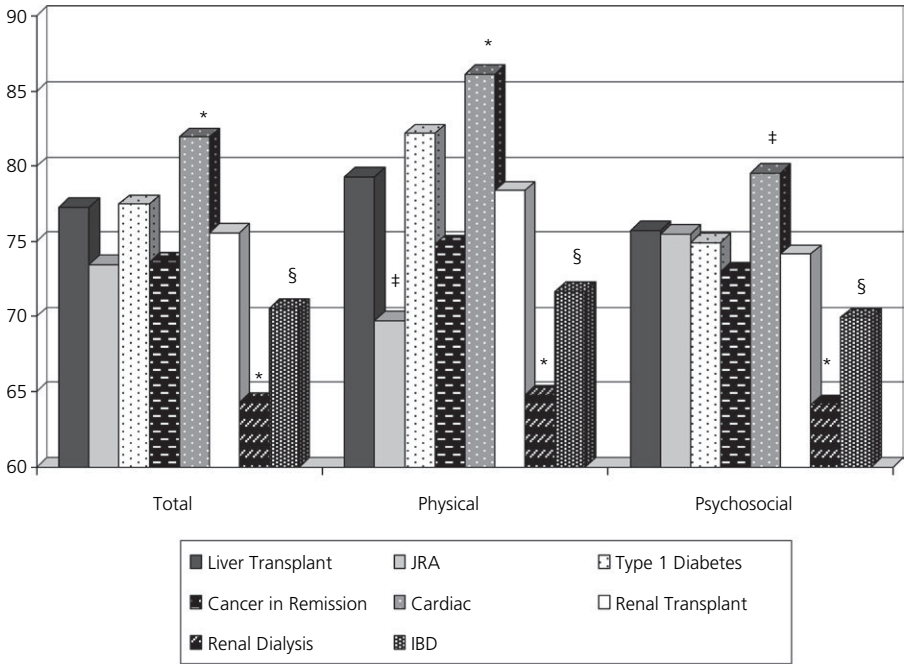

Figure 119.2. Parent proxy-reported PedsQL™ scores for liver transplant sample and chronic disease groups. Reproduced from Limbers et al. Health-related quality of life in pediatric liver transplant recipients compared with other chronic disease groups. Pediatric Transplant. 2011;15(3):245–253, with permission from Wiley.

condition would likely improve the child's HRQOL. This strategy is especially important since patients levels of HRQOL have been linked to adherence behaviors and possibly long-term maintenance of graft function [70].

Heart and lung recipients

Although it is reported that the majority of patients adjust well after transplant, HRQOL has not been systematically studied. A recent study of 23 adult patients who received heart transplants as children reported physical and mental health scores using the SF-36 (QualityMetric) that were similar to the general US population [71]. Seventy percent lived with a parent or family member and had private medical insurance and all had completed high school. Among lung transplant recipients there appears to be a significant percentage that have behavior problems at home, and decreased social competence with their peers, most commonly in male patients. The prevalence of depression has been explored with reported rates of 23% at one year and 13% at the three year follow-up [41,72].

Intestine recipients

Assessment of QOL after intestinal transplant has been limited by small sample sizes and is confounded with issues related to feeding and ongoing gastrointestinal symptoms. Scores for QOL often deteriorate over time in patients on parenteral nutrition, particularly as complications such as liver disease emerge. Conversely, after intestine transplantation, QOL is reported to improve with time [73]. After the initial postoperative course, assessed QOL was similar between parenteral nutrition-dependent patients and intestinal transplantation (IT) patients. In longitudinal follow-up, IT patients reported significant improvement in anxiety, sleep, and impulsiveness and control, which reflected a progressive adjustment to post-transplant status [73]. Parents of intestinal recipients have reported significant limitations in the physical and psychosocial well-being of their children, while the patients themselves reported little impact in most domains compared with other normal school children [74]. These authors concluded despite differences in life relative to peers, these children do not find that these differences impact their functioning.

Function outcome summary

The HRQOL and functional outcomes of children that have received solid organ transplantation are lower than a normative population and appear to be most comparable to that of children with other chronic diseases. Parents of pediatric solid organ transplant recipients experience stress related to their child's illness and tend to report functional outcomes that are lower than that reported by the children themselves. Age at the time of testing may have an influence on reporting of HRQOL with parents of younger children reporting better functional outcomes. It appears that demographic factors have a significant impact on HRQOL and interventions that provide support and education for families caring for organ transplant recipients may improve outcomes for these children.

Health related QOL is a global measure of how well patients feel, how they see themselves in relationship to others and how they function within their environment. Despite good long-term patient and graft survival in solid organ transplantation, many recipients struggle with diminished HRQOL and sub-optimal or incomplete rehabilitation. Data regarding long-term HRQOL in these pediatric

Table 119.2. Suggested screening methods for annual assessment of developmental delay by age

Age	Functional domain	Screening method		Referral***
		Tool	Mode of administration	
1–5 years	Adaptive behaviors: Socialization, motor, communication, daily living skills	Adaptive Behavior Assessment System, Second Edition (ABAS-II) [77] * or Vineland Adaptive Behavior Scales, Second Edition (Vineland-II) [78]*	Parent completed survey (15–20 minutes)	Developmental evaluation (psychologist)
	Developmental milestones: Social, motor, language	Denver-II [79]	Screening in clinic (10–20 minutes)	Developmental evaluation (psychologist)
	Language	Preschool Language Scale, Fifth Edition (PLS-5) Home Communication Questionnaire [80]	Parent completed survey (10–20 minutes)	Developmental evaluation (psychologist) and speech/language evaluation
	Hearing	Audiology screen**		Comprehensive audiology evaluation
6–13 years	General physical and psychosocial function	PedsQL-4.0 Generic Core Scales [81]*	Parent and child completed surveys (5–10 minutes)	Psychological diagnostic evaluation (pediatric medical psychologist)
	Executive function	PedsQL-CF Scale [75]*	Parent and child completed surveys (5 minutes)	Neuropsychological evaluation (neuropsychologist)
		Behavior Rating Inventory of Executive Function (BRIEF) [82]	Parent and teacher completed surveys; child self report survey (age 11+) (10–15 minutes)	
	Attention	ADHD Rating Scale IV [83]*	Parent and teacher completed surveys (10–15 minutes)	
	School performance	Medical clinicians and/or social service support team should assess for school concerns: Does the parent report academic delays? Does the child receive special education services, such as an Individualized Educational Plan (IEP) or 504 plan? Are the child's grades below average, highly variable, or lower than in the past? Does schoolwork require more effort, help, or time to complete than for peers? Is the child excessively frustrated by schoolwork? Does child avoid/resist schoolwork and/or become distressed about going to school? How many days of school have been missed due to illness or medical visits?	Parent interview (10–15 minutes)	
	Hearing	As above at age 1–5 years		
14–18 years	General physical and psychosocial function Executive function Attention School performance	As above at age 6–13		
	Transition planning	Medical clinicians and/or social service support team should discuss plans for advanced education or job placement within context of cognitive function and history of school performance. Cognitive function, especially intellectual and executive function should be considered in developing plans for self-management of medical care/self care.	Parent/patient interview (10–15 minutes)	Transition planning for self management of medical care/self care: Psychological diagnostic evaluation (pediatric medical psychologist). Transition planning for continued education or vocational development: School

* Available in English and Spanish

** Audiology screen is recommended at least once following transplantation. If concerns are noted on screening, refer for comprehensive audiology evaluation. No further screening is needed unless clinical concerns are suspected and/or prior audiology testing indicated problems.

*** Comprehensive neuropsychological, developmental, speech/language, and psychological diagnostic evaluations do not need to be completed annually. If child has already had one evaluation, consult relevant specialist for follow-up recommendations, or refer for re-evaluation if new concerns arise or concerns become more serious following initial evaluation.

groups is lacking and would be best served by a multi-center study design. The primary care provider, through their relationship with the family and other siblings is well poised to assist families in determining needs for rehabilitation and social support. By facilitating evaluation and laboratory monitoring in the community, the primary provider may help to reduce the need for school absences and interruption of family activities. The primary provider can also help the family place their concerns within the context of those of parents of children with other chronic diseases and share intervention strategies that have improved functional outcomes for children with other more common diseases, such as asthma or diabetes.

Summary and implications for long-term follow-up

As we review the various aspects of developmental outcomes in pediatric solid organ transplant recipients some common themes emerge. Although many recipients have completely normal cognitive function and perform well in school, a disproportionate number have below average intelligence, learning difficulties and problems meeting expectations in the classroom. Subtle physical deficits such as delays in fine motor skills and high frequency hearing loss can make it even more difficult for them to keep up with their peers. The stress related to coping with these differences

increases significantly as academic tasks become more complex and children progress through adolescence. The duration and severity of the chronic disease, be it dialysis, portal hypertension or congestive heart failure, clearly impacts outcomes. Socioeconomic and environmental factors likely have an influence as well and factors that limit a family's ability to secure special resources for their child may compound the impact of medical risk factors. For most transplant families, their strongest connection to the medical system is through their transplant team. However, even with input from transplant social service professionals, transplant teams may not always elicit a history of cognitive delay or school problems during routine post-transplant follow-up. Special attention to these aspects of the medical history should be incorporated into follow-up care with both their transplant physician and primary care provider.

Developmental testing is both expensive and labor intensive and thus difficult to justify for all transplant recipients. Yet, patients that would benefit from more rigorous testing to pinpoint deficits can be identified through systematic screening programs embedded into routine post-transplant care. Some suggestions for screening measures and appropriate referrals are included in Table 119.2. If concerns are found, referral for more comprehensive evaluation by the appropriate clinician as indicated is recommended.

Questions regarding physical and social developmental milestones and language acquisition should be routinely included in follow-up evaluation of children less than age four years. The older child, age five and above, should be assessed for difficulties in the classroom and frequency of school absence. In liver transplant recipients, age five to seven years, the question "PedsQL™4.0 Cognitive Function Scale" questionnaire correlates well with more extensive measures of executive function and can easily be administered in a clinic setting [75]. For more comprehensive assessment of executive function, the BRIEF can be used. Adolescents that have required special educational support while in the primary grades may struggle with many of the tasks required for self-management as cognitive function is a vital consideration in individualized transition of care plans. The ultimate objective for pediatric recipients is that they grow into healthy and productive adults. Interventions to help these children circumvent cognitive weaknesses would be expected to improve their functional status as young adults. Likewise, identifying specific problems such as borderline intelligence or diminished executive skills helps patients and their families set realistic expectations for career choices and long-term functional outcomes.

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CHAPTER 120

Nonadherence, Psychosocial Adaptation and Its Effects in Pediatric Transplantation

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Introduction

The World Health Organization defines medication adherence as "the extent to which a person's behavior (taking medications, following a recommended diet and/or executing life-style changes) corresponds with the agreed recommendations of a healthcare provider [1]". As this definition implies, there are numerous recommendations with which a chronically ill transplant patient is expected to comply, including attendance at medical appointments, having blood tests performed, and avoidance of alcohol or illicit drugs. In this chapter, we focus on non-adherence with immunosuppressive medications, with the understanding that if a family or recipient is not following other facets of the recommended care program, this could also have deleterious effects on the recipient's health or well-being. It will be apparent that non-adherence remains a major cause of late graft loss and patient death, and thus remains a critical target for acquisition of new knowledge and for trials of interventions to enhance adherence.

The burden of medication non-adherence on the health of transplant recipients

A Cochrane review, "Interventions for enhancing medication adherence" concluded that improving medication taking may have a far more profound impact on clinical outcomes than any discreet improvement in treatments [2]. Since there are currently no practical, reproducible ways to assure consistent transplant success without immunosuppressive medication, it stands to reason that medication adherence is a critical component of solid organ transplantation success. Indeed, in the overwhelming number of adult organ transplant recipients, medication adherence has been shown to be critical in the prevention of allograft rejection [3,4].

The success of transplantation in the modern era is due in large part to the more potent immunosuppressive agents available to combat rejection. Nevertheless, these advancements in immunosuppression come at a price. All of the currently available maintenance agents are administered orally, and need to be taken for the lifetime of the allograft. Transplant recipients who forgo taking their medication, even if it is only for a few days, run the risk of acute rejection and allograft loss, consigning the patients to the difficult life of dialysis [5–8] or death, in the case of other life-sustaining organs such as the heart.

In addition to the human costs, there are huge financial costs associated with medication non-adherence. It is estimated that the cost of non-adherence in solid organ transplant recipients ranges between $15 million to $100 million annually [9]. The median annual cost of maintaining a functioning kidney transplant is approximately $8500, while the cost after a failed transplant is about $51 000 [9]. Moreover, based on average costs, it takes approximately two and a half years of transplant function for the cost of transplant to be offset by the avoided costs of dialysis [10]. Similar analyses in Europe have come to essentially the same conclusions [11]. Further time of transplant function is a large economic gain to the healthcare system. In this time of concern about healthcare finances, improvements in medication adherence can have an important impact on healthcare spending.

Under-appreciation of the incidence and impact of non-adherence

The frequency and impact of medication non-adherence in solid organ transplant recipients is frequently underappreciated. Only a 2% non-adherence rate was reported in a group of pediatric liver transplant recipients to United Network for Organ Sharing (UNOS) [12]. However, a retrospective review found that 35% of graft loss was attributable to non-adherence [12]. There are other examples of the lack of recognition of medication non-adherence [9], yet scant attention has been paid to improvement in adherence [13,14]. Even the pediatric renal transplant community has been late coming to an acceptance of the impact of medication non-adherence. In the 2010 Annual Report of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS), almost 50% of grafts that failed did so because of "rejection". Yet, graft loss due to patients discontinuing their medicines was recognized in only 4.5% of graft losses [15].

Unique pediatric implications

Solid organ transplantation is widely accepted as the therapy of choice for children and adolescents with end stage organ failure. For example, kidney transplantation yields remarkable improvements in health and cognitive function that far surpass those seen with any form of dialysis [16,17]. The United States Renal Data

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

System (USRDS) data show that kidney transplant recipients live markedly longer than those on dialysis and this effect is most pronounced in pediatric patients, with gains in expected lifetime years of approximately 20 to 30 years [18]. The benefits of successful transplantation include improvement in growth and sexual development [19,20] and the ability to adopt the roles of a "well" person instead of the role of the chronically ill individual. In pediatrics, this translates into the chance to negotiate and enjoy the roles and responsibilities that come with maturation [21–23].

Despite all of these positive results, the incidence of medication non-adherence is estimated to be high in pediatric renal transplant recipients, particularly adolescents [24]. While renal allograft survival at one year is excellent for all children over one year of age, patients who are transplanted as adolescents, or who survive to be adolescents, show a greater decline in renal graft function and allograft survival over three to five years than younger recipients. At five years after transplantation, patients who are transplanted as adolescents have the worst allograft loss rate of any age group, including all adult groups [17]. The increased rate of graft loss in the adolescent transplant recipient appears to be due, in large part, to medication non-adherence.

Definitions of non-adherence

The WHO definition of adherence mentioned previously fails to address a number of issues that underpin failure to take prescribed medication. Non-adherence should not be seen as solely a willful defiance or careless behavior on the part of the patient. Rather, non-adherence represents a limitation in the delivery of healthcare. This may be the result of a failure between the medical provider and the patient to fully agree about the prescription in the first place, or it may represent a lack of identification and provision of the educational, emotional, or financial support that the patients need [25]. Thus, there are nuanced definitions of medication adherence that stress not only the patients' responsibilities, but also the caregivers' approaches.

Compliance

This designates the situation wherein the patient follows the medical team's dictated orders without necessarily buying into the reasons for these orders. This is the situation in which the adolescent transplant recipient often finds himself/herself.

Adherence

Adherence describes a situation in which the patient understands the reasons for the medical teams' orders and participates in their execution by following these orders. In pediatric and adolescent solid organ transplantation, this is often the best situation that is obtained.

Concordance

This is the theoretical goal in the adherence literature. With concordance, the patient's desires and incentives are aligned with those of the medical team. Thus, the conclusion is that the patient wants to do those things that the medical team believes will benefit him/her.

Operational definition of non-adherence in organ transplantation

The issues that surround medical adherence or compliance in solid organ transplant recipients are often difficult for patients and families to appreciate. On the other hand, those issues are more starkly drawn for the healthcare team. Chapman offered the following useful definition: "Non-adherence is the covert failure to take prescribed medication used for the prophylaxis of allograft rejection, which potentially results in impaired graft histology or function [26]". This definition has two important clinical features: (1) The non-adherence must be covert, and in the majority of cases of adolescent non-adherence, this condition is met. Occasionally, however, the adolescent is overtly expressing defiance through refusal to take the prescribed medication. Usually, however, the pediatric transplant physician and medical team cannot accurately identify when an individual is non-adherent;(2) The non-adherence should have a measurable effect on the clinical outcome of the graft for the behavior to be meaningful non-compliance [26].

There are a number of issues that make the literature in pediatric transplant non-adherence difficult to interpret. Matas and Nevins [8] asked pertinent questions: What is a robust, clinically useful definition of medication non-compliance [non-adherence]? How much compliance is enough? What about dosing delays [8]? Is there a true "threshold-effect", beyond which graft damage will occur? Because of individual differences in immune reactivity, organ immunogenicity, histocompatibility matching, drug metabolism and countless other factors (many of them undefined), it is sometimes difficult to apply hard-and-fast definitions.

As an example, consider the various definitions in the peer-reviewed literature about what comprises the quantitative aspect of "medication non-adherence". Greenstein and Siegal defined non-compliance as missing, forgetting, altering or delaying a dose at least once per month (2% of doses assuming twice daily dosing) [27]. Nevins et al. defined non-compliance as missing at least 10% of drug doses, that is, six doses per month [28]. Chisholm et al. and Blowey et al. defined non-compliance as missing at least 20% of doses, or 12 doses per month [29,30]. If taking doses of medications "late" is defined as non-adherence, what is the cutoff for appropriate timing [31]? In recognition of this conundrum, a recent consensus conference defined non-adherence in organ transplantation as "any deviation from the prescribed medication sufficient to influence adversely the regimen's intended effect" [9]. There is no doubt that all clinicians would strive for 100% compliance. Failing that, a clinician must fall back on the operational definition of organ pathology as a true marker of non-adherence and subsequent under-immunosuppression leading to acute or chronic rejection.

Prevalence of non-adherence with immunosuppressive medications in pediatric transplantation

According to a recent meta-analysis [32], approximately 50% of studies dealing with non-adherence in pediatric transplantation were performed in renal transplant recipients. Thirty percent of such studies were performed in pediatric liver allograft recipients, while 13% were reported in heart recipients. Thus, studies in kidney transplantation are by far the most frequent. Across all transplant types, the immunosuppression non-adherence rate was six cases per 100 patients per year. There was no significant difference between immunosuppressive medication non-adherence rates in different organ types [32].

It is difficult to compare the incidence/prevalence of immunosuppression medication non-adherence between adult and pediatric populations. Much of this involves the different methodologies and definitions of non-adherence from study to study. As a result,

meta-analyses containing the sum and substance of "all" the studies in the literature will often come to different conclusions. For example, as noted above, Dew et al. [32] found that the pediatric immunosuppressive medication non-adherence rate was only six cases per 100 patients per year, while in adults, the rate was between 19–25cases per 100 patients per year [33]. Other meta-analyses have come to different conclusions [24,34,35]. Prevalence rates vary greatly from study to study, ranging from 5% to almost 80% [36], reflecting variations in study quality, study methods, and definitions of non-adherence, among other factors. In pediatric kidney transplantation, the weighted mean prevalence across 16 studies dealing with immunosuppression non-adherence was 30.7% [34]. A previous study by the same authors compared the weighted mean prevalence of medication non-adherence over different solid organ types. This study reported a weighted mean prevalence of 32% for pediatric kidney transplant recipients, 30.8% for liver recipients, and 15.9% in cardiac recipients [24].

Numerous studies have identified adolescence as a prime age for non-adherence. The systematic review by Dobells et al. identified adolescence as the most worrisome time for medication non-adherence in pediatric transplantation. There is a significant difference between adolescents and younger children, with weighted rates of 43.2% and 22.4% respectively [24,32,34]. In the review by Dobells et al., adolescence was defined as age $\geq$10 years, unless otherwise designated. Other analyses have also identified that older pediatric transplant recipients (i.e. adolescents) are at higher risk for medication non-adherence [37]. High rates of medication non-adherence are also reported in adolescents with other conditions that require chronic medication such as asthma, HIV infection, rheumatoid arthritis and diabetes [38,39].

Well over 50% of pediatric renal transplants are performed in adolescents, especially when one extends the lower age limit to $\geq$10 years old [15,40]. In most studies, patients are characterized by the age at which they received their kidney transplant [15]. Thus, when patients are transplanted in adolescence, it has been impossible to know at what specific age medical complications occurred that might be due to medication non-adherence.

New data appear to clarify this. Foster et al. [41] have more precisely identified the ages at which adolescents experience graft loss. Using the USRDS database, and controlling for time post-transplant in their analysis, the authors demonstrated that beginning at approximately nine years of age, adolescent patients experienced year to year graft loss at an ever increasing rate until it peaked at an age of 19–20 years. They also demonstrated that in children less than 12 years old the rate of graft loss increases dramatically over the late post-transplant period, that is, when the patients reach late adolescence. The greatest incidence of graft failure occurred in patients aged 17–24 years of age. Foster et al. found that for children who were very young at the time of transplant, the highest rate of graft failure occurred some 13–17 years post-transplant, whereas higher failure rates were found in the years immediately following transplant among those transplanted during adolescence. Taken together, these data strongly suggest that adolescence, in and of itself, is a specific risk factor for graft loss. The reasons underlying these findings are by no means straightforward because the process of graft loss in pediatric transplantation is complex. None-the-less, the most obvious explanation of the findings of Foster et al. is medication non-adherence. Other immunobiological processes, including enhanced alloreactivity with older age in childhood and infection with environmental pathogens, particularly viruses [42–45], may also play an important role in allograft rejection and loss.

Psychosocial and developmental adaptation in pediatric and adolescent transplant recipients

Issues of non-adherence to medication after transplantation vary with the age and developmental status of the transplant recipient [32]. To understand how to facilitate adherence, the transplant team must consider not only the cognitive developmental stage of the recipient but also the developmental tasks for that age group.

Early childhood

Generally, issues of non-adherence for children under the age of seven are considered to be due to parental or social factors and have less to do with the child's level of understanding or co-operation [32]. Children below the age of three live in the moment, and express their pleasure or displeasure with the physical sensations they are experiencing. They do not understand why they need to see doctors or take medication, and can be very resistant. Most parents are comfortable making infants and toddlers take medication, and many are skilled at doing this. These very young children can be traumatized, however, if they have the experience that the adults on whom they depend are emotionally out of control, frequently angry, or physically overpowering for any reason, even if it is ostensibly for their own good [46,47]. Secure attachment (which predicts future emotional stability) is dependent on a predictable, responsive adult. Insecure attachment does not bode well for later adherence, as will be discussed further.

Children between the ages of three and about seven are in what Piaget termed the preoperational stage of cognitive development [48]. Although they are now able to understand relatively complex language, they do not have a good sense of cause and effect and they are unlikely to understand the need for medication. They are developing a sense of their own autonomy, and developing the interpersonal skills they will need for school. Very anxious parents of preschool- age children may understandably view transplant recipients as "vulnerable" and be extremely protective and overly vigilant [49]. Anxiety or even post-traumatic stress disorder (PTSD) on the part of the parents may be (at least temporarily) beneficial in terms of medication adherence [50]. However, the child who is kept in this dependent role when it is no longer medically or developmentally appropriate will be less able to participate in their care in the future. They may be non-adherent in a deliberate attempt to establish independence.

School age

Children ages 7–11 are more able to think logically and to understand the reasons for medication [51]. Piaget called this cognitive stage "concrete operational thinking". Clinicians and parents must be careful in the way they explain things, as school age children do not understand anatomy and tend to take instructions and explanations literally and view guidelines as rules [52]. School-age children can follow instructions, but do not have very good independent judgment, and are generally not well organized or responsible. Studies examining the role of school age children in post-transplant care have found that children under the age of 11 are generally starting to have some responsibility for their medications, but this is limited and often with supervision [53,54]. This supervision is quite appropriate.

Adolescence

Adolescents have the worst transplant outcomes, and much of this has been attributed to non-adherence [41]. This is interesting, because from a purely cognitive developmental standpoint, one would expect recipients over the age of 11 to be the most adherent. They are in what Piaget called the Formal Operational Stage, able to understand multiple causations of events and to reason abstractly [55]. However, the three most prominent characteristics of adolescent behavior are an increase in risk taking, increased sensation seeking, and a move away from parents to greater peer affiliation [56]. These behaviors set the stage for the post-transplant behavioral problems we term as "non-adherence".

Three major themes have emerged based on cumulative neuroimaging research on the developing brain of adolescents [57]. First, there appears to be an increase in associative cognitive activity as various areas of the brain become increasingly integrated [58]. The second structural theme is a general pattern of childhood peaks (such as synaptic proliferation) followed by adolescent declines (known as pruning). Thus the brain becomes "refined" [57]. The third, and perhaps the most important for our discussion, is a changing balance between competing neuronal networks as different cognitive and emotional systems mature at different rates [57]. There appears to be an increase in executive functioning, which encompasses a broad array of abilities including attention, response inhibition, emotional regulation, organization and long range planning. These functions appear to lie in the prefrontal lobe circuitry that is relatively late in maturing [57]. Over time, there appears to be an increasing proportion of frontal as opposed to limbic system activity for a number of cognitive tasks [59].

An additional contribution to non-adherence is the major developmental tasks of adolescence of separation and individuation [60]. Adolescents are even more attuned to what is acceptable to their peers than school-age children. They experiment to see which of their parents' values and expectations they wish to retain and what to reject. People in authority, like doctors and teachers, are questioned as a part of this discovery of what the teens believe and who they are [61]. An adolescent who questions the necessity of daily medication may try to see what happens if they skip a dose here and there. They find that stopping medication, whether deliberately or because they "forgot", has no immediate negative consequences. By the time rejection is detected it is often too late.

Young adults

The developmental tasks of young adulthood can also present challenges for adherence [62]. This is the age when young people are deciding on advanced education, careers, life-partners, housing and parenting. Limitations in any of these areas, perceived or real, may feel devastating to a young person. There is some suggestion, based on data from childhood cancer survivors, that people who were not symptomatic with PTSD as children or adolescents may become so as young adults, as they encounter these developmental tasks [63]. Non-adherence may reflect a desire that everything associated with the illness and transplant now be over, so they can return to the life they would have had if they had been healthy.

Factors associated with medication non-adherence in pediatric patients

Much has been written about the factors that are associated with immunosuppressive medication non-adherence [24,34,64]. Many factors have been identified (Table 120.1). It is important to realize, however, that the research methodologies used to identify the factors in question vary greatly between studies. Thus it is challenging to establish the validity or comparative predicative power of these factors. As a general rule, clinicians (as opposed to researchers in the field of medication adherence) use these factors as a rough guide to uncover non-adherence and address/relieve barriers to adherence that might be modified. Table 120.1 contains a list of risk factors associated with medication non-adherence. Many of the factors that appear in pediatric/adolescent patients overlap with those observed in adults. The factors that appear with the greatest consistency in the literature are discussed further on.

The WHO identified five distinct categories of risk factors for medication non-adherence [1]. These include socioeconomic factors, patient related factors, condition related factors, treatment related factors, and factors related to the healthcare system and team. A number of these factors fall into more than one category.

Socioeconomic factors

Into this category fall such issues as the cost burden of medication [65], low socioeconomic status [65–67], African American ethnicity [68], family instability, poor social support from parents [30,66,69], and single parent homes [66,67]. Other factors are detailed in Table 120.1.

In general, adolescents living in stable, supportive environments show better adherence with medical treatment compared to those growing up in an atmosphere of family conflict with lack of family cohesion [24,67,69–74]. It is also important to recognize that a patient's social support network may change over time. The medical team needs to continually evaluate the social support network as well as the patient him/herself. Caregiver "burn-out" can set the stage for worsening medication adherence [75,76].

Patient-related factors

These include those behavioral factors that would cause an adolescent to forgo medication administration. Some of these are shown in Table 120.1. Greenstein and Siegal [27] established a useful classification of some patient related factors. They identified at least three non-adherence patterns: accidental non-adherence which implies forgetfulness [30]; the invulnerable pattern [30,65], which may be common in adolescents; and the decisive non-adherence pattern, which can involve risk-taking behavior [24,77–79]. In adults, it appears that kidney transplant recipients are more likely to evidence non-adherence due to forgetfulness than to intentional behavior [80].

Other patient-related characteristics appear to be related to the experience of the transplant as a traumatic event or events, with resulting symptoms of PTSD [81]. For these transplant recipients, daily medication can serve as a traumatic reminder of all of their negative experiences. Avoidance of the medication provides some respite from the memories of hospitalization, illness, absence from friends, and blood tests. It can help them to feel "normal", a wish often cited in studies of adolescent transplant recipients [82]. Intervening with the post-traumatic stress may be useful in improving non-adherence in these situations [67]. Interestingly, parents can also experience PTSD [50] in response to their anxiety about their child's wellbeing [24,66]. The stress of the illness and necessary treatment can also result in clinically significant anxiety and depression [66,71,78], particularly if the recipient was depressed before transplantation. Recipients may have low self-esteem [65,71], which can be associated with non-adherence, particularly if the treatment is leading to an altered body image. On the other hand, the ability

Table 120.1. Factors that associate with medication non-adherence (Data from Wolff et al. [64], Dobells et al. [24,34], Butler et al. [123], Denhaerynck et al. [3] and Bunzel and Laedrach-Hofmann [211])

- Socioeconomic factors
  - Pediatric
    - Poverty or poor socioeconomic status
    - Race/cultural background (often a surrogate for low socioeconomic status)
    - Medication costs / inadequate healthcare coverage
    - Family dynamics:
    - **I.** Family instability / poor family cohesion
    - **II.** Single parent family
    - **III.** Lack of parental supervision / social support
    - **IV.** Poor intrafamily communication
    - **V.** Parental anxiety, overprotection, or PTSD
    - Social isolation
  - Adult
    - Younger age
    - Race / cultural background (often a surrogate for low socioeconomic status)
    - Lower educational background
    - Marital problems/divorce
    - Lack of social support
- Patient related factors
  - Pediatric
    - Poor Knowledge of:
    - **I.** Names and doses/ timing of medications
    - **II.** Functions of medications
    - **III.** Inappropriate health beliefs
    - Low self-esteem:
    - **I.** Poor Body Image
    - **II.** Not liking to carry medications with them
    - Forgetfulness:
    - **I.** Busy life style
    - Developmental delay
    - Age:
    - **I.** At transplant
    - **II.** At the time the patient is currently being followed
    - Psychological:
    - **I.** Previous non-adherence [222]
    - **II.** Depression / anxiety
    - **III.** PTSD
    - **IV.** Anger
    - **V.** School dropout
    - **VI.** History of child abuse
    - **VII.** Denial
    - **VIII.** Substance abuse
    - **IX.** Poor coping mechanisms
      - **a.** Feeling overwhelmed by transplant issues
    - Social:
    - **I.** Poor social skills
    - **II.** Problems with social adjustment
    - **III.** Deficient social support
    - **IV.** Reluctance to admit to friends that patient has a transplant
    - Stages of psychological development:
    - **I.** Risk taking behavior
    - **II.** Progression towards independence
      - **a.** Difficulty with resolving dependence on parents versus need for autonomy
  - Adult
    - Poor knowledge about their own healthcare
    - Health beliefs diverge from those of the medical team
    - **I.** External locus of control
    - **II.** Perceived lack of autonomy
    - Forgetfulness:
    - **I.** Poor organizational skills
    - **II.** Interference with life style/ work
    - Pretransplant non-adherence
    - Lack of motivation:
    - **I.** Low self-efficacy
    - **II.** Difficulties with self-care
    - **III.** Substance abuse

(*Continued*)

Table 120.1. (*Continued*)

Disease related conditions
Pediatric
Time:
I. Duration of illness
II. Longer time post-transplant
Feeling of good health:
I. lower perception of vulnerability
Never having been on dialysis
Adult
Depression
Longer time since transplant
Feeling of good health
Physical limitations secondary to infection or rejection
Treatment related factors
Pediatric
Cosmetic side effects
Complexity of medication regimen:
I. Total number of medications/pills
II. Higher number of doses during the day (i.e., thrice daily vs. twice daily)
Other attributes of medications:
I. Taste
II. Size of pills and difficulty swallowing pills
Adult
Concern about adverse effects of medications
Use of non-prescribed drugs
Increasing dosage frequency
Increased number of medications
Evening dosing
Healthcare system and team related factors
Pediatric
Communication between parents and healthcare team
Selective attribution by healthcare team:
I. Healthcare workers consider the non-adherent patient "bad"
II. Healthcare team unaware of the attitudes they may have which can affect the desire of the patient/parent to adhere to regimen
Adult
Lack of insurance
Inability to pay for car
Poor patient-provider relationship
Transition from pediatric to adult oriented care

to have a great deal of hope is associated with improved adherence, in both pediatric and adult patients [83,84].

It is important for pediatric transplant recipients to gradually take control of their own healthcare. However, a decline in adherence has been noted as pediatric transplant recipients assume responsibility for taking their medication, and the role of parents in administering medications is reduced [37,85,86]. Similarly, adherence declines during the transition of transplant recipients from a pediatric healthcare system to an adult healthcare system [87,88].

An individual who believes that health outcomes are controlled by chance rather than by their own actions (external locus of control) are more likely to forgo medications [89]. On the other hand, it has been clearly shown that adolescents who know the name of their medications, and the purpose of those medications, are far more likely to be adherent [90].

Disease condition-related factors

Non-adherence is more likely when the transplant has been functioning for one or more years [91]. This is generally a point when physicians feel that they do not need to see the transplant recipient as frequently. Unfortunately, this reduced interaction with the medical team is associated with decreased adherence by adolescents [92]. While non-adherence can be demonstrated early after transplant, as time passes the urgency of taking medications decreases, and adherence with medications also decreases [93–95]. Ironically, the feeling of good health that the adolescent perceives as a result of a successful transplant helps to encourage, rather than discourage the occurrences of medication non-adherence. Since there are no immediate adverse side effects when a dose of medication is missed, it is easy for the adolescent to assume that there will be no consequences for missing medications.

Treatment related factors

Treatment related factors include the characteristics of the medications administered and the complexity of the medical regimen. In pediatrics, bad tasting medications [86], medications that are difficult to swallow, or medications that cause adverse side effects [85] may be associated with non-adherence. The complexity of the medication regimen is also highly associated with non-adherence after transplantation [96]. This includes both the frequency of dosing and the number of pills that have to be taken.

Healthcare system and team related factors

The approach of the healthcare team is also an important factor in non-adherence. Wolff et al. [64] stress that the team needs to avoid selectively attributing all of the problems leading to non-adherence solely to the patient [34]. The messages sent by the healthcare team must be clear and unambiguous. As an example of one problematic type of interaction [34], a study found that a counterproductive message was sent by scheduling tests or follow-ups at a time which interfered with medication administration [97]. Some evidence

Table 120.2. Barriers to adherence [102]

Barrier Type	Examples	Parent reported barriers/Number/%	Adolescent reported barriers/Number/%
Total number of barriers		76	73
Forgot or distracted	"Not paying attention to how much is left"; "ran out"; "completely forgot"; "doing something else"	13/17.1	21/28.8
Poor planning and scheduling problems	"Keeping 24 hour pill rotation is difficult"; "On weekends, sleeping in"	52/68.4	42/57.5
Physical barriers or medication issues	"Too tired"; "Nauseous in the morning";	4/5.3	7/9.6
Voluntary resistance or attempts to be normal	"When I see my friends don't have to take it, I don't want to take it"; "Teenage lifestyle"; "Just not doing it"	7/9.2	3/4.1

supporting the role of the team is the finding that non-adherence rates appear to differ between transplant centers [34,98]. How the healthcare team relates to recipients and parents plays a key role in determining the presence and severity of medication non-adherence.

Healthcare system factors such as insurance to cover the cost of medications obviously have some interaction with socioeconomic factors. However, there are other examples of healthcare system factors leading to non-adherence. These include the mandatory transition of care from a pediatric to an adult facility at a specific age, and the loss of insurance coverage for a "child" as she/he turns 21 years old (or at whatever age is deemed the "transition" age) [99,100]. There is usually a system of mandatory transition, whether or not the patient is appropriately prepared for this. Since medication non-adherence increases when either a planned or unplanned transition occurs [53,87,88], it is in the patient's and healthcare system's best interest to provide an organized transition program that recognizes the risk for increased non-adherence at this critical juncture. This program must include financial counseling [100].

Barriers to adherence

The factors associated with non-adherence discussed above are those which have been noted in studies examining correlations of specific demographic, treatment and systems variables with a specific measure of adherence. "Another way of understanding what causes non-adherence is to ask transplant recipients and their parents what they see as barriers to adherence". One of the most frequently used measures of perceived barriers to adherence was developed by Simons et al. [85]. The authors queried 80 adolescent transplant recipients and their parents using standardized open-ended questions to identify important categorical dimensions of barriers to adherence, and described them in four categories [101].

The overall number of perceived barriers was significantly greater in the transplant recipients who were classified by the Medical Adherence Measure (MAM) [86,101] as non-adherent. Moreover, patients in this study who experienced a rejection episode reported a greater number of overall barriers. Table 120.2 shows that the two major barrier types described in this study were forgetfulness/distraction and poor planning and scheduling [101]. Non-adherence was also found to be more likely when adolescents, as opposed to parents, were responsible for administering the medication, and when taking morning rather than evening doses (ostensibly because they were late for school). These results are consistent with other reports that describe significant barriers to include cognitive barriers [102,103,104], problematic characteristics of the medication [103,105], and voluntary resistance to medication taking [104].

Simons and Blount have formalized the testing for barriers to medication adherence with two validated questionnaires based on this work. The Parent Medication Barriers Score (PMBS) and the Adolescent Medication Barriers Score (AMBS) [101]. The PMBS consists of 16 items with a Cronbach's alpha of .87 indicating strong internal consistency. There are four factor-analytically derived subscales: "Disease Frustration/Adolescent Issues" with seven items ($\alpha = .84$), "Regimen Adaptation/Cognitive" with five items ($\alpha = .82$), "Ingestion Issues" with three items ($\alpha = .69$), and "Parent Reminder" with one item. The AMBS consists of 17 items with a Cronbach's alpha of .86 indicating strong internal consistency. There are three factor-analytically derived subscales: "Disease Frustration/Adolescent Issues" with eight items ($\alpha = .84$), "Ingestion Issues" with five items ($\alpha = .70$), and "Regimen Adaptation/Cognitive" with four items ($\alpha = .76$). Each item is rated on a five point Likert scale ranging from "strongly disagree" to "strongly agree".

In an important follow-up to the perceived barriers research discussed above, Simons et al. in 2010 [106] found that these perceived barriers did not change over time. More importantly, the adolescent-perceived barriers of "disease frustration/adolescent issues" and parent-perceived barriers of "regimen adaptation/cognitive issues" were associated with poorer adherence to medication taking at follow-up. Medical complications and mortality were significantly associated with both parent and adolescent-perceived "ingestion issues" barriers.

Others have also studied the presence of barriers to adherence and the prevalence of medication non-adherence in pediatric transplant recipients. Gerson et al. [107] found that elevated levels of parental stress, dysfunctional parent–child interactions, and child behavior problems were associated with poorer medication adherence based on electronic monitoring of adherence. They also found evidence to support the relationship between subjective dissatisfaction with appearance and poorer medication adherence.

Tielen et al. proposed an alternative to identifying adolescent patients at risk for non-adherent behavior by investigating their attitudes about their post-transplant lifestyle [108]. They utilized a robust hybrid technique, which combines qualitative and quantitative measurements. This technique allows for the systematic study of subjectivity, beliefs, feelings, and opinion [109]. This type of Q methodology has been used in adolescents to study their health lifestyle attitudes [110]. Four distinct health lifestyle attitudes among young adult transplant recipients were identified: (a) concerned and controlled, (b) appearance orientated, (c) opinionated and independent, and (d) easy going and pliable [108]. This

self-categorization of these attitude types appeared to be of use in predicting non-adherence. While attitudes a, b and c were positively associated with adherence, it was suggested that attitude types b and c called for close medical professional monitoring. Bullington et al. [111] also used Q-methodology and found that three factors — medication issues (taste, side of pills, frequency of dosing and scheduling), "troubled" adolescent (poor home life, overwhelming medical situation, depression) and deliberate non-adherence (attention seeker, infallible attitude) helped to identify those at risk for non-adherence as well as informing management approaches. Pai reported the efficacy of the Allocation of Treatment Responsibility (ATR) scale, a brief measure of the distribution of treatment tasks across the family members of a child with a kidney transplant. In its initial report, this standardized and validated instrument measured the modifiable family processes, allowing for the development of targeted interventions [54].

The cost of immunosuppressive medications is a barrier to non-adherence that is common to both pediatric and adult transplant recipients. In the US, lack of continuous insurance coverage for outpatient immunosuppressive medications is a distinct barrier to successful medical management. In 2010, a joint project involving the American Society of Transplantation (AST), the United Network for Organ Sharing (UNOS) and the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) launched a descriptive study of transplant programs to establish the scope and magnitude of the problem [112]. Over 70% of all programs reported that at least 20% of their patients encountered difficulties in their ability to pay for immunosuppressive medications. Over 43% of all programs reported that at least 10% of their patients were not taking their medications because of cost issues. This issue is particularly acute in adults, since the main source of funding for adult recipients is Medicare, and Medicare only covers immunosuppressive medications for three years after transplantation. This problem of medication cost may be less acute in pediatric transplant recipients, because of a patchwork of aid programs in many states. Since the coverage of immunosuppressive medications is cost-effective, it has been argued that indefinite extension of Medicare benefits should be granted [113,114].

Consequences of medication non-adherence

The consequences of medication non-adherence are quite serious. In pediatric renal transplant recipients, the overwhelming data suggests that medication non-adherence is likely the major contributor of poor late allograft outcome [5,6]. The majority of studies establish the association between medication non-adherence and disastrous clinical outcomes including rejection and graft loss [6,32,115]. A recent literature review found that 14% of renal grafts were lost because of immunosuppression non-adherence [24]. In other pediatric solid organ transplant recipients, almost 16% of pediatric liver transplant recipients and 35% of heart recipients lost their grafts due to medication non-adherence [24]. Again, there was a preponderance of graft loss in adolescents [5]. In studies focused solely on adolescents, non-adherence accounted for almost 32% of allograft losses; in other studies examining a mixed pediatric/adolescent population, the rate was 14.1%. Immunosuppression adherence appears to be an important predictor of outcome in pediatric renal transplantation [115].

Studies have reinforced the message that medication non-adherence takes a huge toll in transplantation, both in clinical outcomes and economic burdens. Two studies have used large databases and calculated the "medication position ratio", a metric that is defined as the number of days that medication is supplied over a one-year period. This metric utilizes pharmacy records to compare the number of doses supplied to the number of doses that should be taken if adherence were perfect [116]. Pinsky et al. found that patients aged 19–24 years old had the lowest level of non-adherence by this metric [117]. Somewhat surprisingly, Chisholm-Burns et al., using the same metric, found that adolescents were more adherent than younger patients [115]. Both studies confirmed the poor clinical outcomes and the vast economic consequences that accompany medication non-adherence. In the model constructed by Chisholm-Burns, a 10% improvement in the medication position ratio would lead to an 8 % reduction in graft failure among children [115].

Late acute rejections often appear to be the result of medication non-adherence [4]. In adolescents, non-adherence was responsible for 23.2% of late rejection episodes, which are highly predictive of chronic graft failure [24]. Thirty-three percent of late acute rejections in pediatric liver transplant recipients [118–120], and over 70% of late rejections in pediatric heart recipients [121] are associated with medication non-adherence. Prospective studies in pediatric liver [79] and kidney transplants [122] have borne out these findings. Similar findings are present in studies of adult recipients [3,123]. Pediatric renal allograft recipients with outstanding adherence appear to have significantly better long-term outcomes than do adults [115]. In adult recipients, immunosuppressive medication non-adherence is significantly associated with progressive worsening of renal function [14] and late acute rejection [124]. The worsening of renal function over time occurs even in the absence of overt acute rejection [125].

Another consequence of non-adherence has recently come to light. In kidney transplantation, anti-HLA antibodies directed against the antigens of the donor, so called donor specific antibodies (DSA), have a strong relationship to antibody mediated rejection [126,127] and poor graft outcome [128]. Antibody mediated rejection, along with interstitial fibrosis, tubular atrophy and cell mediated rejection, in turn are prominently associated with medication non-adherence [125,128]. Thus, it seems likely that de novo DSA development might be a good indicator of medication non-adherence [129]. This has been suggested in our work in pediatric renal transplant recipients [130], and other recently published work [128,131].

Measuring non-adherence

There is no agreed upon definition of how much non-adherence is necessary for adverse clinical outcomes. The assessment of the damage done by medication non-adherence is confounded by the difficulty in defining and documenting clinically meaningful non-adherence. There also is also no currently agreed-upon measurement to precisely determine the outcomes of non-adherence. Transplant professionals need to discover non-adherence in "real time" which would thus permit intervention before the graft is irretrievably damaged [9].

Shemesh and Fine established some characteristics that would provide a practical and useful measure of adherence [132]. These recommendations include measures that: (1) would be easily integrated into clinical practice; (2) pose no additional burden on patients; (3) are directly measuring the ingestion of medication; (4) rely on as simple a procedure as possible; (5) are correlated with

an adverse outcome, such as rejection; (6) have the discriminatory power to raise a valid concern about a patient's behavior; and (7) provide evidence in real time (as well as post hoc) so that an intervention can be undertaken.

There are two broad categories of non-adherence detection methods: Direct and indirect [133].

Direct methods

These include directly observed therapy (DOT), measurement of concentrations of a drug or its metabolite in blood or urine, or detection of a biologic or electronic marker added to the drug formulation. As a general rule, direct measures of medication adherence are cumbersome, expensive, burdensome to the healthcare team, and possibly susceptible to manipulation by a resistant patient [133]. Without doubt, DOT is the most accurate method of assuring adherence. Often the parents can be the observers for a pediatric patient, but as children become adolescents, there may be less willingness to submit to DOT. Besides its general impracticality, DOT is susceptible to children "cheeking" the medication and then discarding the pills. Pediatric patients can become so adept at this behavior that they avoid medications even under direct observation in the hospital.

The use of drug levels of immunosuppressive medications has become standard practice. Calcineurin inhibitor (CNI) drug levels are routinely performed at clinic visits, but accurate adherence assessment using a single level is challenging [133]. It is not advisable to use a single aberrant level to make a clinical judgment. This is particularly true when one is assessing 12 hour trough levels. Patients may mistime their taking of medications, and the resulting level maybe abnormally high or low. Perhaps more importantly, non-adherent patients may try to disguise their non-adherence by taking a large dose on the evening before their clinic visit and/or blood level determination. All of this activity has been termed "white coat compliance" [134].

Thus, it is more logical to measure the fluctuation of medication levels over time to assess adherence. This has been tested in multiple pediatric liver transplant centers [67,132,135–137]. Specifically, these centers determined the standard deviation of a series of tacrolimus levels over time, and found that a standard deviation (SD) above a given threshold was significantly associated with late rejection [132,137]. This approach correlated well with results of electronic monitoring (see further on) [138]. Pollack-BarZiv et al. extended the SD approach to children with other solid organ transplants, and found similar results with regard to late rejection [139]:

> We utilized this standard deviation (SD) technique to examine its use in 46 renal transplant recipients. We found that the median SD was 5.3 in patients with rejection and 3.5 in patients not experiencing rejection (P = NS). We then used the coefficient of variation (CV% = SD/mean multiplied by 100) as a marker for variability in tacrolimus levels [122]. In the 46 adolescent recipients, the median tacrolimus CV% was significantly higher in patients with rejection (53.4%) than those without rejection (30%) ($P = 0.005$). There was no overlap of discriminatory values. Importantly, we were not able to find any similar relationship when studying mycophenolic acid levels, and so this technique appears for now to be restricted to tacrolimus [122]. Follow-up studies solely in adolescents validated this approach and established that a CV% $\geq$ 31% had a significant association with late rejection [140].

Perhaps the most direct, albeit the most invasive and impractical method for diagnosing clinically significant non-adherence is the renal biopsy. Protocol biopsies performed at standardized times can reveal "subclinical" rejections [141,142] and, as noted above, this may be the first evidence of medication non-adherence. While the usefulness of protocol biopsies in kidney transplantation is outside the scope of this discussion [143], the presence of biopsy proven acute rejection (BPAR) is the *sine qua non* for measuring allograft damage and thus a direct mode of identifying clinically significant non-adherence with immunosuppressant medications [26,144]. The use of protocol biopsies is also standard of care in many heart transplantation programs [145].

A new technology holds promise as a direct measure of adherence [146]. Ingestible event markers (IEMs) are tiny, digestible sensors made from food ingredients, which are activated by stomach fluids after swallowing. The IEMs are manufactured at "wafer scale" on silicon and are therefore extremely economical to produce, costing a few cents per sensor in large quantities. Once activated, the IEM creates an ultra-low-power, private, digital signal detected by a microelectronic recorder configured as a small bandage style skin patch. The patch is easily placed over the stomach. The detector date- and time-stamps, decodes, and records information. This information can then be uploaded to a patient's cell phone. From there, the information is further uploaded to a "Cloud" system, which is then downloadable to investigators. This technology is currently in use in clinical trials, and has been approved by regulators in the European Union and the FDA. As of this writing, the information about this technique is only available in abstract form [146]. Clinical trials in transplantation have been in progress and the first publications are awaited eagerly.

Indirect methods

These assessments of medication adherence are generally recommended for use as sets of measures to better identify ("triangulate") the presence of non-adherence [31,147,148]. Currently, the approach of using a multidimensional "toolbox" may not be fully practical or evidence-based [149], but it is fair to say that there is a consensus in the literature that this is an effective way to study and monitor medication non-adherence [31,150,151].

Patient (and parent) self-report scales

These are perhaps the easiest and simplest measures of medication non-adherence [152]. Their advantages include: (1) simplicity, (2) low cost, (3) ease of administration, (4) usefulness in clinical situations, (5) patients who are self-reporting are likely to be truthful, and (6) self-report can give insights into social, situational and behavioral patterns that are leading to non-adherence [31,153–157]. Disadvantages include: (1) self-report overestimates adherence, (2) inaccuracies due to recall bias, social and medical desirability ("telling the doctors what they want to hear"), (3) wording of questions can be confusing, (4) timeframe of adherence recollection can affect accuracy, and, (5) requires a non-judgmental approach [153,154,156–158]. The inherent difficulty with self-report, overall, is that the patient or parents may not represent their experience accurately [152]. In comparing methods, self-report tends to underestimate the actual incidence of non-adherence, as assessed by other measures [151].

There are many adherence self-report questionnaires in the literature, and no single measure that is universally regarded as the

most useful. As might be expected, there is much more literature that deals with adults in this area. An exhaustive meta-analysis identified three self-report instruments that achieved appropriate reliability, validity and responsiveness in adults and could be recommended for clinical transplant practice [31]. These tests included the Basel Assessment of Adherence to Immunosuppressive Medication Scale (BAASIS) [159] [31,160,161], The Brief Antiretroviral Adherence Index [162–164], and the Medication Adherence Self-Report Inventory (MASRI) [165,166]. These latter two indices are validated for AIDS, and would need to be validated for transplantation as well. These instruments were considered best for transplantation clinical care because [31]:

1 These instruments take into account both the taking and regularity of medication intake.
2 They are simple to use and easy to score, making it appropriate for busy clinical settings.
3 All instruments have been validated and have shown good psychometric properties.

The BAASIS appears to be particularly appropriate for use in pediatric transplant recipients because it was designed for transplantation. This questionnaire can be finished in approximately five minutes, rendering it extremely practical. Interestingly, this instrument has also been used successfully in a pediatric renal transplant trial [167].

Reliable instruments specifically for self-report in pediatric transplantation have been slower to develop. The MAM, mentioned above, assesses adherence over a wide range of health behaviors and barriers [168]. It has been reported to be particularly useful since it focuses on the timing of medication administration as well as the medication-taking itself [34].The Basic Medical Questionnaire (BMQ) also assesses self-efficacy, making it useful in the post-transplant clinical setting [169,170]. It asks directly who is responsible for medication administration (the patient vs. the parent/guardian vs. a shared responsibility). This questionnaire thus yields information about the administration (or lack thereof) of medications and the possible reasons for immunosuppressive medication non-adherence.

Pill counts

Despite their apparent simplicity, pill counts are notoriously unreliable as true measurements of adherence [152]. It is easy to discard medications and return empty medication bottles, falsely suggesting good immunosuppressive adherence [171]. When measured against electronic monitoring devices, pill counts have been shown to underestimate non-adherence [172]. In certain low resource areas, pill counts and pharmacy refill records (see further on), have been used with HIV treatment, but it has been shown that these measures must be supplemented with direct measurements of adherence to be effective [173].

Prescription refill rates

These can be powerful tools in adherence research when a large database is available [115,117]. It can also be useful in individual patients [174], particularly if there is a closed pharmacy system and/or the use of mail ordering of prescriptions and refills [175]. On a local level, one can use refill rates in as many as four different pharmacies [176]. However, in an environment of multiple managed care organizations, the logistics may be challenging. This could change with more extensive use of electronic medical record systems that communicate with one another. A larger concern lies with the defiant adolescent, since the absence of a pill does not indicate the ingestion of that pill.

Electronic monitoring

Electronic monitoring systems have been touted as the "gold standard" for measuring medication non-adherence, and are recognized as the best of the indirect methods to detect non-adherence [97,122,146]. A continuous microprocessor, usually attached to the cap of a medication container, records each container opening as a presumptive dose and documents the time and frequency of the medication event by date and time stamping [28]. Recorded data can then be retrieved and an assessment of adherence made on the assumption that these medication events correspond to drug ingestions and that absent openings correspond to missed medications [28,94,152]. While a number of electronic monitoring systems are currently in use, the one most commonly cited in the pediatric non-adherence literature in the Medication Event Monitoring System (MEMS, Aardex Ltd). MEMS utilize an on-line transfer of the dosing history at each clinic visit to a secured server. The data is processed according to several predefined and validated algorithms. The Web portal displays reports (e.g. calendar plots, chronology plot) with adherence information. At the end of the study a report is generated that a study statistician can analyze.

Electronic monitoring has been used in studies with renal transplant recipients [28,94,152,177]. These data have successfully identified early non-adherent patterns, which carried through to the late post-transplant period [94]. This method is thought to provide the most accurate way to detect non-adherence [123,177,178] in that, when used correctly, the dynamics of medication taking are clearly recorded. This modality has been used successfully in pediatric patients to measure adherence [107]. Unfortunately, this methodology is expensive and difficult to implement in clinical practice (see further on). Its greatest use has been in clinical trials examining possible interventions for medication non-adherence to implement in clinical practice.

A recent study meticulously examined several factors that could jeopardize the validity of electronic monitoring studies in adult transplant recipients [179]. The first factor is the requirement that the equipment functions properly. Early studies in kidney transplant recipients did not permit an assessment of the exact number of non-functional caps [98]. In the study of Denhaerynck et al., the system was functionally reliable, with a failure rate of $\leq$0.5% [179].

The second factor in electronic monitoring reliability is the assumption that each time the patient unscrews the EM-bottle cap he/she also ingests the prescribed dose, and does so immediately. This may not be the case however. More or less can be removed, and pills ingested then, later or never, thus underestimating non-adherence [178]. It is also possible for patients to correctly ingest the medication from a source other than the MEMS bottle, or from a supply of pills previously removed from the MEMS bottle (perhaps because of impracticality or embarrassment), resulting in overestimation of non-adherence [98,180,181]. While this can be ameliorated to some degree by asking the patient to keep a diary of possible discrepancies [4,181] patients who are non-adherent, and particularly adolescents, are likely to keep poor records [182] or deliberately obfuscate the process. Denhaerynck et al. evaluated this approach with the electronic monitoring system and found that with adults who are accurately keeping a diary, accurate identification of adherence occurred in over 96% of the correctly dosed days [179].

The third factor to consider in assessing MEMS reliability is the "Hawthorne" effect [183,184]. This is the tendency for a patient's behavior to change in response to the awareness that he/she is being studied. The observed assessment cannot be assumed to reflect behavior when the patient is not being studied. Medication adherence may be enhanced because of study participation [181]. However, it is also possible that medication adherence is hampered because the patient who uses a daily pillbox is prevented from doing so by the constraint of the MEMS bottle [185]. Previous investigations of the impact of MEMS on adherence by Denhaerynck and others have been inconclusive [179,186].

The last concern about the validity of electronic monitoring regards selection bias. The use of MEMS requires commitment, and because of that, only motivated patients with increased adherence would join or persevere in a MEMS study. Thus, an accurate sampling of the population would be impossible [182,185]. Denhaerynck et al. could not confirm or deny the presence of such selection bias [179].

Overall, it is appropriate to conclude that the MEMS system is a potentially powerful tool, yet several factors can impact the devices' utility [185] and validity. It is therefore important to recognize the limitations of the "gold standard" for adherence measurement [187]. MEMS caps are generally inappropriate for younger patients who are receiving liquid medication. In the study by Shellmer and Zelikovsky [187], 41% of patients reported that it was onerous to transfer their medication to the designated pill bottle, 27% felt that the pill bottles were a burden to carry, 22% thought it changed their routine, and 10% worried it would cause them to miss their medications [185,187]. This led 51% of patients approached to refuse to participate in the MEMS study. Nevertheless, only one of 27 recipients who were using the MEMS technology stated that it could increase the possibility of medication non-adherence. One study experienced difficulty with MEMS as a measure of adherence, since 40% of adolescents simply did not return the caps with the memory chips [84]. Strategies for achieving greater acceptance of MEMS are described by Shellmer and Zelikovsky [187].

A number of studies investigating non-adherence have used multiple measures to refine their accuracy [147,148,151]. The combination of self-reported non-adherence/or at least one clinician reporting non-adherence with assessment for sub therapeutic blood levels had a sensitivity of 72% and a specificity of 43% [151]. It will be important in future prospective studies to utilize and validate direct and indirect measurements such as DSA, the IEM, anti-HLA antibodies and renal biopsy, alone and in concert, to determine the most accurate combination of measures.

Interventions to prevent or improve non-adherence

There are a very large number of descriptions in the medical, psychological, behavioral and nursing literature describing interventions to avert or improve medication adherence in chronically ill populations (both pediatric and adult). Numerous types of interventions in transplant populations have been proposed. As a general rule, these interventions are tailored and targeted to address the factors that are associated with non-adherence and the barriers that are identified as impairing medication adherence. Interventions include, but are certainly not limited to: educational approaches [53,69,188,189]; behavioral approaches [39,190] including contracting [191]; removal of financial barriers to the purchase of immunosuppressive medications [192]; and multiple studies utilizing electronic and digital technology [187,193–196].

Disappointingly, there is only a weak evidence base for identifying successful interventions to prevent or correct immunosuppressive medication non-adherence in transplant recipients [34,197,198]. In adult transplant recipients, a few medication non-adherence studies with a methodologically sound and randomized controlled design have been published [24,198,199]. This having been said, there are a number of interventions available which have been used to address non-adherence with other types of medications or treatments. But even here, the rigorous evidence base is often lacking.

The lack of an evidence-based approach in transplantation is illustrated by considering the KDIGO (Kidney Disease Improving Global Outcomes) Guidelines for Care of Transplant Recipients [200]. The expert panel could not even grade the quality of evidence for their recommendations for the management of medication non-adherence [201]. Many of the interventions described in the KDIGO Guidelines or elsewhere represent "common sense", based on the recognition of the factors that are associated with non-adherence as opposed to an evidence base [202].

When evaluating interventions for success in medication non-adherence, there are two basic types of outcome measures. The focus can be on evaluation of the true adherence behavior to the medical team's directives. However, if the concern is primarily with clinically significant non-adherence, biological outcomes will be the most useful measure [203]. This is a common procedure in pediatric HIV research [204,205] where adherence can be inferred by improving CD4+ T cell counts or decreasing viral loads. The success of kidney transplantation adherence interventions could be evaluated using frequency of late rejection episodes, allograft survival, or biopsy results.

As a guiding first principle, it should be clear that there are different patterns of medication non-adherence, from occasional mistiming of medications, to severe timing infractions, through occasional missed doses, to drug holidays/frequent severe timing infractions, all the way to outright refusal to take any doses [28,206]. Therefore, it should be clear that with numerous types of non-adherent activity, many behavioral "predictors", and multiple barriers to adherence, a "one size fits all" approach is impractical and doomed to failure. Interventions must also be tailored for the developmental stage of the recipient [207,208]. The lack of immediate consequences for a missed dose and the individual variations in immune responsiveness contribute to a tendency of adolescents to question the instructions of their parents and the healthcare team, as the teen strives to be an independent decision-maker [209]. There is a strong tendency for adolescents to focus on the "here and now" [206]. They have difficulty in abstract thinking about the long-term. Specifically, there is no immediate pain or suffering that the adolescent experiences when an immunosuppressive medication is missed. And since immune responsiveness differs from individual [208] to individual, there is also no current biological test that can give reliable immediate feedback. In kidney transplantation, the medical team still relies on serum creatinine levels, which are both insensitive and nonspecific. The lack of reliable immediate feedback hampers the effort to support immediate detection of immunosuppressive medication adherence and prompt intervention.

Even within adolescence, interventions must be constructed that take into account the specific developmental stage of a given patient [205]. Regardless of chronological age, different adolescents may function differently along the developmental spectrum. Adolescents are striving to become independent decision makers, but they often develop negative behaviors in an attempt to become

decision makers independent from their caregivers/parents [209]. Further problems may develop because different members of the healthcare team may differ in their assessment of an adolescent's competence to be a constructive and motivated autonomous decision maker [209,210]. It is therefore incumbent on the healthcare team to carefully craft the non-adherence intervention — it should have a grounded base in theory and specifically address all of these challenges. With these caveats in mind, we will describe a series of classifications of interventions that have been discussed in the literature, and their practicability in clinical practice.

Pretransplant evaluation and behavior modification

It would appear intuitive that pretransplant medication non-adherence, as well as other behavioral and psychosocial issues, might predict post-transplant non-adherence [211]. Surprisingly, there is a paucity of data on this subject [212]. There appear to be conflicting views on the subject [212]. A report examining the predictive value of previous non-adherence was limited by its retrospective nature and reliance on weak criteria to define pretransplant non-adherence [213]. On the other hand, in theoretical models designed to explain health behavior, past behavior can be a powerful tool to predict future behavior such as medication non-adherence [212,214]. Pretransplant evaluations are the first clinical opportunities to examine possibilities of medication non-adherence. Even if pretransplant behavior is predictive, it is not entirely clear that pretransplant clinical interventions will significantly modify post-transplant non-adherent behavior [215–219].

On the other hand, a recent prospective study by Dobbels et al. [220] in adults suggested that pretransplant medication adherence, poor social support for medication taking, low scores on the personality trait "conscientiousness", and higher education levels predicted post-transplant non-adherence and poor graft outcome. The finding of *higher* education level as a predictor of non-adherence came as something of a surprise, since other studies suggested that *lower* educational levels were associated with non-adherence [221]. Most notable was the finding that pretransplant non-adherence was a strong predictor of late acute rejection episodes [220].

The KDIGO guidelines on kidney transplantation [200] recommend preventative interventions for non-adherence, including the provision of "education, prevention and treatment measures to minimize non-adherence to the taking of immunosuppression medications, and to increasingly screen those patients at increased risk for non-adherence" [215]. However, the specific ways that these general pretransplant interventions might be implemented are not detailed. The KDOQI commentary on the KDIGO Guidelines for pretransplant interventions comments on the need to address factors suggestive of post-transplant non-adherence. However, they point out that KDIGO offers little guidance or evidence except to state that it is important to involve multiple disciplines in the provision of appropriate interventions [216]. It has been proposed that the pretransplant evaluation for adult kidney transplantation should consider non-adherence to the dialysis program and medication taking, and should delay transplantation until suitable adherence is achieved [14]. However, non-adherence in one area does not guarantee non-adherence in another. For example, studies in liver transplantation have found that substance abuse (viewed as a non-adherent activity) in the pretransplant period did not necessarily eventuate in medication non-adherence [217,218].

The Transplant Program at the University of Minnesota has reported their experience with a protocol whose results may inform the construction of pretransplant assessments and interventions. The protocol describes the steps that must be considered for a second renal transplant for patients who have failed a first transplant because of non-adherence. This protocol was first described in 1995, and updated in 2009 [218,219]. While these reports do not describe a clinical trial, they utilized a historical control group and represent the only large published clinical experience. The 114 patients who lost their first renal allograft to medication non-adherence, documented by self-report, underwent a complete re-evaluation, which included an educational class and a visit with the full transplant team. The specific evaluation components were: (1) an individualized discussion with the candidate and the transplant coordinator, nephrologist and surgeon, dealing with the reasons for the prior non-adherence and how to prevent subsequent non-adherence; (2) an evaluation by the transplant social worker and neuropsychologist to identify additional factors that could lead to non-adherence; and (3) a point by point discussion with the candidate laying out the conditions that must be met before transplantation is offered [219]. Major criteria that had to be fully met in the Minnesota protocol included: (i) full adherence for at least six months to the entire dialysis, medication/laboratory and other testing, and clinic visits; (ii) completion of all recommendations by the social worker and neuropsychologist; (iii) the amelioration of those factors that were identified as contributing to the prior non-adherence; and (iv) in cases where a living donor is planned, full discussion of the entire protocol with the prospective donor. A final consultation was scheduled to assure the medical team that the candidate fully understood and planned to implement the requirements and terms for transplant. This entire pretransplant intervention is not unlike that practiced at many transplant centers.

Seventy-five of the 114 (66%) patients initiated the pretransplant screening and intervention protocol. Of these 75, 48 were able to satisfactorily complete the evaluation/intervention protocol, and 37 of the 48 had received kidney transplants at the time of this report. The authors found no significant differences between these patients and the historical control group with regard to patient survival rates, death-censored graft survival, or chronic rejection-free survival, although there was a suggestion of a trend towards differences in the latter categories. There was a significant increase in acute rejection episodes in the patients who had manifested non-adherence with their first allograft. However, there was no increase in the incidence of late acute rejection, a hallmark of medication non-adherence. This appears to be contrary to other reports [220]. Unfortunately, there appeared to be some important durability in the non-adherent behavior, notwithstanding the pretransplant interventions. In the group of previously non-adherent patients, 14% lost their retransplant to non-adherence, while only 2% of the control retransplants lost their grafts to non-adherence [219]. Moreover, 57% of the originally non-adherent retransplant recipients exhibited non-adherent behavior.

This study raises some important questions about the extent to which pretransplant interventions can be effective. On the one hand, it is disappointing that non-adherent behavior persisted in those patients with prior non-adherence after retransplant [222]. On the other hand, we are not given sufficient information from the Minnesota study (e.g., the presence of anti-HLA DSA) and the study did not really have enough power, nor was it sufficiently controlled, to fully answer the question of whether their pretransplant interventions were effective. Indeed, the clinical outcomes were acceptable, if somewhat less than optimal.

Taken together, what conclusions can be drawn about pretransplant interventions to prevent non-adherence? It seems reasonable to conclude that a necessary part of the pretransplant evaluation is the investigation of candidate's adherence status. Every pretransplant candidate should be considered at risk for non-adherence [222]. Prior to transplant, the patient's adherence should be repeatedly assessed, ideally by individuals who are skilled in identifying non-adherence and making use of available time, such as while the patient is on dialysis. Interviewers should explore the underlying reasons for a patient's problematic behavior. The pretransplant team (particularly the social worker, psychologist and transplant coordinator) should implement tailored interventions to target any underlying issues that can be identified, using the factors outlined previously [222] (see Table 120.1).

In the pediatric/adolescent candidate, pretransplant interventions that seem to be appropriate (notwithstanding the absence of a strict evidence based evaluation) should include:

- Educational intervention methods to instruct adolescent and caregiver(s) about post-transplant medical regimen and importance of consistent, and long-term adherence.
- Interventions to remediate skill deficits (e.g. organizational strategies, public transportation use, thermometer reading, and lab value interpretations).
- Treatment of identified emotional and/or behavior problems.
- Interventions to improve provider/patient relationship and communication.
- Interventions to increase/optimize social/emotional support for adolescent.

Pretransplant educational interventions should also follow the following guidelines:

- Information should be given by several different educators.
- Specifically address issues of medication knowledge, side-effects, and timing.
- Emphasize reconciling health beliefs between patients/parents and health providers.
- Approach the patient in an understanding manner.

We use an approach that acknowledges that adherence is sometimes difficult ("I know you must find it difficult to remember to take your medications when you are out with friends or are in school"), and work with him/her to acknowledge the barriers, so that they can be addressed.

Siegal and Greenstein have identified three patterns of medication non-adherence [223] that have briefly been described above. The following recommendations about the type of intervention are tailored to address these specific patterns or circumstances:

Accidental

These individuals tend to have strong beliefs that the immunosuppressive medications are effective and understand that the medication administration should not be missed or delayed. They tend to believe that the mode of action of the immunosuppressive agent in question is limited to 12 hours. These patients need interventions that work to remind the patient to take the medication in question. Pillboxes for each day that are filled once a week can be helpful, as can cueing techniques. They also require counseling on integration of their medication with their lifestyle.

Invulnerables

These patients tend to be younger, less well educated and have had their kidney transplants for a shorter period of time than the other groups. These patients have a high tendency not to believe in the efficacy of the medications or the need to time their medication administration accurately. Educational interventions need to address the long-term effects of medication non-adherence.

Decisive non-adherers

These patients tend, on average, to be more highly educated or, if adults, have white-collar jobs. They are accustomed to making independent decisions and understand the need for the immunosuppressive medications, but believe that the effect of the medication continues for >24 hours and further believe that an occasional late or missed dose does not have great import. Interventions with these individuals need to take into account their independent decision making habits.

Psychological distress

A number of patients may not fall into any of these categories, but instead may suffer from psychological problems such as depression about their disease or PTSD [67]. These issues must be identified and addressed before transplant if possible. It is possible that caregivers, such as close family members, may suffer similar disorders and require care as well [50].

Peritransplant and post-transplant interventions

As with pretransplant interventions, every transplant recipient should be assumed to be at risk for medication non-adherence [92,222]. Thus, it is important to utilize every post-transplant clinic visit to reinforce adherent behavior, both with medication administration, and with other health activities, clinic visits, and healthy lifestyle choices. The medical team must continually educate, as well as to introduce other measures to maximize adherence because results are more pronounced in the near term after a discreet intervention [39]. As time goes on, the results of a discrete intervention diminish with time. This is certainly in keeping with findings that interventions, particularly psychological treatment effects, tend to weaken over time [224]. Some overall guidelines for preventative interventions are listed below.

1. Interventions involving the medical team's communication with the patient/family:
   - Adopt an "Interactional Model", not a "we/they" approach; *Listen* to the patient/family.
   - Assume a non-judgmental approach.
   - Avoid selective attribution (the "fault is the patient's") [64].
   - Stress a "team" approach — parents are part of a team to help "coach" (remind), but not to control; healthcare professionals are part of team.
   - Have different team members develop "personal chemistry" with specific patients. These connections should be fostered during the interactions between medical staff and patients, especially adolescents
2. Interventions after transplantation attempting to forestall non-adherence:
   - Continual education with every visit.
   - Written instructions that are modified as time goes on.
   - *Recognize and address* patient and parent psychological and social problems — depression, anxiety, insurance changes, school counseling.
   - Essentials for discussion:
     i. medications have an effect only for 12–24 hours
     ii. delay or postponing medication will not have immediate consequences, but will in the long run. Damage is not immediately noticeable, but it is slowly cumulative.

The descriptions of interventional studies in the literature are often incomplete [225–227]. In a recent review by De Bleser et al. [198], 12 studies were identified that dealt with research on interventions for non-adherence in the transplant recipient. The majority of these studies merited only a weak or moderate ranking [198]. We will review those transplant-specific interventions below. Only peer-reviewed publications will be outlined. Interventions were classified as either: (1) educational/ cognitive: conveying information; (2) counseling/behavioral: the changing of behavior to empower patients to participate in their care and develop new skill sets of self-care; (3) psychological/affective: strategies dealing with feelings, emotions or social relationships; and (4) mixed interventions which utilize a combinations of these [188,197].

Educational/cognitive interventions

In the non-adherence literature dealing with pediatric patients taking medication for chronic diseases (but not transplantation), a recent meta-analysis found that educational interventions were an important component of every adherence enhancing intervention [228]. A study of 21 pediatric transplant recipients, 17 of whom were adolescents, was not as positive [72]. Twenty-one patients and/or parents were counseled at every clinic visit, which averaged about every two months. Written reinforcement of the counseling was provided, as were medication calendars, schedules and pamphlets. The authors concluded that the medical education program improved patients and parents knowledge of their medications, but did not increase compliance significantly. However, since this study contained a small sample size, and utilized a methodologically flawed measure of medication adherence, this report should not be taken as a blanket indictment of education as a tool to improve adherence.

Annunziato et al. [53] evaluated an educational intervention targeting the transition from parent-provided care to self-care. Twenty-two pediatric liver transplant recipients were enrolled for a two session educational study. Ten were enrolled because of a perceived difficulty in transitioning care from caregiver to patient, while 11 patients were enrolled sequentially without any predetermined entrance criteria. As judged by improvements of the standard deviation scores for tacrolimus levels, the educational interventions significantly improved adherence in the patients who were perceived to have had difficulty in transitioning from parental-care to self-care. However, adherence was not significantly improved in the group who entered the study without any perceived difficulty in the transition process. While the study sample was small, and there was no control group, the report did suggest that a targeted educational intervention aimed towards patients and families who were having difficulty in transferring responsibility to adolescents from parents has potential merit. In another study, verbal prompts by the care giver and verification that medication was taken were both related to higher adherence rates [229]. Klein et al. [188] examined educational strategies building on previous studies that suggested that clinical pharmacy services could positively impact medication compliance in renal transplantation [224], as well as other areas of medicine [230]. In a prospective, randomized controlled trial, the authors studied the effect of a 12 month pharmaceutical care program addressing medication adherence in 20 liver transplant recipients, compared to 21 control patients. Patients in the intervention groups received educational counseling about the immunosuppressive medications one week before the transplant and every one to three months after transplantation. Non-adherence was measured by electronic monitoring (MEMS), "immunosuppressive medication levels", pill counts, a Morisky questionnaire [231] and self-reports. The intervention group showed statistically significant improvements in adherence in virtually all measures, particularly in the electronic monitoring group. The number of patients was small, and there were patient dropouts because of the cumbersomeness of the MEMS. In addition, the authors could not correct for a Hawthorne effect. However, when taken in concert with a previous study [224], these findings strongly suggest that pharmacists can play a valuable role in an educational adherence intervention.

By contrast, Dean et al. found that in children and adolescents, educational interventions alone are insufficient to promote or sustain optimal adherence [232]. This was confirmed by the analysis of Kahana et al. [39]. Another, meta-analysis of adherence in chronic disease concluded that educational programs alone did not in themselves improve adherence or positively affect outcomes [233]. It is appropriate to conclude that educational interventions are necessary components for success, and can be effective when targeted, but are not sufficient to prevent medication non-adherence [234–236].

Counseling/behavioral interventions

Hardstaff et al. [190] studied the impact of a strictly behavioral/ counseling intervention with 75 kidney transplant recipients who were >1 year out from surgery. All patients used the MEMS system. The patients were randomized to either receive feedback about their MEMS results after their first clinic visit, or receive no feedback. Neither group received any further feedback for the rest of the 12-month trial. The one session of feedback resulted in no improvement in medication adherence. It may be that the intervention was too brief, or not targeted. A number of other negative studies dictate that more research is necessary [231,236–238]. However, the pediatric HIV literature strongly suggests that a behavioral component may successfully enhance outcomes [39,237]. It would appear from the literature that continued assessment and feedback to the patient has the highest likelihood of success as a behavioral intervention [233], and such strategies should be strongly considered in mixed interventions. In the adult chronic disease literature, there are successful interventions using operant conditioning with financial reinforcement principles [238]. In other words, patients are paid for being adherent. A similar concept was attempted in substance abusing adolescents, where prizes were awarded for desirable behaviors. This proved effective [239]. It is reasonable to suggest that such positive reinforcement could be a part of a successful adolescent adherence intervention, so long as the appropriate reinforcement moieties are chosen. It has been demonstrated that for the chronically ill adolescent, the personal significance of the illness and the treatment, and the degree of individual motivation, markedly affect adherence [240].

Psychological/affective interventions

There have not been any significant intervention studies with pediatric transplant recipients using only psychological/affective interventions [196], although a pilot study suggested that a targeted intervention for PSTD was successful in improving adherence in pediatric liver transplant recipients [65].

Mixed interventions

In examining interventions in chronic disease adherence management, multicomponent and behavioral interventions have produced particularly marked effects on adherence behaviors [39,241].

Most combined interventions included educational and behavioral interventions, with a small number using social support strategies with educational or behavioral elements [233]. It is thus reasonable to expect some success with mixed interventions in transplantation. The review by De Blesser et al. [198] identified five studies that adopted a mixed intervention approach combining educational/cognitive, counseling/behavioral and affective/psychological approaches [198].

De Geest et al. [150] conducted the highest quality study of these five. They identified low self- efficacy [242] and depression [243] as targets for a randomized controlled trial in adults undergoing kidney transplantation who had been identified as non-adherent. Patients were randomized to an "enhanced usual care group" and an intervention group. Electronic monitoring using MEMS tracked adherence for the three-month intervention/control period and for six months of follow-up. The authors used a structured interviewing technique [4] to evaluate the MEMS validity and reliability. A comprehensive home visit was made for each of the patients (and family member, when available) to assess the specific barriers to adherence that might be targeted. The "intervention nurse" discussed and inaugurated a series of interventions including behavioral elements to enhance self-efficacy (e.g. motivational interviewing [244]), other behavioral interventions to remind the patient to take the medications, tailored education modules to reinforce the patient's knowledge about adherence, and helping the patient organize social support to aid in medication taking. Eighteen non-adherent patients were selected to participate in this pilot. Six were included in the intervention group and 12 in a comparison group. Medication adherence improved in both groups, and it appeared to improve more in the intervention group, although the effect did not reach statistical significance. The randomized controlled trial was very underpowered. In addition, the multiple elements of the intervention were very time and labor intensive. The authors suggested that although their model was impractical in the usual clinical setting, an ongoing set of psychosocial and behavioral elements could be used in an ongoing continuity of care model or a chronic disease-management model [245,246]. This is an approach that is used in many pediatric transplant clinics, where the patient and family are seen in a multidisciplinary fashion so that not only medical, but psychosocial and behavioral approaches can be utilized.

The first mixed interventional study with pediatric renal transplant recipients [70,247] used combined educational and behavioral approaches, and involved the entire family, as is appropriate in a pediatric intervention. In addition to educational materials, the intervention involved parents giving weekly awards for adherent behavior. Utilizing a multi-parameter measurement system, recipients in the experimental group were more knowledgeable about transplantation. Moreover, adherence with azathioprine and prednisone improved by approximately 60%, while the control group experienced only a 33% improvement.

Shemesh et al. [248] identified non-adherent pediatric liver transplant recipients and used an approach featuring increasing the frequency of clinic visits and providing a targeted educational program to the patient and family about the importance of adherence. They followed the standard deviation of tacrolimus levels in conjunction with the increased visits. After the intervention, the number of patients with high alanine aminotransferase levels decreased significantly from eight before the intervention to four afterwards. Other outcomes, including the number of rejection episodes and adherence to tacrolimus, also improved, but did not achieve statistical significance [248]. Since this type of intervention is feasible for clinical practice [192] it will be important to test it with a randomized and more fully powered trial, perhaps adding in a more formalized behavioral module (e.g. reviewing adherence measurement by electronic monitoring).

Another small study utilized a mixed interventional approach with 12 patients [236]. Clinical pharmacists employed monthly educational counseling and medication reconciliation, direct feedback by telephone, and behavioral motivational counseling. Adherence, as measured by pharmacy refills and immunosuppressive blood levels, was statistically significantly better in the patients receiving the clinical pharmacists' interventions than in 12 controls. The study was small in size and the assessments of adherence utilized measures that were methodologically weak. Nevertheless, this mixed intervention supports the concept that multiple interventions that are targeted to the specific patient have great merit. A four month educational support program using web-based electronic communication with the healthcare team resulted in improved medication adherence for those who were most actively involved, but was not statistically significant for the overall group [194].

It should be acknowledged that most of these reports have enough important weaknesses to mandate a good deal of circumspection about the evidence based foundation of interventions for non-adherence. Nonetheless, it would appear that multidimensional interventions involving not only education, but behavior modification, encouragement of self-care, the development of stable social support and appropriate therapy for psychological issues such as depression could play an important role in constructing successful programs to prevent or improve medication non-adherence.

Other interventions for improving medication non-adherence

Simplifying the drug regimen

Simplified drug regimens are associated with improved medication adherence in patients with chronic illnesses [134,233,249] with a large effect on adherence [250–253]. Eisen et al. demonstrated that adherence improves incrementally as the medication dose frequency decreases [254]. This effect has also been demonstrated in adult renal transplant recipients [98]. Since the drug regimen for transplant recipients is quite often complex, simplification has an obvious advantage. As an example, recent studies using a new once daily sustained release formulation of tacrolimus [255] have found a positive effect on medication adherence [248,256] although the method of measuring adherence was not robust. This approach is not without problems [257]. The pharmacokinetics of the single drug in question (in this case tacrolimus) may not be equivalent to twice daily dosing, necessitating dosage changes [258,259]. In addition, if the patient is non-adherent and misses his/her once-daily dose, there may be increased clinical repercussions [257]. Nevertheless, with appropriate monitoring of adherence, a simplification of the immunosuppressive medication appears rational, although definitive data on the specifics of the simplification require validation in appropriately conducted studies.

Avoidance of medications with noxious side effects

The unacceptable side effects of certain immunosuppressive medications can lead to medication non-adherence, particularly in adolescents. A prime example of this is the use of corticosteroids in post-transplant immunosuppression. The noxious side effects of steroids on body image [260] and growth [261,262] (among other

side effects) are important reasons why patients are non-adherent with these medications [263]. The use of steroid-free and steroid-sparing protocols have been shown to be successful [19,264,265]. By minimizing or totally avoiding corticosteroids, it is reasonable to assume that at least one important barrier to adherence can be overcome.

Additional possible medication modifications

Clinical tolerance

By definition, the state of immune tolerance refers to the ability to maintain a well functioning allograft and a competent immune system to third party invaders with no additional immunosuppression [266]. While this may not modify other risk-taking behaviors characteristic of the adolescent transplant recipient, the state of tolerance would represent an answer to medication non-adherence [267–269]. Unfortunately, it is not possible to reliably produce tolerance in the human clinical setting, and this is particularly true in the pediatric setting [270]. Spontaneous or operational tolerance is occasionally observed when an organ survives unscathed in the face of withdrawal of immunosuppression, either under medical supervision or as a result of non-adherence. If biomarkers can be identified that identify the state of operational tolerance, some transplant recipients could theoretically be withdrawn from immunosuppressive medications [271]. This could be of benefit to some adolescents, and might be particularly common in liver transplant recipients [271]. In this regard, it is interesting to note that in pediatric patients, recipients of combined liver-kidney transplants have improved renal allograft survival compared to those receiving only kidney transplants [272].

Long acting parenteral immunosuppressive agents

The use of safe, long-acting immunosuppressive medications has appeal in the context of medication non-adherence, in that the administration of such a medication is simple and can be directly observed [133]. This strategy prompted us to examine the use of Figolimod (FTY 720), which depresses peripheral lymphocyte count for up to one week [273].There is even greater theoretical appeal for such a medication to be administered parenterally by medical personnel. In pediatric renal transplantation, daclizumab, a humanized monoclonal anti-CD25 antibody has been used successfully for over six months with excellent results [265]. In non-infectious ocular inflammatory disease, daclizumab has been used successfully for over three years [274]. Prolonged, subcutaneously administered daclizumab is currently being evaluated for use in relapsing-remitting multiple sclerosis [275], but is not currently being evaluated for use in renal transplantation.

One strategy that has attracted considerable interest is the use of belatacept. For a T cell to become fully activated, signals must be transduced through the T cell receptor and through a costimulatory molecule or molecules. Interruption of this costimulation renders the T lymphocyte anergic. There are a number of dyads (a molecule on the antigen presenting cells ligating with an antigen on the T lymphocyte), and the specifics of these are discussed in detail elsewhere in this book. One of the first reported and most prominent of these costimulatory pathways is the CD 28/B7 (i.e., CD80 and CD86) ligation. The engagement of CD28 on the T cell by B7 on the surface of antigen presenting cells, in conjunction with the signaling of the T cell receptor by the MHC antigens results in marked T cell activation and proliferation. CTLA-4, another T cell surface receptor, binds 100 times more avidly to the B7 antigens, thus blocking the CD28 costimulatory signal. Belatacept is a fusion protein containing CTLA-4. This causes strong binding to B7 and blockade of the CD28 signal [276]. Of importance for this discussion, belatacept can be given parenterally monthly, with resulting excellent and probable safe immunosuppression. This agent has been studied in phase III trials, with durations of administration of over three to five years [277,278]. Formal clinical trials have not yet begun in pediatrics as of this writing. One can be cautiously optimistic that if adolescent patients are getting at least one effective immunosuppressive agent reliably, this may result in some amelioration of the impact of medication non-adherence [279]. Two notes of caution must be struck. Belatacept is recommended only for use in EBV seropositive patients, to protect against the generation of PTLD [280]; thus belatacept may not be appropriate for every adolescent. Additionally, the successful administration of belatacept may not be accompanied by good adherence with the other oral immunosuppressive agents.

Addressing underlying psychological problems

Adult patients with chronic illness [281,282] and specifically end stage renal disease, commonly manifest depressive symptoms. In one study, adults beginning chronic hemodialysis underwent psychometric analysis, and 44% had scores which indicated significant depression [283]. The psychological distress appears to persist, although to a somewhat decreased extent, after kidney transplantation [284]. The same is true with pediatric patients. Children with chronic illnesses are over twice as likely to develop psychological and behavioral problems when compared to healthy children [285]. Children undergoing dialysis and transplantation have classically demonstrated depression, school problems and emotional distress [279,286,287]. Later studies using more sophisticated instruments have confirmed these observations [77]. Illness-related uncertainty appears to play an important part in this [84,288] while hope appears to moderate depressive symptoms [84].

There appears to be a strong linkage between emotional problems such as depression/anxiety and medication non-adherence [289,290]. This occurs in a significant number of chronically ill patients, including recipients of renal and liver transplants [291–295]. Similarly, in adolescents with chronic illness, there is an association between poor adherence and emotional problems [83,296–298]. Penkower et al. [77] studied 22 adolescent transplant recipients, and found a significant association of non-adherence with anger, but not with depression or anxiety. Others have suggested that there is an association between compromised emotional functioning and non-adherence in pediatric transplant recipients [72,299]. It has been strongly suggested that illness-related-uncertainty can mediate depression and this in turn mediates medication non-adherence [84]. On the other hand, the presence of hope, by mitigating depression, appears to be associated with immunosuppressive medication adherence [84]. Interestingly, it appears that the development of anxiety after transplantation may not foreshadow increased levels of medication non-adherence, but in fact may be associated with more stable adherence patterns [300]. Finally, the development of PTSD, as detailed earlier in this chapter, is associated with medical adherence difficulties.

Summary

It is not overstating the issue to suggest that medication non-adherence represents one of the most important unmet needs in solid organ transplantation. The problems associated with medication non-adherence are under recognized, both in pediatrics and

adults. The research in this field is plagued by difficulties in uniformity of definitions, detection techniques, agreement on relevant barriers and precipitating factors, and proven interventions. Long-term transplant outcomes, particularly in kidney transplantation, have not changed dramatically over time, although short-term improvements have been made. It is quite likely that some or most of the long-term barriers to improved graft outcome can be realized with a more thoughtful and concerted effort to improve immunosuppressive medication adherence.

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CHAPTER 121

Management of the Organ Transplant Recipient in the Transition from Pediatric to Adult Care

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Introduction

In the 1960s and 1970s, pediatric transplantation was in its infancy. Pediatric transplant rates were low and mortality high [1]. The number of recipients reaching adulthood was small and the consequences of their move to adult care received little attention. Since the 1980s, with remarkable medical, pharmacological and surgical progress in pediatric transplantation, ten-year recipient survival rates for some organs approach 95% [1,2]. Both the absolute number of pediatric transplants and the proportion of children transplanted in infancy and early childhood has increased and an ever-expanding number of pediatric recipients are emerging into adulthood. Pediatric providers are challenged to help them transition from being children with chronic illness to young adults with complex medical issues, equipped with the knowledge and skills to effectively function in an adult healthcare system. This chapter will discuss facilitators and barriers to successful transition and review recommendations for best practices to enhance the quality of care for transitioning patients. Additional discussion of adherence issues germane to this topic can be found in Chapter 120.

Background

It is vital to consider transition and transfer as two separate entities. The former is a process and the latter an event. Transition is best defined as "the purposeful, planned movement of adolescents and young adults with chronic physical and medical conditions from child-centered to adult-centered healthcare systems." [3] This definition incorporates the broad range of psychosocial, biological, and developmental changes which occur during adolescence. The provision of transitional care is a process which occurs over a prolonged period of time with transfer being a discrete point along the continuum of care. Importantly, transition does not end at the time of transfer. The concept of transitioning youth is not specific to pediatric organ transplantation. Indeed, many of the current practice recommendations and guidelines for optimizing adolescent and young adult care are drawn from studies of patients with a wide range of chronic illnesses [3,4].

Addressing the transition needs of adolescent and young adult transplant recipients is crucial not only to their graft function but also their survival. Several large studies have observed higher rates of graft failure during the time frame and the age range of transfer to adult care. Using a Canadian registry, Samuel et al. examined whether increased graft loss occurred during the peri-transfer adaptation phase [5]. They considered the "adaptation period" to be the time encompassing 6 months before and 2.5 years after the first recorded adult visit. Compared with the period immediately prior, the adjusted hazard ratio (HR) for graft loss within the adaptation period was 2.24 (95% Confidence Interval (CI): 1.19–4.20). When they narrowed the adaptation period to six months before and six months after transfer, the adjusted HR for graft failure was even higher at 5.42 (95% CI: 2.83–10.39). Similarly, a US Government report by the General Accountability Office examined rates of kidney transplant failure among different age groups, pediatric vs. "transitional" vs. adult [6]. The study classified "transitional" transplant recipients as those who were transplanted as children and turned 18 years of age during the seven year follow-up period. By five years post-transplant, the percentage of transitional recipients who experienced a transplant failure (33%) was double the percentage of pediatric recipients (16%) and slightly higher than adult recipients (28%). It is assumed that this transitional group would have transferred medical care during the follow-up period. In contrast, a single centre Canadian study of 115 kidney transplant recipients found no increase in graft failure following transfer and a study from the Netherlands showed no increase in acute rejection rate among patients transferred to adult care at a mean age of 18 years [7,8]. Thus, transfer does not inevitably lead to rejection or graft loss. Further research is needed to clarify which patient and healthcare system factors may protect from vs. predict allograft dysfunction or loss surrounding the time of transfer.

Age has been suggested as an independent risk factor for increased risk of allograft loss during the transition of adolescents and young adults. Smith and colleagues reported a higher risk of graft loss, late acute rejection and incomplete rejection reversals among US adolescent renal transplant recipients aged 13–17 years compared with younger transplant recipients in the North American Pediatric Renal Transplant Collaborative (NAPRTCS) [9]. Using a larger database, the United States Renal Data System (USRDS), Foster et al. found that age-standardized graft failure rate was 12.9 per 100 person-years among patients who transferred at less than 21 years of age compared with 8.7 per 100 person years among those who transferred $\geq$21 years of age [10]. In Canada,

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Kiberd compared graft survival rates between pediatric patients <18 years who were transplanted in a pediatric hospital, with young adults transplanted in an adult hospital, in two different age ranges: 18–24 years and 25–35 years [11]. Those in the 25–35 year age range had significantly better graft survival compared with the pediatric and younger adult (18–24) groups (HR 0.45; 95% CI: 0.25–0.82). However, graft survival rates were similar between the pediatric patients and the young adults aged 18–24, regardless of transfer of care. In a recent and very important USRDS study, Foster et al. examined both age at transplant and current age as risk factors for allograft loss [12]. Among first kidney transplant recipients younger than 40 years of age, older adolescent and young adults ages 17–24 years had the highest risk of graft failure, compared with the younger and older age groups, irrespective of age at transplant and time since transplant.

The normal developmental processes of adolescence and emerging adulthood likely contribute to the increased graft loss observed during this period (Table 121.1) [13–17]. The "emotional" brain and "executive" brain have different developmental trajectories, with executive functions being slowest to mature [18]. Elegant neuroimaging studies have shown that full brain maturation is not achieved until young adults are in their mid-to-late twenties [19–22]. Risk-taking normally peaks in young adulthood; this can be seen in both sophisticated neuropsychological experiments and in morbidity-mortality statistics [18,23]. Young adults (age 18–25 years) have the highest rate of accidental death, substance abuse and sexual risk taking [23]. This peak risk taking age coincides with the time most youth transfer to adult care. It is often a period when other stressful events may occur, such as leaving home, moving to another city for work or education, and intense emotional or romantic relationships. The confluence of all these factors, in a young person with a chronic illness, may play a role in medication non-adherence and missed medical appointments.

Regardless of whether age or transfer matter more, the need to prevent allograft loss in a transitioning young adult transplant recipient is critical not only for the individual patient but also for society. Every potentially preventable organ loss that occurs due to problematic transfer or transition adds to a higher demand for organs in a world of limited supply. In addition, there is substantial economic burden when a patient loses an allograft and returns to dialysis treatment. A patient on dialysis is generally less able to work than one with a transplant and the healthcare costs of dialysis are substantially higher than those of transplant [6].

Although non-adherence is believed to contribute to allograft loss during the transition period, adherence is the focus of a separate chapter in this book; the current chapter will concentrate on more systematic issues related to the provision of transitional care.

Recommendations and guidelines for the provision of care to the transitioning adolescent and emerging adult transplant recipient

There have been numerous reviews providing recommendations for the provision of transitional care but there are few data to support specific evidence-based guidelines [17,24–29]. Bell et al. reported transitional care recommendations for solid organ transplant recipients from an international collaborative effort of multiple transplant societies [17]. This report reflected expert opinions from a wide variety of pediatric and adult transplant physicians and nurses, psychologists, social workers, and administrators. Webb et al. later conducted a similar conference in the UK but also included patients and parents [30]. In general, diverse expert groups have suggested several major themes and critical transition components (Table 121.2) which are in concert with the basic principles and fundamental steps of transitional care provision promoted by the Society

Table 121.1. Developmental stages of adolescence and young adulthood and corresponding clinical approach suggestions

	Early adolescence (ages ~ 10–13 years)	Mid adolescence (ages ~ 14–16 years)	Late adolescence (ages ~ 17–21 years and beyond)
Cognitive, affective and moral	• Concrete thinking • Beginning of abstract thought in some • Cannot grasp long-term outcome of decisions • Heightened emotional arousability • Sensation seeking • Reward oriented	• Increased abstract thinking • Concrete thinking in stressful situations • May begin to perceive future consequences of actions but not utilize in decision making • Heightened vulnerability to risk taking • May have difficulty regulating affect and behaviour	• Complex abstract thinking (some) • More future oriented: can act on long term plans, delay gratification, set limits • Greater emotional stability • Idealism but sometimes absolutism, with intolerance of opposing views
Self-concept/Identity formation	• Fantasy and present-oriented • Self conscious about appearance	• Increased introspection • May have intense feelings of inner turmoil • Concern with attractiveness	• Progression of personal identity • More stable body image; attractiveness may remain important
Family and peers	• Beginning of emotional separation from parents • Need for privacy • Beginning of strong peer identification and affiliation	• Further emotional separation from parents and family • Struggles and conflicts over autonomy • Strong peer identification and involvement	• Separation from family emotionally and physically • Peer group/peer values diminish in importance • Formation of stable relationships
Relationship to society	• Adaptation to middle /early secondary school years	• Assessment of skills and opportunities • Early vocational plans • Realistic role models critically important	• Career decisions central • Progress of vocational capability and financial independence
Suggested subjects to explore and discuss at clinic visits	• Introduce concept of private confidential visits to parents and patient • Simple directed interactive questions to patient • Straightforward interactive counselling	• Enquire about friends, free time activities, home life, school • Ask in non-threatening, non-judgmental manner about any experimentation with drugs, alcohol or sexual activity or any trouble with law • Discuss healthy living	• Discuss healthy living habits and personal interventions for improving overall wellness • Identify unhealthy living habits and their consequences • Discuss personal, educational and vocational goals • Build upon questions from early age ranges (to left)

Table 121.2. Critical components for the provision of transitional care to adolescents and young adult transplant recipients

1	Start discussion of transition early in adolescence.
2	Transitional care must be flexible, individualized and developmentally appropriate. Care must address common health and lifestyle concerns of adolescents.
3	Care must be directed at enhancing patient autonomy, increasing a patient's sense of personal responsibility and facilitating self-reliance in concert with a patient's potential for independence.
4	There must be a designated healthcare professional who takes responsibility for the transition process. Each patient and family should have a transitional care coordinator and advocate.
5	There should be a detailed written transition plan that is developed and updated with each young person and his/her family.
6	There must be a concise, portable medical summary accessible to both the patient and the receiving healthcare provider.
7	There must be attention given to supporting continuous health coverage for the transplant recipient during transition and transfer.
8	There must be communication between the pediatric and adult health care providers preceding, during and subsequent to transfer of care.
9	Transfers should not occur in times of crisis and should be agreed upon jointly by the family, patient and pediatric and adult providers.
10	Guardianship and legal competency issues should be clarified before the age of majority.

for Adolescent Medicine [3]. Many of these recommendations are supported by small qualitative studies from diverse transition stakeholders' perspectives. Herein we will discuss each of these critical components. It is crucial to keep in mind that "the optimal goal of transition is to provide healthcare that is uninterrupted, coordinated, developmentally appropriate, psychosocially sound, and comprehensive" [3].

Start early

Most consensus guidelines and recommendations suggest that preparation for transition begin early in adolescence, generally between the ages of 12 and 14 years [31–33]; some advise earlier, in order to foster progressive independence both socially and medically [17,25,34]. There is no one age, time or method that is known to be best for introducing the concept of transition. Early introduction may mean simply acknowledging that one day the patient will be of adult age and will need to transfer. It may be a relief for parents to realize that the physician expects their child to reach adulthood, despite their illness, and that they need to plan for his/her future. Early introduction of the concept of transition can help parents establish age and developmentally appropriate tasks and responsibilities for their child with a chronic illness and reinforce the need for regular school attendance and social activities. Table 121.3 illustrates how parents and their child can share healthcare tasks and gradually shift the major locus of responsibility from parent to child [17,35,36]. These are key elements in helping a child gradually develop self-responsibility and autonomy.

During medical visits the pediatric nurse and physician should spend part of the interview directly communicating with the child, encouraging him/her to answer some of the medical history questions and to understand the treatment plan. This can help the patient feel a member of the team and reinforce his/her active role in the therapeutic process.

In addition, even in early adolescence, pediatric providers should consider interviewing patients independently for part of the visit. Private one-on-one time during healthcare visits plays a significant role in the quality of care perceived by youth, and is recommended as a component of quality healthcare for adolescents by the American Academy of Pediatrics [37]. In early adolescence this independent interview may be very concrete, with the provider asking, "What is your favorite subject at school? What do you like to do for fun?" The content is not crucial but the relationship and trust that begin to build between the provider and patient can be. This private interview (no caregiver present) provides a chance for the patient to hone communication skills vital to adult health self-management and affords opportunities for the patient to communicate confidential information, which might otherwise remain unrevealed. Patients have reported that being seen independently is an important component of preparing them to transfer [38,39]. Some centers place posters in the waiting room which explain confidentiality rights and may serve as a reminder to patients and families that the primary relationship lays between the healthcare provider and the patient, not parent. It is important for the healthcare provider to know and understand the state, provincial and/or federal laws that govern confidentiality and consent issues for minors, as these vary by jurisdiction.

Flexible, individualized and developmentally appropriate care

Organ transplant recipients may have neurocognitive and maturational delays and sometimes these are not readily apparent [40–43]. Thus transition of care strategies require flexibility. It is important to develop a transition plan in the context of the patient's neurocognitive function. This may necessitate involvement of a psychologist to formally assess the patient's potential for healthcare self-management and independence and to avoid the pitfalls of unrealistic expectations, either set too high or too low. The aims of transitional care include helping patients with goal setting, self-advocacy, healthcare self-management, and problem solving. The expectations of the patient, family and healthcare team must be in concert with the ability and potential of the patient.

Provision of developmentally appropriate care also refers to adolescent-centered care. Providers need to address common health and lifestyle concerns of adolescents and young adults, such as reproductive health, negative health behaviors (drinking, smoking, unprotected sex), as well as positive health behaviors (healthy diet, exercise), and mood disorder screening. In the US, it is estimated that over 13% of high school adolescents have seriously considered suicide and 47% have had sexual intercourse, of whom 12.9% did not use birth control [44]. Violence is also prevalent amongst US high school adolescents, with 32.8% reporting having been in a physical fight and 8% reporting sexual abuse [44]. A broad psychosocial interview, including sexual history, is equally important for both sexes. Recent studies suggest that the sexual and reproductive health needs of male adolescents may often go unrecognized [45]. A common psychosocial interview mnemonic for adolescents is HEEAADSSS (Home, Education and Employment, Eating, Activities, Affect, Drugs, Sexuality, Suicide and Depression, Safety) [13]. Although transplant subspecialists may feel that these adolescent themes are best deferred to the general pediatric provider, adolescents generally have very low primary care usage rates [46,47]. Thus, adolescent transplant recipients may not have an established primary care provider with whom they have built trust and rapport. In addition, they may benefit from discussing their psychosocial concerns in the context of their transplant and medical regimen and have special areas in need of more attention, such as optimal vaccination, health related travel risks, sexuality, and genetic counseling around reproduction.

Another component of comprehensive, individualized care is a vocational and educational assessment, guidance and support.

Table 121.3. Suggested approach to development of child and adolescent's leadership /self management skills

Stage/age	Child capabilities/actions forming basis for leadership skills	Parent(s)' leadership actions supporting child's increasing capabilities
Toddler (1–3 yr) Parent as "CEO"	• Cooperates with routine treatment with limited acting out • Understands firm limits of parents • Helps hold equipment and supplies (e.g. oral medication dispensers, enteral feeding supplies)	• Rituals for treatment: child knows what to expect and can learn by repetition • Identify possible treatment roles to share with child • Emphasize hygiene in prevention of infection (e.g. hand-washing, teeth cleaning, safe handling of pets)
Pre-school (4–5 yr) Child begins to learn basic self care	• Participates further in self-care (e.g. helping gather medications or treatment supplies) • Medical play: such as nurse or doctor kits, doll that undergoes similar treatments to patient (e.g. venipuncture, peritoneal dialysis, feeding tube, etc) • Begins to identify body parts important to his/her health condition • Learns name of health condition (e.g. heart, kidney, liver transplant, etc) • Learns simple terms for transplant related problems and medication side-effects (e.g. for feeling unwell, nausea, painful urination, skin lesions)	• Explain in simple concrete terms what will happen at clinic/hospital visits • Set fair and appropriate limits (e.g. taking medication on time, teeth brushing, dietary and fluid requirements, infection prevention strategies) • Allow choices (where appropriate); such as which medicine to swallow first • Model acceptance of management routines and limits • Reassure child he/she is not to blame or being punished when treatment causes discomfort (e.g. injections, blood drawing)
Early school age (6–9 yr) Child provides some self care	• Recognizes one or two major internal body cues of a problem (e.g. fever, abdominal pain, graft pain, dysuria, swelling) • Participates actively in concrete care and monitoring of condition (e.g. oral hygiene, fluid intake, recording medications on personal medication record) • Increases concrete level of understanding of the health condition, cause of original organ failure, requirements of treatment	• Negotiate with child "rules" for working together to get all treatment done (e.g. taking medication on time in parent's presence) • Encourage healthy living (regular exercise, healthy food choices) • Support normative activities (sports, other organized activities) to encourage fitness, teamwork, and sense of achievement • Discuss and model approach to telling teachers, friends, and coaches about health condition, and the amount of detail to share
Early Adolescence (10–13 years) Parent becomes "Manager" vs. "CEO" Adolescent performs greater self care and some self management	• Increases understanding of the transplant condition and its affect on daily life • Learns signs/symptoms of transplant organ rejection • Begins to communicate directly with health care provider, initially in parents' presence • Independently completes some medical self management tasks (e.g. alarm watch for medication, agenda with appointments and tests, learn medication names, doses and times, and allergies) • Prepares medication dose boxes, with parental supervision • Learns how to respond to peer pressure and yet still take care of self	• Provide tools so child can self-manage (e.g. notebook and/or agenda for record keeping of treatments, medication taken) • Observe process closely, to offer immediate corrective feedback • Remain present and involved in care, decision making, and monitoring • Be there for new symptoms, changes in treatment and emergencies • Ensure child has told important others (e.g. friends, teachers, coaches) of his/her transplant and what assistance they could provide if needed • Further encouragement of healthy living and normative activities as above
Mid adolescence (14–16 years) Parent becomes "Supervisor" Adolescent becomes main "Manager" of daily routine care	• Achieves more abstract knowledge of treatment requirements, complication prevention and transplant organ rejection • Develops strategies to complete routine management tasks, such as taking medication on time (alarm watch, cues linked to daily routine) • Learns medications' roles, side effects, consequences of taking irregularly • Participates in appointment making • Effectively asks for assistance in complex situations • Reads more about transplantation and his/her underlying condition	• Negotiate and renegotiate who does what • Discuss new issues (e.g. sex, drugs, alcohol), related peer group activities/pressures and any effects on transplant condition • Begin to discuss vocational and educational goals and insurance issues • Encourage community involvement (e.g. club, volunteer work, extra-curricular activity groups) • Consider adolescent's participation in limited part-time or summer employment
Late adolescence Parent becomes "Consultant" and resource person to adolescent	• Achieves sense of self as capable manager of the transplant condition • Integrates realities of his/her transplant care with risk taking of peers • Can appreciate the benefits of transplant despite constraints of management • Continue to develop more independent health and community support network with transition to adult based services • Make commitment to lifetime of treatment	• Develop flexible way of communicating with the adolescent to remain informed but not interfering • Remain present for support and problem solving • Provide encouragement and guidance as the youth transitions from paediatric to adult care services • Encourage vocational, college or university training and/or integration into the work force

Education is an important determinant of health and is linked to health related behaviors, self-determination and empowerment, employment, socioeconomic status, and health insurance [48,49]. Optimizing the education potential of pediatric transplant recipients can contribute to their long-term health and well being and their adaptation in adult life. A social worker is a vital team member to help address these issues in a multi-disciplinary transition service.

At times, patients may bring peers or significant others to clinic appointments. These are rich opportunities to assess and engage the patient's support system. Including peers in the conceptualization of the patient's support system is important. Patients have also expressed the desire to have a "buddy" with similar experiences to help transition [50]. In the UK, youth workers who serve as peers and role models have been incorporated into a transition clinic model [51]. Possibilities for peer support include encouraging adolescent and young adult transplant recipients to engage with each other on the days of their clinic visits and facilitating regularly scheduled social events for them to interact outside of the medical setting [51].

Enhance patient autonomy

Young adult transplant recipients often express a preference to be treated as adults prior to transfer [33,38]. It is essential for the healthcare provider to collaborate with the patient and family to identify specific tasks for which the patient assumes increasing responsibility. For example, the patient may listen to the parent call the pharmacy for refills; then do it him/herself with parent

supervising; then do it independently. Making a healthcare appointment is another learned skill. The patient can listen to the parent make the appointment by phone or in person after a clinic visit. The patient can do it together with the parent several times and then independently thereafter. An analogy is when a teenager learns how to drive. A parent or guardian may walk the teen through it, show them were the brakes and accelerator are, how to turn the key, how to accelerate, etc. The parent then has the teen drive with the parent in the car, providing feedback. Finally, after practice, the teen is comfortable enough to obtain a driver's license and drive independently. Although there is no driver's license equivalent to healthcare self-management, the steps involved in learning to drive parallel many of those in learning to be an independent health advocate. These task-based accomplishments take time, practice and supervision, and the parent or guardian should be an active partner in the process. Suggestions for progressive enhancement of autonomy are found in Tables 121.1 and 121.3.

Designated healthcare professional

Guidelines suggest that there should be a transition "champion" to lead transitional care efforts [17,31]. Generally, this responsibility would fall to a physician in either the adult or pediatric center, but ideally both. There is also a need for a transition advocate or coordinator for each patient, usually someone in direct service, such as a transplant coordinator, nurse, social worker or case manager. In some centers, he/she may be a youth worker [51]. This person is the primary contact for assessing the patient's transition progress and helps the patient navigate the healthcare system during transfer. Ideal site resources include the transplant physician(s), and surgeon(s), designated transplant nurse(s), nurse coordinator, social worker, psychologist and designated transfer liaison; in the adult centers these may also include a reproductive specialist and urologist [17,51]. Additionally, it is highly recommended that the patient have an identified primary care provider who will provide continuity of general care.

Written transition plan

It is important for the provider, patient and family to work together to construct a patient-specific written transition plan. A center may start with a general plan for all patients and tailor the plan based on patient and family feedback and the patient's developmental stage and potential for independence. A written plan encourages expectations and goals to be clearly set and systematically assessed. It should be regularly reviewed and updated based on patient progress and challenges. Some programs conduct a formal transition evaluation yearly. The interval needed may depend on the patient and the program's resources, but at a minimum it should be annually.

Portable medical summary

The pediatric healthcare provider should formulate a concise but comprehensive medical summary for both the patient and the accepting healthcare provider to facilitate the transmission of knowledge when the patient transfers and in the case of emergencies. At a minimum, it should include the primary diagnosis, date of transplant, major transplant events (e.g. biopsies, rejection, medication changes, infections, etc.), an updated medication list, drug allergies and intolerances, psychosocial status, healthcare insurance status, the most recent laboratory studies and comprehensive contact information for health providers to reach the patient and/or caregiver. An excellent example of a patient-oriented portable medical summary is "MyHealth Passport" from the Hospital for Sick Children in Toronto, Canada [52]. It is readily accessible on the internet, includes a transplant-specific model and can be completed by the patient with assistance from a healthcare provider. The data entered are transformed into a printable, wallet-sized medical summary. The electronic health record can also be tailored to produce a concise medical summary. A portable, concise summary may come in many forms and include pictures, audio, video or storage on USB drives. The use of information technology to enhance the transfer of health information among medical providers and across various healthcare settings has been promoted by the American Academy of Pediatrics, as a means to improve the quality of care for transitioning patients [53].

Healthcare insurance coverage

Transfer to adult care should take place with due consideration of the adolescent/young adult's financial factors. If a patient is at imminent risk of losing health insurance, delay of transfer should be considered. Young adults have the lowest rates of health insurance compared with any other age group [54]. Between 2000 and 2010, the percentage of American adults 18–64 years of age with private health insurance coverage decreased while the numbers of uninsured increased [54]. There are widespread advocacy efforts in the US to extend immunosuppressive coverage for kidney transplant recipients, in order to avoid graft loss due to insurance gaps. It is estimated that extension of immunosuppressive coverage would actually produce significant cost savings [55]. While absence of insurance is a major concern, no less important is underinsurance. Many insured adolescents suffer adverse health consequences due to inadequate coverage for needed services, including preventive, reproductive and behavioral health [47,56,57]. Regardless of the national healthcare system in place, it is crucial to ensure that transitioning young adults can access and afford to continue receiving necessary medications and treatments that promote graft and patient survival throughout the transfer period and beyond.

Communication between adult and pediatric providers

In some transplant centers, pediatric and adult providers participate jointly in seeing patients, either alternating visits or seeing patients concurrently. In other centers, transition efforts are focused in the pediatric or adult center respectively. The model of transitional care implemented depends greatly on the available resources and structure of the healthcare system. None is currently known to be superior to another. Regardless of the model of care, it is essential for pediatric and adult providers to communicate with each other to ensure optimal continuity of care for the patient. Patients have expressed frustration with insufficient communication between pediatric and adult medical providers [33]. Communication should ideally take place before, during and after transfer, and should occur through multiple modalities, such as written communication as well as phone calls or live meetings. The communication should also focus on provision of a concise but comprehensive summary of the pertinent medical and psychosocial issues.

It may be very helpful for a pediatric healthcare team member to do a site visit of the adult transplant center during a typical clinic day so that transitioning patients can be told more accurately what to expect. This may include the content of the physician and nurse interaction, duration of appointment, how blood testing is arranged, what the waiting room looks like, how long the wait usually is,

where to park, etc. Many patients express lack of understanding of the differences between the pediatric and adult healthcare centers [50]. Some centers provide tours of the adult facility prior to the first formal adult visit and this is generally very well received by patients [38,50].

Another important communication aspect is the sharing of information on treatment protocols. It is important for both the adult and pediatric transplant professionals to be familiar with each others' approaches. Ideally any modifications in immunosuppression regimens should occur prior to transfer and allow for a period of drug level stability before the patient changes sites. Medication revisions occurring early after transfer can lead to confusion, dosing mix-ups and sometimes lack of trust.

Avoid transfer in crisis

Ideally, the timing of transfer should be anticipated and agreed upon by the patient, family and providers [17,30,50]. There is no ideal age to transfer although many healthcare systems mandate specific age cut-offs. However, if a patient is in an unstable or evolving medical situation at the required transfer age (for example experiencing a serious complication like rejection or post-transplant malignancy), every effort should be made to postpone transfer until a reasonable period of stability has been achieved. Transferring a patient during an evolving treatment plan, or when the patient's condition is deteriorating, may undermine the patient and family's confidence in the new treating team. Similarly transfer should be avoided during a psychosocial crisis. In some cases, unexpected events may necessitate an urgent or unplanned transfer; for example, some pediatric centers may oblige transfer for female pregnancy whereas others have an adolescent medicine service that can assist in following the patient jointly with a high risk obstetrical center, before and after delivery. When urgent transfer is required, the best approach is to secure good communication between the pediatric and adult providers, and have a transfer plan that is transparent to the pediatric and adult teams, and to the patient and family. This will help avoid the problem of "bounce-backs". It is important to reinforce to the family that care will be taken over by the adult team if that is the plan. In such cases, the patient should be discouraged from ricocheting back and forth, as this can create problems of discontinuity of care and increase the risk of inadvertent negative health outcomes.

Guardianship, consent and confidentiality issues

Prior to transfer and before the age of majority, a provider should discuss the patient's legal rights with the patient and his/her family, as well as any age related changes in confidentiality and informed consent. In some jurisdictions, minors can provide consent before the age of 18 for defined procedures or conditions. It is important for the healthcare provider to be aware of state, provincial and/or federal laws that govern age-related confidentiality and consent issues, as these vary considerably by jurisdiction [58–62]. Some patients may feel overwhelmed by the responsibility of self-consent and some parents may feel left out or that their sense of control is abruptly severed. Anticipatory counseling is important to help prevent confusion, anxiety, and resentment.

Guardianship issues are particularly important when cognitive limitations exist; in transition planning it is crucial to consider the developmental stage and capacity for independence of the individual patient. A neurocognitive evaluation may be necessary. This is discussed further on in this chapter.

The developmentally challenged patient

Patients with cognitive delays often face healthcare disparities and increased health risks beyond those of the healthy adolescent with a transplant [63–65]. Challenges related to their transition planning and preparation include heightened anxiety about transfer, adaptability to changes in routine, capacity for independent living, sexuality, vocational issues, modes of communication, decision making and consent, guardianship and substitute decision making.

A key component in the transfer document of these patients is a summary of their potential for self-management and medical decision-making [64]. Intellectual capacity and ability to perform activities of daily living are not synonymous entities. Although an IQ less than 70 is traditionally considered cognitively delayed, IQ testing comprises only part of the assessment for independent function and test results can be misleading, particularly when comorbidities such as learning disabilities or depression exist. Labeling a teen's ability by education level, developmental age or IQ may not reflect his/her ability to perform self-care or follow directions. Assessment of adaptive behaviors such as daily living activities, communication and social skills is essential. A clinically practical method is to ask a parent or caregiver to describe a typical day for their teen, inquiring specifically about what he/she does for self-care, how he/she communicates, and how he/she solves problems [64]. Clinical observation alone may be deceptive and potentially either overestimate or underrate their abilities. Teens may behave differently in the clinical setting and limited time may restrict the range of adaptive behaviors observed. Furthermore, unless one probes deeply, it may be difficult to appreciate that a young person doesn't fully comprehend the questions posed or instructions given.

Issues of consent for different types of procedures and treatments and whether substitute decision making is needed can be complex and relate in part to the type of procedure (e.g. starting intravenous antibiotics versus complicated elective surgery), level of understanding needed to comprehend benefits and risks, and medical or surgical urgency. It is best to evaluate and plan for these contingencies well in advance of the patient's age of majority. It is also very important to relay to the adult provider how best to communicate with the developmentally challenged young person. One must assess his/her ability to both speak and understand, and tailor the approach to that level. There is a need for the dialogue to be calm and clear. Generally one should include caregivers in the interview but also offer time to the patient to be interviewed alone.

Vocational and educational issues can be of great concern for cognitively challenged youth and options may be more limited. Yet work can provide structure, stability and insurance coverage. In early adolescence, the transition team should inquire about how the patient is being prepared for a vocation. A social worker can serve as an excellent liaison with the educational system to ensure a modified educational plan is put in place early [66]. It can be helpful to have the patient meet another cognitively disabled individual who is employed. Additionally, forming social networks may be challenging for the patient, but there may be community organizations that can help the patient learn social skills and engage in social networks in a safe, supervised way.

As with other adolescent patients, it is very important to provide developmentally appropriate sex education for these patients. One cannot assume that a cognitively challenged patient will not engage in risky behaviors or not be sexually active; similarly it should not be supposed that sexual activity is non-consensual. Another factor to consider is living arrangements. Parents may suppose that the patient will live with them or with another caregiver forever and

may not fully appreciate the patient's ability or desire for independent living.

Because cognitively delayed adolescents usually do well with structured routines, their adherence may be better than in those with normal intellect. However they may be less able to adapt to changes in routine, such as figuring out how to take medications when not at home or how to deal with missed doses due to illness. Specific organizational skills can be addressed with the patient during the transition process as well as after transfer, and contingency supports put in place.

It is valuable to give cognitively challenged patients opportunities to take more responsibility in their care and allow them to develop as much autonomy as they are able to have. A longer transition period may be needed and more done to engage community support. A proper assessment of cognitive delay is also important to help families access potential social and financial resources. These are all issues that need to be considered throughout the transition process.

Barriers to smooth transition and assessment of transition readiness

There are many potential barriers to young adults experiencing smooth transition. To overcome barriers, one must first identify and understand them. It is helpful to consider three potential barrier sources: providers/healthcare systems, parents/caregivers, and patients themselves.

Healthcare provider and healthcare system-related barriers

Pediatric providers have often developed long-term relationships with patients and their families and may experience difficulty relinquishing their care. This may result in patients being retained in the pediatric healthcare system longer than necessary. It also may result in pediatric providers fostering feelings of dependency by their patients and families. Pediatric providers may unintentionally communicate distrust to patients about the adult healthcare environment. Improving communication between adult and pediatric providers may help overcome pediatric providers' hesitation to transfer ownership of their patients.

Currently no formal "transition of care" competencies exist for adult or pediatric providers and both may feel inadequately trained to handle issues that usually fall more into the other's realm. Adult providers have expressed a need for better training in congenital and childhood-onset conditions as well as concerns about adolescent and young adult patients' psychosocial issues and maturity [67]. Pediatric providers may help by providing insight into special inherited conditions, congenital urologic anomalies and other syndromes that need careful follow-up or consideration of other organ involvement. Conversely, adult providers may impart useful information about their practices of anticipatory guidance, with more attention to reproductive healthcare, cancer prevention and screening, cardiovascular disease and other later-onset complications of transplantation.

By improving communication between the adult and pediatric providers opportunities will be gained to learn from each other. Rather than operating in silos, pediatric and adult providers can mutually benefit through engaging in discussions of transitioning patients, whether informally or in scheduled case conferences. Shared review of patient cases could help mitigate several barriers to smooth transition. For example, adult social workers could be engaged early to prevent impediments to access of care, such as gaps in insurance or transportation obstacles. For patients with significant cognitive delays, shared discussion may help set realistic expectations by the adult healthcare team in terms of the extent to which the patient can be independently engaged in his/her healthcare self-management.

Improved training for healthcare professionals who look after adolescents with chronic diseases is greatly needed. In the UK, the National Health Service (NHS) sponsors an adolescent health programme of e-learning modules for healthcare professionals, to support the delivery of quality care to adolescents and young adults [68]. These modules cover a wide range of adolescent health issues that are non-disease specific, including issues of consent and confidentiality. They also provide feedback to participants and are eligible for continuing education credit. This innovative program may serve as a model for creating metrics for quality in adolescent healthcare delivery.

Healthcare system barriers are a key area to address and include insufficient healthcare provider time and resources and often an inefficient healthcare system. Young adult patients may need longer visits to ensure comprehensive care but providers may feel time constraints [67]. Obtaining an appointment in an adult hospital setting may be challenging for the transitioning patient, with longer delays due to higher volumes of patients. In addition, there is often less ancillary support to capture missed appointments or laboratory tests. Each of the above factors may contribute to loss to follow-up for young adult patients not yet comfortable managing independently in the new medical environment. In addition, incompatibility or lack of electronic medical record keeping systems can impair transfer of medical information, even when pediatric and adult care facilities are within the same healthcare system. A fixed inflexible age of transfer, with no room for adaptability for health crises or special needs, can also be a major deterrent to success.

Historically in the US, timing of transfer and the timing of public insurance termination for transplant immunosuppressant medication have often coincided, leaving young patients vulnerable to insurance gaps. Recent healthcare reform measures may mitigate this particular barrier but further study needs to be undertaken.

Parent/caregiver barriers to smooth transition

Parents of transplant recipients may experience difficulty relinquishing control of healthcare responsibility to their adolescent and feel hurt and angry if excluded from healthcare consultations at the adult center, particularly if they have been very involved in each pediatric visit. In the literature, there is no definite consensus about whether parents should be excluded from the consultation in the adult center [30,31].

Parents of liver transplant recipients have reported recognition of the importance of transitional care but poor knowledge of the process [69]. Pediatric providers need to encourage parents to gradually promote their child's independence and self-responsibility from an early age (Table 121.3). This includes supervised participation in healthcare routines at age appropriate levels with gradual increase in responsibility and skills. In early to mid-adolescence, this may include the parents giving the patient increasing responsibility to call in prescriptions, starting with those that are non-essential medications. A parent can then move on to another specific task, such as making appointments for healthcare visits. At the same time, the child needs to be encouraged to participate in other normal age related activities and tasks, so that an age-appropriate sense of self-reliance can develop. If parents are hesitant

to share health responsibilities with their teen, it can be helpful to make parallel comparisons between healthcare expectations and other age-appropriate life expectations, such as doing homework independently and learning to drive. As adolescents learn new tasks, parents should remain available to monitor and support them. Parental modeling of positive health behaviors is also important to emphasize.

Patient barriers to smooth transition

Many patient-related barriers to the transition process result from the normal developmental phases of adolescence and emerging adulthood, or the consequences of having a complex chronic illness from an early age. In addition, transfer to adult care usually occurs during emerging adulthood, the age range of 18 to 25 years, when risk behaviors peak [70].

The period of adolescence/emerging adulthood is one of exploration, identity formation, boundary testing, risk-taking, intense emotion, and creativity [70]. The imagination, energy and idealism of youth have led to important advances in many domains. However, the burden of a chronic illness can play havoc with this potentially carefree time of life. This is compounded by the difficulty adolescents have in perceiving future consequences of present actions, making it hard for them to understand the relevance of current limitations for future health [71,72]. Attempts by adolescent/emerging adult transplant recipients to emulate the more casual lifestyles of their peers may lead to treatment non-adherence, and in turn graft failure. Adherence is believed to be the major factor in the high graft loss rate during adolescence and emerging adulthood, and during transition to adult care [5,12,73].

The experience of chronic illness as a child can lead to cognitive, emotional and social challenges, which pose potentially important barriers to successful transition to adult care [63–76]. A recent study by Berney-Martinet and colleagues used semi-structured psychiatric diagnostic interviews and questionnaires (Youth Self Report) to examine the psychological and psychiatric profiles of 40 adolescent renal transplant recipients (mean age 15.2 years) and 40 healthy adolescent controls, matched for age, gender, socioeconomic status and ethnic background. The parents of the patients were also interviewed and the parents of both groups filled out questionnaires (Child Behaviour Checklist). School delays or educational problems were striking. Sixty percent of transplant recipients had repeated at least one school grade (vs. 12.5% controls, $P < 0.001$). The prevalence of learning difficulties was 30% (controls 7.5%, P 0.03) and was associated with younger age at renal disease diagnosis, hemodialysis treatments, and lower socioeconomic group. Almost all those with learning difficulties were in a special class at the time of the study, for behavioral problems and/or disability (27.5% vs. 2.5% in controls, P 0.005). Psychiatric co-morbidity was high, with 65% of recipients meeting DSM-IV criteria for a lifetime psychiatric disorder and 50% having two or more concurrent psychiatric diagnoses. Included were depression (35%), attention deficit hyperactivity disorder (ADHD)(23%) and phobias (20%). The transplant adolescents were also significantly more withdrawn and socially isolated than their age matched peers. Another report, a systematic review of qualitative studies involving more than 300 adolescent transplant recipients, revealed that they had lowered self-esteem, and perceived negative reactions from their peers, a loss of belonging, and uncertainty about their life expectancy [77]. Both these studies illustrate the importance of pro-active assessment of pediatric transplant recipients' psychological health.

Education is an important social determinant of health and of success in later life [48,49,78]. As a group, pediatric organ transplant recipients have more challenges with school achievement than their peers and a higher unemployment rate in adulthood [70,79]. Although many of them wish to set long-term academic and vocational goals, extensive school absenteeism may cause some to fall behind and be overwhelmed with the struggle to achieve satisfactory grades [77]. Repeated or extended school absences not only lead to missed opportunities to master core skills, but can also make young people lose confidence in returning to school and less at ease socially, as they experience the challenge of rebuilding friendships that may be perceived to, or actually, have "moved on" [70]. Pediatric health professionals need to be vigilant, routinely screening transplant recipients for educational and social difficulties, and guide their parents to obtain appropriate supportive/interventional resources. Possible means to optimize the educational and vocational potential of transplant recipients include educational intervention programs, school-reintegration programs for those who have had prolonged absences, and greater coordination between hospital and school settings [80]. A well-timed intervention is key because students who show low levels of school engagement at the beginning of adolescence are more likely to drop out [81]. Wolf–Branigin et al. developed an innovative program to help integrate youth with disabilities into adulthood. It encompassed after-school activities, job "boot camp", summertime employment preparation, self advocacy skill development, a six-week "Summer Institute" of work experience and classroom activities, monthly workshops, parenting support classes, a youth leadership forum, and an intensive individualized education program focusing on high school completion [82]. Participants ranged in age from 12–20 years, with the mean age 14.8 years. Physical health, school functioning, and total functioning improved during the two year program, as measured by the Pediatric Quality of Life Inventory 4.0. The Career Maturity Inventory—Attitude Scale (CMI-AS) was used to assess employment readiness and showed a mean improvement over two years only for those in the youngest-age/grade cohort, suggesting a need for intervention early in adolescence.

Additional barriers to transition may result from adolescents' lack of engagement and lack of knowledge in the process of transition. Findings are mixed on how adolescents and young adults view the importance of transitional care. Anthony and colleagues conducted a qualitative study examining the perceptions and beliefs of 14 adolescent heart transplant recipients (mean age 15.7 years, range 11.7–17.8) [83]. They found the adolescents to be disinterested and apathetic toward the transition process, in striking contrast to the fear and anxiety of their parents. None of the teens were interested in becoming more involved or autonomous in their care. The authors speculated that caregiver overprotectiveness might have encouraged the adolescents to disengage from the transition process. Fredericks et al. studied the transition perspectives of adolescent/emerging adult liver transplant recipients using a comprehensive questionnaire [69]. The cohort consisted of 31 liver recipients between the ages of 12–17 years, and 15 between 18–21 years, as well as the parents of the younger group. These investigators found a low level of prior thought about transition, a very low level of knowledge about the process, and only moderate interest in learning about it. When dividing the patients by age group, those over 16 years had more interest in learning about transition. These two studies corroborate recommendations to begin discussing transition at an early age and to sensitize parents to the importance of promoting their child's involvement in his/her care, with supervised

progressive self-responsibility [17,25,31–34]. Some have expressed concern over the extent of responsibility adolescents should have for their medications. Simons et al. examined barriers to medication adherence in adolescent kidney transplant recipients. It appeared that non-adherence was more likely when the adolescents were responsible for taking their medications, rather than their parents; they did not report how the transition of medication responsibility from parent to adolescent occurred [84]. Studies have also shown that abrupt transitions of healthcare responsibility from parent to adolescent may cause distress to both. Nonadherence correlates with lower family cohesion and parental feelings of distress, and family conflict can negatively affect mental health outcomes of adolescent transplant recipients [85–87]. Healthcare practitioners need to be sensitive to these risks and provide counsel and support to patients and their families throughout the transition of healthcare responsibility.

In summary, patient level barriers to successful transition include the normal neurocognitive developmental processes that occur during adolescence and emerging adulthood, adherence issues, learning difficulties, missed educational opportunities, reduced social and work experience, psychiatric co-morbidities and lack of integrated patient preparation. Timely, proactive and multilevel interdisciplinary processes are needed to enhance integration of adolescent transplant recipients into adult care and to optimize their quality of life in adulthood.

Transition readiness

A major challenge in achieving successful transition is identifying the right time for transfer. Some centers have a specific chronological age by which transfer must occur. However patients of similar age may be very different developmentally, with some ready to transfer much earlier than others. The transition process includes not only the transfer of care but also transition of healthcare responsibility from parent to patient. Many consensus recommendations and guidelines advise that transfer should occur when the patient, caregivers and the pediatric and adult healthcare teams agree that the patient is ready [17,31]. Yet, how can providers best assess a patient's transition readiness? To truly understand what is required to optimize a young person's engagement in the adult healthcare system, standardized tools and metrics to assess transition readiness need to be developed and investigated. This is a burgeoning area of interest with several recently developed tools showing promise for clinical and research use. When assessing transition readiness tools for organ transplant recipients, there are four primary principles to apply:

1 Assessments should include multiple perspectives, including input from both the patient and the family. Several studies suggest discordance between patient and parent reporting [69,88,89]. In general, patients tend to report more healthcare independence than their caregivers do [69].
2 Assessments of transition readiness should be theoretically informed, reliable and valid. Achieving transition readiness requires progressive behavior changes on the parts of the patient, caregiver and provider. Using behavioral theories and models to inform the design of assessment tools can lead to better understanding of the successes or failures of behavioral interventions [90].
3 Assessments should be repeated on an ongoing basis throughout the transition process. The ideal transition readiness measure is sensitive to change and can be applied longitudinally.

Table 121.4. Transition milestones

1	Understand and be able to describe etiology of organ failure.
2	Be aware of long and short-term complications of transplant for overall health.
3	Understand impact of illness on sexuality and reproductive health.
4	Have sense of responsibility for own healthcare. This includes calling in refills, having medication knowledge, preparing pill box, adhering to medical regimen and managing healthcare appointments.
5	Demonstrate capacity to provide most self-care.
6	Express readiness to move into adulthood.
7	Own medical information in the form of a concise summary.
8	Be a knowledgeable health consumer. Be able to relay personal medical history. Understand health insurance coverage. Know how to seek emergent aid. Know how to communicate health concerns to providers independently.

4 Assessments should include proficiency in self-care, healthcare decision-making and self-advocacy, in addition to disease-related skills and knowledge [91]. Milestones for transition readiness have been suggested in various literature reviews and consensus statements [17,92] (Table 121.4)

The goal of a planned coordinated transition from pediatric to adult healthcare is to "optimize health and facilitate each person's achievement of his or her maximum potential." [93] Sawicki et al. developed the Transition Readiness Assessment Questionnaire (TRAQ) to identify areas in which emerging adults with special healthcare needs require additional preparation to achieve healthcare self-management [94]. It is based on the "Stages of Change" model which requires evaluation of a person's stage of readiness to adopt specific behaviors. TRAQ is a 33 item patient-completed questionnaire which measures skills needed to successfully transition in health, education, work, and daily life. It is reported on an ordinal scale and focuses on two domains, self-management and self-advocacy. The self-management questions include items about managing medication, medical appointments and health insurance. The self-advocacy questions include items about communicating with health providers as well as self-advocacy in education and employment. This questionnaire does not include caregiver or provider perspectives. It was validated in patients ages 16–26 years with a diverse range of special healthcare needs. It has not yet been tested as a longitudinal measure with predictive validity.

Another disease-generic questionnaire, the Developmentally Based Skills Checklist, was used to assess healthcare self-management skills in liver transplant recipients. It consists of 22 questions about understanding one's medical condition, communicating with providers, managing medications and appointments, and living a healthy lifestyle. Patients respond "Never, Sometimes or Always" to each specific behavior [95]. In liver transplant recipients, it identified significant baseline deficits in areas such as making appointments and understanding insurance. However, it is not known if it relates well to transition readiness or medical or behavioral outcomes and was not used as a longitudinal measure. Like TRAQ, it is a patient-focused measure with no parallel caregiver assessment.

Pai et al. created the Allocation of Treatment Responsibility (ATR) scale, to assess healthcare responsibility of adolescent and emerging adult transplant recipients and their caregivers [89]. This 18 item measure incorporates three subscales: oral medication, clinic attendance and laboratory attendance. It is completed separately by the patient and caregiver, and assesses who is primarily responsible for treatment-related tasks in the home. It was validated in 39 pediatric renal transplant recipients, ages 7–18 years, and their

caregivers. Discrepancy between patient and caregiver reports of total patient responsibility was associated with greater nonadherence. The ATR may have potential as a metric of healthcare self-management but has not yet been shown to reflect transition readiness.

Fredericks et al. has developed a transition readiness assessment tool for pediatric liver transplant patients 11–20 years of age [96]. It consists of 38 items and four healthcare provider administrated questions. Responses are either Likert scale (questions) self-report or direct demonstration of skills. There are four domains: self-management, medication knowledge, demonstrated skills and psychosocial adjustment. There is a separate 36 item survey for caregivers. The authors compared transition readiness scores with age, medication adherence, clinic attendance and liver function in 71 patients. There were no significant associations between transition readiness with adherence, clinic attendance or health outcomes. Older patients reported higher self-management skills but notably their perception did not equate with better ability to demonstrate those skills, such as being able to describe one's medication regimen. Moreover, greater healthcare self-management by the adolescent or emerging adult was associated with increased non-adherence, suggesting that decreased parental involvement may be associated with more medication indiscretion by the patient. This finding was also supported in a study of pediatric kidney transplant recipients who were more likely to be non-adherent when they had primary responsibility for administering their medications [84]. These data emphasize the need for providers to help patients and parents transition healthcare responsibility in a step-wise, systematic way, rather than having a parent relinquish total responsibility abruptly or, conversely, maintain total control as an adolescent matures.

In another study of kidney transplant recipients aged 15–21 years, Gilleland et al. tested the Readiness for Transition Questionnaire (RTQ), a 22 item tool which separately surveys patients and caregivers about (1) the patient's responsibility in healthcare self-management behaviors and (2) overall parental involvement in these behaviors [88]. In addition, patients and caregivers separately report on the patient's readiness (1) to assume full responsibility in healthcare and (2) to transfer to adult care. Patient and parent reports of overall transition readiness correlated positively with their reports of patient responsibility but inversely with reports of parental involvement. Thus, the patients most ready to transition had increased healthcare responsibility and decreased parental involvement. These patients also reported more medication knowledge, greater likelihood to call in their own refills and a better relationship with their parents. Reported increased transition readiness was linked with fewer medication barriers, but surprisingly was not associated with medication adherence. The RTQ is robust in that it considers multiple perspectives, is theory-based and valid, and considers medical skills and knowledge as well as personal health advocacy. This measure, however, like the others mentioned above, has not yet been tested longitudinally.

Thus, although the above peer reviewed published measures of transition readiness and healthcare responsibility are currently in use, none is perfect. They have not yet been tested longitudinally nor shown to be sensitive to change. Additionally, they are either self- and/or caregiver reports but do not include healthcare providers. Healthcare providers' input is also integral in the transition process and their objective assessments need to be interwoven with patient and parent reports. There are also several additional web-based tools that the reader may wish to refer to but none has been rigorously assessed for reliability, validity and ongoing predictive value [97–99].

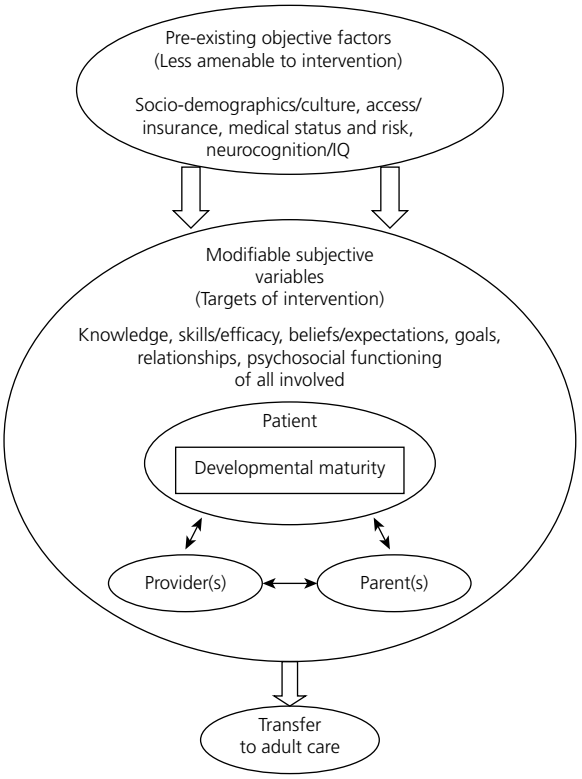

Figure 121.1. Social-ecological model of adolescents and young adults readiness for transition.
Reproduced from [91] Schwartz *et al.* with permission from Wiley.

A theoretical model proposed to operationalize assessment of transition readiness is the Social-ecological Model of Adolescents and young adult Readiness for Transition (SMART) shown in Figure 121.1 [91]. This model incorporates the complex interactions of patients, family and providers within the context of the patient's socio-demographic status and health experience. One component considers factors that may not be readily amenable to intervention, such as age, race, ethnicity and family structure, as well as potential transition barriers, such as impaired healthcare access and neurocognitive impairment. Another component focuses on the patient's developmental maturity, health knowledge, healthcare management skills, transition beliefs, goals, relationships and psychosocial functioning. This model is being tested in cancer survivorship but could also provide a comprehensive foundation for the development of future transition readiness assessment tools for transplant recipients. It adds to current measures by providing a comprehensive theoretical model which considers the partnership between providers, patient and family, within the context of the healthcare system and the patient's personal life outside the medical setting. This model shows promise to inform, build and test future transition readiness tools and interventions.

While establishing metrics of transition readiness remains challenging, much progress has been made in the last decade and current research efforts will continue to advance the science of transition.

Transition outcomes and research needs

The overarching goal of adolescent transition to adult care, originally published in 2002 and reiterated in numerous journal articles since, is to "maximize lifelong functioning and potential through high-quality, developmentally appropriate uninterrupted healthcare services that are patient-centered, flexible, coordinated, responsive, and comprehensive" [100]. In this section, we will discuss transition outcomes and research needs in the context of the above objectives.

Two published large database studies have clearly shown that the adolescent/emerging adult age group of transplant recipients is a very high risk demographic for graft loss and that the time period surrounding the event of transfer to adult care, especially the first-year, is associated with a remarkably high risk of graft failure [5,12]. These two reports provide important evidence of the need to be particularly vigilant in care of recipients who are in the 17 to 24 year age range and to improve the process of transition and transfer. They are important studies, as previous evidence for transition being a risky time was largely based on small single center series.

How should one proceed to improve the process of transition and evaluate the effects of interventions? A priority and a challenge are to define reliable measures of outcomes, what to measure and how to measure it. To assess the transition goal of "maximizing lifelong functioning and potential" we need to look at a wide range of elements, including graft function, graft and patient survival, overall physical and mental health, and psychosocial elements. However graft and patient survival may not be adequate for the purposes of many transition studies. Clearly defined rigorous endpoints that are sensitive (e.g. biomarkers of graft function such as in the areas of metabolomics, proteomics, and genomics, rather than graft loss) but also specific, with clear delineation of normal and abnormal ranges are needed. We need to discern changes in graft function early, before significant damage occurs. A confounding factor in assessing for changes in graft function following transition is that, for most organ transplants, there is a normal slow decline in function over time. Normal function may also have small day-to-day variations, so measures in current use may not be sufficiently sensitive and specific.

Evaluation of health and medication insurance adequacy and continuity of coverage throughout the transition period and into early adulthood are critical elements to assess for "uninterrupted care" and may be considered as good outcomes measures. However, assessment of "uninterrupted healthcare services" requires accurate databases of transitioning patients and their relevant transplant data both pre and post-transfer to adult care. A major limitation of current national databases is that they do not define the point of transfer to adult care, making it very difficult to evaluate large-scale effects of transition and transfer. Elements important to study include whether transitioning patients maintain their follow-up after transfer, have defined accessible healthcare providers for both transplant and primary care and attend clinic and laboratory appointments as expected. Hospitalizations and emergency room visits can also be examined but practice variation and policies of hospitalization and emergency room use may differ between pediatric and adult centers, making it difficult for a patient to serve as his/her own control.

Health related quality of life (HRQOL) is central to maximizing a patient's potential, and is an essential element to assess in transitioning patients. In evaluating patients prior to transfer it is important to keep in mind that parent/caregiver and patient reports may differ, and that it is the patient's evaluation that will be followed over time [87,101]. Factors that contribute to HRQOL, such as healthy nutrition, physical exercise and conditioning, and social integration can be actively incorporated into transition programs and evaluated. Attention is also needed to optimize the potential for education, a major determinant of health, and the preparation for satisfying employment, important in long-term quality of life. Thus, educational and vocational training outcomes are critical to study. Currently there is a paucity of HRQOL measures that can be applied and interpreted longitudinally as patients mature from childhood to adulthood. One unique tool to measure HRQOL over time across conditions and age ranges is The National Institutes of Health' Patient Reported Outcomes Measurement Information System (PROMIS), a reliable, valid, precise and responsive assessment tool that measures patient-reported health status [102]. This tool is not disease-specific however.

Once outcomes are defined, exposures must also be considered. An important component of outcomes assessment for success or failure of transition must include treatment-specific factors that contribute to optimal graft function and patient survival, such as medication adherence (discussed separately in this text) and treatment sequelae (e.g. malignancy, diabetes, hyperlipidemia, altered immunity).

When considering various study designs, both qualitative and quantitative studies are valuable, but study designs and objectives must be clear and reproducible. Qualitative research is important to assess elements such as, "developmentally appropriate, flexible and responsive" care. Patient experiences can be more richly described and deeply analyzed with carefully conducted qualitative studies and these works can help define questions to subsequently evaluate in a more widespread quantitative manner. In addition, patient experiences can inform quality assurance at a program level [17].

Transition research must be collaborative. On an institutional level, research efforts should be multi-disciplinary to incorporate the various biopsychosocial aspects of transition. While single center studies are important to inform the feasibility and design of various approaches, multi-center collaborations are necessary to ensure sufficient power and generalizability. To date, there are few publications on outcomes of transition processes and most are descriptive small cohort analyses, often single-center, sometimes with historical controls [7,51,103]. Although the results are encouraging, studies with historical controls generally cannot account for subtle but significant adjustments in treatment protocols, changes in healthcare personnel or variations in disease characteristics, particularly with the small patient numbers in pediatric transplantation.

Transition research should ideally be longitudinal. One challenge to longitudinal data collection is securing patient consent to permit long-term follow-up [17]. It has been suggested that national registries collect more granular data to better assess long-term patient outcomes [104].

Beyond patient outcomes, future transitional research efforts should examine the education and training of healthcare professionals around transitional care to promote service delivery that is comprehensive and systematic. In the UK, it has been proposed to establish a set of performance standards for operating a transition service so that performance may be assessed [30]. In 1993, Blum et al. reported that "while we can define the concept and goals of transition, there is simply too much that is not known about the

process" [3]. At that time, Blum cited lack of knowledge regarding which care models are most effective and for whom, and whether, health outcomes change as a result of formal transition programs. These deficits in knowledge still exist 20 years later. If the provision of transitional care is systematic and theoretically informed, it will be easier to understand clinical interventions and interpret observed results. Systematic quality assurance and improvement studies as well as cost-effectiveness analyses are crucial to inform and support research approaches.

Summary

- Transition is a dynamic process with many stakeholders. Care must be planned, accessible, coordinated and continuous as well as psychologically/developmentally appropriate. Think about transitional care for patients starting from the age of 10–12 years and possibly even earlier.
- Consider various sources of barriers to smooth transition, including the patient, caregiver, provider and healthcare system. Once barriers are identified, work with patients and families to develop targeted approaches to promote successful transition.
- To evaluate "high-quality, developmentally appropriate uninterrupted healthcare services that are patient-centered, flexible, coordinated, responsive, and comprehensiveness," there are a multitude of factors to study, some easier than others. A major domain is preparing patients and families for transition and transfer. We need clearly defined tools for patient preparation, longitudinal assessment of progress and metrics to measure readiness for transfer. It is important to use behavioral theory in intervention design and the most effective efforts to make change may result from collaborations between clinicians, psychologists, social workers, educators, and behavior theorists.

Conclusion

The goal of transition programs for adolescent and emering adult transplant recipients is to improve long-term health outcomes, including graft survival and psychosocial elements, such as gainful employment and health related quality of life. The need to prevent allograft loss in transitioning transplant recipients is critical not only for the individual patient but also for society. Every potentially preventable organ loss that occurs due to problematic transfer or transition adds to the burgeoning demand for limited organs. Although multi-disciplinary care may appear to require substantial resources, the potential benefits are great.

Smooth transition need not remain an elusive concept when providers, patients and families are engaged in the process of healthcare self-management early, and on an ongoing basis. Important components of advancing improvements in transition are education and training of healthcare professionals, to promote systematic and effective service delivery. Research is essential to evaluate efficacy of interventions and future research efforts must be multidisciplinary, comprehensive and longitudinal.

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SECTION 9

Transplant Administration

CHAPTER 122

Transplant Coordination

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Introduction

The successful management of a transplant patient is due in part to the multidisciplinary team approach to their care before, during and after their transplant. Transplant Coordinators are essential members of multidisciplinary transplant teams and their role in all phases of transplant care has become established in essentially all robust transplant centers. Transplant coordinators have numerous roles, but in general help patients navigate the complexities of transplant care, and assist physicians in ensuring that the required monitoring and care is delivered. This chapter will focus attention on the evolution of transplant coordinators as invaluable members of a transplant team, detail their numerous roles and responsibilities, and summarize the current training models employed for their certification. Additional details regarding the roles of coordinators can be found in Chapters 124 and 125, discussing inpatient and outpatient service structures respectively.

History and the evolution of the role of the transplant coordinator

Currently a nursing role, transplant coordination was developed out of necessity in the early days of transplantation to "coordinate" any aspect of the donation and transplant process. Early coordinators were from many disciplines including nursing, surgical technicians, and laboratory personnel. They were involved in organ recovery, preservation and post-operative management of the transplant patient.

The role has evolved to an advanced nursing role with a defined scope of practice, competencies, and a certification process. Additionally it is divided into two types of coordinators; procurement coordinators managing the organ donation process, and clinical transplant coordinators caring for the transplant patient. The clinical transplant coordinator role has further specialized into organ specific roles as well as pre and post-transplantation. Emerging is the utilization of the advanced practice nurse functioning as a transplant coordinator and a transplant coordinator role specifically for the care of the live organ donor.

The inception of the role of the transplant coordinator

As the early physician pioneers of transplantation worked on the surgical and immunological ways of making solid organ transplantation possible, in the background were people performing many of the tasks to support the efforts of the physician. This was the role of the transplant coordinator. These tasks included helping with recovery, preservation, and implantation of the donated graft, to selecting and evaluating patients and assisting in developing ways to allocate the organs available. Essentially, it was any non-physician role that needed to be done to assist in making the miracle of transplant happen. "The term, "Transplant Coordinator" was likely coined by transplant surgeons who looked for people who could, and would, need to multi-task, would be asked to provide skills from several disciplines, and to coordinate what was then a work in progress" [1].

The first transplant coordinators in the US. worked in the early 1960s, in Europe in the mid 1970s and in Asia in the 1980s [1]. Initially as a "jack of all trades", Transplant Coordination has evolved into a profession and has become very specialized over time, with more than one type of coordinator involved in the success of transplantation. Early procurement coordinators started with the consent process working with families to understand the concept of organ donation and assisting surgeons in the surgical retrieval of organs, preservation and pumping organs, and transporting them to the destination safely. The first clinical coordinators, often dialysis nurses, transitioned from the procurement role to maintain waiting lists, assisted patients getting evaluated for transplant, and managing post-operative issues such as complications and immunosuppression management, while surgeons were in the operating room. In fact, the utilization of a transplant coordinator to work with physicians and surgeons is likely one of the first instances where a multidisciplinary team was critical to the success of the surgery.

In 1979 the North American Transplant Coordinators Organization (NATCO) was formed as a national society where those in the role could work together to establish the transplant coordinator as a profession, define a scope of practice, identify educational needs of future transplant coordinators. This early group of transplant coordinators also needed to collaborate to address the many allocation, legal, medical, social, financial and religious issues which had arisen as the success of transplant has improved.

Before the inception of the United Network for Organ Sharing (UNOS), and The Organ Procurement and Transplant Network (OPTN), early allocation of kidneys was accomplished by the Southeastern Procurement Foundation (SEOPF), but many don't realize that it was the transplant coordinators through the

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

NATCO that manage a "24 hour alert system" to allocate extra-renal organs [1]. Initially as an answering machine and eventually a computer system that allocated organs 24 hours seven days a week. It is with the persistence, drive and passion for the success of transplant that these early pioneers helped to define the scope and practice of our current transplant coordinator.

Transplant certification

As the field of transplant grew and more people chose transplant coordination as a profession it was determined that a national certification process was needed to enforce standards for those who care for donors and recipients. A task force of various transplant professionals who assumed the role of the transplant coordinator was created to perform a job analysis, look at certification of other allied health groups, and consult other professions involved in organ donation and transplant. In 1988 The American Board for Transplant Certification (ABTC), an independent, non-profit organization, was founded [2]. Initially offering procurement and clinical transplant certification, currently the ABTC is the certifying agency offering four transplant certifications: Certified Procurement Coordinator (CPTC), Certified Clinical Transplant Coordinator (CCTC), Certified Clinical Transplant Nurse (CCTN) and the Certified Transplant Preservationist (CTP). These are voluntary accredited examinations in the field of organ transplantation. Its goals are to:

- Establish educational and competency standards for the transplant professional.
- Define transplant coordination, nursing and organ preservation as a profession.
- Credential transplant professionals.
- Maintain a list of credentialed practitioners.
- Promote continued professional growth of practitioners through education and recertification [2].

Currently, transplant coordinators must work in the field for at least one year prior to sitting for the exam and maintain currency through continuing education credits. In 2010, there were over 2100 certified transplant coordinators in the US [3].

Procurement coordinators

Today's procurement coordinator functions in many capacities and may assume one or all of the following roles depending on the organ procurement agency's organizational structure [4]:

- Facilitates hospital development and professional education.
- Evaluates organ and tissue donors.
- Approachs families about organ donation and obtain informed consent.
- Obtains extensive medical and social history by interviewing the family and available resources.
- Ensures that all medical and legal requirements are met prior to organ donation and recovery.
- Assists with organ donor management to maintain optimal organ function.
- Ensures allocation is efficient, timely and proceeds according to UNOS policy.
- Assists the surgical team with organ recovery and tissue preservation.
- Maintains donor records.
- Communicates and supports the donor family.
- Communicates with collaborates with and supports the donor hospital staff.

Clinical transplant coordinators

Just as the role of the procurement coordinator has changed over time, the clinical transplant coordinator has evolved as well. The reason for this is multifaceted. First, patients are more complex, many sicker and older with more co-morbidities, there are more regulatory requirements for programs to comply with, there are more transplants being performed, medication regimens are more elaborate and programs have become organ specific. Additionally both UNOS/OPTN and Centers for Medicare Conditions of Participation (COP) have requirements for the types of professionals who can be clinical transplant coordinators and roles they must perform.

The OPTN describes the clinical transplant coordinator as a member of the transplant team who will be responsible for coordinating clinical aspects of candidate's care. This includes working with patients and their families to be listed, and followed, through transplantation. The transplant coordinator must be a registered nurse or other licensed clinician who performs or oversees other healthcare personnel [5].

The Medicare COP require a clinical transplant coordinator to ensure continuity of care of patients and live donors during the pretransplant, transplant, and discharge phases of organ transplantation and live donation [6]. This includes addressing elements identified in the assessment and care plan, educating patients, live donors and families about treatment options, post-operative care and therapies, monitoring medical, surgical and psychosocial status and providing feedback to other team members. Kidney transplant coordinators must document communication with dialysis facilities. Additionally, all coordinators must be a registered nurse or clinician, licensed in the state in which they practice, and have experience and knowledge of donation and transplantation [6].

Furthermore, the COP requires adequate training of nursing staff and clinical transplant coordinators that should include; thorough orientation program, documented review of policy and procedures, structured continuing education, and evaluation of the coordinators training needs [6].

Neither regulatory body dictates if the same personnel or different personnel perform the various roles pre, peri and post-operatively. Individual transplant programs implement the roles differently. Some have a pre and post-transplant models, others have the same transplant coordinator care for a patient throughout the entire process. Additionally some programs utilize registered nurses only while others have a mix of other healthcare professionals working together to incorporate the various roles. Examples of care models are shown in Table 122.1, and core competencies for clinical transplant coordinators are found in Table 122.2.

Pretransplant

Evaluation

The transplant referral and evaluation process is the initial step to treat end organ failure by determining suitable candidates for a successful transplant. A clinical transplant coordinator must understand the purpose of and help coordinate the transplant evaluation [7]. This includes the medical, psychosocial, laboratory, radiological, nutritional and financial evaluation. They must

Table 122.1. Transplant coordinator care models utilized at transplant programs

Staffing
- Registered Nurse(RN) only Transplant Coordinators
- Advanced Practice Nurses(APN) and/or Physician Assistants hired to perform traditional role of Transplant Coordinator with no autonomy
- Combined APN/RN model
- APN only model

Care models
- Separate pretransplant and post-transplant coordinators with on call responsibilities
- Continuity of care model: same coordinator pre and post-transplant
- On Call teams
- Single organ teams and multi organ teams
- Adult and pediatric combined or separate

Table 122.2. Competencies for the clinical transplant coordinator (CTC)

Pretransplant

Transplant referral and evaluation
- CTC must understand the purpose of and help coordinate the transplant evaluation
- CTC must follow UNOS/OPTN regulations for listing of the candidate

The waiting period
- CTC must have knowledge and ability to monitor and coordinate care of the patients awaiting transplant
- CTC must be knowledgeable of CMS and UNOS/OPTN requirements
- CTC must maintain complete documentation of the pretransplant phase and education

Perioperative period
- CTC has the knowledge and ability to facilitate the transplant process when an organ is available for the intended candidate
- CTC must adhere to current UNOS/OPTN allocation policies

Post-transplant

Inpatient period
- CTC is knowledgeable of post-transplant management including medications, complications, interventions
- CTC is involved in discharge planning
- CTC collaborates effectively with the multidisciplinary team

Outpatient period
- CTC is knowledgeable and is able to coordinate the care of the outpatient recipient
- CTC facilitates a recipient's ability to achieve optimal physical, social and emotional health

Professional practice
- CTC is proficient in and continues to advance his/her knowledge of transplant
- CTC is responsible for sound judgment and administration of quality care to donors, recipients and families

Adapted from the NATCO Core Competencies for the Clinical Transplant Coordinator [7].

collaborate with the multidisciplinary team to determine blood type and immunological data for compatibility, serological data to assess for the presence of infectious disease, screen for evidence of cancer and identify co-morbidities that may increase the risk of or preclude transplantation as an option for the candidate [5–7].

The psychosocial, psychiatric and financial status of the candidate must be assessed and the clinical transplant coordinator must work with the team to determine the educational level, cultural background, ability to comply and evidence of adequate support for a successful transplant. Additionally they must collaborate with referring physicians, consultants, and the transplant team regarding the candidacy of the patients.

Table 122.3. Critical components of education and informed consent

- Transplant evaluation process
- Options for transplant:
  - living donation
  - standard criteria deceased donation
  - expanded criteria deceased donation
- Alternatives to transplant
- Organ allocation
- OPTN regulations
- Waiting for a transplant
- Requirements for re-evaluation when needed
- Need for support throughout the process
- Option to multiple list for transplant
- Transplant process
- Risks and benefits to transplant
- CDC high risk factors
- Risk of recurrent disease
- Center specific patient and graft survival and compare it to national survival rates
- Financial aspects of transplantation
- Postoperative course:
  - monitoring of vital signs
  - diet and activity
  - medication, purpose, dosing and side effects
  - need for biopsy and laboratory tests
  - health maintenance and adherence to treatment regimen
- How to communicate with the transplant team
- Need for and frequency of follow-up
- Method for accessing psychosocial services and local medical care
- How to identify complications:
  - rejection
  - infection

Data from UNOS/OPTN Policy, CMS conditions of participation and NATCO Core competencies [5–7].

The clinical transplant coordinator must understand and comply with all UNOS/OPTN and CMS regulations for listing of the candidate [5,6]. Lastly, and most importantly, the clinical transplant coordinator must educate the patient, family and support team about all aspects of transplantation and document in the medical record. Critical elements of the education for transplant patients are shown in Table 122.3.

The waiting period

A clinical transplant coordinator designated to waitlist management is a crucial role especially in large abdominal transplant programs, due to the length of time it takes for an organ to become available and the demand to keep evaluation tests current, thus ensuring that candidates are ready for transplant when an organ is allocated. Depending on the age, disease and organ needed, re-evaluation testing varies in type and frequency. The clinical transplant coordinator must understand policies and procedures related to wait list management and be familiar with the national recommendations for retesting to ensure ongoing candidacy. They must have the knowledge and ability to monitor and coordinate care of the patients awaiting transplant, maintain relationships with patients and collaborate extensively with local physicians and dialysis centers [7]. The entire pretransplant medical record must be maintained complete with documentation of the testing performed during the pretransplant phase, education and informed consent process, and any correspondence between patient and referring team required for regulatory compliance.

Perioperative period

The clinical transplant coordinator works closely with the Organ Procurement Agency (OPO) surgical team and patient when an

organ is offered. They must have the knowledge and ability to facilitate the transplant process when an organ is available for the intended candidate [7]. This includes, but not limited to, understanding what makes an organ suitable for transplant; communication with the OPO and transplant team about organ suitability, logistics, and policy; compliance with organ allocation policy; education and support of the candidate about the process and specifics of the particular organ allocated (Table 122.3). Various other roles including coordinating transportation, obtaining crossmatch results, list management, verifying vital data between the donor and recipient, arranging for presurgical procedures such as dialysis and documentation of the process may also be performed by the clinical transplant coordinator. It is imperative that the coordinator comprehends and adheres to current UNOS/OPTN allocation policies during the perioperative period.

Post-transplant

Inpatient period

At the time of transplant, a patient and their support system may be overwhelmed with the enormity of receiving a new life saving organ. Whether the recovery is smooth or complications arise, the clinical transplant coordinator is integral to working with the multidisciplinary team to care for the patient in this transition period. The clinical transplant coordinator must be knowledgeable of post-transplant management including medications, complications, and interventions [5–7]. Communication is essential between the team and the referring physician, consultants and payers. A coordinator can be a facilitator of this communication. Additionally, the coordinator must be involved with discharge planning to anticipate any medical psychosocial and financial needs prior to leaving the hospital. Education is of utmost importance and it must be culturally and education level appropriate (Table 122.3).

Patients need to learn the medications needed post-transplant, this includes their purpose and dosing as well as side effects. They need to be able to identify signs of rejection and infection. They need education about diet; exercise, restrictions and how to comply with post-transplant protocols. This can be overwhelming and therefore knowing the transplant coordinator is accessible and available to assist with questions after discharge is key [7]. Lastly, the coordinator needs to communicate verbally and in writing to any medical team that will be involved in the post-transplant care to ensure optimal care.

Outpatient period

A clinical transplant coordinator caring for a transplant recipient after discharge must be knowledgeable and able to coordinate the care after organ transplantation. These patients are very complex and are at risk for complications, medication interactions and side effects. The transplant coordinator must monitor a patient, often in person and by telephone, and be competent to determine needs and risk factors for complications. They must provide ongoing education and reinforcement of education to ensure comprehension. Additionally, they must assess for any evidence of non-adherence that may affect graft function. This monitoring may include review of lab values that determine organ function, assessment of therapeutic range for medications and identification of complications. It is often the coordinator that reviews these labs and makes adjustments in medications according to agreed upon post-transplant protocols. They must collaborate with the physicians and surgeons on the team to make appropriate changes in regimens. They are also involved in coordinating follow-up and consultant appointments and work with other disciplines to facilitate a recipient's ability to achieve optimal physical, social and emotional health [7].

Professional practice

A clinical transplant coordinator's role is an essential role in transplant and as the field changes so to must the coordinator. They must be proficient in and continue to advance his/her knowledge of transplantation. One way to do this is to become certified in transplant coordination and attend continuing educational seminars to remain current. Additionally one must review professional literature in transplant journals and incorporate this into the care of the patients. Often a coordinator is asked to participate in, or facilitate transplant related research, and publish finding as appropriate.

To ensure ongoing quality care, experienced transplant coordinators must mentor new staff to assist in the development of new professionals. Adherence to regulations, allocation policy, professional conduct and sound judgment is essential for the administration of quality care to donors, recipients and families.

Live donor coordinator

As the regulatory requirements for transplant programs have increased, so has the requirements surrounding the care of the live organ donor. Transplant programs that perform live donation must have a separate team independent of the recipient to care for the live donor. Each donor must be assigned an independent donor advocate and a Live Donor Transplant Coordinator is involved in the multidisciplinary process to coordinate the care pre and post-transplant donation [6,8].

The live donor coordinator roles are similar to that of the clinical transplant coordinator caring for transplant patients, however, as the field of live donation has evolved, the needs of live organ donors has become very specialized making it a new subspecialty of transplant [9]. Coordinators caring for live donors need a unique set of competencies to delivery quality care. These are shown in Table 122.4. It is important that a live donor coordinator maintains an understanding and working knowledge of transplantation and related policies and procedures because potential donors need to understand the recipient process, risks and chance of surviva,l and rely on a knowledgeable donor team to prepare them for donation. They must identify appropriate living donor candidates upon referral and coordinate the evaluation process of the living donor. It is of utmost importance that donor safety remains a priority and those with contraindications are excluded early in the process [10]. This coordinator must have the sophistication and experience to

Table 122.4. Role of the live donor transplant coordinator

- Maintains an understanding and working knowledge of transplantation and related policies and procedures
- Demonstrates knowledge of OPTN/UNOS policies and listing requirements
- Identifies appropriate living donor candidate upon referral
- Coordinates evaluation process of the living donor
- Education of patients, families and the public about live donation, process, risks, benefits and need for follow-up
- Assesses donor motivation, ability to comprehend risk and desire to proceed with donation
- Assists in determination of donor suitability
- Collaboration with the multidisciplinary team involved in donor care and the live donor advocate
- Provide direct patient care, support and education post donation both in the perioperative period and through long term follow-up.
- Provides Living Donor Advocacy before and after donation and in the setting of choosing not to donate

assess a donor's motivation, ability to comprehend risk and desire to proceed with donation. Often the coordinator will be involved in the education of patients, families and the public about live donation; process, risks, benefits and need for follow-up. They collaborate with the multidisciplinary team involved in donor care and the live donor advocate to determine donor suitability. If unsuitable or if a donor decides not to proceed with donation, the coordinator ensures donors are comfortable with the outcomes and provides support as needed. They provide direct patient care, support and education post-transplant donation both in the perioperative period and through long-term follow-up. Ultimately a live donor coordinator provides care and advocacy before and after donation and in the setting of choosing not to donate.

Role of the coordinator in education

Likely the most important role a clinical transplant coordinator holds is that of educator [11]. Although much more common place than years past, transplantation remains a specialty that has a limited number of clinicians competent to care for this complicated patient populations. Regardless of which organ specialty they work in or which aspect of the care they deliver, a transplant coordinator must educate their patients and families about transplantation. The specific topics have been discussed previously (see Table 122.3). Additionally it is often necessary to educate other nurses, consultants, referring physicians, residents, medical students, and fellows rotating through a transplant service. Outside the medical team a clinical transplant coordinator may need to educate payers, regulatory agencies, financial coordinators, pharmacies, and the public about the nuances of transplantation in order to advocate for their patients. Therefore it is important for them to understand all aspects of transplantation.

Role of the advanced practice nurse as coordinator

The traditional role of the clinical transplant coordinator is an advanced role with significant autonomy required. For this reason more transplant programs are utilizing the advanced practice nurses (APN) in the care of transplant patients, either in combination with the traditional transplant coordinator or in its place. The reasons include:

- APN are educated about physical assessment, diagnostic testing and trained to independently manage complex disease processes.
- APN are licensed to practice autonomously.
- APN have prescriptive authority in most states.
- APN have the ability to bill for services thru Medicare, Medicaid and many private payers.
- Transplant centers are overburdened with the volume of patients requiring healthcare and there is shortage of house staff and physicians entering the field of transplant to care for them, APN fill this void.

An APN in transplantation has been described as a healthcare provider with an advanced level of education in their profession who directs care for patients who are potential/actual organ donors (living or deceased), transplant candidates or transplant recipients. APNs are medical providers who are not physicians, but who are licensed to diagnose and treat patients in collaboration with a physician, extending the availability of health care [12].

Roles performed by the APN as transplant coordinator include pretransplant education and evaluation, waitlist management, collaboration with referring physicians, assisting the surgical team in the operating room, inpatient management of pre and post-transplant patients, outpatient post-transplant management of immunosuppression and complications, long term management or the transplant recipient, care of the live organ donor, care of the pediatric transplant recipient, management of outreach clinics, psychological management pre and post-transplantation, and professional development and leadership [9,12–17].

APNs complement existing staffing models. Care models utilizing the APN vary but the ability to reimburse for care is a financial incentive [18]. They can relieve an overburdened medical team and provide quality care and education to the patients. Figure 122.1 shows an example of how ANP can be incorporated into the current

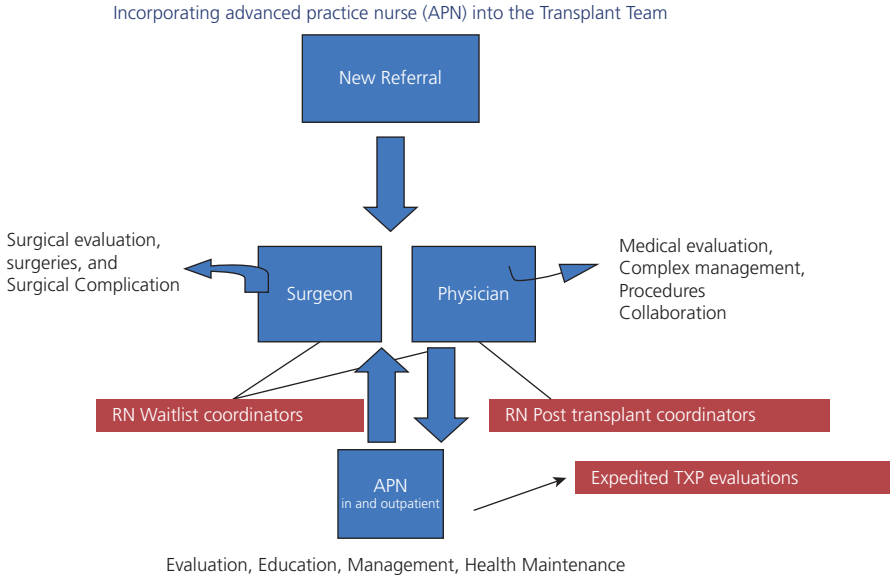

Figure 122.1. Care model utilizing an advanced practice nurse.

transplant team. They can work in combination with or in place of pre and post-transplant coordinators to care for patients in collaboration with the physicians and surgeons. Broad cost analysis and data on successful utilization is needed to understand the true benefit to programs and the transplant patients.

Summary

Regardless of which aspect of organ donation or transplant the transplant coordinator works, it is a critical role whose key components are to support, clinically manage, educate, and work collaboratively with the other members of the transplant team to achieve successful outcomes. Traditionally a generalist coordinator role who was a registered nurse, current transplant coordinators have many specialties and includes advanced practice nurses. As healthcare reform continues to change transplantation, so too will the role of the coordinator need to evolve to meet the demand of our patients.

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CHAPTER 123

Transplant Pharmacy Services

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Introduction

The success of organ transplantation is arguably as dependent on the proper use of an expanding array of pharmacological agents as on any other factor. Indeed, management of immunosuppressive drugs, anti-infective agents, and an often daunting list of medications required to address recipient co-morbid conditions occupies a substantial amount of a transplant teams' efforts, and often involves navigation of significant drug interactions and incompatibilities. As such, pharmacists have become integral members of most transplant teams, and accordingly, the expectations of transplant pharmacists have become increasingly defined and codified. This chapter will describe the role of a transplant pharmacist as part of a broader multidisciplinary transplant service, provide the reader with a general understanding of the training necessary to become a transplant pharmacist, and the manner in which they can improve patient outcomes and care. Specifics of the pharmacology of transplantation are covered in Chapters 98–102.

Evolution of transplant pharmacy

Pharmacists have been part of the multidisciplinary transplant care team for almost a half a century. The first publication detailing the pharmacists' role on the transplant team was published by John Mitchell from The Ohio State University in 1976 [1]. Mitchell describes the pharmacists' role in the 1970s as one that is very similar to how many pharmacists practice today. In 2007, Dr. Gilbert Burkart published reflections on the past 30 years of transplant pharmacy [2]. In this paper much credit is given to Dr. Thomas Starzl at the University of Pittsburgh and Dr. John Najarian at the University of Minnesota for being the first surgeons to include pharmacy faculty members as part of the transplant team recognizing their pharmacotherapy expertise. Burckart and colleagues Venkataramanan and Ptachcinski facilitated therapeutic drug monitoring of cyclosporine into clinical practice, and published detailed cyclosporine pharmacokinetics in 1986 [3]. Since the mid-eighties, pharmacists have continued to make significant contributions to the transplant literature with publications on the pharmacokinetics and pharmacogenomics of immunosuppressive therapy in addition to numerous publications focusing on clinical outcomes in transplantation [4–10]. The direct impact of the transplant pharmacist on patient outcomes has been demonstrated in publications showing improved medication compliance and blood pressure management with transplant pharmacy services [11–13].

The pharmacist's membership within the transplant team has transitioned from a novelty to an expectation for today's transplant clinicians. Most notably, the first publication outlining role of the pharmacist as part of the multidisciplinary transplant team was published in the *American Journal of Transplantation* in 2011 [14]. This paper outlines the various roles the pharmacist can have in the inpatient, ambulatory and research setting and describes the necessary training required to be competent in these roles.

In addition to contributions to the literature, transplant pharmacists have become increasingly recognized by various professional pharmacy and medical societies. The membership within these professional societies has grown exponentially over the past five years. Since its inception in 1994, the American College of Clinical Pharmacy (ACCP) Immunology and Transplantation Practice and Research Network has grown from 75 members to over 330 members, most notable is the increase in membership from 2004 to 2011 where the group grew from 170 to 332 members [15]. The majority of this growth has been related to the increased need for transplant pharmacists subsequent to the requirements set forth by Centers for Medicare and Medicaid Services (CMS), which will be discussed in length later in the chapter.

The ACCP and the American Society for Health System Pharmacists (ASHP) now identify transplant pharmacy as a discipline worthy of recognition commensurate with the highly specialized patient population served. The ASHP now has specific accreditation standards for postgraduate year two (PGY2) transplant residency programs and has begun to accredit transplant pharmacy residency programs across the country [16]. This is an important step in the evolution of transplant pharmacy training, as it ensures that pharmacy residents are meeting specific standards and receiving high quality training. The number of PGY2 transplant residency programs has also increased dramatically in the past ten years growing from less than six programs to nearly twenty in 2012, with the majority now being ASHP accredited programs. This will allow the profession to continue to grow, and maintain high standards of excellence for pharmacy services provided to all transplant donors and recipients.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

In October 2008, the board of directors within the American Society of Transplantation (AST) incorporated transplant pharmacists into the community of practice (CoP) model. This CoP was initiated in 2009, and as of November 2011 had over 160 members, including a chair, chair-elect, past-chair and four members at large. This formalized development of a specific practice group within the AST was followed by the appointment of a transplant pharmacist onto the Editorial Board of the *American Journal of Transplatation*, the latest step in recognizing pharmacists as vital to the overall success of transplantation and significant contributors to the ongoing research into optimizing drug therapy in this high risk patient population.

Governing body support for transplant pharmacy

In 1984, the US Congress under the National Organ Transplant Act (NOTA) established the unified transplant network known as The Organ Procurement and Transplantation Network (OPTN). The act called for the network to be operated by a private, non-profit organization under federal contract. The OPTN has two primary goals: (1) Increase the effectiveness and efficiency of organ sharing and equity in the national system of organ allocation, and (2) Increase the supply of donated organs available for transplantation. The OPTN is a unique public-private partnership that links all of the professionals involved in the donation and transplantation system. The OPTN is administered by the United Network for Organ Sharing (UNOS) under contract with the Health Resources and Services Administration of the US Department of Health and Human Services [17].

A set of bylaws developed by UNOS specifies the exact criteria that each transplant program must follow in order to be compliant with the standards. These bylaws were amended in 2004 to recognize and identify the roles and responsibilities of the pharmacist as an essential member of the transplant team. Specifically, these bylaws mandate that "all transplant programs should identify one or more pharmacists who will be responsible for providing pharmaceutical care to solid organ transplant recipients" [18]. The transplant pharmacist should be the designated member of the team who plays a key role as the drug information expert on the team and is responsible for compliance to institutional protocols, screening and anticipating drug interactions, patient and caregiver education, along with responsibilities outlined in later sections of this chapter [18].The CMS currently provides accreditation for transplant programs allowing them to receive payment for services rendered. CMS performs formal site visits at all CMS approved transplant centers every three to four years to ensure the transplant centers are meeting the established conditions of participation. On March 30, 2007, CMS published the *Medicare Requirements for approval and Re-Approval of Transplant Centers to Perform Organ Transplants*. This document outlines the specific requirements set forth by CMS for accreditation. With regards to the role of the pharmacist on the transplant team, the document states "a transplant center must identify a multidisciplinary transplant team (composed of individuals from medicine, nursing, nutrition, social services, transplant coordination, and pharmacology) and describe the responsibilities of each member of the team". The document does not specifically state that the pharmacist must be the team member with the pharmacology knowledge. However, in an open comment session CMS did refer to the UNOS bylaws for details on the specific role of the pharmacist on the transplant team [19]. An updated version of the interpretive guidelines was published in June of 2008 [20]. Important updates outlined in this document include the need for all members of the multidisciplinary team to document all activities in the medical record as well as the need for the multidisciplinary team to be involved with the care of both the donor and recipient throughout all distinct phases of the donation and transplantation process. These phases include the pretransplant phase before listing, the perioperative period, the immediate post-transplant hospital admission and the discharge process.

In addition to the updated interpretive guidelines, CMS also published a set of documents in September 2008 to assist centers in planning for surveys [21]. This document specifies that during the CMS visit the inpatient pharmacy will be toured and the designated transplant pharmacists will be interviewed. This document provides examples of the typical questions that will be asked of the pharmacist. The pharmacist personnel files are also reviewed to evaluate the pharmacist's transplant specific training, current competency and continuing education related to transplant.

Education and training of the transplant pharmacist

Transplant pharmacists have various levels of training and education. A recent survey of the ACCP transplantation and immunology Practice and Research Networks (PRN) reveals that the majority of practicing transplant pharmacists have advanced training beyond their PharmD degree. Sixty six percent completed at least a postgraduate year 1 (PGY1) residency and an additional 45% completed advanced training in transplantation through a transplant residency and/or a fellowship. Even though the majority of transplant pharmacists are pursuing advanced training, the minimum qualifications for the transplant pharmacist vary from center to center. Thirty-seven percent of survey responders indicated that their employers required a minimum of a PharmD degree. Another 35% required at least a PGY1 residency; 29% required at least five years of transplant experience or a postgraduate year 2 (PGY2) residency in transplant; and 26% required a PGY2 in transplant.

Historically, a four year Bachelor of Science degree was required to become a registered pharmacist. However, in the 1990s the degree was converted from a bachelor's to a doctorate degree. Currently most schools of pharmacy subscribe to a four year (three year accelerated year-round) doctor of pharmacy program following completion of prerequisites or a four year bachelor of Science in Pharmacy, followed by a two year doctor of pharmacy program. The final year of the doctoral degree program consists of a series of clinical rotations focused on patient care. After obtaining the PharmD, all individuals must pass a mandatory national (competency) and state (law) exam along with completing a specified number of internship hours prior to receiving licensure to practice pharmacy as a registered pharmacist.

Transplant pharmacists practicing prior to the advent of formal postgraduate training

Many of the current mentors in the transplant pharmacy field entered practice without formal residency training in solid organ transplant. These pharmacists are responsible, along with their non-accredited trainees, for developing the principles and current educational standards of new transplant pharmacists and their accredited residency programs.

Postgraduate residency training

The complexity of transplantation and limited exposure to transplant during didactic PharmD course work has led to the need for formalized postgraduate clinical training. The first stage of postgraduate training is often completion of a PGY1 pharmacy residency program. This residency should be accredited by ASHP. Completion of a PGY1 residency allows one to rotate through various clinical patient care settings such as internal medicine, surgery, intensive care, specialties such as transplant, infectious disease, cardiology, ambulatory care and others. In addition to the clinical rotations, PGY1 residents are exposed to administrative practices, teaching activities and some gain experience in conducting clinical research through their training. This introduction serves as a foundation for developing skills and competence in providing pharmaceutical care in multiple practice settings.

Following the PGY1 residency, transplant pharmacists should complete a PGY2 specialty residency in transplantation. The majority of these PGY2 training programs are accredited by ASHP. The required educational outcomes, goals, and objectives for PGY2 pharmacy residencies in solid organ transplant are designed to transition PGY1 residency graduates from generalist practice to specialized practice focused on the care of solid organ transplant recipients and, in some instances, living organ donors. The graduates are equipped to participate on interdisciplinary teams assuming the responsibility of medication related aspects of patient care including education and research in both the inpatient and outpatient settings.

Transplant fellowship training

Although fewer in number, some transplant pharmacists, have undergone specialized fellowship training. The numbers of programs suited for this type of training are limited. These programs are typically two years in length and are usually completed following a PGY1 or PGY2 residency. Unlike a PGY2 transplant specialty residency, where the emphasis is patient care, the emphasis during a transplant fellowship is basic and/or clinical research in the field of transplantation. The goal of a fellowship is to train the pharmacist to become an independent researcher in the field of transplantation.

Additional formal education

Master of Science in clinical research (MCR) programs have surfaced around the country with the intent of providing clinicians from multiple disciplines the skills and education necessary to enter into clinical research as successful independent and collaborative investigators. These programs augment the research potential of the novice transplant pharmacist specifically in the areas of research design, epidemiology and biostatistics. Additionally, some transplant pharmacists engage in other types of additional formal education, including Master of Science in public health (MPH) or business administration (MBA). The options are limitless and are often pursued based on the individual transplant pharmacist's practice setting and professional goals.

Description of roles and responsibilities on the patient care team

Inpatient transplant pharmacists roles and responsibilities

Clinical transplant pharmacists combine the principles of several sub-specialties to be effective members of the multidisciplinary inpatient transplant patient care team (also see Chapter 124). This includes optimization of pharmacotherapy across the phases of care from the intensive care unit to the time of discharge for adult and pediatric transplant recipients and living donors. Knowledge of drug delivery systems, pharmacoeconomics, drug information and drug literature evaluation, statistics, immunology, pharmacokinetics, pharmacology, pharmacogenomics, pathophysiology, pharmacotherapy, pharmacovigilance, regulatory standards, and medication safety are a necessity. Transplant pharmacists have substantial expertise in the management of novel and traditional immunosuppression and incorporate this with other subspecialties such as infectious diseases, cardiology, hepatology, nephrology, pulmonology, endocrinology, hematology, pediatrics, and critical care in order to manage patients with multiple co-morbid conditions.

While serving on the inpatient clinical team, the transplant pharmacist has a number of responsibilities during the peri and post-transplant period. These include continual assessment of the appropriateness of drug therapy prescribing practices as well as outcomes of therapy through active monitoring of safety and effectiveness parameters. Daily activities include therapeutic drug monitoring, pharmacokinetic and pharmacodynamic surveillance, drug (drug and food) interaction management, and optimization of drug administration, delivery and costs. In addition, the transplant pharmacist assumes the role of facilitating admission and discharge medication reconciliation in collaboration with the nurse coordinator, midlevel practitioner, social worker and other members of the patient care team.

The transplant pharmacist is also typically involved in development of transplant medication use protocols, ensuring adherence to the protocols during the transplantation process, and then proactively measuring the protocol related outcomes, collecting data that can be used to make informed modifications to the protocols over time. The data collection involved with evaluating the effectiveness of the protocols also often goes hand-in-hand with research efforts of the transplant center, with the transplant pharmacist taking an important lead in quality improvement. The results often prove meaningful to peer institutions and have contributed to the growing literature describing methods for optimizing patient outcomes [22].

These protocols typically require agreement among the transplant physician provider and approval from drug use oversight committees within the healthcare organization, for example, the Pharmacy and Therapeutics Committee. In this role, the transplant pharmacists ensure the protocols are consistent with institutional guidelines and when necessary secure the required approval from oversight committees. These protocols may be implemented and enforced in conjunction with collaborative practice agreements.

Collaborative practice agreements between physicians and pharmacists exist in which one or more pharmacists and one or more physicians enter into a medication therapy agreement that may occur in any setting in which a pharmacist-patient relationship can be established [23]. A collaborative practice agreement provides the pharmacist authority to manage medication therapy within a scope of practice. In the transplant setting, such an agreement authorizes the pharmacist to deliver patient care services, including but not limited to the following, according to the individual needs of the patient:

- Establish provider-patient relationships with patients outside of the practice of medication dispensing.
- Perform assessments and interviews to obtain complete medication, medical, social, and other pertinent patient information.

- Evaluate and monitor medication therapy through obtaining and assessing objective and subjective markers of effectiveness and/or tolerability.
- Initiate, modify and discontinue medication therapy.
- Order laboratory tests.
- Administer medications, including injectable agents, for example, vaccines.
- Educate patients and/or caregivers on medications, medical conditions, relevant non-medication interventions, and health promotion factors
- Document and communicate the encounter within the patient's health record.

Another major emphasis of transplant pharmacy practice is patient and caregiver education. A significant portion of the education patients and family members receive regarding medication occurs in the post-transplant period during the initial hospitalization. The pharmacist provides or coordinates much of this education and assesses the ability of the patient and family to independently manage the medications upon discharge. As a result the transplant pharmacist, in conjunction with the rest of the multidisciplinary patient care team, plays a vital role in coordinating drug therapy as they follow the patient throughout their continuum of care.

In addition to educating patients and caregivers about the appropriate administration of post-transplant medications, there is an increasing need for pharmacists to focus education on risks and side effects of immunosuppressive therapy. The heightened awareness of such risks arises from new Food and Drug Administration (FDA) regulations regarding risk evaluation and mitigation strategies (REMs). In March 2008 the FDA was granted authority to require drug manufacturers to submit proposed REMs as part of the drug approval process if it is determined that REMs are necessary to ensure a drug's benefits outweigh its risks [24]. The required components of REMs vary based on the severity of perceived risk. REMs may require any or all of the following:

- a medication guide and/or patient package insert;
- a communication plan;
- or elements to assure safe use (ETASU).

REMs programs are required to undergo regular assessment by the manufacturer and the FDA. These assessments take place at least 18 months, 3 years and 7 years post-REMs approval. Transplant providers must stay on top of the ever changing REMs requirements to ensure patients are properly educated about the risks of their medication therapy. The mTOR inhibitor everolimus was the first immunosuppressive agent approved by the FDA with a REMs program in place. The original REMs program included both a communication plan and a medication guide; the medication guide was removed from the REMs a year and a half after its approval. The REMs for sirolimus (medication guide) was added 10 years after it was approved by the FDA only to be removed 6 months later [25]. Belatacept is a first in class biologic immunosuppressive agent approved by the FDA in June 2011. Belatacept was approved with a REMs program that requires use of a medication guide and a communication plan. Typically medication guides are required to be dispensed by retail pharmacies with the initial prescription fill and all subsequent refills. Belatacept is unique in that it is the first intravenous maintenance immunosuppressive agent. Given its route of administration and the fact that the first dose of belatacept is given in the perioperative phase of transplantation, there has been some debate as to when it is necessary to provide the patient with a medication guide. In November 2011, the FDA published guidance surrounding the provision of medication guides to inpatients [26]. This document states that a medication guide should be provided:

1. any time a patient or caregiver requests the medication guide;
2. if the drug is subject to an elements to assure safe use (ETASU) REMs that includes specific requirements for providing and reviewing a medication guide;
3. the first time a drug is dispensed to a healthcare professional for administration to a patient in a clinic or infusion center;
4. the first time a drug is dispensed to a healthcare professional for administration to a patient in a clinic or infusion center after a medication guide is materially changed; and
5. any time a drug is dispensed directly to a patient or caregiver in an outpatient setting.

Gabardi and colleagues published a detailed review in 2011, of the REMs system, and its implications in transplantation. These reviews should be consulted for a more detailed overview of REMs in transplantation [27,28].

Transplant pharmacists develop tools and individualize strategies and systems to improve drug adherence. Some of these range from educational techniques to use of reminder tools to help ensure adherence with complicated medication regimens. Examples include: development of educational material such as written, video or other visual aids; provision and instruction on the use of pill boxes or other storage devices; electronic methods such as automatic text message or email reminders; implementation of web-based medication lists and organization programs; and self-medications programs where the patient manages their new medication while in the hospital, building confidence and demonstrating skill at assuming responsibility for their medication management while still under the direct supervision of the transplant team.

In addition to educating patients and family members the transplant pharmacist is also responsible for education of other transplant care providers and other pharmacists within the medical center who may manage aspects of pharmacotherapy for the transplant recipient. The transplant pharmacist in the inpatient setting also communicates and documents all of the activities described above in the medical record to ensure practitioners at all levels of the care team understand the plan of care for transplant pharmacotherapy. Furthermore, it is essential for the transplant pharmacist to articulate key issues regarding changes in medications and therapy plans with both the patient and their family or caregivers. Hereby, pharmacists make a vital contribution in creating a smooth and safe transition of care [14].

Integration of the transplant pharmacist into the ambulatory care setting

Implementation of transplant pharmacy services into the ambulatory clinics has been a recent addition to the variety of services impacting patient outcomes. Transplant pharmacists provide a broad spectrum of clinical and operational services complementing the numerous responsibilities handled by the outpatient transplant services (see Chapter 125). Several studies have evaluated the impact of the transplant pharmacist in the ambulatory setting. Chisholm and colleagues evaluated the cost savings associated with implementing a clinical pharmacist lead patient assistance program in a kidney transplant clinic [29]. A total of 61 patients were enrolled which lead to $124 793 in cost avoidance in the first year of the program secondary to acquisition of immunosuppressants through industry sponsored patient assistance programs. The cost incurred for the pharmacist's time was estimated at $16 650. The impact of clinical pharmacy services on transplant outcomes and

adherence was also evaluated in a subsequent study lead by Chisholm and colleagues in 2008 [30]. This group investigated two groups of kidney transplant recipients. The intervention group (n = 12) were provided intense clinical pharmacy services aimed at compliance, adherence, medication education and access to medications while the control group (n = 12) received standard care. Results indicated that the intervention group had a higher mean adherence rate (96.1% ± 4.7%) versus the control group (81.6% ± 11.5%, $P < 0.001$). In those patients who eventually became nonadherent improved adherence lasted longer in the intervention group versus the control group (mean 11 months vs. 9 months in the control group, $P < 0.05$) [30].

The return on investment from the transplant service lines for implementation of dedicated pharmacist's services include improved patient and allograft outcomes, along with increased reimbursement for specific therapies (e.g. basiliximab, antithymocyte globulin, intravenous immune globulin) by facilitating the appropriate coding and billing procedures. Additionally, transplant pharmacists are well positioned in the clinic to identify patients at high risk for non-compliance secondary to financial issues. Pharmacists are able to refer these individuals to patient assistance programs or modifying their regimens to maximize cost effectiveness.

Several centers have also implemented ambulatory care transplant pharmacy services to increase their outpatient pharmacy revenue and improve patient outcomes through developing additional disease state management services (e.g. pharmacist run collaborative diabetes, anticoagulation, hypertension, or hepatitis C) allowing for quick, concise referrals yielding immediate results or utilizing that pharmacist to provide the pathway for patients to return to the outpatient pharmacy to fill their prescriptions beyond their immunosuppressants [31]. Several ambulatory transplant pharmacist models exist which can aid in improving the continuity of patient care from the inpatient to the outpatient stay. Specifically, when the inpatient and outpatient pharmacists rotate between the services, a dedicated ambulatory pharmacist can provide inpatient discharge counseling for patients they are going to see in clinic. Additionally, utilization of an electronic note system accessible by all providers can facilitate communication of patient issues, such as the need for patient assistance, adherence barriers, or variables which may contribute to infections or rejection.

More recently, from a regulatory standpoint, the transplant pharmacist is required by Centers for Medicare and Medicaid Services (CMS) to evaluate patients proceeding through the pretransplant work up. This is an additional opportunity for the team to utilize the ambulatory transplant pharmacist to reconcile medications prior to transplant, identify any barriers to post-transplant medication compliance and provide recommendations for perioperative medication selection if necessary.

The impact of a comprehensive inpatient and ambulatory pharmaceutical care program on post-transplant medication compliance was published in 2009 [32]. This study was a prospective, randomized controlled trial, designed to evaluate the impact of a 12 month pharmaceutical care program on post-transplant compliance in 50 liver transplant recipients. Methods used to assess compliance in this study include medication event monitoring systems (MEMs) caps, immunosuppressant concentrations, pill counts, self-reports and the Morisky questionnaire. In addition to routine clinical care, patients randomized to the intervention group received pharmaceutical care services provided by a dedicated hospital pharmacist. The pharmacist began meeting with patients approximately one week prior to discharge, at this meeting they discussed dosing instructions, possible side effects, monitoring and discharge instructions. This review occurred three to four times prior to discharge. Additionally, on discharge, these patients were given written information about their medications, a discharge plan and diary for documenting vital signs and labs. During the first year, post-transplant patients met with the pharmacist on a quarterly basis to review medication changes, labs and drug related problems. Results indicated that patients randomized to receive pharmaceutical care had a higher dosing compliance rate ([# of days with correct number of bottle openings/# of monitored days] × 100) measured by electronic compliance monitoring compared to controls (90.2% ± 6.2% vs. 80.8% ± 12.4%, $P = 0.015$). Compliance as measured by pill counts showed large intra and interpatient variability, especially in the control group. However, the median compliance rate was still found to be significantly higher in the patients who received pharmaceutical care compared to the controls. Target serum immunosuppressant levels were achieved in a significantly higher proportion of patients receiving pharmaceutical care compared to controls (78% vs. 51%, $P < 0.001$) [32].

Despite the need for pharmacy services in both the inpatient and ambulatory care setting, the results of a recent survey indicate that 43% of transplant pharmacists focus their time on the inpatient setting. Thirty six percent are able to split their time between inpatient and outpatient and another 3% split their time between inpatient, outpatient, research and transplant administration. The majority of effort is focused on kidney (79%) liver (63%) and pancreas (61%) transplant recipients. A growing number of pharmacists are also providing care to heart (34%) and lung (29%) transplant recipients. As composite tissue allograft programs are becoming more prevalent 5% of pharmacists responding to the survey state that they are incorporated as part of this multidisciplinary team.

Determining the ideal patient to pharmacist ratio remains a challenge. The survey captured both the number of pharmacists per site and the annual transplant rate, which allowed quantitation of the current pharmacist to patient ratios at responding centers (Figure 123.1). Transplant centers performing over 200 transplants

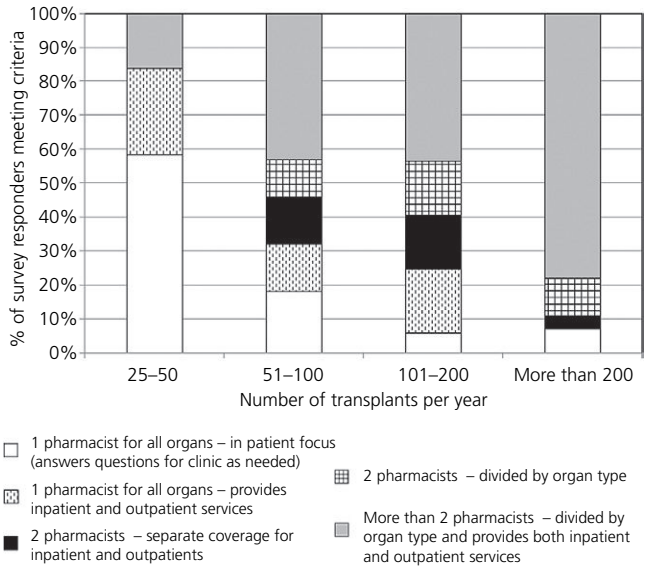

Figure 123.1. Transplant pharmacist coverage by transplant volume.

a year are most likely (78%) to employ more than two pharmacists who cover all organs and provide coverage to both the inpatient and ambulatory setting.

The role of the pharmacist in clinical research

Understanding the pharmacist potential for expertise as a clinical scientist

Clinical and translational research is not a singular activity but rather a continuum of efforts. By virtue of its interdisciplinary nature, pharmacy aligns extraordinarily well with this continuum [33]. Specifically in transplantation, many transplant pharmacists serves as a liaison between the inpatient and ambulatory care team [14]. The advanced perspective of the transplant pharmacist positions them well to develop, facilitate and analyze transplant related outcomes through clinical and translational research.

A unique set of challenges exists for pharmacists within immunosuppressive pharmaceutical research. They are increasingly required to understand drug development processes, best clinical practices, federal regulations and guidelines, and clinical research protocol development, implementation, and monitoring. In addition, extensive training and experience in writing protocols, including background, rationale, and methods sections, are required as part of any clinical research protocol. Pharmacists have a strong technical background in areas such as dosage form technology, medicinal chemistry, pharmacology, and therapeutics that are critical to the drug development and commercialization process and are thus effective investigators for clinical research related to immunosuppressive drug therapy. In the areas of discovery and translational research, pharmacists with specific training can provide input into preclinical studies that assess new technologies and methods applicable to pharmacogenomics and design approaches to develop clinically useful biomarkers. They may also oversee and plan clinical development strategies; design studies; write research protocols; oversee study implementation; analyze data, author reports, and publications; and review the scientific content of informational materials. Individuals with specialized training in pharmacokinetics and drug metabolism are typically required to design and analyze data obtained from a variety of transplant studies. Focus on the safety and efficacy components of phases II and III studies that support regulatory approval, or oversight of phase IV post-marketing research that addresses critical questions regarding novel immunosuppressant combinations and optimal treatment strategies are all areas of research in which transplant pharmacists are involved [34].

Rarely in transplantation is a large study powered appropriately to evaluate the use of all agents in the various transplant organ types or regimen combinations. With such a rapid transition from research to practice, the pharmacist is uniquely educated to combine their knowledge of therapeutics with the clinical knowledge of transplantation to critically evaluate such therapies with focus to efficacy and toxicity. While this should never replace well controlled clinical trials, this practice is inevitable in transplantation. The pharmacist is uniquely positioned to critically evaluate the smaller pilot experiences and cautiously transition these to practice while never loosing site of our responsibility to "first do no harm".

With the introduction of comparative effectiveness research, a new research endpoint has developed focused on overall healthcare cost containment. While the cost effectiveness of many drug therapies are debated, drug therapy costs are seen as one of the "easiest" ways to contain healthcare costs. Pharmacists have a unique understanding of the overall impact of drug costs which may provide an influential perspective away from drug costs alone to overall healthcare cost management. This is especially important in transplantation due to the costs associated with lifelong immunosuppressive therapy.

Pharmacist's role in the continuum of clinical research

Success in the arena of research typically requires productive collaborations with physician or multidisciplinary investigator teams. The collaboration amongst a multidisciplinary team is well established within transplantation, however recently the role of the pharmacist within this team has strengthened. The role of the pharmacist may take a variety of forms primarily related to the knowledge base and strengths of the individual combined with the institutional needs. Likewise, the pharmacist roles and responsibilities on the research may be varied.

As a clinical researcher, pharmacists have been involved in all phases of clinical trials, Phase I–IV. The pharmacist's delineation of responsibilities within the various studies can include principal investigator, co-investigator, sub investigator, coordinator, investigational pharmacist, research coordinator, research assistant, etc. The pharmacist serving as principal investigator, is the individual who is responsible and ultimately accountable for design, conduct, reporting, and monitoring of a study.

Pharmacists participating as co- or sub investigators work in concert with physicians to carry out the regulatory and reporting requirements needed to maintain compliance with these increasingly complex studies. As co-investigators pharmacists have the ability to use their own unique knowledge of immunosuppression to consent and enroll patients in these trials, then follow their progress throughout the post-transplant course. Pharmacists participate on daily rounds and in clinic, to aid clinicians and study investigators with trial procedures, as well as, provide expertise with drug therapy management.

Interface between transplantation and the investigational pharmacy

The principal investigator is responsible for the plan to control the investigational product. For example, how it will be stored, controlled, and dispensed so that only qualified investigators and the participants may use the investigational drug. Many times this responsibility is delegated to an investigational drug pharmacist. Investigational pharmacy services can be very complex for studies in solid organ transplant due to the fact that most transplants are not scheduled procedures thus requiring around the clock services with most transplant studies require long term study drug administration to assess long term outcomes. A dedicated investigational pharmacy service with experience in solid organ transplant can facilitate the conduct of the study on many levels. For many transplant studies, randomization and/or administration of the investigational product must occur prior to or during the transplant procedure. Assuring this service and delivery of the investigational product in a timely manner so as not to delay the transplant procedure is of vital importance. This requires close collaboration and communication with the investigational pharmacist and the clinical research team. Facilitating the dispensing of chronic medications over a long-term period of time in a timely manner that meets regulatory compliance standards for investigational products is challenging. This must be done in the context of the already complex post-transplant pharmacotherapy regimen. Understand-

Table 123.1. Stages of coordination with investigational pharmacy

Phase	Stages
I Planning	Time in which the investigator/designee is aware of a potential study which administers an investigational drug. During this phase activities may include defining individual drug services (IDS) scope of work, identifying desired IDS services, constructing an accurate budget for IDS services, establishing a timeline for subsequent study phases, meeting with study monitors for site evaluation visits, etc. At this point, the study protocol may or may not be finalized. If the intent of the investigator is to manage the storage, inventory control, and distribution of any substance used as part of a study, the investigator or designee must submit appropriate documentation of this process for review. This plan is in the context of other institutional regulations for substance storage and distribution
II Start up	Time in which the investigator/designee deems there is a high likelihood the study will be initiated. During this phase activities may include, IRB submission, contract review, notice of grant award, etc.
III Open enrollment	Time when IRB, contract and institutional approvals are complete. Study drug is available on site and ready to dispense
IV Close enrollment	Time when enrollment is completed, but patients remain in follow-up
V Study closure	Time after last patient, last visit when all study documents are finalized and corrected

ing the complexity of the individual study and the coordination of investigational drug services (IDS) can be maximized by effective communication. Effective communication at each study phase is optimal. Table 123.1 outlines the phases of communication necessary to ensure optimal results.

With the extensive use of off label medication, one of the basic challenges in transplantation is determination if your study includes an investigational drug. To determine if your study meets the definition of an investigational drug you may ask, "Is the drug used in human research study for the primary intent of accumulating scientific knowledge?" If yes, your study could be considered using an investigational drug. Whether or not investigational drug services may be necessary can be facilitated by the diagram in Figure 123.2. The decision tree includes the basic questions, however, implementation must be tailored to specific institutional requirements. Use of investigational drug services does not necessarily require dispensing by a pharmacy.

Pharmacists as directors of clinical research

Several pharmacists have become Directors of clinical research programs. In this role, the pharmacist combines administrative and clinical knowledge to facilitate the overall conduct of transplant clinical research within a given institution. While many universities tend to provide infrastructure for National Institutes of Health (NIH) sponsored research, many do not provide the necessary infrastructure for an active clinical research program. Independent of the study's scientific component, successful clinical research programs require expertise from additional areas such as regulatory, laboratory, clinical skills, financial, administrative, and collaboration between several institutional departments, such as anesthesia, surgery, nephrology, pathology, etc.. Strength in these areas is equally important as the scientific expertise to facilitate overall success in the clinical research area. In today's economic environment, industry needs centers which will enroll many patients quickly and provide quality data. Likewise, universities can no longer financially support clinical research teams who are not financially viable. This is not possible without a focus on not only science and patient care, but on business and finance also.

Financial justification for clinical pharmacy services

Integration of clinical pharmacy services

It is well established that clinical pharmacists are an integral part of a hospital and health system's patient care team. Hospital and health systems with outpatient pharmacies and clinical pharmacist involvement are able to take the patient's care one step further, following the continuum of care directly to the outpatient prescription setting at time of discharge, as well as post-discharge. In addition to enhancing quality of care, an outpatient pharmacy with the ability to bill Medicare Part B (health insurance)and specialty pharmacy services allows additional revenue for hospitals and health systems currently looking for avenues to reduce cost and maximize revenue. By capturing this outpatient revenue and referring patients to a robust internal pharmacy program, expense of pharmacist resources can be offset, allowing for a win-win for all parties involved. With support from transplant and hospital leadership, and an educated pharmacy staff willing to embrace the opportunities available through a transplant specialty pharmacy service line, significant revenue can be realized. When institutions develop a transplant specialty pharmacy service line and are able to maintain patients within their own system they have the opportunity to utilize this revenue as further financial justification for their clinical pharmacy services.

Another potential opportunity for transplant pharmacists to financially justify their positions is to bill for their services. Although there are a limited number of transplant pharmacists that are currently pursuing this route, Maldonado and colleagues demonstrated that pharmacists in one year (208 pharmacist visits) were responsible for increasing the outpatient reimbursement by nearly $10 000 or roughly $100/visit. This increase in reimbursement along with other factors was utilized to substantiate the case for clinical pharmacy services [35]. In the current healthcare environment any opportunity to offset/justify resources must be evaluated. New technology, electronic health records, electronic prescribing, and medication therapy management will continue to allow pharmacists to expand their roles, and ensure that medication needs of patients are appropriately addressed during all stages of inpatient and outpatient care.

Establishment of mail order transplant pharmacy services — impact of 340B drug pricing program

The 340B Drug Pricing Program was established in response to the passage of Section 340B of US Public Law 102–585, the Veterans Health Care Act of 1992. Section 340B of this law limits the cost of drugs to certain grantees of federal agencies and other entities identified in the statute. This program is administered by the Office of Pharmacy Affairs (OPA) of Health Resources and Services Administration (HRSA), under the federal US Department of Health and Human Services (HHS).

The federal 340B Drug Pricing Program provides access to reduced price prescription drugs to over 16 869 (as of September 30, 2011) healthcare facilities certified by the HHS as "covered

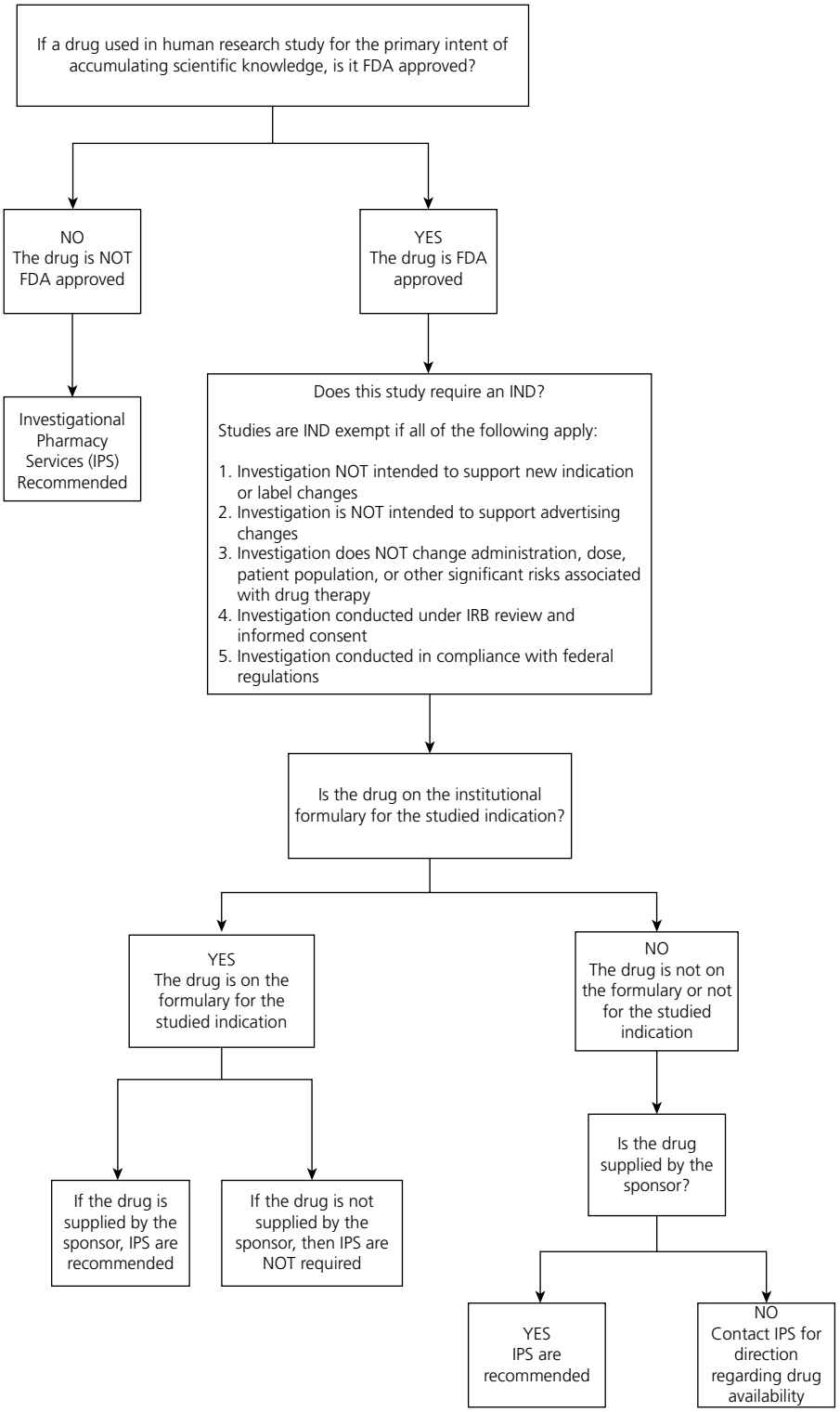

Figure 123.2. Evaluation of the need for investigational pharmacy services.

entities". These clinics, hospitals, and centers in turn serve more than ten million patients in all 50 states, in addition to commonwealths and territories.

Maximizing participation in 340B pricing under the federal Public Health Act allows for increased savings and expanded access to prescription drugs for states and clinics. This represents a significant opportunity for transplant programs to capture revenue as the 340B price is 19% below the average Medicaid "best price" net or rebates, 39% below the average insurance reimbursement, and 51% less than average wholesale price (AWP). Strategies to maximize savings are to target the most costly aspects of pharmacy programs, or most costly patient populations and/or disease states, such as

mental health, transplant, hemophilia, HIV/AIDS, and other categories of patients with expensive and chronic disease states. Transplant institutions that pursue setting up an outpatient pharmacy system and maintain their patients in their system have the opportunity to recognize significant financial gains again financially justifying a transplant pharmacist salary.

Specialty pharmacy establishment

The high cost of medications and the complexity of pharmacy billing and reimbursement have discouraged many independent and small retail pharmacies from participating in the Medicare Part B and "specialty" market, and have allowed the business to be taken over by larger, more "sophisticated" retail giants and mail order pharmacies. Over the last 30 years, "specialty" pharmacy has grown from a notion to an industry approaching $100 billion annually, and has become a major force in healthcare quality and innovation [36].

Specialty pharmacies offer services for providers and insurers by streamlining the delivery process and regulating the challenges in healthcare delivery. Many specialty pharmacies focus on pharmaceutical and biologic products with high acquisition cost, that are difficult to manage from both a patient care and reimbursement standpoint. Patient's prescription drug benefit plans often require that patients obtain these specialty medications from a mail order pharmacy participating with the insurer's specialty drug network. Medical supervision and/or instruction is often required for certain injectable medications, and many medications considered "durable medical equipment". As a result, many medications may be covered by *either* pharmacy or medical benefits, thus making care even more complex for providers, patients, and pharmacy staff.

As medications for high cost therapies may be covered under pharmacy and/or major medical benefits, it is important to understand how to identify and properly bill for these medications. Medications that cross over to medical coverage include, but are not limited to injectable and infusion therapies, and therapies that require complex care such as: cancer, HIV/AIDS, organ transplantation, hepatitis C, and rheumatoid arthritis.Facilities and pharmacies with existing transplant and oncology patient populations have the potential to develop work flow plans and processes around these patients to allow for a seamless encounter for patients, along with personalized medication management. The creation of a robust mail order pharmacy to retain patients outside the pharmacies immediate area, and to allow patients to remain in the system for maintenance medications and long term medication needs, adds substantial profit to a hospital/facilities bottom line thus again offering an opportunity to offset the expense associated with a transplant pharmacist and other much needed resources.

Impact of medicare coverage

Thousands of individuals receive a Medicare covered kidney transplant every year through the Medicare End Stage Renal Disease (ESRD) program. Kidney failure, or ESRD, is the only disease specific Medicare benefit, providing coverage of dialysis, transplantation, and immunosuppressive medications, among other services. Individuals with ESRD automatically qualify for Medicare if they or their spouse or parent have paid into Social Security for at least ten years.

Medicare established an immunosuppressive drug benefit to allow for coverage of costly immunosuppressive medications post-transplant that has continually expanded since the establishment of coverage in 1993. The Benefits Improvement and Protection Act of 2000 eliminated the previous 36 month limitation for Medicare aged and disabled transplant recipients who were Medicare eligible at the time of their transplant. Currently, if a patients eligibility is based solely on ESRD status, coverage will end 36 months post-transplant. However, if a kidney transplant recipient had Medicare ESRD status at the time of their transplant and their 36 month time limit expired, if they later attained Medicare coverage based on age or disability status, Medicare coverage of their immunosuppressive medications would resume. Legislation to remove the 36 month coverage limitation for immunosuppressive medication has repeatedly been introduced to Congress. Passage of this legislation to law is both fiscally and medically responsible.

For outpatient prescription drugs, Medicare has two distinct programs with a maze of complex policies. Drugs that are billed by pharmacy suppliers and administered through durable medical equipment are covered by Medicare Part B, as are some oral medications billed by pharmacy suppliers and self-administered by the patient, such as immunosuppressive medications and some oral anti-cancer medications. For Medicare Part B medication coverage in a physician's office, the medication must be furnished "incident to" a physician service. This typically means the medication is physician prescribed and dispensed or physician prescribed and administered during the course of an office visit. Medicare Part B drug coverage is usually limited to drugs or biologicals administered by injection and infusion, unless the injection is generally self-administered. Medicare uses a combination of local and national coverage determinations, and in absence of a CMS national coverage determination, local coverage determinations are made by individual Medicare carriers.

Medicare Part B defines several take home medications as "durable medical equipment". Those items include: nebulizer solutions, immunosuppressive medication, diabetic supplies, and certain oral anti-cancer medications [37–39].

Medicare cost report and organ acquisition discussion

The largest primary payer of organ transplantation in the US today is Medicare. Transplant programs must account for all allowable direct and indirect expenses to the appropriate Medicare acquisition cost center in order to receive appropriate reimbursement from Medicare, and in a market of continually dwindling reimbursement, capturing all Medicare allowable expenses is critical for success.

Medicare reimburses hospitals that are certified transplant centers for certain costs associated with the acquisition of organs for transplant to Medicare beneficiaries. Medicare requires that claimed costs be reasonable, properly allocated, and supported. Medicare allows as organ acquisition costs all costs associated with the organ donor and recipient before admission to a hospital for the transplant operation (i.e. pretransplant services) and hospital inpatient costs associated with the donor. At the end of each year each hospital files a cost report which is reconciled by Medicare to insure that all costs are allowable as defined in Medicare regulations and policy.

Medicare requires that transplant hospitals allocate only the portion of costs that relate to time spent on allowable organ acquisition activities as organ acquisition costs on the Medicare cost report. Institutions are required to use a reasonable basis to allocate costs to appropriate cost centers for pretransplant, post-transplant, and non-transplant related activities. For every

Table 123.2. Transplant pharmacist activities

Fundamental activities

1 The pharmacist's time is dedicated to transplant patients, with few commitments to other patient populations/services.
2 The pharmacist has dedicated hours for the clinic and inpatient transplant service.
3 The pharmacist regularly makes rounds as a member of the multidisciplinary transplant team to provide pharmacotherapeutic management for all transplant patients.
4 The pharmacist coordinates the development and implementation of drug therapy protocols and/or transplant pathways to maximize benefits of drug therapy.
5 The pharmacist prospectively evaluates all drug therapy for appropriate indications, dosage, drug interactions, and drug allergies; monitors the patient's pharmacotherapeutic regimen for effectiveness and adverse drug events; and intervenes as needed.
6 The pharmacist ensures adequate reconciliation of the medication record between the hospital and clinic at the transplant center.
7 In conjunction with the clinical dietician, the pharmacist recommends modifications to the nutritional regimen.
8 The pharmacist identifies adverse drug events and assists in their management and prevention, and develops process improvements to reduce drug errors and preventable adverse drug events.
9 The pharmacist uses the medical record as one means to communicate with other health care professionals and to document specific pharmacotherapeutic recommendations.
10 The pharmacist provides pharmacokinetic monitoring of immunosuppressive medications and other medication when such monitoring is necessary.
11 The pharmacist provides drug information to the transplant team.
12 The pharmacist participates in training pharmacy students, residents, and fellows through experiential transplant rotations, where applicable.
13 The pharmacist maintains transplant protocols and appropriate references.
14 The pharmacist provides medication therapy-related education to transplant team members.
15 The pharmacist participates in reporting adverse drug events to institutional quality assurance committees and to the FDA's MedWatch program.
16 The pharmacist provides updates and education to the transplant team surrounding the REMs requirements of immunosuppressive therapy.
17 The pharmacist provides and documents medication use education to all transplant recipients, certifies that patients are capable of managing their medications and reconciles home medications with post-transplant drug list.
18 The pharmacist reviews patient drug history to determine which medications should be continued after transplantation:
  (a) The pharmacist clarifies previously effective dosages, dosage regimens and provides recommendations for dosage adjustments as needed following transplant
  (b) The pharmacist assures that any drug-drug interaction is managed appropriately.
  (c) For all suspected drug-related hospital admissions, the pharmacist assesses the patient drug history for causality and documents in the medical record any findings that will impact the patient's management.
19 The pharmacist ensures the patient has access to all required transplant medications prior to discharge from the hospital.
20 The pharmacist documents clinical activities that include, but are not limited to, disease state management, general pharmacotherapeutic monitoring, pharmacokinetic monitoring, adverse drug events, education and other patient care activities.
21 The pharmacist acts as a liaison between pharmacy, nursing, and the medical staff to educate health professionals regarding drug-related procedures, policies, guidelines and pathways.
22 The pharmacist contributes to the hospital newsletters and drug monographs on issues related to transplant medication utilization.
23 The pharmacist implements and maintains departmental policies and procedures related to the safe effective use of transplant medications.
24 The pharmacist collaborates with nursing, medical staff, and hospital administration to prepare the transplant program for The Joint Commission (TJC), Centers for Medicare and Medicaid Services (CMS), and United Organ Sharing Network (UNOS) surveys and responds to any identified deficiencies.
25 The pharmacist provides consultation to hospital committees, such as Pharmacy and Therapeutics, when transplant pharmacotherapy issues are reviewed.
26 The pharmacist identifies how drug costs may be minimized through appropriate use of transplant medications and through implementation of cost-containment measures.
27 The pharmacist will ensure safe utilization of generic narrow therapeutic index medications.
28 The pharmacist participates in quality assurance programs to enhance pharmaceutical care.
29 The pharmacist is involved in non-patient care activities including multidisciplinary medical review committees and educational in-services.

Desirable activities

1 The pharmacist regularly meets with patients in the transplant clinic to provide follow-up education and ensure proper adherence to prescribed medication regimen.
2 The pharmacist maintains knowledge of current primary literature pertinent to transplant pharmacotherapy.
3 The pharmacist provides didactic lectures to health professional students in transplant pharmacology and therapeutics, where applicable.
4 The pharmacist uses a documentation program that attaches both a clinical significance and an economic value to clinical interventions.
5 The pharmacist is actively involved in transplant pharmacotherapy research by assisting in the screening and enrollment of patients by serving as a study coordinator or contact person, where applicable.
6 The pharmacist participates in research design and data analysis, where applicable.
7 The pharmacist contributes to the medical literature, for example, case reports, letters to the editor, and therapeutic, pharmacokinetic, and pharmacoeconomic reports.
8 The pharmacist promotes awareness in the community about the importance of organ donation.

Optimal Activities

1 The pharmacist assists physicians in discussions with patients and/or family members to help make informed decisions regarding treatment options.
2 The pharmacist provides formal accredited educational sessions, such as grand rounds, for medical staff, students, and residents.
3 The pharmacist develops residencies and/or fellowships in transplant pharmacy practice.
4 The pharmacist identifies and educates lay groups and medical personnel in the community about the role of pharmacists as part of the multidisciplinary transplantation team.
5 The pharmacist independently investigates or collaborates with other transplant practitioners to evaluate the impact of guidelines and/or protocols used in transplantation for drug administration and management of disease states associated with end-organ disease.
6 The pharmacist uses pharmacoeconomic analysis to prospectively evaluate existing or new pharmacy services and the place of new medications in transplant pharmacotherapy.
7 The pharmacist is proactive in designing, prioritizing and promoting new pharmacy programs and services for transplant patients.
8 The pharmacist seeks funds for conducting research.
9 The pharmacist reports results of clinical research and pharmacoeconomic analysis to the pharmacy and medical community at regional and national meetings.
10 The pharmacist publishes in peer-reviewed pharmacy and medical literature as a result of any of the following activities:
  (a) Clinical research or other original research that quantitatively or quantitatively evaluates drug therapy and the provision of pharmacy services.
  (b) Investigator-initiated grants and contracts.
  (c) Pharmacoeconomic and outcomes research.

transplant center the capture of pretransplant related cost associated with personnel salaries is vital. All transplant centers are very familiar with utilizing tools, such as time studies, to capture the amount of time that transplant coordinators, social workers, financial coordinators and administrative staff spend working in the pretransplant phase. These pretransplant salary costs are Medicare allowable and should be captured for partial reimbursement via the cost report. As both Medicare and UNOS now require the participation of a transplant pharmacist in the pretransplant phase, it is vital that transplant centers include pharmacists in these time studies. As centers document and appropriately capture pharmacist pretransplant time for inclusion on the cost report a substantial portion of a pharmacist's salary can be offset. The amount of offset will vary by each institutions Medicare organ percentage and the amount of pretransplant pharmacist involvement [40–42].

Summary

Recognition of the role of the transplant pharmacist by governing bodies such as CMS and UNOS has transitioned the role of the transplant pharmacist from a novelty to a necessary member of the multidisciplinary transplant team. The value of the transplant pharmacist as a member of the inpatient, ambulatory, research and transplant administration team is becoming evident. The various transplant pharmacist activities performed in the inpatient, ambulatory and research setting are outlined in Table 123.2. *Fundamental activities* are defined as expected activities to ensure safe and effective pharmaceutical care and access to medications for the transplant recipient. Fundamental activities should not be considered the minimum standards of activity of a transplant pharmacist. *Desired activities* include fundamental activities in conjunction with enhanced transplant related pharmacotherapy responsibilities and transplant related research. *Optimal activities* include fundamental activities and desired activities and incorporate the roles and responsibilities of the transplant pharmacist as the leader of transplant related pharmacotherapy research and education. They also incorporate the highest levels of expertise to optimize patient and allograft outcomes. Transplant pharmacists should strive to become the drug therapy expert on their perspective team and optimize pharmacotherapy at their practice site in addition to their numerous other responsibilities. The list of activities is not all inclusive and is constantly evolving with the practice of pharmacy and medicine.

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CHAPTER 124

Inpatient Transplant Unit Management

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Introduction

Care of the solid organ transplant candidate or recipient requires specialized skills and high level competencies in the staff on transplant units. The complexity of care required of this patient population is best guided through multidisciplinary planning on daily rounds. Surgeons, physicians, nurses, transplant coordinators, nurse practitioners, pharmacists, social workers, dietitians and clergy comprise the multidisciplinary team members collaborating and planning individualized care of each transplant recipient or living donor. The bedside nurse implements the plan of care, evaluates outcomes, communicates results to transplant team members and educates patients and families. The role of a nurse practitioner on an inpatient unit has evolved over time and varies among transplant programs. It is becoming more common for units to have three or more nurse practitioners functioning in the role of a hospitalist on transplant units and in the Intensive Care Units [1]. Some nurse practitioners take calls for the transplant unit.

Pre transplant candidates for heart or liver transplantation are often cared for in an intensive care unit or a specialized progressive care unit as they await a suitable organ for transplantation. Over the last ten years there has been a sharp rise in the use of mechanical circulatory support devices such as a left ventricular assist device or total artificial heart as a bridge to heart transplantation. These devices require highly specialized and competent staff to ensure safe, efficient care for patients. Many transplant centers have designated, specialized units for care of the post transplant recipient due to the complexity of care required of this population. Most units are further specialized with either thoracic or abdominal transplant recipients. Immediately following surgery, liver, lung or heart recipients are taken to their specialized intensive care unit (ICU) for care. Many hospitals care for kidney recipients and living kidney donors in progressive care units rather than in an ICU environment. However, some surgeons prefer all kidney recipients are recovered in an ICU for at least the first 24 hours. This chapter addresses structure, management and staffing issues as they relate to the inpatient care of candidates and recipients on thoracic and abdominal transplant units. Outpatient clinic management and the roles of transplant nurse coordinators are specifically covered in Chapters 122 and 125, respectively.

The physical environment and resources — infection control

It is a fortunate few who experience the luxury of creating a physical environment from scratch for our transplant units. Most of us inherit the space in which we practice and we make accommodations for our unique population. Private rooms for every patient top the list of our transplant wishes. When only a semi-private room configuration is available, decisions to maximize infection control are essential for patient placement. For the patient who has just received a solid organ transplant, and is newly immunosuppressed, the private room scenario seems obvious. The same private room can be just as important to a pretransplant patient, as any infection may make that patient temporarily unable to receive an organ, effectively lengthening the time on a waitlist. This consideration applies to the ICU as well as the progressive or general care unit. On a unit where there are multiple semi-private rooms, decisions often need to be made to block one bed in a double room in order to create the private room scenario. Support from hospital administration for this practice can present a challenge when overall facility census is high. Discussions and planning for this potential situation should be held prior to the actual event. The hospital epidemiology experts are helpful in such discussions.

Another serious consideration for infection control on any transplant unit is the quality of the airflow. High efficiency particulate airflow (HEPA) should be readily available, if not a constant state of the unit ventilation system. The merits of true positive pressure ventilation (ppv), implying a sealed room with an extremely high air exchange rate per hour, have long been debated but have never been proven scientifically [2]. An outbreak of aspergillosis on a transplant unit can be deadly. It is then that a discussion of the merits of ppv are most often heard. Prevention is the best intervention. Frequent rounding to assess potential breakdown in the physical environment is the key to avoiding problems. For example, dialysis on a transplant unit is a common occurrence as not all patients are able to travel to a central dialysis area. The dialysis procedure requires water, and a lot of it. Secure machine connections to a source of water and a place to drain that water after the treatment are required. Poor connections can lead to constantly moist areas, a condition which leads to mold and mold begets aspergillosis. Less common than dialysis, but also a source of moisture buildup, is a window that chronically leaks in a hard rain. The

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wise manager of a transplant unit develops an ever vigilant posture and promotes the same vigilance in the bedside nurse and support staff, including environmental services and plant operations workers.

The human resource — staffing

While the thoracic transplant/mechanical support device unit are comprised of a multidisciplinary team of cardiology physicians, cardiothoracic surgeons, nurse practitioners, advanced practice nurses, registered nurses (RNs), care partners, a dietician, physical therapists, occupational therapists, pharmacists, social workers, care coordinators (utilization review/discharge planner), the unit's budget is largely comprised of nursing personnel. The nursing budget is comprised of clinical nurses, care partners, unit secretaries, clinical coordinator, clinical nurse specialist and nurse manager. The nurse manager prepares the unit's budget with input from all staff on the unit, and advocates for the necessary resources to provide safe and competent care to complex cardiothoracic surgery patients.

To prepare an operational budget for an inpatient unit, a nurse manager must be familiar with the financial structure and goals of the organization. There must be a strong understanding of the organization's priorities, so that the unit's plan is aligned with the organization's strategic direction. A budget is the plan for a year that allows the organization to prepare activities and control costs within its means. Nurses and nurse managers are key to cost control, and more importantly, they are instrumental in maintaining patient safety and ensuring positive quality patient outcomes.

The level of training and knowledge of nurse managers varies among various types of organizations. The Chief Nurse Executive holds ultimate accountability for nursing's budget development and expense management. Front line managers must have skills to plan the budget, control costs, and make effective decisions regarding resource allocation [3]. The budget identifies the unit's level of service and workload that determine personnel and non personnel resources required [4]. Nursing's personnel budget is a large portion of the healthcare organization's labor expense. A strong plan for allocating resources and the leader's ability to manage those resources are key to operating a successful inpatient transplant unit.

Nurse staffing is traditionally measured in one of two ways, hours (of care) per patient day, or nurse to patient ratio. The staffing plan for inpatient transplant units is established by the organization utilizing hours per patient day (HPPD). This measure, HPPD, was developed by the American Nursing Association for the National Database of Nursing Quality Indicators (NDNQI) [5]. Nursing HPPD refers to the number of nursing care hours needed relative to the nursing workload. The HPPD values are determined from hospital direct care hours and unit census data, individualized per unit based on historical census data. Census data is determined each night at midnight. Nursing workload varies based on the type of unit. The nursing workload is greater in an ICU than an acute care unit. Acute care units may be intermediate or progressive care or medical-surgical telemetry units. As nursing workload increases, the nurse to patient ratio must decrease. Each year, the staffing budget is determined based on historical average daily census (patient days). Patient days are projected for the year, based on historical census data.

Staffing a nursing unit for transplants is an important concept to understand, so that managers can advocate for sufficient resources to care for complex patients. Hours per patient day and staffing resources have been studied in recent years to assess the relationship between staffing numbers and patient/hospital outcomes [6,7]. Results of those studies have shown mixed results regarding the relationship between staffing and patient outcomes. Studies have shown a correlation between nursing care hours and patient outcomes [6–8]. Increasing nurse staffing resources is costly to organizations, as nursing budget is already a large portion of the hospital's budget. However, a reduction in adverse events and improvement of nurse sensitive indicators can be shown to be cost beneficial to the organization. HPPD accounts for volume. Increased levels of nursing staff have been shown to improve outcomes and studies continue to evaluate the validity and reliability of this measure of workload [6–8].

Once the unit's budget has been established, it is the responsibility of the nurse leader/manager to effectively manage those resources. Careful consideration must be given to the patient acuity and the skill mix of staff working on the unit. At the authors organization, a staffing grid guideline is developed based on HPPD and a nurse to patient ratio established for the unit to provide front line staff a guideline of how many RN's should be working, based on current total unit census.

The Labor Management Institute, a consulting, education and research firm provides services in the areas of workforce management and operations. They conduct and annual survey of 360 hospitals from 45 states regarding hours per patient day and nurse to patient ratio. According to the Labor Management Institute 2009 Annual Survey of hours, intermediate care units (stepdown) HPPD and total hours per patient day, averaged between 9.6–11.1 (total HPPD). Direct hours per patient day averaged between 9.3–10.8. Nurse to patient ratio for RN's is 1:3.6–1:3.8 for teaching hospitals [9].

Once equipped with a well designed plan for staffing resources, it is the leader's challenge to allocate the nursing resources to provide safe and expert care to complex heart surgery patients. A grid can be developed as a guideline for determining the number of staff members needed based on current patient census. This tool can be helpful in maintaining expenses within framework. As census fluctuates, staff can be reallocated to areas of need within the organization.

The thoracic transplant unit

On the thoracic transplant/mechanical circulatory support device (MCSD) unit, competency is determined in three phases: at hire, during orientation, and on a continual basis. At the time of hire, RN competency is assessed through verifying licensure, registration, certification, behavioral interviewing, and previous experience. The initial competency phase focuses on the skills and abilities necessary to care for the patients during the first year of employment [10].

During the orientation competency period, RN staff on the thoracic transplant/MCSD unit are introduced to a variety of experiences to prepare them for independently caring for both transplant and MCSD patients. This initial orientation period is usually 12 weeks and includes didactic classroom content, hands on orientation to a variety of MCSDs, as well as observational experiences. The RN is assigned to a primary and associate preceptor who guides them through care of patients on our unit. They are immediately assigned to take care of MCSD patients, including left ventricular assist device patients (LVAD) and total artificial heart (TAH) patients, under the supervision of their preceptor. The didactic

Table 124.1. Mechanical circulatory support device (MCSD) competency testing (Reprinted from [11] Savage L, Murphy J, Joyce K. Developing and maintaining competency with circulatory assist devices: How to meet the challenge. *Progress in Transplantation* 2010;20:125–128 with permission from InnoVision Communications, Inc)

Topic	Reviewed (date)	Demonstrated (date)	Date/Signature/Method S = simulated C = cognitive D = demonstrated
1. TAH-t/LVAD Implant			
A. Preoperative teaching			
B. Equipment required			
i. what to take to OR			
ii. backup required			
iii. how to obtain additional equipment			
C. Set-up PBU/system monitor			
2. Patient Care			
A. Site care			
B. System check/data card			
C. Batteries			
D. Hand pumping			
E. Documentation			
F. Special lab draws TAH-t			
3. Troubleshooting			
A. Low flow states (etiology and actions			
B. Changing system controller			
C. Alarms			
D. yellow wrench			
E. red heart			
F. Defibrillation/Cardioversion			
G. Air tanks			
4. Discharge planning LVAD			
5. Diversional activities LVAD/TAH-t			
6. Resources			
A. TAH-t Syncardia			
B. VAD coordinators			
C. Heartline 24/7			

portion of their orientation is usually provided at the end of their orientation. Providing hands on review and training with their preceptor in advance of the classroom content allows for the orientee to better understand the classroom content. Prior to the end of orientation, the orientee completes a hands on competency assessment including emergency measures to change batteries, respond to alarms and in some cases switch out drivers (total artificial heart and freedom driver-portable TAH-t (Table 124.1) [11]. Staff on this unit review and complete a transplant competency test on an annual basis as well as MCSD competency testing.

The didactic content includes indications for left ventricular assist devices, components of the devices, postoperative management of the patients, alarm management, and pump malfunction, drive line care (care of the exit wound), battery changes and charging, complications, and equipment management. A competency checklist is completed for each device used in the environment. New staff observes and performs wound care to the exit drive line care under the observation of their preceptors. Meticulous, sterile technique must be used, and a immobilizer device should be placed on the drive line to minimize the movement of the lines at the exit site.

Other concepts important in the care of the transplant/MCSD patients include care of the postoperative heart surgery patients. Once transferred out of the ICU, nursing priorities include medication management, preventing pulmonary complications, progressive mobilization of patients, wound care, pain management, and addressing psychosocial aspects of caring for patients with MCSD.

For small programs, it is challenging to maintain competency when these procedures are low volume. Nursing leaders may consider limiting the number of staff oriented to care for the MCSD and transplant patients, in order to maintain competency. For other programs, where implantation of mechanical circulatory support devices and thoracic transplants are routine, all nursing staff should be trained and validated annually for competencies. Ongoing competency assessment should include new procedures, technologies, policies or initiatives or changes in initiatives, procedures, policies. High risk responsibilities that could cause harm, death or legal action should also be included in the ongoing competency assessments [10]. A variety of competency verification methods should be utilized in order to assess different aspects of the job skills including technical, critical thinking and interpersonal. A variety of methods can be used to demonstrate competencies including post tests, exemplars, observation of skills, case studies, peer reviews, mock events [10]. Review of case studies is an excellent way to incorporate critical thinking skills and to apply knowledge to specific patient scenarios. Actual patient events are described. The employee reflects on the event and answer questions as to appropriate interventions.

The abdominal transplant unit

An abdominal transplant unit provides care for the patient population who receive kidney, kidney-pancreas, pancreas alone, liver, intestines and/or multivisceral organs. Frequently, they suffer from multiple system complexities with disease processes that are seldom limited to one organ. In some cases, patients are receiving a combination of two or more of these organs. Resources required to sustain a successful abdominal transplant inpatient unit include both an appropriate physical environment and a patient care staff with knowledge, skill, and professional behavior.

The inpatient unit may be as small as ten or as large as 80 beds. If the unit is small, it may provide care for only the immediate postoperative, sometimes known as freshly transplanted, patients. A larger unit provides care for patients all along the transplant continuum from the evaluation phase, the waiting period, perioperative period and for any post-transplant admissions as well. In a unit such as the latter, many patients may be admitted and discharged several times throughout the course of their chronic, acute, and recovery phases. These repeated visits over time can create a very personal environment for the patients and the care givers, in particular the nursing and adjunct therapy staff. This is one component of transplant care that staff find rewarding.

An additional cadre of patients for the abdominal transplant unit may be the living donors of kidneys or sections of a liver. We are aware of programs that routinely place related and unrelated, known and confidential, donors on the same unit as the recipients. A related or a non-related donor, whose identity is known to the recipient, would not generally be placed in a bed in the same room or an immediately contiguous room. Physical separation allows for privacy but also is intended to protect each patient from the stress of worry over the other. On the unit within which we practice, the separation of a donor from a recipient, when the two know each other, is often the impetus for one or the other to take that first walk down the hall to visit. These walks often provide moments of joy and humor for the patients and gratification for the staff.

In the case of a confidential donation, also known as a Good Samaritan or altruistic donor, it is possible to place the donor on the same unit as the recipient; however, extra care must be taken to ensure that the identity of the two patients is protected. The size and design of the physical space of the unit and the number of staff dictates how that confidentiality is accomplished. For example, it would be unwise to have one RN caring for both the donor and the recipient. In some programs, either all donors or just the confidential donors are placed on separate post surgical units with the specific intent to maintain confidentiality.

Staffing the abdominal unit

Similar to the thoracic transplant area, the abdominal transplant team consists of nephrologists, transplant surgeons, pharmacists with a transplant specialty, transplant coordinators, nurse practitioners, RNs, a dietitian and social worker, unlicensed assistive and clerical staff. The support services of physical and occupational therapy are also involved in the daily planning of care. The responsibility for staffing the unit falls to the unit manager and includes only the direct care RNs and unlicensed assistants.

The mechanics of staffing numbers, as presented previously, are at the core of the staffing model for any hospital inpatient unit. The number of full time equivalents (FTEs), spread among licensed and unlicensed assistive positions, is established annually and forms the structure within which a unit manager must work. The day to day, shift to shift, story of staffing is much more fluid, and particularly so on an abdominal transplant unit due to variations in census and variations in acuity, which are the norm.

Transplant surgery is an uncertain event to a great degree. If the program performs living kidney and/or living liver donation, the surgeries are scheduled with bed and staffing need being planned in advance. However, the availability of organs from deceased donors cannot be predicted. The size of the center's waiting list, the medical demographics of those that are waiting (ABO type, severity of illness etc.), the size and quality of the donor pool, and the number of transplant centers within a geographic area all have an impact on the number and frequency of the organ transplant event and thus the patient census on a transplant unit. Staffing must be accomplished not only with baseline numbers but also a plan to flex up or down with changes in census and acuity.

A 20 bed unit with three newly transplanted liver recipients transferred from the ICU, five newly transplanted kidneys on vasoactive drips, and ten patients at varying stages of procedural intervention must be staffed at a higher nurse patient ratio than a 20 bed unit with ten empty beds and no recent transplants in the other ten beds. This kind of variation in staffing need has the potential to force reassignment, usually referred to as "floating" staff to other units when they are not needed on the "home" unit or forcing them to use paid time off. Neither of these options is palatable for the bedside care giver and leads to dissatisfaction, turnover, and staff vacancies. All of these outcomes can ultimately lead to the unintended consequence of poor patient outcomes [12].

One strategy to maintain a full and stable census on such a unit is to identify non-transplant service patients that fit well on the unit without compromising infection control or staff competency. The unit we are most familiar with has been able to safely accept overflow from neurology and cardiology areas, as well as some individual surgical specialties, for example parathyroidectomies. Key to this strategy is the ability to screen all patients quickly for signs of infection prior to accepting them for admission to the transplant unit. Placing these patients on the transplant unit has a mutual benefit to the organization's throughput needs and can stabilize the fluctuations in census and staffing for the abdominal transplant unit.

Staff training, education, competencies, and certification

The nursing staff chosen to work on the abdominal transplant unit range from novice to experienced nurses. Basic competency is determined at hire based on licensure, behavioral interviews, certifications and core nursing skills. Through an extensive orientation, approximately 12–14 weeks of both didactic and hands-on work, increased competency is developed. Each RN orientee is assigned to a primary and a secondary preceptor. The preceptors have all successfully completed a course that trains them in general preceptor skills. It is the primary job of the preceptors to guide and coach the orientee through the entire training period. On our particular unit this process is overseen by the unit Nurse Clinician who is an advanced practice RN.

On the abdominal transplant unit, initial competencies are looked at from the perspective of clinical knowledge and practice. Those specific core competencies to be mastered before a nurse finishes orientation on the abdominal transplant unit include:

- Administering immunosuppressive medications, intravenous (IV) and oral.
- Monitoring lab values for therapeutic levels.
- Monitoring patient response to these medications and quickly identifying adverse reactions.
- Recognizing signs and symptoms of rejection or infection.
- Administering and titrating vasoactive IV medications to achieve tight blood pressure control.
- Monitoring of fluid balance with early recognition of need to adjust fluid intake, ability to accurately replace fluid as needed by use of an established protocol.
- Monitoring for signs and symptoms of bleeding, at surgical sites, in drains, or in hemodynamic stability.
- Monitor central venous pressure (CVP); ability to interpret readings and identify cause of changes.

Table 124.2. Competencies for transplant staff nurses (Data from [13])

Category	Competency	Activity to demonstrate competency
Assessment	Systematically and comprehensively collects, prioritizes, and documents data on a given patient, including past and current history Assesses and documents all aspects of family and care giver support systems (tasks, stressors, etc.) Assesses adherence to therapeutic recommendations in the past and present.	Identifies impact of history on current condition Identifies impact of systems on patient and potential outcomes Identifies risk factors for future adherence. Identifies educational needs of patient, family, etc.
Nursing sensitive diagnosis	Makes nursing diagnosis based on the assessment. Refines and revises diagnoses continually, based on new data Documents and discusses the diagnoses with the patient, family, other caregivers, and members of the multidisciplinary team.	Differentiates variations between normal and abnormal for the transplant population
Outcomes identification	Identifies expected outcomes that are patient oriented and developmentally appropriate, evidence-based, attainable, and realistic Modifies outcomes based on ongoing evaluation	Ensures the expected outcomes are mutually acceptable to the patient and caregivers. Documents outcomes in measureable goals
Planning	Develops an individualized plan of care, aligned demographically with the patient, utilizing strategies and alternatives, to meet the expected outcomes Defines the plan to reflect current regulations and standards of transplant nursing practice	Collaborates with patient, care givers, and team in development of plan and setting priorities Considers the economic impact of the plan on the patient. Documents the plan, using standardized terminology and technology
Implementation	Implements the identified plan in a safe and timely manner Uses evidence-based interventions specific to the diagnoses Coordinates delivery of care Employs strategies to promote health and a safe environment	Collaborates with the interdisciplinary team to implement Documents implementation and any modifications or changes Documents coordination clearly Creates an environment in which learning can take place and provides health teaching
Evaluation	Conducts systematic, ongoing, criterion-based evaluation of the outcomes	Evaluates the effectiveness of the planned strategies
Quality of practice	Systematically enhances the quality and effectiveness of nursing practice Participates in nursing practice QI activities	Evaluates the practice environment in relation to existing evidence, identifying opportunities for new research Considers access, safety, cost, satisfaction, and effectiveness
Education	Attains knowledge and competency that reflects current nursing practice and standard. Obtains professional certification	Utilizes self-reflection and inquiry to identify personal learning needs.
Professional practice evaluation	Evaluates own nursing practice in relation to professional practice standards and guidelines, current regulatory environment	Obtains feedback from peers, patients, and colleagues. Takes action to achieve goals identified in evaluation process
Collegiality	Contributes to the professional development of peers and colleagues	Shares knowledge and skill with peers and colleagues, including feedback Mentors others
Ethics	Integrates ethical principles in all aspects of practice Contributes to the resolution of ethical issues by participating on interprofessional teams, ethics committees, etc.	Maintains patient and donor confidentiality Informs patients of the risks, benefits, alternatives, and potential outcomes of healthcare regimens
Research	Integrates research findings into practice	Uses the best available evidence in practice; actively participates in research activities
Resource utilization	Considers factors related to safety, efficacy, cost, and impact on practice in the planning and delivery of services	Assists the patient and family to become informed consumers about options, costs, risks, and benefits of care
Leadership	Provides leadership in nursing practice and in the nursing profession Shows respect for the inherent dignity and value of other people	Engages in teamwork as a team player and a team builder Willingly accepts mistakes by self and others and uses them as opportunities for learning

- Monitoring, interpreting, and completing a 12 lead electrocardiogram (EKG).
- Recognizing electrolyte levels which require heart monitoring.
- Caring for central lines, including peripherally inserted central line catheters (PICC) lines and dialysis catheters.
- Monitoring signs of metabolic disorders, such as diabetes.

There are broader and more comprehensive competencies which are demonstrated on an all encompassing behavioral level. Those competencies have been identified by the International Transplant Nurses Society and are summarized in fifteen domains [13]. Table 124.2 outlines those domains and summarizes some of the observable practices within the domains.

Certification

Certification is a process which validates the qualifications and knowledge of an individual nurse in a specifically defined area of practice [14]. The organization which grants the certification is non-governmental and the award is based on a set of predetermined standards [15]. Licensure for a nurse is based on minimum professional requirements. Certification denotes a recognized higher standard of knowledge and practice. While requirements vary according to the particular nursing specialty, all certifications include elements of practice, a known body of required knowledge, and a testing blueprint [16].

For the nurse working in a transplant area, there are many options for certification:

- Progressive Care Certified Nurse (PCCN)
- Critical Care Registered Nurse (CCRN)
- Certified Clinical Transplant Nurse (CCTN)
- Certified Clinical Transplant Coordinator (CCTC).

Why would a transplant nurse want to be certified? The American Board of Nursing Specialties (ABNS) published a white paper on nursing certification in 2006 which stated that certification is perceived by nurses and by the public as influencing accountability,

professional accomplishment and growth, and specialized knowledge [17]. The paper noted that nurses felt greater personal accomplishment, professional challenge and professional credibility.

Why would a nurse manager, nurse leader, promote and encourage specialty transplant certification on the inpatient unit? There is strong evidence that specialty nursing certification has a positive impact on a unit, on the organization as a whole, on the profession of nursing, and locally on nursing satisfaction and retention [16].

Discharge planning

Preparing patients for discharge begins on admission and requires considerable individualized planning. Fragmented care in the discharge planning process can result in rehospitalization [17]. Multidisciplinary planning for a patient's discharge requires communication to ensure the highest quality of preparations for patients to be successful at home. Transitioning patients from a controlled hospital setting to home provides opportunities for adverse events and errors with medication and self care [18]. According to the Institute of Medicine report, "To Err is Human", the majority of medical errors are a result of systemic problems [19]. Because discharge planning is a systemic process, errors are likely to occur. With the multidisciplinary approach, planning for a patient's discharge must be well documented to maintain continuity of care and communication among staff. Multidisciplinary rounds must demonstrate that each member of the team has signed the plan of care to indicate coordination of care. A discharge that is well planned and coordinated among the disciplines serves as a platform to prevent errors.

Structured, individualized discharge plans have been shown to decrease length of stay and readmission rates [20]. Some centers have a discharge pathway that is used by multidisciplinary team members [21]. Each discipline signs off on sections assigned to teach in preparation for each patient's discharge. Others also use a discharge pathway and document their education in the patient's chart. During multidisciplinary rounds a patient's progress is discussed to determine readiness or obstacles to discharge.

Patient and family education is one of the most important roles of transplant coordinators, nurse practitioners and staff nurses in preparing patients for discharge. Patient education actually begins in the pretransplant phase and continues throughout the admission, discharge and in the ambulatory care follow-up processes. Probably the most complex process for patients and families to grasp is the medications required post-transplantation. Educating patients and asking them to demonstrate self-medication is challenging within a busy hospital environment. Medications provide many opportunities for errors as patients begin the self-medication process. The frequency and changes in dosages, sometimes on a daily basis, adds to the complexity. As medications and dosages are individualized, it is not unusual to change immunosuppressive agents, thus adding to the confusion. Even though patients have learned about the many medications they will be required to take after transplantation, the reality sets in as they prepare for discharge.

Many transplant programs use an on line discharge planning program for medications called MedActionPlan [22]. This program can be accessed by patients once they are home and by providers during the patient's hospitalization and in the ambulatory care setting. It is an excellent resource for clinicians and educational tool for patients. Medications can be updated daily and printed out in a format that can be carried in a wallet or on a clipboard. Pictures of most medications are available to guide patients through the complex process of learning each new medication. Access is also available for iphones and Android devices with reminders being sent to patients by email or text messaging [22].

Case managers and social workers often help with the discharge planning process and transition to the home care environment by educating patients and families about community resources. The transition to ambulatory care can be frightening to some, especially those being discharged with a mechanical circulatory support device. While patients and families may have individualized education prior to discharge, once they are home there are questions and concerns that need answering. All transplant and device programs have an on call system where patients and family members can call to ask questions and seek guidance. An important part of the discharge planning process is providing patients with the number to call where a physician or nurse is available 24/7 to respond to concerns. Written protocols and guidelines are available to nursing staff on call to respond to patient questions with physician back up for more complex cases. This on call system helps patients transition from inpatient to ambulatory care. On call staff members must also maintain competencies that are assessed annually for each organ system and for mechanical circulatory support devices.

Quality assessment and performance improvement

Until 2007, most transplant programs reviewed their outcomes internally. Few had quality improvement programs. Today, transplant programs must demonstrate to surveyors and insurers that they are monitoring their outcomes and sharing their data with hospital Quality Assurance and Performance Improvement (QAPI) programs. The regulatory environment has led to many transplant programs developing a role for a Quality Improvement Coordinator with larger programs actually having a Transplant QAPI Department. Centers of Medicare and Medicaid (CMS) has provided the transplant community with guidelines and a surveyor's worksheet for reviewing quality programs. During the initial CMS surveys between 2007 and 2010 surveyors evaluated programs for their adherence to program policies and procedures. On the second survey of transplant centers, the focus is clearly on QAPI. Programs are being asked to provide minutes from quality team meetings, from QAPI meetings and to demonstrate the relationship to a hospital's quality program. As surveyors review a center's quality program they are requesting evidence of process improvement projects underway or completed in addition to reviewing adverse events experienced in the program.

Establishing performance measures that reflect the transplant process, pretransplant through postoperative follow-up, is key for each program. While programs utilize reports from the Scientific Registry for Transplant Recipients, this data represents cohorts that provide outcomes for up to five previous years to determine three year outcomes [23]. To stay abreast of a program's current outcomes, real time data must be maintained in key areas such as death on the waitlist, graft loss, patient death. While most QAPI programs for transplant are followed by the ambulatory care clinics, a clear relationship to the inpatient units that care for transplant recipients and candidates is needed. Staff nurses, intensivists and operating room (OR) staff should be members of QAPI teams. Results of quarterly QAPI reports should be presented to staff on each unit as well as to the (OR) staff.

Communication is a key component to capturing important data for QAPI programs. Adverse event reporting requires communication with the OR, inpatient units and ambulatory care clinics. Having members from each organ system that cares for transplant candidates and recipients on QAPI teams assists with ensuring data is complete. Transplant programs must maintain an adverse events monitoring system. Each unit should maintain an adverse events log. Morbidity and mortality meetings should include a review of adverse events. Committee meeting minutes are maintained for each QAPI meeting. Agenda items are developed based on unit events as well as following up on plans and problems identified in the previous meetings.

Process improvement projects are developed based on needs of each program. Patient satisfaction scores, patient safety issues or an increase in graft losses are projects that may be selected by transplant programs to improve. Projects should also align with hospital goals each year. For instance, patient safety is a major focus for hospitals at this time. Staff members are invited to participate in projects to improve practice in their areas. Working together on projects such as this allows staff an opportunity to take a leadership role on the transplant team. Co-chairs are selected and provide updates at staff meetings and at organ specific quality team meetings.

Regulatory issues

The CMS established Conditions of Participation (COP) for organ transplant programs on March 30, 2007, and with an effective date of June 28, 2007 [24]. With the advent of the new CMS Transplant COP, a new regulatory process for transplant programs was set in motion that would define further the future of transplantation. A transplant program is defined by CMS as a component within a transplant hospital that provides transplantation of a particular type of organ. All organ transplant programs must be located in a hospital that has a Medicare provider agreement. In addition to meeting the transplant COP the transplant program must also comply with the hospital's Medicare COP specified in 42 CFR 482.1 through 482.57 which is a separate document from the Transplant COP [25].

Transplant programs that were previously Medicare-approved prior to June 28, 2007 were required to apply for continued participation to CMS by December 26, 2007 [24]. Some of the key points with the COP included notification to CMS of significant changes to a transplant program, evaluation of compliance with Medicare's requirements, and the process for requesting consideration of mitigating factors in CMS' determination of Medicare approval of organ transplant centers [24].

In notification to CMS of significant changes to a transplant program, centers are required to provide notice within seven days of any changes that could affect its compliance with Medicare's requirements 42 CFR 482.74. This includes changes in key staff members such as the primary surgeon or primary physician of a specific program, change in the transplant administrator, a significant decrease in the volume or outcomes of a program, termination of an agreement between the hospital and the Organ Procurement Organization, and inactivation of the transplant program [24].

With implementation of transplant center COP program outcomes are monitored by regulatory agencies through data submitted by each program and analyzed by the Scientific Registry for Transplant Recipients (SRTR) [23]. Data is submitted through forms submitted electronically to the Organ Procurement and Transplantation Network (OPTN) for each candidate and recipient. Data submission is monitored to ensure 95% of a center's data is submitted no later than 90 days after its due date (COP 482.8). Failing to submit data in a timely manner may result in a deficiency accessed by the surveyors [23].

Candidates are registered with the OPTN and listed for a new organ through the computerized system. Once transplanted, follow-up forms are submitted regularly through the first year, then annually for the life of the recipient. Graft and patient outcomes are analyzed by the Scientific Registry of Transplant Recipient's (SRTR) based on forms submitted to the OPTN. These outcomes are publicly reported every six months and provide 30 day, one year and three year outcomes for both graft and patient survival. CMS, insurers and the OPTN all monitor these outcomes. Centers falling below expected outcomes are subject to a Systems Improvement Agreement (SIA) issued by CMS. This document requires a transplant program to improve their practice during a one year probation period. If the program does not improve, it may lose Medicare and Medicaid coverage for transplantation.

The process for requesting consideration of mitigating factors in CMS determination of Medicare approval of organ transplant centers describes three general areas that will be evaluated to determine whether or not a program can be approved based on the following mitigating factors: (1) the extent to which outcome measures are met or exceeded; (2) the availability of Medicare-approved transplant centers in the area; and (3) extenuating circumstances that may have a temporary effect on meeting the COP [23].

In reviewing outcome measure failure, CMS evaluates the extent and nature of the outcome measure failure and to what extent has the risk-adjusted performance departed from the standard. Important trends are reviewed for improvement, status quo or worsening along with the length of time the center has been below standard. One of the key factors measured by CMS is the risk-adjustment anomalies. CMS evaluates the evidence to determine the extent that performance has been adversely affected by transplant risks not captured in the SRTR risk-adjustment methodology. Experimental protocols would also be included in the review. Other factors included are the evidence of access to care and unusual access to care issues, patient population, organ type and factors beyond the control of the hospital, natural disasters and other factors.

Transplant programs function in both the inpatient and ambulatory care settings of a hospital. Unannounced surveys by CMS occur every three years, but CMS may survey a program earlier if patient and graft outcomes consistently decline in a program. Surveyors use tracer methodology to evaluate compliance with documentation and to determine if practitioners are following their program policies and protocols. This process involved both inpatient and ambulatory care documentation. During the survey, daily inpatient census of patients admitted to the hospital are provided to site surveyors. They may interview patients about their care during this time. Surveyors also request the names of all patients with ambulatory care visits during the site survey. Records from this population are also reviewed and patients in the clinics may be interviewed about their care [26].

The OPTN also performs site surveys, however, they provide transplant programs with the dates of their planned visit to a program and also give the center a list of patient charts they plan to audit during their one to three day survey [27]. In 2011, the OPTN began surveying living donor programs separately from the deceased donor transplants surveys. Thus, a transplant program that performs living and deceased donor kidney transplants

could have three surveys in one year: A CMS site visit as well as two separate OPTN site surveys. While the regulatory aspects of transplantation have created a more standardized structure for transplant programs, the staffing requirements have increased to oversee the data management, quality and compliance issues. Inpatient and ambulatory care settings are working more closely together to ensure safe, quality programs recognizing that volume and outcomes are being monitored and are tied to payment.

In addition to the CMS regulations and OPTN policies, the Joint Commission conducts surveys for disease specific certifications such as the Ventricular Assist Device (VAD) programs associated with heart transplant programs in the US [28]. Mechanical circulatory support devices such as the various VADs have become a large component of most heart transplant programs as either a bridge to heart transplantation or as destination therapy [29]. Surveys are conducted every two years by The Joint Commission to maintain a certification that verifies a program has met safety and quality standards established by The Joint Commission [28]. Nurses responsible for the care of patients on mechanical circulatory support devices in the intensive care and progressive care units are interviewed by surveyors to evaluate their knowledge of patient care and to discuss their orientation to the devices. A review of staff competencies is often requested along with documentation of continuing education of staff.

Joint Commission surveyors conduct tracers on patients with mechanical circulatory support devices from the emergency room to ambulatory care settings. Once discharged, patients are followed in the ambulatory care clinics by pretransplant staff including cardiologists, surgeons, RNs and nurse practitioners. Nurses and staff in ambulatory care settings are also interviewed by Joint Commission surveyors and reviewed for their updated competencies. Surveyors request documentation of nurses' orientation and continuing education about device therapy to ensure they are current with their knowledge and expertise in the care of this population.

Several heart transplant programs also implant the total artificial heart (TAH). Nursing staff in the intensive care and progressive care units who care for this patient population are interviewed by The Joint Commission surveyors on their knowledge, expertise and competencies in the care of TAH patients. In 2011 the portable TAH, an investigational device, allowed patients to be discharged on the TAH.

Disease Specific Certification for VADs requires that programs develop quality indicators to follow while patients are implanted with devices. The QAPI program must include at least four performance measures that address both outcomes and process. At this time the performance measures are not prescribed by The Joint Commission and can be identified by each program based on clinical practice guidelines. Once data from all certified programs has been analyzed, The Joint Commission plans to standardize performance measures. Data is submitted to the Joint Commission on a quarterly basis. All certified VAD programs are required to participate in a national database registry called INTERMACS. Data is collected and submitted to INTERMACS on both inpatients and ambulatory care patients as long as they remain on a VAD. Outcome reports from this database are published annually [29].

Summary

Transplantation is a highly regulated area of healthcare due to its high costs and high risks. Transplant programs have become more standardized and focused on quality outcomes within this regulatory environment. Many transplant programs have expanded staff to include a quality and compliance experts and additional data coordinators to monitor real time outcomes. The frequency of regulatory surveys by CMS, the OPTN and The Joint Commission has resulted in additional staffing requirements to be ever ready for those unannounced surveys. In large transplant programs, candidates and recipients are often cared for in specialty units allowing the entire staff of nurses to become familiar with the complexities and competent with the skills needed to care for these patients and their families. In smaller transplant programs, patients may be integrated with non-transplant patients. In such cases a specialized core group of nurses provide care for transplant candidates and recipients. Competency testing for nursing staff may be semiannual or annual. Certification is highly recommended for staff nurses as well as transplant coordinators to further ensure they maintain current knowledge in their specialty area. The multidisciplinary team approach is very effective in caring for this complex patient population with the different perspectives from each team member culminating in the comprehensive care these patients require and deserve.

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CHAPTER 125

Transplant Clinic Management

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Introduction

The field of transplantation has evolved far beyond the fabled 13th century operating room of Saints Cosmos and Damien to sophisticated multi-program transplant centers. Surgical skills and medical advancements aside, over time it has become clear that additional clinical resources are needed to provide long-term care to a population of increasingly complex patients. Outpatient transplant clinics staffed with clinical experts have been developed to provide comprehensive, cost-efficient care to transplant candidates, recipients and living donors. Major challenges facing transplant programs today include high acuity candidates facing increasingly long waiting times, an aging transplant recipient population confronted with many chronic disease issues, and shortened pre and post-transplant lengths of stay.

Little information is found in the literature regarding the development or evolution of the early outpatient transplant clinics, but the value of organized delivery of care is commonly discussed. Organized delivery of care yields improved clinical quality of care and control of chronic disease, improved patient satisfaction, and a reduction of hospitalizations, emergency visits, and prescription drug expenses [1]. Pioneering transplant efforts have been largely that of a "team", typically consisting of surgeons, physicians and transplant coordinators. Much is written about the early days of transplant coordination from the surgeons' perspective, but few publications focus on the role of early transplant clinics [2]. Therefore, the aim of this chapter is to describe current outpatient transplant clinic management operational strategies. This overview is applicable to all phases of transplantation: evaluation, candidate/wait-list management, and recipient/living donor long term care management.

In the US, transplantation is highly regulated by the federal government. The operational strategies discussed in this chapter are often based on these regulations, which will be introduced to the extent that they impact clinic management. More detailed treatment of governmental oversight can be found in Chapter 128. Detailed treatment of inpatient unit design and management is found in Chapter 124.

Role of regulatory agencies

Organ Procurement and Transplantation Network/ United Network of Organ Sharing (OPTN/UNOS)

Until the 1980s, relatively few medical centers performed transplants, and organ allocation was managed locally. With the improvement of transplant outcomes, increased number of existing transplant programs, and as more patients became transplant candidates, the federal government recognized the need for a centralized, national organ distribution system assuring all patients an equal chance to receive donor organs. With the enactment of the 1984 National Organ Transplant Act (NOTA), a national Organ Procurement and Transplantation Network (OPTN) was mandated to build a system for the equitable allocation of donated organs and to increase the supply of available donated organs for transplant that is operated by a private, non-profit organization under federal contract. The United Network of Organ Sharing (UNOS) currently holds the OPTN operational contract. UNOS' mission is to advance organ availability and transplantation by uniting and supporting transplant communities for the benefit of patients through education, technology, and policy development [3]. At the same time, a national Scientific Registry for Transplant Recipients (SRTR) was designed to compile and analyze data on all transplants performed for identification of potential improvements to enhance the lives of transplant patients [4].

The OPTN Final Rule was implemented March 16, 2000 and resulted in a substantially different regulatory environment throughout the US for solid organ transplantation. The Final Rule established a regulatory framework for operation of the OPTN. Several factors impact organ transplantation, of which the most profound is the national shortage of donor organs. As a result of this shortage a need developed to mandate transparency in assessment of transplant programs to enhance the supply, allocation, and management of donor organs. The Organ Procurement Transplant Network (OPTN) is a private, not-for-profit entity with expertise in organ procurement and transplantation whose role is to maintain an equitable system for organ allocation, maintain a wait-list of potential recipients, match potential recipients with organ donors

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

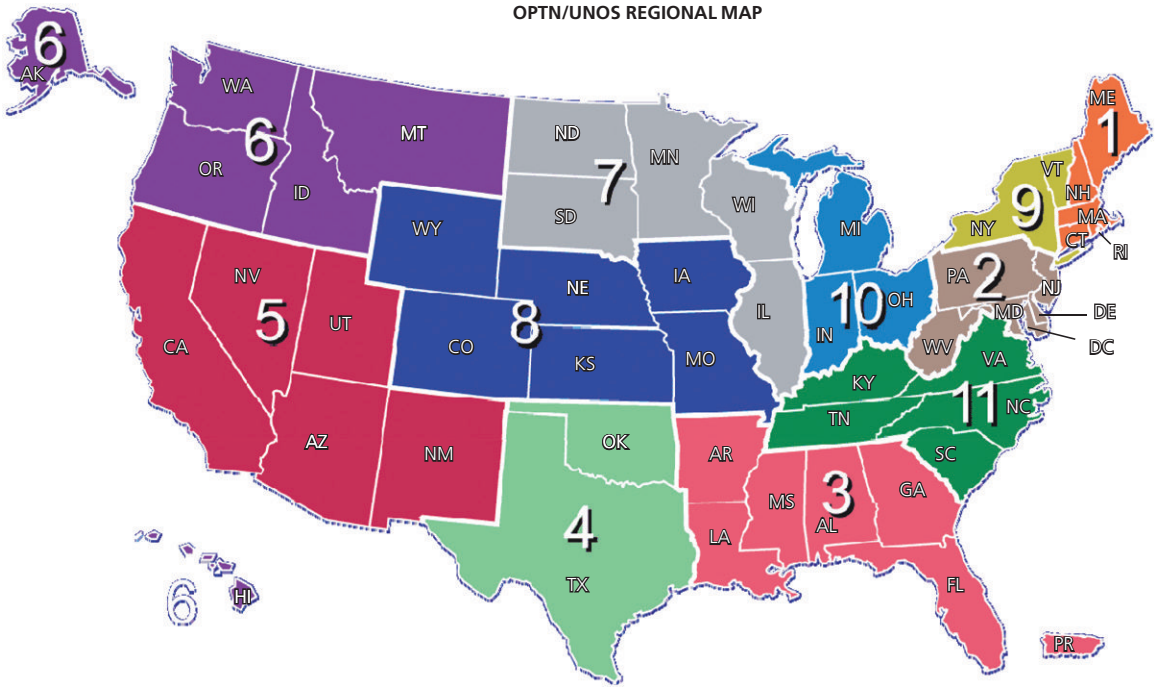

Figure 125.1. UNOS regional service areas map. Reproduced from [6] UNOS Regions Map. http://optn.transplant.hrsa.gov/ (accessed 1 December 2011).

according to established criteria, and list and remove transplant patients. The OPTN also collects, analyzes, and publishes data on pre and post-transplant events to advance the fields of organ transplantation, organ procurement, organ preservation, and immunogenetics. These data is managed through an online database system called UNet. UNet contains data regarding every donation and transplant event since October 1, 1987. The OPTN has established policies and bylaws to administer the bureaucratic aspects of policy development, allocation of donated organs, and collection of transplant data throughout the US. The OPTN is composed of 22 national committees, 23 Regional and National Review Boards, and a board of directors to develop policies. OPTN/UNOS has defined the country into 11 Regional Service Areas; each region is subdivided into Organ Procurement Organization (OPO) Donor Service Areas (DSA) to support fair and equitable allocation of donor organs [5] (Figure 125.1).

The OPTN conducts ongoing and periodic surveys and evaluations of each OPO and transplant hospital for compliance with the OPTN Final Rule and policies. For compliance monitoring, the OPTN uses defined processes and protocols, applications for program membership and key personnel, specific required notification elements, and onsite and desk performance audits, all developed by the OPTN Contractor in accordance with the federal contract. The OPTN makes every effort to bring members into compliance through the development of corrective plans, use of sanctions including prompt corrective action as necessary and confidential peer review through the Membership Professional and Standards Committee (MPSC). This process encourages participation in quality improvement using the following disciplinary sanctions: Letter of warning (confidential peer review), Probation (public notice), and Declaration of "Member Not in Good Standing" (public notice) [7,8].

Centers for Medicare and Medicaid Services (CMS)

Conditions of Participation (CoPs) for organ transplant programs were established in 2007. The Final Rule set forth expectations for safe, high quality transplant services delivered by Medicare-participating facilities. Areas of regulation include compliance with federal, state, and local laws, and laws related to patient care plans, patients' rights, utilization rates, service requirements, and reasonable and necessary national coverage decisions.

In 2004 the Office of the Inspector General (OIG), in what appears to be the initial steps to the current CoPs, recommended that CMS should develop criteria for recipient volumes and survival rates, develop guidelines and procedures to deal with transplant programs that do not meet the volume and survival rates criteria, and oversee improved data collection related to volume and survival of transplant recipients.

Over time, other program quality and service specific issues, as well as complaints, demonstrated that self-reporting of significant changes was not adequate to ensure high quality and safe transplant services. In addition explicate criteria for de-certification had not been identified or established by the OIG. As a result, The Final Rule and subsequently CoPs established requirements for the initial approval and re-approval of Medicare approved hospitals that provide transplantation services for adult and pediatric patient and living donation services.

The Department of Health and Human Services (HHS), with CMS as the responsible federal agency, launched the transplant program approval and re-approval application process in March 2007. The CMS survey certification process assesses a transplant program's initial and ongoing compliance with the CoPs. Transplant hospitals are expected to provide notification to CMS of any significant changes to the transplant program which includes key staff changes, changes in clinical experience and outcomes,

termination of Organ Procurement Organization (OPO) agreement, and/or inactivation of a transplant program. After self-reporting, transplant centers must apply for "consideration of mitigating factors" when they knowingly do not meet one of the CoPs [9].

Other agencies

The HHS Advisory Committee on Organ Transplantation (ACOT) was established as an advisory committee acting through the Health Resources and Services Administration (HRSA) in regards to organ donation, procurement, allocation, and transplantation issues as directed by the Secretary of Health. The mission of this committee is to review significant proposed OPTN policies when submitted to the Secretary for approval on a variety of transplant related topics.

Donation and Transplantation Community of Practice, under the direction of HRSA, established the Organ Donation Breakthrough Collaborative in 2003 with the goal to end transplant waitlist deaths by increasing the number of deceased organ donors, increasing number of organs available per donor, and creating best practice guidelines. These collaborative efforts have continued and broadened to include all aspects of donation and transplantation. In 2007 the HRSA Transplant Center Growth and Management Collaborative Best Practice Final Report was issued. This report presents best practice observations from selected high-performing transplant centers that have achieved high organ transplantation rates and efficiency in recovered organ use, while maintaining expected or higher than expected patient and graft survival outcomes [10].

Design/layout of outpatient transplant clinics

Goals and purpose

Outpatient clinics can range from a simple medical office providing primary care, to highly specialized, complex facilities that are essentially hospitals without inpatient beds. Goals of outpatient transplant clinics are incorporated into each individual center's mission statements, but usually are broad in scope and maintain a shared vision and commitment of optimization of wellness and wellbeing of candidates, recipients, living donors, and their families. The clinics' purposes are to provide evaluations of transplant candidates and living donors, optimize medical management of transplant candidates and recipients, including prescribing, monitoring and adjustment of immunosuppression; diagnosis and management of acute and long term complications of transplant, and living donor follow-up. To ensure optimal patient care and continuity of care, transplant teams need to have a collaborative approach.

Operations

Transplant clinic operations and designs can vary. Most are based on the needs, volumes, and patient population of the transplant center in which they exist. All exist as part of the larger medical center of which they are affiliated, with the commonality of integrating continuity of patient care from inpatient to outpatient setting, therefore becoming the patient's primary center of transplant-related care delivery.

To meet expanding patient needs and enhance access to transplant related services, off-site satellite clinics are also being utilized to facilitate patient care. Satellite clinics have emerged as part of integrated healthcare systems that accommodate large academic centers, community centers, and rural centers for ease of patient care delivery. This is an opportunity to utilize diagnostic and surgicenters off-site but still within the larger healthcare system for pre and post-transplant follow-up care. These clinics reduce the travel and financial burden for patients and may ultimately enhance their adherence to the prescribed transplant plan of care. Satellite clinics may also help increase referral bases for transplant centers and ultimately expand the program. These clinics are staffed with designated personnel in laboratories, radiology, and procedure areas; however, the multidisciplinary teams have the ability to travel within the system to see transplant patients at these facilities as well as the transplant center [11].

Governance and regulation of outpatient transplant clinics can either fall under hospital-based clinic management or ambulatory care clinic management. Operational regulations and reimbursement schedules vary according to type of clinical management and state accreditation structures. Briefly, hospital-based clinics function as Physicians' offices providing services specific to the population of patients served; however, they operate under the hospital's licensure and are accountable to the same federal and regulatory bodies as the hospital, that is, The Joint Commission on the Accreditation of Healthcare Organizations (JCAHO). Attention must be paid as to how the various types of clinics are surveyed by local, state, and federal regulatory agencies and national accreditation bodies relative to their respective requirements.

Design

Specific to transplant clinic operations, a dedicated transplant structured clinic is optimal. The concept behind a transplant structured clinic is the ability of specially trained transplant personnel to provide comprehensive transplant-specific services within a space designed solely for the care of transplant candidates, recipients, and living donors. The function of this type of clinic ranges from the coordination of an extensive candidate or living donor evaluation to long term follow-up of transplant recipients and living donors.

Hospital and transplant administration, along with physician leaders, provide the building blocks to any clinic development, design, and management. Structural logistics and limitations must be considered in the physical design of clinic space. Varying size and types of services offered are common attributes of all outpatient transplant clinics. Transplant patients are coming to the medical center for complex services and by nature this patient population may be accompanied by multiple family members plus medical equipment such as oxygen tanks, walkers, or wheelchairs. In order to enhance the functionality of the work environment, the clinic design team should ideally include administration, medical architecture personnel, and transplant team personnel who are providing services within the given space. Patient and family input must be soliaccessed as well. General themes to be considered in structuring an outpatient clinic include:

- ability to offer services in a cost efficient manner
- flexibility and expandability to meet the current and future needs of the program
- environmental quality and safety
- visibility and accessibility to include American's with Disabilities Act regulations
- therapeutic, nonthreatening environment for patients
- sustainability
- Health Insurance Portability and Accountability Act of Privacy and Security (HIPAA) compliance [12].

Ideally, facilities management would adequately support the needs of the transplant program without operational limitations.

Clinically, an adequate number of clinic rooms for consultation and evaluations would be available in addition to dedicated workspace for the medical team to review records and complete documentation. The number of clinic rooms required is dependent on the size of the program and annual clinic volumes. Dedicated transplant specialty laboratories, staffed with personnel who are familiar with the extensive transplant-specific blood tests ordered, should also be available within or near the clinic space. Amenities such as: registration areas for patient check in and discharge; private consultation rooms; patient education facilities for individual or group teaching; a patient library; and spacious waiting rooms, all need to be considered in the physical design and layout of the transplant clinic. Despite physical limitations of any clinic design, the primary efforts of the transplant team should be on excellent customer service delivery with the long-term goal of measurable, sustained quality patient outcomes.

Clinic scheduling models

Models

There are various types of clinic scheduling models available that can be the basis for the transplant clinic to develop as their standard. No matter which model is incorporated as the foundation for the clinic structure, the ability to offer any needed transplant service in a patient and family centered approach with sustained quality outcomes is the common goal of all transplant programs. Basically there are two types of patient service delivery models — physician driven or clinic/service line driven approaches. Traditional physician driven clinics are scheduled per provider with designated clinic times. In a clinic/service line driven clinic model, patients are generally scheduled with common arrival times according to organ type, and transplant status (pre/wait-list/post/living donor), without a direct provider specified.

Regardless of which clinic model is adopted, clinic staffing patterns can vary greatly. One staffing pattern is to have fully dedicated clinic staff. This type of staffing would include support staff, phlebotomists, medical assistants, and transplant clinic nurses functioning under the direction of a dedicated clinic nurse manager and office manager with the sole purpose of treating patients presenting to clinic. An alternative staffing pattern is one in which the clinic space is staffed by service lines, including the transplant coordinators and support staff. Regardless of the staffing pattern selected, efficient quality patient care must be maintained as well as regulatory compliance and ongoing patient satisfaction monitoring.

Types of clinic visits: Pretransplant and post-transplant

Pretransplant clinic visits

Typically, the first encounter the potential candidate or living donor will have with the transplant center is at the time of evaluation for transplantation or donation. The goal of a transplant candidate evaluation is to determine the medical need for organ transplantation for patients in end-stage organ failure when traditional medical options have been exhausted. Potential transplant candidates will be referred to the transplant center at various stages of end-stage organ failure. Prior to the actual transplant evaluation, medical records are reviewed by members of the multidisciplinary team to determine the appropriate level of evaluation to be scheduled. Once the patient is medically approved and financial authorization is in place, a formal transplant evaluation is scheduled. During the course of the initial transplant evaluation, the cause of end-stage disease, and the indications and contraindications for transplant will be determined. Further work-up and management of co-existing medical conditions may be required in order to complete the evaluation. A dedicated transplant clinic affords the ability to coordinate a comprehensive testing schedule. Typically, each candidate's evaluation will be tailored to their medical needs, as well as program and organ specific directives. A dedicated transplant clinic and personnel provides a venue for the comprehensive multidisciplinary assessment of potential transplant candidates. Members of the clinical transplant team must partner with the referring physician to keep him/her abreast of the patient's progress throughout the evaluation. This begins at the point of referral and becomes the foundation of life-long mutual patient care team collaborations (Figure 125.2).

From the patient's perspective, the clinic appointment starts before the actual visit. A welcome packet of information is sent to the patient outlining the upcoming visit. This packet typically includes driving directions, information on local housing, medical center directions, patient evaluation schedule, specific test preparation instructions if any, organ-specific education materials, regulatory documents (e.g. UNOS materials, Informed Consent), and other center specific items. A confirmation of the candidate's upcoming evaluation should be communicated to the referring physician also. Items for schedulers to be mindful of include obtaining alternative telephone numbers for patients and lodging location during the evaluation for those patients who are traveling. Schedulers should also confirm appointments 24–48 hours in advance. Due to the complexity of transplant candidates' medical conditions, frequent hospitalizations are not uncommon but contribute to a higher rate of "no-shows" or missed appointments. This also contributes to lengthened time between referral and transplantation, which ultimately may affect overall patient outcomes.

From a transplant team perspective, the number of clinic appointments scheduled on any given day is dictated by the number of clinic rooms available, provider availability, and length of given appointment type. Coordination of testing and consultations among ancillary departments is crucial to the flow and success of the visit.

Prior to the patient's arrival, accessibility of parking and navigation through the system must be facilitated. Amenities such as a designated patient drop-off area or valet parking with handicapped accessibility provide a caring first impression to an anxious, overwhelmed population. Wheelchairs and/or scooters should be readily available for patients use upon arrival, in addition to hospital concierge and transport specialists. Hospital signage must be up to date and easy to follow.

Upon arrival at the transplant clinic, check-in and registration will take place. Due to the extent of the transplant evaluation, it is expected that these patients will be in the clinic for a prolonged period, perhaps over several days. The actual length of the evaluation is variable and dependent upon each transplant center's organ-specific selection criteria requirements and the patient's overall health status. The clinic team will guide the patient through the evaluation process. This would encompass services such as phlebotomy, medical assessment, medication reconciliation, consultations, patient education, and additional testing specific to the transplant candidate's medical status and history. The staff member who completes the outlined tasks may vary per facility: medical assistants (MA), registered nurses (RN), transplant coordinators (TC), advanced practice nurses (APN), physician assistants (PA),

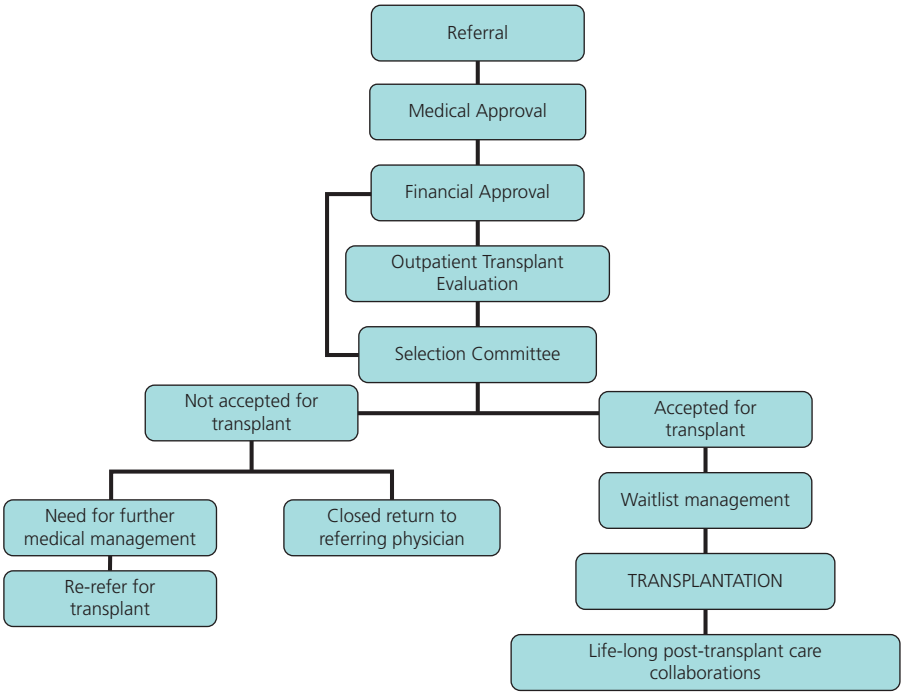

Figure 125.2. Transplant process chart.

or transplant physicians. At the end of each visit, reinforcement of the upcoming prescheduled evaluation should be reviewed with the patient and family.

Any additional needs are also addressed upon checkout prior to the patient's departure from the clinic. Ideally at the end of the evaluation a "wrap up" visit after all consultations and testing have been completed should be conducted. Preliminary test results and patient concerns and questions should be addressed at this time. Once a patient has completed the evaluation and has been waitlisted, return visits to clinic will be determined by listing status and individual needs.

The transplant evaluation process must adhere to both UNOS and CMS regulations. The CMS CoPs identify the transplant professionals who are required to participate in candidate and living donor evaluations. Included in the multidisciplinary team are: medicine (transplant surgeon and transplant physician), nursing, social service, clinical transplant coordinator, nutrition, and pharmacology. Specific to evaluation of living donors is the Independent Living Donor Advocate evaluation. Additional members of the multidisciplinary transplant team potentially include: administrative support staff, schedulers, medical records staff, UNOS data coordinators, transplant financial coordinators, and research staff. Each of these identified team members possess a unique skill set and expertise required to comprehensively provide life-long care to transplant patients across the transplant continuum. CMS regulations require that institutions develop individual policies that specify the multidisciplinary evaluation team members, as well as their qualifications, credentials, and roles within their transplant center [13].

Medicine (transplant surgeon or physician)

Transplant physicians and surgeons are responsible for directly overseeing transplant surgical and medical services. A designated surgeon must be available immediately when an organ is offered for transplantation. The primary physicians and surgeons must be approved by the OPTN [13].

Nursing

Nurses provide care to transplant patients at all points along the transplant continuum. From referring physician offices, to outpatient transplant clinics, intra-operatively, intensive care units, general nursing units, rehabilitation units, long term care facilities, skilled nursing facilities, home care nurses, research teams, nursing educators, and nursing administrators are all various types of nurses involved in the care of transplant patients. Levels of nursing education range from licensed practical/vocational (LPN/LVN), registered (RN), or advanced practice nurses (APN). Specialty certification in transplantation is available to transplant nurses with one year of transplant specific nursing experience [13,14].

Social services

Social services are an inherently important part of the transplant team, providing continuity of care to the patient throughout the entire process. Their focus is to convey to the patient that they are not experiencing the transplant process alone, but as members of the team. Transplant social workers provide support, education and counseling to help reduce the stress of undergoing a transplant. Performing evaluation assessments is mandated by CMS CoPs with the goal to ensure that the patient and team have all information necessary to achieve a potentially successful outcome for both the patient and the program. Topics addressed in a social services evaluation include:

- risk and benefits of transplantation acknowledged
- adherence assessment
- mental health history, including substance and alcohol use/abuse
- assessment of available coping abilities
- assessment of financial capabilities, resources

- assessment of adequate social, transportation, lodging support [13].

Clinical transplant coordinator

Transplant coordinators (TCs) must be registered nurses or clinicians licensed in the state of practice that possess knowledge and experience in transplantation issues. TCs ensure continuity of care and coordinate the clinical care of candidates/recipients/donors throughout all phases of transplantation/donation and also act as a liaison with all members of the multidisciplinary team involved in the patient's care [14,15]. Transplant coordination has evolved into a critical component of essentially all transplant teams and is covered in depth in Chapter 122.

Nutrition

Nutritional assessments and diet counseling must be available at the transplant center by qualified dietitians for all transplant patients, candidates and living donors, in the evaluation phase. Education is provided to the patient specific to their individual needs. Medical nutritional therapy is provided when indicated. Nutritional services are also available to recipients and donors post-operatively to discuss nutritional concerns and issues (i.e weight gain, weight loss, electrolyte imbalances, dietary restrictions due to medications). Individual transplant center's policies must outline the utilization of nutritional services including nutritional assessment, diet counseling, or nutritional interventions [15].

Pharmacology

Transplant pharmacists help to manage the everyday care of the transplant patient. They assess and suggest plans of care based upon the individual not just specific to the transplanted organ. They provide valuable insight into immunosuppression management and rejection therapies. Throughout the transplant continuum, transplant pharmacists monitor medications prescribed specifically looking for interactions and therapeutic drug levels. Consideration must be given to over the counter medications and supplements the patient may use as well. Transplant pharmacists are often members of transplant research teams [15,16]. The role of the transplant pharmacists is discussed in detail in Chapter 123.

Living donor advocate

Transplant centers that perform living donor transplants must identify an independent living donor advocate to ensure protection of prospective living donor's rights. This individual should function independently from the transplant team [15].

UNOS data coordinators

Some large, multi-program transplant centers have UNOS data coordinators on the multidisciplinary team. UNOS data coordinators are individuals that manage UNOS/UNet related data entry. Completion of Transplant Candidate Registration (TCR), Transplant Recipient Registration (TRR), Transplant Recipient Follow-up Forms (TRF), activations, in-activations, listing status recertifications, and removals are examples of tasks UNOS data coordinators perform. These coordinators are also important members of quality improvement (QAPI) committees and are participants in quality control auditing.

Transplant financial coordinators

One of the first members of the transplant team a new patient meets is the Transplant Financial Coordinator (TFC). This person functions as a counselor to the patient as well as an insurance liaison/coordinator for the provider. After an in-depth review and verification of insurance benefits, a consultation appointment is scheduled with the patient during the evaluation to review all eligible insurance coverage benefits and limitations. The TFC explains what the financial impact for the high cost transplant related services could potentially be, and guidance is given to those who need additional assistance due to coverage and benefit limitations. The TFC also acts as a liaison for the medical and administrative departments, alerting the team to limits or restrictions of coverage and when authorizations for services are required [17].

Post-transplant clinic visits

Post-transplant visits tend to be shorter, but equally important in promoting successful long-term patient outcomes. The goals of this type of visit include optimal management of immunosuppression, monitoring and diagnosis of both acute and chronic complications associated with transplantation, as well as overall health promotion. These visits may or may not include lab draws, but would include patient assessment, medication reconciliation, consultation, education, and transplant specific testing as needed. Of note, one can expect the initial post-transplant visit to be more in-depth and longer due to the level of re-education involved for the patient and the family by both the nurse and the physician.

Transplant clinics alone are not able to provide all services transplant patients require. Coordination of services must be available within the medical center system. Integration of care with other members of the multidisciplinary transplant team including but not limited to pathology, radiology, infectious disease, nephrology, hepatology, cardiology, pulmonary, anesthesia, endocrine, pharmacology, psychiatry, and palliative care is essential to the long term care of the transplant recipient. Standing protocols or pathways may be developed by the transplant team, in conjunction with the identified specialties, to facilitate implementation of patient care.

A dedicated transplant outpatient procedure unit is also critical to the care of this complex patient population. This type of unit provides an intermediate level of organ specific pre and post-transplant services and treatments, and other invasive procedures unique to the transplant population requiring more intensive medical management than the outpatient clinic can provide. Included would be delivery of interventions and treatments that do not require hospitalization but do require patient oversight to assure patient safety. Typical nurse to patient staffing ratios are one nurse to three or four transplant patients. Services that an outpatient procedure unit can offer include daily monitoring post hospital discharge, phlebotomy, biopsies, blood product administration, medication administration, invasive radiology testing, and plasmapheresis. Associated cost-saving benefits of this type of service include promotion of early discharges, prevention of readmissions, and delivery of an intermediate level of care that cannot be provided in the home.

At some point in time, the care of transplant recipients may be transitioned to a healthcare provider outside the transplant team for long-term healthcare services such as a primary care physicians (PCPs) or other non-transplant related specialists. Coordination of care between the transplant team and other members of the multidisciplinary team must be built on open communication and collaboration to deliver the appropriate services to this complex patient population. Long-term transplant follow-up must be coordinated through the transplant office to ensure compliance with regulatory reporting requirements.

Staffing

CMS CoPs have stipulated which multidisciplinary team members must participate in a transplant patient's care. The CoPs clearly state that each patient must be under the care of a multidisciplinary team. This team is to be coordinated by a transplant physician who must be involved in all aspects of patient care throughout transplant and discharge phases, including evaluation and routine follow-up care [13]. However, how individual transplant centers integrate these roles varies greatly among programs. Center- specific policies regarding the multidisciplinary team must address the composition of the team members, roles, qualifications, and supporting documentation. The complex, chronic nature of the transplant candidate, recipient, or living donor coupled with the need for specialized medical management further compounds the staffing dilemma. Additional limiting factors include the number of examination rooms available, number of providers and levels of support staff available, and annual volumes of the clinic population served.

Two models of general staffing practice in existence include the "Medical Model" and the "Nursing Model." In the "Medical Model" the traditional provider (physician, PA), medical assistants (MA), and support staff run the clinic. This type of provider-run clinic has lower operating costs but consequently offers a limited scope of services. In the "Nursing Model" led clinic, this environment increases utilization of nurses with the provider (physician, PA) in addition to the MAs and support staff. This type of nurse led clinic supports the acuity of a specialty care outpatient environment by enhancing the scope of available patient services and education, and enhancing quality patient outcomes [18].

In response to staffing dilemmas, in 2003 UNOS developed the Transplant Administrators Staffing Survey through UNet to help determine optimal staffing levels given the size and characteristics of the transplant program. This survey identifies roles of members of the multidisciplinary transplant team. The Transplant Administrators Committee Staffing Survey examines the size and scope of each transplant program. It also identifies the type of personnel working at the center, as well as how many perform in each role. An interactive report permits a transplant center to compare staffing levels to other transplant centers of similar size on a confidential basis. Again, this tool is to be used as a staffing guide as there currently are no measurable, agreed upon metrics for transplant clinic staffing. Data reported on this survey is only available to transplant centers that have participated in the survey [19].

Competencies

Transplant professionals provide highly specialized care to candidates, recipients, families, and living donors. As technological, surgical, and pharmacological advances continue, transplant professionals must continually update their knowledge base and demonstrate expertise in their field to care for an increasingly complex patient population. All healthcare providers are held to some level of competence. "Competencies" is an all-inclusive term used to capture broad elements of performance. Competencies include assessment of behaviors, skills, technical knowledge, and proficiencies at a healthcare system, department specific, and regulatory level. Annual healthcare system competency training can be facilitated electronically for global competencies to ease completion and assure consistency in the delivery and completion of organizational core competencies. Departmental specific competencies and regulatory competencies are conducted in addition and individualized to the discipline and the healthcare provider delivering the care. Transplant coordinators would be expected to participate in clinical competencies unique to the patient population they are providing care for, as well as regulatory specific transplant related competencies. UNOS data coordinators or other support staff would be expected to participate in competencies associated with clerical and administrative tasks. This could include UNet and regulatory specific transplant related tasks.

In 2009 The International Transplant Nurses Society (ITNS) in collaboration with the American Nurses Association (ANA) developed the *Transplant Nursing Scope and Standards of Practice*. The function of these standards of practice and professional performance provide specific measurable elements that can be used by nursing professionals to measure professional performance [20]. Transplant centers can refer to this document when establishing transplant specific annual competency assessments within their department.

Electronic medical record

Over the past 20 years there has been a growing impetus for electronic medical records (EMRs). With stricter federal government oversight and the ongoing healthcare reform, most centers are seeing integration of EMRs into practice. EMRs are longitudinal electronic records of patient health information generated by one or more encounters in any care delivery setting. EMRs provide immediate access by authorized providers to patient records that enhances quality, safety, and efficiency of patient care [21].

Key capabilities of EMR according to the American Health Information Management Association (AHIMA) are the ability to capture data at point of care in real time, integrate data from multiple internal and external sources, and to support clinical decision making. Core components/capabilities of any EMR include health information and data storage, efficient results management, order entry management, clinical decision support, communication and connectivity enhancing continuity of care among all providers, patient support and access to their protected health records, and direct interface with regulatory reporting [22].

There are many commercial EMRs available to healthcare systems. EMRs have the ability to generate a complete patient record, as well as supporting other care related activities directly or indirectly via interface. This would include such activities as evidence-based decision support, quality management, and outcomes reporting. Providers need to choose the system that best fits the program's needs. Transplant specific applications are available as well to support daily operations, research data collection, and regulatory reporting integration.

Patient education/informed consent

Education of transplant patients has been recognized as a contributing factor in the success of transplantation. Comprehensive patient and family education begins at the time of transplant referral and is ongoing. Transplantation is overwhelming and requires an open and trusting relationship between the patient and transplant team to ensure success. Transplantation involves major lifestyle changes and the transplant center becomes the primary source of education for patients and their families. All members of the multidisciplinary team are involved in the education process, which

begins as soon as a patient is informed of the need for a transplant. Ongoing education throughout the transplant process is necessary to enable patients and their families to make informed decisions about their care and successfully manage their health.

Informed consent

The CMS CoPs provide the foundation for all transplant education programs. The CoPs clearly outline patient and living donor rights, including the process for obtaining Informed Consent and documentation of patient education. Topics to be included in the Informed Consent include the evaluation process, surgical procedure, alternative treatments, medical or psychosocial risks, outcome results, donor risk factors, right of refusal, and limitations of not having transplant performed at a Medicare approved transplant center. During the evaluation phase laboratory and diagnostic tests results, selection criteria, psychosocial issues identified that may impact transplantation, financial concerns, and necessity of strict adherence to a medical regime post-transplant are discussed in detail. The surgical procedure discussions must occur on multiple occasions prior to the transplant. Detailed discussions of the surgical procedure, anesthesia risks, risks associated with use of blood products, post-transplant recovery, and associated risks and benefits of the surgery must be discussed and any limitations imposed by the patient must be documented in the EMR. Options for alternative treatments will vary per organ type and patient's overall medical condition and must be presented to potential candidates prior to listing. Medical risks discussions need to include postoperative complications specific to wound infections, pneumonia, blood clots, rejection, need for retransplant, need for lifelong immunosuppression, multi-organ failure, and death. Psychosocial risk discussions need to include topics related to depression, post-traumatic stress disorder (PTSD), and anxiety. CMS CoPs require national and transplant center-specific outcomes from most recent SRTR reports be made available to all potential candidates. This is to include the transplant center's observed and expected one-year patient and graft survival, national one-year patient and graft survival, and notification of all Medicare outcome requirements not being met by the transplant center. Updated outcome reporting must be made available to all candidates in the evaluation and wait-list phases every six months upon release. Discussions of organ donor risk factors that could affect the success of graft function need to include medical/social history, age, condition of the organ, risk of HIV, hepatitis B virus, hepatitis C virus, or cancer. Candidates need to be informed of their right to refuse transplant at any time during the process [23].

Informed consent for living donation must include all of the above and also specifically address the possibility that future health problems related to the donation may not be covered by the donor's health insurance, the possibility that the donor's ability to obtain health, disability, or life insurance may be permanently affected, and discussion outlining the donor's right to opt out of donation at any time during the donation process [23].

During a CMS survey, auditors will ask to see copies of all educational materials provided to patients by the transplant center as part of the outlined Informed Consent process, as well as supporting documentation in the patient's medical record that educational materials were provided to the patient and family. Transplant centers must develop organ-specific informed consents and policies pertaining to who is responsible for discussing the Informed Consent process with the patient, who is responsible for obtaining informed consent, and methods and documentation of patient education per CMS guidelines [23] (Figure 125.3)

Multiple listing

During the initial evaluation potential transplant candidates must be informed of the options for "multiple listing". According to current OPTN/UNOS policy 3.2 Organ Distribution: UNOS Patient Waiting List for 2011, patients may be listed as transplant candidates at multiple centers. Multiple listing is a way of increasing chances of receiving an organ offer. Patients must be made aware of this option as well as some of the associated responsibilities and costs. Transplant center documentation that the patient has been notified of multiple-center listing option is required per OPTN policy and subject to audit [24].

Ongoing education

As stated, education of a transplant patient is a continuous process and must occur on many levels. For most patients and families, transplantation is a highly stressful and life-changing process. Patient and family education focuses on the maintenance of optimal health while undergoing transplant evaluation and waiting for an organ, as well as maintenance of a healthy organ post-transplant. Each encounter with a patient should be viewed as a teaching opportunity. Clear, concise, consistent education at every patient encounter ensures understanding of current treatment plan of care and the patient's responsibilities. All patients learn differently. Stress and emotional status, physical status, learning styles, willingness/readiness to learn, in addition to any identified barriers to learning, all must be incorporated into individualized education plans. Because all learners learn differently, a multisensory approach should be taken. Written materials should be provided at a reading level and in layman's terms so that those with a basic education will comprehend them. Among educators this is agreed upon to be a sixth grade educational level. Materials also need to be available in foreign languages or interpreters must be available to translate. Visual aids such as pictures and posters to reinforce the written materials should be incorporated into teaching plans. Computer based educational tools should be provided for group or individual viewing. Structured group education sessions can be built into candidate evaluations and postoperatively prior to discharge from the hospital. Individual education reinforcement occurs during clinic visits and during any form of communication with the patient. Accessibility to the transplant center via phone must be offered 24 hours a day, seven days a week. This service must be offered to answer patient questions regarding treatment plan and associated complications.

Education of the transplant candidate initiates the life-long transplant learning process. The content presented to candidates varies based on transplanted organ involved and among transplant centers. Patients must learn to manage their health by adhering to treatment plans, following dietary guidelines, taking their medication correctly, and knowing when to call the center for help. These can be major lifestyle changes for patients, and they look to the transplant center as a primary source of education. It is important to begin patient education at the point of referral. This includes organ specific education on the process of transplantation and life after transplant. Key information must be reviewed as necessary to promote patient and family retention. Providing clear, consistent, and continuous education during every patient interaction ensures

Education Documentation Tool Samples

Outpatient Interdisciplinary Patient/Family Educational Record

Patient: _____

Coordinator: _____

Pre-transplant: Organ

PRE-TRANSPLANT

- New patient packet sent _____ (date sent)
  - Teaching materials included
  - Multiple Listing and Waiting Time Transfer (UNOS) brochure
  - Informed Consent
- Initial Evaluation Clinic Appointment _____ (date)
  - Medical Consult _____ (date)
  - Surgery Consult _____ (date)
  - Social Services Consult _____ (date)
  - Nutritional Consult _____ (date)
  - Pharmacology Consult _____ (date)
  - Transplant Financial Coordinator Consult _____ (date)
  - Living Donor Coordinator Consult _____ (date)
  - Transplant Coordinator Consult _____ (date)
    - Role of the Transplant Coordinator
    - Required evaluation testing education
    - Overview of the transplant process
    - Overview of organ specific allocation system
    - Patient responsibilities during waiting period
    - What to expect when called for transplant
    - Overview of surgical procedure
    - Overview of hospital stay and post-transplant follow up
    - Immunosuppression medications
    - Post-transplant complications: rejection, infection, malignancy
- Group Teaching Class _____ (date scheduled/attended)
- Additional individual teaching sessions _____ (as needed, center specific initiatives)
- When to call the transplant center
- Transplant Center Phone Number _____

I understand the material presented and have had the opportunity, and ongoing opportunity, to have any questions answered by the transplant team pertaining to my candidacy for _____ transplantation.

Patient Signature: _____ Date: _____

Educator Signature: _____ Date: _____

(A)

Education Documentation Tool Samples

Outpatient Interdisciplinary Patient/Family Educational Record

Patient: _____

Coordinator: _____

Post-Transplant: Organ

POST-TRANSPLANT

- Initial Post-Transplant Clinic Appointment _____ (date)
  - Physician Consult _____ (date)
  - Transplant Coordinator Consult _____ (date)
    - Role of the transplant team
    - Copy of post-transplant book given _____ (date)
    - Attendance at Transplant Education Class _____ (date)
    - Identified caregiver
    - Medication review
    - Laboratory review
    - Wound care
    - Diet and exercise
    - Quality of life issues
    - Long term follow-up requirements
    - Surveillance for rejection, infection, malignancy
    - Short and long-term complications review
    - Role of local physician
    - Regulatory reporting requirements UNOS
    - Patient responsibilities
- When to call the transplant center
- Transplant center phone number _____

I understand the material presented and have had the opportunity, and ongoing opportunity, to have any questions answered by the transplant team pertaining to my _____ transplantation.

Patient Signature: _____ Date: _____

Educator Signature: _____ Date: _____

(B)

Figure 125.3. Education documentation tool samples for pre and post-transplant education.

Table 125.1. Patient education table [13,26,27]

Topic	Pretransplant	Discharge	Post-transplant
Overview	X	-	-
Disease process	X	-	-
Transplant process	X	-	-
Role of transplant team	X	-	X
Evaluation process	X	-	-
Diagnostic tests	X	-	-
Blood work	X	-	-
Consults	X	-	-
Informed Consent	X	-	-
Staying healthy	-	-	-
Waiting	X	-	-
Diet and exercise	X	X	X
When to call	X	X	X
Organ Allocation	X	-	-
Surgical procedure	X	-	-
Post-op recovery	X	-	-
Length of stay	X	-	-
Wound care	-	X	X
Medications	-	-	-
Immunosuppression	X	X	X
Side effects	X	X	X
Over the counter	X	X	X
Herbal medications	X	X	X
Postop complications	-	-	-
Rejection	X	X	X
Infection	X	X	X
Malignancy	X	X	X
Long term			X
Psychosocial issues	X	X	X
Follow-up visits	X	X	X
Frequency of visits	X	X	X
Required lab work	X	X	X
Financial issues	X	X	X
Quality of life post	X	X	X
Return to work	-	X	X
Dental care	-	X	X
Sexual issues	-	X	X
Travel	-	X	X
Vaccinations	-	X	X
Pets	-	X	X
Ongoing health maintenance	-	-	X

patients and their families understand their roles and responsibilities [25] (Table 125.1).

Outcome measures reporting

The field of transplantation, including living donation, is highly regulated by OPTN/UNOS, HRSA and CMS. CMS CoPs clearly identify outcome reporting requirements. Transplant outcome measurements include reporting across the full transplant continuum, beginning at activation on the transplant list through graft failure or patient death.

OPTN/UNOS data submissions from the transplant centers directly create the SRTR program specific reports. Program specific reports are available for the entire transplant community including patients and families, payers, transplant centers, and the federal government. These program specific reports include many features of the transplant program such as:

- wait-list activity — additions and reasons for removal
- characteristics of wait-listed patients
- transplant and mortality rates for wait-listed patients
- waiting times
- number of transplants performed
- characteristics of recipients, donors and transplant procedures
- graft and patient survival rates compared with expected rates reported at one month, one year, and three year outcomes for a 2.5 year cohort of patients.

These reports are published every six months in January and July with revised data about each transplant program in the US. The outcomes are adjusted to reflect the specific center's donors, recipients, and practices. Risk adjustment permits individual centers to compare their reported outcomes to the national outcomes for similar patient among transplant programs across the country. Adjusting for recipient and donor characteristics ensure that transplant centers are not penalized for accepting hard to treat patients and aggressively using expanded donors. Transplant centers need to aggressively and proactively learn to manage their data for accuracy, identify the risks for failure, and develop best practices for quality outcomes. Accurate and on-time submissions of "transplant candidate registrations", "transplant recipient registrations", and "transplant recipient follow-up" forms is essential for individual programs to independently monitor their outcomes as well as demonstrate compliance with regulatory reporting submission requirements [28]. All of the data is important to monitor the success of programs and development of best practices, however; the three year graft and patient survival data is most reflective of the ongoing outpatient management.

Patient satisfaction measures

A fairly new approach to measuring the level of quality care provided to patients is to request feedback from the patient themselves regarding their experience [29]. Developing strong patient relationships with high levels of satisfaction is a challenging, but realistic goal. A culture of mutual patient and provider trust, commitment and understanding of individual patient's needs promote quality patient care. Administration needs to address the following when discussing patient satisfaction; what is the utility in measuring patient satisfaction; how is it best measured for a specific patient population; and what to ultimately do with the results? Clinicians, medical centers, and payers all have a vested interest in measuring patient satisfaction for practical, professional, and ethical reasons. Measuring patient satisfaction is not clear cut, but rather a qualitative measure of a patient's perception of their healthcare experience.

There are numerous fee-for-service organizations available to monitor patient satisfaction. One of the most popular is Press Ganey Associates, Inc. Press Ganey Associates, Inc. has been in the business of helping providers improve the delivery of healthcare for more than 25 years. Press Ganey Associates' Inc. philosophy is that patients have a valuable perspective to offer regarding the quality of care they received and work to ensure that patients were heard by healthcare organizations looking at ways to evaluate and improve quality of care. With the use of specialized tools, services, consulting, and data analysis, Press Ganey Associates, Inc. provides information to healthcare providers on how well they are performing and ways to improve their performance [30].

In 2010 Press Ganey Associates, Inc. in conjunction with UNOS Transplant Administrators Committee (TAC) developed a specialized inpatient and outpatient transplant survey. Items specific to the outpatient transplant survey include generalized background questions addressing phase of transplantation, organ transplanted, and waiting time in clinic. Non-transplant specific issues associated with registration and the transplant facility are addressed. Transplant specific care and education items addressed include medications, diet, wound care, blood work, when to call the transplant

team, how to contact the transplant team, associated costs, and patient ratings of the multidisciplinary team members. Transplant centers can use these results to develop performance improvement initiatives directed at enhancing patient satisfaction [31] (Figure 125.4).

Financial considerations for transplant

Transplant is considered an elective life-saving procedure, sometimes the only treatment remaining to patients with end-stage organ disease. Transplant institutions need to maintain fiscal strength in a competitive market. Monitoring of the program's financial strength, reimbursements, performance, outcomes, and volumes assist in negotiating contracts and maintaining overall fiscal strength of the transplant center. Transplant centers need to competitively offer services efficiently and effectively to remain profitable. Due to the complexities and chronic nature of transplantation, the associated costs for services can be astronomical and life-long. Financial limitations can be imposed on the patient's level of benefits and coverage for transplant services. Prior to initiating any transplant service a thorough evaluation of the patient's benefits must be determined by specialized transplant financial coordinators.

There are four basic types of coverage for most patients: Medicaid/Medical Assistance (MA), Medicare (MC), commercial payers, and private/self-pay. CMS includes MA/MC plans and is the largest single payer in the US. Most commercial payers follow CMS regulations regarding reimbursement. Transplant centers must develop policies clearly outlining acceptable levels of coverage and reimbursement for transplant related services. The following is a brief overview of the common insurances seen in transplantation. There are patient assistance foundations for pharmacological assistance, as well as foundations that assist patients with fundraising and charitable care options.

Medicaid fee-for-service

Most states require the MA recipients to receive medical care within their home state when available. All providers of care, including the hospital and each individual physician who treats a patient, must be assigned (enrolled) with the state in order to be reimbursed. State funds are limited; this impacts the amount available to pay for transplant associated medical care costs. Depending on the MA reimbursement schedule, providers may experience a net loss in revenue. Transplant financial coordinators must be diligent when reviewing what transplant services are and are not covered by the state as well as the packaged coverage a patient may have. The MA fee-for-service programs are strictly regulated and not open for the negotiation of additional benefits.

Managed medicaid organizations

Managed Medicaid Organizations (MMO) products are similar to MA products. MMO's may not require providers to be enrolled with the state for reimbursement but the recipients are managed by primary care physicians (PCPs) who must coordinate all care. This creates the challenge of obtaining authorizations for referrals and post-transplant services not ordered by the PCP. If a patient is referred out of network and/or out of state, the transplant provider may negotiate an acceptable reimbursement rate for services with the MMO.

Traditional medicare

Traditional Medicare (MC) can be considered universal as there are no assigned networks or states and reimbursement rates are geographically determined. Almost all providers are enrolled with MC and no referrals are required. Patients may be MC eligible through age, disability, or end-stage renal disease (ESRD). However, an individual must be qualified to receive MC benefits. Qualification is based on work history; therefore, the TFC must review this with the individual.

Medicare components

There are four components to MC: Parts A, B, C, and D. Individuals who qualify for MA are automatically enrolled in MC Part A at no cost. MC Part A covers inpatient hospital care; however, a deductible and co-insurance applies as determined by the length of stay. An individual must elect and pay a monthly premium for MC Part B, which covers 80% of the outpatient hospital and professional charges. In addition to MC Parts A/B, the individual should be counseled to purchase MC Part D, which covers prescription medications. A co-payment applies to most prescriptions.

As noted above MC does not offer full coverage, exposing individuals to potential financial hardships. MC recipients should be advised to purchase a "medicare supplement". And it is just that, it supplements the MC payment to the extent an individual would be considered fully covered eliminating the liability of the deductibles and co-insurances.

Managed medicare/medicare advantage

Many companies offer a Managed Medicare/Medicare Advantage (MC Part C) product in the form of health maintenance organizations (HMOs) or preferred provider organizations (PPOs) but individuals must be enrolled in both MC Part A and MC Part B. The disadvantage to MC Part C is that there are assigned networks and an individual cannot choose where to seek care. Much like MMO's, referrals are needed. In some cases the MC C company will work with the providers to negotiate an agreeable reimbursement which also includes waiving the member's higher out of pocket liability [33].

Commercial insurance

Individuals can obtain commercial insurance either through an employer group or through individual purchase. In either case not all plans are the same, even if the coverage is through the same company. When individual insurance is purchased, the amount of member deductible and/or co-insurance responsibility is determined by the amount of premium paid. A higher premium means a lower out-of-pocket member responsibility; likewise a lower premium means a higher out-of-pocket member responsibility. Coverage through an employer group in most cases offers the best coverage.

Whether the insurance is obtained on an individual or group basis, patients must be aware of the differences in coverage and must be very careful and understand the level of coverage they select. When considering commercial insurance, there are choices of indemnity plans, preferred provider organization (PPO) plans, and a health maintenance organization (HMO) plans. An insurance company can offer any or all of these plans, so again it cannot be assumed that the coverage is the same for all members of the same company. Indemnity plans are the least restrictive plans offered commercially. PPO plans have both in-network and out-of-network benefits. If the member uses an in-network provider the amount of financial liability incurred would be less.

In some cases an insurance company and provider may consider an agreement on behalf of an individual often called a Single Case

CLIENT LOGO

OUTPATIENT SERVICES TRANSPLANT SURVEY

We thank you in advance for completing this questionnaire. When you have finished, please mail it in the enclosed envelope.

BACKGROUND QUESTIONS

1. Have you had your transplant surgery yet?..... ○ Yes ○ No
2. How many **minutes** did you wait after your scheduled appointment time before you were called to the test or treatment area? ..... ☐☐☐ minutes
3. How many **minutes** did you wait in the test or treatment area before your test or treatment began?..... ☐☐☐ minutes
4. On what day was your most recent visit?
   ○ Monday ○ Thursday ○ Saturday
   ○ Tuesday ○ Friday ○ Sunday
   ○ Wednesday
5. At what time of day was your most recent visit?
   ○ 6:00 am - 8:00 am ○ 2:01 pm - 4:00 pm
   ○ 8:01 am - 10:00 am ○ 4:01 pm - 6:00 pm
   ○ 10:01 am - Noon ○ 6:01 pm - 8:00 pm
   ○ 12:01 pm - 2:00 pm ○ 8:01 pm - 10:00 pm

INSTRUCTIONS: Please rate the outpatient transplant service you received from our facility. Select the response that best describes your experience. If a question does not apply to you, please skip to the next question. Space is provided for you to comment on good or bad things that may have happened to you.

Please use black or blue ink to fill in the circle completely. Example: ●

REGISTRATION

	very poor 1	poor 2	fair 3	good 4	very good 5
1. Helpfulness of the person at the registration desk	○	○	○	○	○
2. Ease of the registration process	○	○	○	○	○
3. Waiting time in registration	○	○	○	○	○
4. If you spoke with the Transplant Center by phone, helpfulness of the person you spoke with	○	○	○	○	○
5. Degree to which you were informed about delays	○	○	○	○	○

Comments (describe good or bad experience): _____

FACILITY

	very poor 1	poor 2	fair 3	good 4	very good 5
1. Comfort of the waiting area	○	○	○	○	○
2. Ease of finding your way around	○	○	○	○	○
3. Cleanliness of the facility	○	○	○	○	○

Comments (describe good or bad experience): _____

YOUR TEST OR TREATMENT

	very poor 1	poor 2	fair 3	good 4	very good 5
1. Friendliness/courtesy of the staff who provided your test or treatment	○	○	○	○	○
2. Explanations from the staff about what would happen during your test or treatment	○	○	○	○	○
3. How well the risks of your tests or treatments were explained to you	○	○	○	○	○
4. How well your options for alternative treatments were explained to you	○	○	○	○	○
5. How well your right to refuse tests or treatments was explained to you	○	○	○	○	○
6. Your perception of the skill of the staff who provided your test or treatment	○	○	○	○	○
7. Staff's concern for your comfort	○	○	○	○	○
8. Staff's concern for your questions and worries	○	○	○	○	○

Comments (describe good or bad experience): _____

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continued...

Figure 125.4. Outpatients services transplant survey. Reproduced from [32] with permission from Press Ganey Associates, Inc.

TRANSPLANT CARE & EDUCATION

	very poor 1	poor 2	fair 3	good 4	very good 5
ANSWER ONLY IF THE QUESTION APPLIES TO YOU.					
1. How well the education you received about the following prepared you to feel more comfortable caring for yourself at home:					
a. Medications	O	O	O	O	O
b. Diet	O	O	O	O	O
c. Incision care	O	O	O	O	O
d. Blood work	O	O	O	O	O
e. When to contact the transplant team	O	O	O	O	O
f. How to contact the transplant team	O	O	O	O	O
g. Costs associated with your Transplant care (e.g. housing, transportation, medication)	O	O	O	O	O
2. How well the transplant team worked together to meet your needs	O	O	O	O	O

Comments (describe good or bad experience): _____

PERSONAL ISSUES

	very poor 1	poor 2	fair 3	good 4	very good 5
1. Our concern for your privacy	O	O	O	O	O
2. Our sensitivity to your needs	O	O	O	O	O
3. Quality of staff's response to concerns/complaints made during your visit	O	O	O	O	O
4. Extent to which staff wore identification badges	O	O	O	O	O
5. Extent to which staff introduced themselves to you	O	O	O	O	O
6. Upon entering your room, extent to which staff sanitized their hands	O	O	O	O	O
7. Degree to which you were able to participate in decisions about your care	O	O	O	O	O
8. Degree to which your family/significant other were able to participate in decisions about your care	O	O	O	O	O

Comments (describe good or bad experience): _____

OVERALL ASSESSMENT

	very poor 1	poor 2	fair 3	good 4	very good 5
1. How well clinic staff worked together to provide care during this visit	O	O	O	O	O
2. Overall rating of the care you received during this visit	O	O	O	O	O
3. Likelihood of your recommending this facility to others	O	O	O	O	O

Comments (describe good or bad experience): _____

RATING

Using any number from 0 to 10, where 0 is the worst experience possible and 10 is the best experience possible, what number would you use to rate the transplant outpatient clinic?

Worst possible **Best possible**

0	1	2	3	4	5	6	7	8	9	10
O	O	O	O	O	O	O	O	O	O	O

Patient's Name: *(optional)* _____

Telephone Number: *(optional)* _____

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PRESS GANEY

This survey was current at the time of printing and distribution to you. If you would like to confirm that it is still the most recent version, please contact your Account Manager or CRE.

Figure 125.4. (*Continued*)

Agreement (SCA). This type of agreement is used when it is necessary to seek treatment from an out-of- network provider. The SCA provides for a specified reimbursement; therefore, balances above the agreed upon rate and the actual charges are waived in the same way as if the care was provided in network.

HMO plans are the most restrictive of the choices because the member is limited to seeing only in-network providers, consequently this type of plans have the lowest premium and member liability. If a member must use an out-of- network provider, the company must give authorization and negotiate a SCA.

Self-pay/private-pay individuals

The uninsured patients are the most challenging. TFCs should consider if the patient would be eligible for public assistance programs, or low cost insurance coverage plans if available. There are also numerous funded assistance programs available for qualifying transplant patients. Eligibility rules vary according to individual programs as well as amount of available assistance. Patients without insurance should be strongly encouraged to contact assistance programs. Transplant programs must have financial policies pertaining to the eligibility for transplant services specifically for the uninsured population of patients.

Foreign national patients

UNOS policy 6.0 Transplantation of Non-Resident Aliens permit foreign national patients to seek transplant services in the US. Current UNOS policy 6.0 from 2005 permits individual transplant programs to transplant 5% of their overall volumes to foreign national patients annually [34]. Individual transplant centers must develop policies pertaining to the management of foreign national patients. A dedicated international department within the health system facilitates the logistics of arranging services, including the cultural needs, for this unique population throughout the transplant continuum. These services may include prearrival assistance and ongoing assistance with obtaining VISAs and other required travel documents for patients and accompanying family members; lodging arrangements while undergoing evaluation, waiting time on the transplant list, and post-transplant care; 24 hours a day, seven days a week availability of interpreters to assist patients throughout the transplant process; coordination of outpatient clinic visits with the multidisciplinary transplant team; inpatient admissions/hospital stays; transitioning patients to home country when medically discharged; and assistance with facilitating longitudinal care monitoring in the patient's home country. By offering personalized services to this group of patients and reducing the barriers of navigating a healthcare system in a foreign country can help eliminate some of the obstacles encountered during the transplant process.

Some challenges unique to this population of transplant patients include patient and family expectations regarding the transplantation process and further patient and family expectations regarding limitations of care. This can be particular difficult when the expectation does not equal the services offered to the patient's satisfaction due to lack of understanding of organ allocation systems and extended waiting times. Reluctance of patients to return home when medically discharged and coordination of care upon discharge to home can be particularly challenging, especially if transplant services are limited in the patient's home country. Based on available funding and sponsorship of transplantation, the ability to return to the transplanting center for follow-up care may also be limited. Negotiations must be clear from the point of acceptance to the transplant program of the extent of covered services and agreed upon costs to the patient and sponsor, excluding complications. All aspects of patient transplant education and the Informed Consent process must be provided in the patient's native language prior to listing.

Hospitality houses

Transplantation can draw patients from around the world to transplant centers. The financial costs of accommodations associated with transplantation can be devastating to patients and their families. As a result of this void, hospitality houses emerged nationwide. "The National Association of Hospital Hospitality Houses, Inc. (NAHHH) is a nationwide professional association of nearly 200 unique, non-profit organizations that provide lodging and support services to patients, families and their loved ones who are receiving medical treatment far from their home communities." [35]. These houses can be small, accommodating just a few people, to facilities that can hold almost 200 residents. At any given house in the US approximately 20–25% of current residents are transplant related patients. Current US facilities can vary as to whether the facility is for children or for adults; and then again whether or not the patient can stay at the location or if it is just for family while the patient is hospitalized. Since the first house opened in Philadelphia in 1981 hospitality houses have grown as a direct result of compassion, determination, and generosity into so much more than just a place for patients and families to sleep in.

These houses are strictly residential facilities and part of the hospitality industry and as such they follow hotel regulations/standards, provide no direct medical care, and are not held to hospital regulations. They have become an integral part of healthcare delivery for patients away from home. Just as if the patient returned to their home following discharge from the hospital post-transplant or waiting for transplant, they would need to call 911 for any medical emergencies. Home health staff visit these facilities to provide needed support in home treatment, durable medical equipment, and assistive devices are permitted. Infection control standards, generally from the hospital, are implemented in the homes and become a learning tool for the patient and their family before returning to their home. They receive hands on training on how to safely clean and live with others as they prepare for the transition to their homes.

These houses provide a community setting for the constantly evolving return to "normality" for the patients and their families. At any given time, the house's current residents become a support group for each other as they learn to navigate the medical system together on the same journey to wellness. Facilities like these are able to provide a holistic approach to the day- in and day-out activities while decreasing financial and emotional strain on the families. The reduced cost of housing and, if needed, financial support from 501(c)(3) fundraisers may be available to supplement what a family can afford. These homes are a fee-for-service which covers approximately 65% of the expenses with the remaining revenue coming from donations and fundraising.

Typically activity within the house picks up in the evenings following visiting hours. Families gather in common areas to share food and more so to talk about their day and learn what to anticipate from others who have been going through a similar experience for a longer period of time. Some facilities utilize volunteers to help provide experiences throughout the month (activities or meals) while attempting to maintain as normal a lifestyle as

possible. Hospitality houses are continuously evolving to meet the guests' needs, as well as developing life long bonds of spirit and strength.

Summary

As the field of transplantation continues to evolve, so does the clinical management of the transplant candidate, recipient, and living donor. More care by an extended multidisciplinary team is being provided in outpatient transplant clinics. Every transplant center functions differently but must be held accountable to the same federal rules and regulations, which provide the basis for policy development. Transplant centers must have a vision and mission statement that guides their practice while demonstrating regulatory compliance, transparency, and producing measurable quality patient outcomes. The topics included in this chapter are not meant to be all-inclusive but to serve as a reference guide to be incorporated into the development of an outpatient clinical practice.

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CHAPTER 126

Transplant Center Management and Leadership

A Case for Structured Intrapreneurship

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Introduction

An entrepreneur is an individual who translates innovation into economic goods using financial and business acumen. Such an initiative may require forming a new organization, or alternatively, the revitalization of an existing organization in response to an opportunity. Although the term has traditionally referred to business initiatives, it has taken on broader applications; social, political, and more recently, knowledge entrepreneurships have emerged. The study of entrepreneurship reaches back to Richard Cantillon and Adam Smith in the late 17th and 18th centuries, but a resurgence of interest in the dynamics of business and economics in the past few decades has refocused our attention on the significance of entrepreneurs. Entrepreneurship is a process by which a new idea or invention is converted into a successful, and typically profitable, innovation. Innovations, in this context, are more likely to represent incremental improvements, rather than quantum discoveries. Entrepreneurs may bear risk, putting their financial security or careers on the line in the name of an idea, demonstrating initiative in the face of uncertainty.

The role for innovation and entrepreneurship in transplantation stems from the fact that no other viable alternatives to end-stage organ failure, particularly kidney failure, existed. The first successful kidney transplant was performed at a time when dialysis was so primitive as to be considered ineffective. Perhaps because surgical solutions to the technical challenges of transplantation preceded effective immunosuppression by several decades, surgeons are considered to be the original innovators in transplantation, while immunologists and scientists were concomitantly defining the very essence of transplantation immunology. It took the entrepreneurial spirit and perseverance of surgical pioneers to incrementally convince primarily academic institutions (medical schools and partnering hospitals) to become laboratories for these unprecedented experiments. Surgeons took personal risk by putting their careers on the line in the name of an idea, and institutions aligned themselves with the surgeons by putting both reputational and financial security at risk. As it became clearer that transplantation was a viable therapeutic option for end-stage organ failure, more institutions joined the ranks of those with existing transplant programs, demonstrating a commitment to the transplant initiative in the face of decreasing uncertainty. As transplantation evolved, the entrepreneurial spirit that marked its birth and growth remained an essential aspect of the transplant culture, allowing transplant centers to continue to be focal points of innovation within medical centers, converting new ideas into therapeutic approaches. The past three decades in particular have witnessed a transition of transplant procedures from experimental and investigative exercises to proven therapeutic interventions.

As transplant programs have evolved, so has the complexity of the administrative structures necessary to support them. At their onset, transplant teams typically consisted of one or two surgeons, collaborative physicians, and one or two nurse coordinators assigned to a low- to mid-level hospital administrator, with varying levels of oversight from the hospital's leadership. During this entrepreneurial phase, vibrant enthusiasm was palpable, primarily from those intimately involved in direct patient care, and passion was fueled by a clear and compelling vision, usually communicated by the surgical leader. Attitudes from others in the medical center were generally supportive, but might have ranged from indifference to benevolent curiosity. In this early phase, the team usually enjoyed strong support from the Chairman of the Department of Surgery (who, in all likelihood, recruited the lead surgeon), and varying levels of enthusiasm and support from the Chairman of the Department of Medicine. As transplant centers have evolved further as the transplant experiment has become successful, administrative structures have also evolved to accommodate the needs of the programs. This has occurred in parallel with a need for further investment of resources and personnel, commanding a higher level of attention from hospital administrators. In a typical scenario, the surgical team continues to drive the agenda, recruiting more collaborative physicians, forfeiting some of the pre and post-transplant care to their respective specialties, while the non-physician components of the team also expand to meet the needs of the growing number of patients evaluated, listed and transplanted. Again, the surgical team leads these efforts, but incrementally forfeits administrative and leadership functions to hospital administrators, relinquishing some decision points regarding both operational and budgetary issues. Although the growth in the physician component of the program may require some structural and organizational

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changes in both departmental (medical school) and practice plan hierarchies, such as creation of a transplant surgery division within a Department of Surgery, growth in the non-physician components is likely to be associated with more significant changes in hospital administrative and management structures, with inherent and requisite sets of rules and operational procedures.

Together, these changes in organizational structure will inevitably introduce an element of corporate culture and discipline, particularly as the transplant program gets increasing attention from institutional leaders. Though genuinely interested in the success of the program, they also have their own respective agendas, as they are often responsible for the broader issues of budgets and compliance. As the program grows, its main oversight will shift incrementally from the medical school and physician practice to that of the hospital, which assumes the mandated responsibility for the program's regulatory compliance. As a direct consequence, the program, coincident with its growth, needs to fit into a more corporate organizational structure, abounding in administrative silos, each with its own set of processes and rules. An unintended consequence of this evolutionary development is a transition from the entrepreneurial spirit responsible for the success of the program, to a more corporate discipline, replete with confining hurdles and constrictive boundaries that inevitably curb the freedom to innovate. Therefore, institutional demands for adherence to well-defined organizational charts and reporting lines, manuals of policies and procedures, compliance with both institutional and regulatory requirements, and discipline directed at fiscal responsibility may ultimately stifle the energy and passion required for continued innovation. As the center of gravity of the program transitions from the entrepreneurial spirit to the corporate culture, the physicians are unavoidably held to the same corporate norms and standards as their non-physician colleagues. The institutional uniform becomes the business suit instead of scrubs and a white coat. In fully integrated healthcare delivery systems, a common set of rules governs all components of the program, which nearly always lean more towards the corporate. In less integrated systems, both medical schools and practice plans must also abide by, and be responsive to, an increasingly regulatory environment, such that the academic leadership is likely to welcome administrative filters imposed by the hospital leadership. Thus, it is inevitable that as programs grow and mature, they are, in a sense "victimized" by their own success, sacrificing their nimbleness and limiting their ability to be responsive to some of the more novel approaches to the challenges of transplantation.

The administrative structures dictated by the corporate culture are essential for achieving high reliability, improving efficiency, meeting financial goals, and maintaining full regulatory compliance, but at the same time their inherently process-oriented and risk-averse culture can cast a shadow on entrepreneurship and innovation. Thus the challenge for transplant leaders consists of maintaining the "esprit-de-corps" and innovative energy that first galvanized the team, while realizing the benefits associated with a corporate culture and structure. The successful transplant enterprise will adapt to the requirements of the corporate culture, while remaining at the margins of discovery, leveraging the strengths and benefits of both, to assure a highly reliable, efficient, and compliant center that remains an engine of innovation. In this chapter, we will discuss further the evolution of transplant centers, outline the critical elements of leading and administering established transplant service lines, and explore mechanisms proven in other areas that transplant leaders can use to establish and defend this essential middle ground.

Evolution of culture in transplant centers

The early days of transplantation were a clear example of the "tough-guy macho culture", marked by risk taking and rapid feedback and reward [1]. In the Deal and Kennedy model, organizational culture was defined by two axes: tolerance for risk, and immediacy of feedback. Starzl expounds on the two ends of the spectrum as he highlights the polemic clash of "surgeons-against-the-bureaucrats" in *The Puzzle People* [2]. As transplantation has evolved from the investigational realm to become a widely accepted therapy, transplant centers find themselves transitioning toward less risk-taking and less immediate feedback and reward. This "process culture", as defined by the Deal and Kennedy framework is marked by bureaucracy, safety and corporate culture. The affinity of large healthcare organizations toward self-protection is sensible, given both financial and reputational liabilities. The lure of innovation is foreshadowed by the realities of corporate responsibility and accountability. As a result, institutional bureaucracy, and policies and procedures designed to contain risk and increase predictability will trump any entrepreneurial desire to assume risk, especially when high profile fiascoes are even remotely possible.

This tendency for hospitals in particular to avoid risk has been magnified by the recent focus on transplant center regulations and public policy. The National Organ Transplantation Act (NOTA) first regulated the allocation of deceased-donor organs as a public good at the federal level [3] and established both Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR) contracts, giving Health Resources and Services Administration (HRSA) regulatory oversight of transplantation. Included in the provisions of NOTA was a requirement for public disclosure of center-specific patient outcomes [4]. In addition, the End-stage Renal Disease (ESRD) entitlement gave the Centers for Medicare and Medicaid Services (CMS) additional authority in regulating transplant centers by withholding reimbursement [5]. In the early part of the 21st century, a number of high-profile sentinel events attracted much government bureaucrat and politician attention. These events included the death of a living liver donor at a center in New York [6], a blood-type incompatible heart transplant in North Carolina that led to the death of a pediatric patient [7], and a series of articles by Charles Ornstein of the Los Angeles *Times* detailing problems at several California transplant centers. Ornstein highlighted the failure of United Network for Organ Sharing (UNOS) and CMS to detect and address those problems [8]. These events led to an Office of the Inspector General (OIG) investigation and Congressional hearings, which found CMS lacking in their oversight responsibilities. The result was the creation and publication of Medicare Conditions of Participation specific to transplant [9]. These regulations created prescriptive requirements mandating transplant hospitals to create transplant-specific policies and procedures and meet certain volume and outcome requirements. CMS implemented triennial compliance audits of transplant centers to enforce these requirements. In response, transplant centers have raised the issue of regulatory compliance to the highest levels of leadership within hospital structures. In similar fashion, HRSA has escalated its oversight by means of the OPTN contractor. The scope and authority of the OPTN was significantly enhanced with revision of NOTA in 2000 and the codification of that revision in the Final Rule in 2005. The OPTN contractor has policy or is proposing policy to create oversight over a wide scope of transplant operations; including, but not limited to, patient education, staff qualifications, the evaluation and selection of living donors and the prevention of disease transmission. The

OPTN exercises this regulatory oversight by both monitoring performance and outcomes and on-site surveys of policy adherence. A perennial cycle of external surveys and compliance audits complicates daily transplant clinical operations.

The threat to innovation by regulations has grown exponentially in the past decade. Both HRSA and CMS share the laudable goal of improving outcomes for transplant patients and living donors [4,10], but the rigid enforcement of the policies and regulations combined with the potential of overreaching interpretations by the bureaucrats and surveyors tasked with enforcing them may lead to the kind of mindless compliance that actually decreases transplant programs' reliability and resilience, stifles innovation and creativity, and drains resources away from clinical activities. Several centers volunteered, or were forced to, withdraw from Medicare participation and reimbursement due to either low volume or poor outcomes [8,11] in the first round of CMS surveys, and other transplant centers report changing practice to avoid similar fates [12]. More oppressive than any hospital corporate structure, over-regulation threatens the overall vitality of transplantation. The challenge to transplant leadership at both the program and national level will be to advocate with government leaders and bureaucrats to keep regulators in their proper role: promoting good self-governance as objective external reviewers [10] and providing peer review guidance [4]; not dictating practice or creating unsustainable administrative burdens. Hospital administrators have become increasingly aware of the need for compliance with these regulations, particularly outcomes requirements. Therefore, the evolution of transplant centers to corporate structures and the rapid deployment of federal regulations with potentially punitive actions have created the perfect storm with respect to risk averseness. As a result, transplant programs have witnessed significant convergence between the stated goals of bureaucratic institutional structures and those of regulatory oversight.

There is great merit to the implementation of evidence-based best practices, and the standardization of processes and improvement of outcome measures espoused by both corporate structures and regulations, as it is well established that standardized processes are a key element in increasing reliability and decreasing the risk of error. The implementation of the principles of high reliability [13] has become topically important in the initiative to improve quality in health care impelled by the Institutes of Medicine's landmark reports in 1999 and 2001 [14]. These principles include, not only structured processes and roles, but a "preoccupation with failure", defined as a constant vigilance for sources of error and accident, and the operational sensitivity to successfully manage the unexpected. The highly reliable organizational (HRO) culture dictates that established therapies be delivered safely and consistently, with efforts to reduce the risk and consequences of individual human failures. These principles, developed to prevent clinical errors, can also be successful in mitigating financial and legal risk. HRO principles have been shown to help manage the evolution of proven procedures into highly reliable service lines, and they require administrative oversight by professionals familiar with the business principles that make large organizations reliable, efficient, and adaptable, as well as with the many complexities and nuances of transplant practice, business operations, and compliance. As a result, this culture is becoming more common in, and being embraced by, transplant centers. Therefore, the widespread risk-taking, typically exhibited by those faced with few, if any, viable treatment alternatives for their desperate patients previously accepted in the earlier days of transplantation, are no longer tolerated. The culture of "never events" dominates in deliberations about healthcare delivery, and may lead to a clash of cultures between the more innovative entrepreneurial spirit of the transplant leader, and the more risk-averse corporate administrative leadership. Although everyone desires the avoidance of risk, most transplant clinicians would agree that the only way to never have significant complications is to never perform transplants. Whether the specific issue is live donor death, donor transmission of communicable conditions, or a novel approach to immune tolerance, implementation of best practices and processes cannot possibly guarantee a complete avoidance of potentially disastrous complications. And if there is little room for mitigating circumstances to be taken into consideration in addressing complications, the resulting risk-aversion may not allow the potential complication related to such a complex set of procedures. Therefore, throughout the application of HRO principles, it is essential that the center not allow itself to blindly adopt a risk-averse culture. Rather, center leadership needs to be vigilant in setting expectations and mitigating potential risks, and then embrace the opportunity to learn from both failure and success. Preoccupation with failure should not be misapplied to stifle entrepreneurship. It should instead empower innovation by adding rigor to the exploration of new approaches to care. All involved should fully understand and accept the risks of innovation, should work to mitigate them to the greatest extent possible, and should support these efforts, despite some inevitable setbacks. To this end, stringent informed consent processes must be deployed.

Intrapreneurship — balancing the entrepreneurial spirit with the corporate culture

The challenge for transplant leaders is to successfully leverage the strengths of corporate cultures, while preserving the entrepreneurial spirit. Service lines of care must be made as safe, reliable, and efficient as possible. These lines benefit from standardization, reproducibility, and transparency. At the same time, development of new ideas and creation of new programs will require the culture of entrepreneurship that has made transplantation an engine of innovation. In *Great By Choice*, Collins and Hansen propose that the most successful companies (10Xers) combine creativity and discipline: "The great task, rarely achieved, is to blend creative intensity with relentless discipline so as to amplify the creativity rather than destroy it. When you marry operating excellence with innovation, you multiply the value of your creativity" [15]. Many businesses have tackled the problem of maintaining an entrepreneurial spirit within a corporate culture, dubbed "intrapreneurship", and these models may be instructive for transplant centers. The terms intrapreneur and intrapreneurship, first described in the late 1970's by Gifford and Pinchot [16], were popularized in 1982 by Macrae [17]. The terms were first used in the popular media in 1985 by *TIME* magazine in an article entitled "Here come the Intrapreneurs" [18], and in the same year, in a quote by Steve Jobs, then Apple's Chairman, in *Newsweek*. In that article, Jobs was quoted: "The Macintosh team was what is commonly known as intrapreneurship, only a few years before the term was coined — a group of people going, in essence, back to the garage, but in a large company" [19]. In 1992, the American Heritage Dictionary acknowledged the word intrapreneur [20]. Intrapreneurs are encouraged to behave as entrepreneurs within the context of the resources, capabilities and security of a larger organization, and this allows for the dynamic and energetic nature of entrepreneurship,

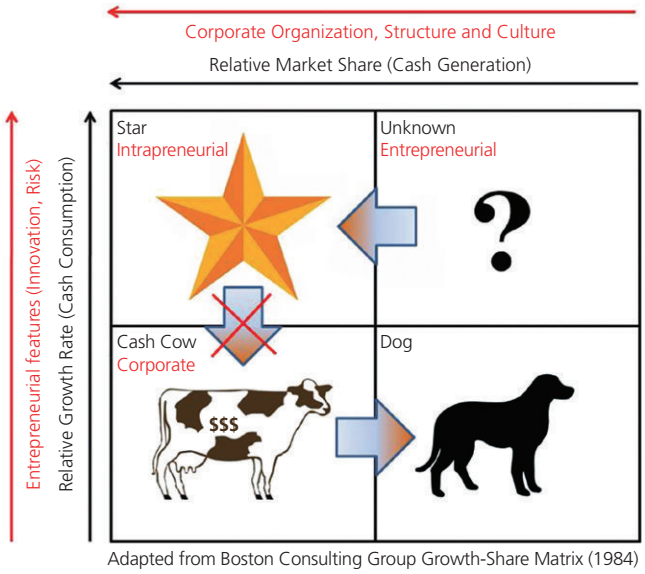

Figure 126.1. Adaptation of Boston Consulting Group (BCG) growth-share matrix to transplant cultures. To build transplant programs that last, it is essential to develop highly reliable corporate organizations to preserve market share during product growth, and to use the strengths of entrepreneurial culture to assure differentiating innovation and continued growth — as well as introduction of new product lines with the potential to become stars themselves — to avoid becoming cash cows destined for commoditization. Simultaneously maximizing both strong corporate organization and aggressive innovation is the hallmark of intrapreneurship.

while providing protection against the risks and accountability normally associated with entrepreneurial failure [21]. Thus, intrapreneurship combines the ability of individuals to transform an idea into a profitable finished product through assertive risk-taking and innovation, with the protection and backing of a large corporation allowing for the application of corporate management practice within "mini-firms".

Contemporaneous with the development of the intrapreneurship concept, the Boston Consulting Group (BCG) developed the Growth-Share Matrix (Figure 126.1) to evaluate product lines within organizations being targeted for investment or acquisition. The authors used the matrix to suggest that as market share increased, so did cash generation, and that as growth rate increased, so did cash consumption. This matrix illustrated a natural business cycle, in which a new idea (represented by a question mark) consumed significant resources without generating much revenue. These "question marks" represent risky targets, but if they progressed successfully to high market share by establishing competitive success, they become "stars", continuing to require investment, but also generating the revenues and margins to offset the investment. As the product is recognized to be profitable, investment in further innovation of the product decreases and the product becomes a "cash cow", continuing to generate revenue while requiring little cost. Although this may intuitively appear to be the best product position from a financial standpoint, as the innovation that differentiated these products from competitors and commanded a premium from consumers and producing healthy margins fades, such product lines inevitably become commodities. Competitors duplicate and successfully compete, and the product lines quickly become dogs, competing for small market share in a saturated market now based more on who can produce the product most cheaply. We propose that the corporate culture is a positive force along the same axis as market share, allowing increased volume reliably and efficiently. On the other hand, entrepreneurial culture is a positive force along the growth axis, allowing the product line to continue to evolve and expand. Therefore in this analogy, much like every attempt should be made to maintain a product line in the "star" category (and avoiding its natural tendency towards a cash cow) by continuing to differentiate the product through investment in innovation, every effort should similarly be made to keep the entrepreneurial and innovative spirits alive and well, while leveraging the benefits of the corporate organization. This push to this particular quadrant of the 2 × 2 grid can occur in one of two ways as illustrated in Figure 126.1. Obviously, question marks can become stars (that is, after all, the natural cycle), or alternatively the transition to cash cows can be avoided by reinvesting in innovation and by allowing the entrepreneurial spirit to remain in the context of the corporate structure. Both represent examples of intrapreneurship.

Intrapreneurship can be implemented in two models. In the first, it is deployed as part of a broad culture change. An example of this is 3M, which allowed its engineers to uniformly spend up to 15% of their time on their own projects [22]. This initiative led to the development of innovations such as Post-It notes. Similar environments produced the Sony PlayStation and the JAVA programming language. The second model consists of establishing a small entrepreneurial group within the larger corporate culture. This group is empowered to adopt a culture of innovation, is freed from many constraints of the larger corporate culture, but is still able to leverage its resources and capabilities. The classic example of this model is the Skunk Works™ at Lockheed Martin, which was formed to rapidly design new aircraft. Led by a young engineer named Kelly Johnson, the group was tasked with developing an entirely new aircraft design in the midst of World War II. They did so in 143 days. Lockheed Martin attributed this to allowing Johnson the autonomy to truly create a different culture. "What allowed Kelly to operate the Skunk Works so effectively and efficiently was his unconventional organizational approach. He broke the rules, challenging the current bureaucratic system that stifled innovation and hindered progress" [23]. It is this model of intrapreneurship, in essence allowing the team the ability to function as an entrepreneurial group within a corporate environment, that we propose as the most relevant to transplantation.

Govindarajan and Trimble studied this specific intrapreneurial model by chronicling initiatives in diverse organizations (e.g. New York Times Company, Corning, Hasbro, Cisco, Kodak, Johnson & Johnson, Thomson Corporation) to assemble best practices for what they called "managing strategic experiments", defined as high-risk, potentially high-return innovation requiring unique managerial approaches. They identified three key strategies:

1. "forgetting" — breaking from the core business, even in areas that are critical to the core business' success;
2. "borrowing" — leveraging the experiences and assets of the core business, giving intrapreneurial initiatives a marked upper hand over independent entrepreneurial competitors; and
3. "learning" — required to fill in those things that cannot be borrowed [24].

We believe that these three strategies are relevant to adopting an intrapreneurial culture in transplant centers. These same authors also described errors made in implementing this model across industries that we believe should serve as important cautionary

notes for transplant leaders wishing to adopt this model. They observed that core businesses often projected their way of doing things, from organizational structure to process management, onto the new ventures. This led to a lack of adaptability, as the new initiatives needed greater freedom to change and required reacting more nimbly to new information. The same business standards that made the large organizations successful and efficient stifled the new ventures. At the same time, many new ventures failed to adequately leverage the advantages of being part of large, established organizations. They cited as an example the New York Times Digital group that had worked so hard to make itself independent of the original organization, that it failed to leverage the content and advertiser base that should have been available to it, and even fought with the print side of the business. And as a failure of learning, the authors examined Hasbro Interactive, whose leaders became so focused on growth and vision that they lost the introspection to identify and learn from their own mistakes. The leaders tracked the wrong metric and set unrealistic targets, which ultimately led to their demise [24].

Leaders of transplant centers will be most successful if they heed the experiences of these initiatives. Successful centers will balance forgetting, borrowing, and learning to leverage the best of both worlds. On one hand, they will leverage the assets, experience, data, planning, human resources, process improvement, etc., available to them as one small piece of a large, successful healthcare organization. On the other, they will maintain the spirit of innovation and entrepreneurship, the capacity for rapid change, and the willingness to take risks that made their programs successful during their development and growth phase.

Transplant center management: structuring intrapreneurship

Most of us have heard someone say: "You've seen one transplant center, and you've seen one transplant center". This statement intimates that the organizational structures that govern transplant programs vary significantly among institutions. And there are two main reasons for this. First, the academic medical centers that typically house transplant programs, themselves, vary greatly in structure. In fact, there are even a small number of transplant programs that do not reside in academic institutions. Within academic institutions, there are a number of models that encompass the hospital, the medical school and the physicians [25]. Second, even within two almost identical academic medical centers, transplant programs may have developed differently over time, and therefore their structure may vary widely. For instance, some may be heavily anchored within a departmental structure, typically a department of surgery, whereas others may have evolved into centers or institutes [26]. And even between centers or institutes, there may be significant variation as to the functions that are governed by these structures. The purpose of this section of the manuscript is not to list every combination and permutation possible, nor is it to recommend one structure over another, but instead we propose to espouse the tenets of Intrapreneurship, as they apply to transplantation, while exploring potential structural frameworks.

Medical directors and administrators must work together to lead and manage the complex and diverse components of a transplant program, while they individually resist both internal and external pressures to operate at either end of the Deal and Kennedy spectrum. HRO expert Karl Wieck (1987) described this as "requisite variety" and recommended embracing complexity, and resisting the corporate tendency to oversimplify and centralize [27]. Different functions within a transplant center require specialized skills and knowledge (Table 126.1). Early on in the evolution of transplant programs, the surgical team is primarily focused on the clinical delivery of transplant services, and management skills and process are not as essential as is clinical excellence. As the programs grow, and as the regulatory and financial considerations become more significant, administrative and management issues take on increased importance and relevance, and transplant leaders must defend against the corporate tendency towards centralization of functions and must become champions for transplant-specific interests and resources, especially preserving the freedom to innovate.

The transition to a more formally structured HRO described previously is essential as the complexity of operations increases, particularly in the setting of a highly regulated healthcare specialty. However, the program's physicians often struggle with the resulting attachment of formal processes and layers of approval attached to decision-making, growth, and change. Although hospitals tend to have well-defined organizational charts, with certain areas

Table 126.1. Administrative functions in a transplant center

Function	Specialized knowledge required?	Comments
Financial		
Accounts payable	Yes	Cost report compliance
Revenue: Billing	Yes	Managed care transplant phases/Medicare secondary payments
Revenue: Collections	No	Low volume/high dollar
Revenue: Pricing	Yes	Managed care contracts, Standard organ acquisition charge (SAC)
Cost report preparation	Yes	
Budget preparation and management	Yes	Acquisition cost centers
Contracting	Yes	Managed care transplant phases
Analysis	Yes	Cost report reimbursement, SAC
Patient counseling	Yes	Managed care, ESRD Medicare, required by OPTN/UNOS
Marketing/outreach	Yes	Specialized product knowledge
Human Resources		
Benefit and payroll administration	No	
Compensation structure	Yes	On call compensation, compensation for outreach/ marketing activities
Recruitment (non-physician)	No	Need targeted advertising
Staff (physician and non-physician) management	Yes	Clinical knowledge valuable; CMS, OPTN knowledge essential
Non-physician staff: Nursing, social work, mid-level providers, dietary, non-licensed professionals	Yes	Specialized knowledge and training for most professionals required by CMS, OPTN
Quality Assurance / Performance Improvement/Regulatory Compliance	Yes	Knowledge of CMS, OPTN regulations and reporting requirements essential
Data Management/IT	Yes	Use of transplant-specific database/EMR
Overall Program Administration	Yes	Management of the requisite complexity of the entire program regardless of where each function is housed

reporting to specific Vice Presidents who are focused respectively on operations, finances, human resources, regulatory compliance, etc., physicians are typically embedded in academic departments and in practice plans that may not be structured to deal with the complexities of transplantation, particularly its interdisciplinary nature. Therefore, as transplant programs grow, it is not uncommon for their administrative center of gravity to shift towards the better organized structure and to adopt its inherent organizational behavior and culture. Hospitals provide excellent homes for this organization, and are well-equipped to support large, complex service lines including transplantation. In contrast, the physician infrastructure tends to be less rigid and more nimble. Unfortunately, academic departments and physician groups are typically not in a position to offer much in terms of resources. Transplant physician practices often require support from their hospitals since a significant amount of work done on behalf of the program is not billable through the standard relative value units (RVU) mechanisms, and is therefore not recognized when medical schools and practice plans calculate physician productivity. It is difficult enough for the physician employers to justify the salary support of their physicians and surgeons. Departments of medicine in particular, are often in need of support for their transplant physicians. The result is that the hospital initiates a transfer of services agreement that is compliant with Stark and other anti-kickback laws on a contractual basis. The inevitable consequence is that some control of the physicians shifts to the hospital. The hospital, fulfilling its responsibilities to both regulators and its governance, will need to exercise some form of oversight on physician activities that relate to the program. In highly integrated healthcare delivery systems, especially those where physicians are employed by the hospital, these circumstances work against the entrepreneurial origins of transplantation. For instance, it may be difficult for the hospital to quantify and incentivize academic efforts such as innovative research. Research is often underfunded by extramural granting agencies, especially for surgeons and other specialists, whose market-based salaries exceed the federal cap for inclusion on National Institutes of Health (NIH) and other federal granting agencies, threatening the financial viability of an academic transplant physician practice. Defined compensation plans that reward clinical activities over and above research activities create an incentive for transplant surgeons to fill any time not occupied by their clinical transplant responsibilities with well-remunerated general surgery operative cases, and a disincentive to participate in research and teaching activities. Regardless of the structure that underpins the delivery of care, as transplant programs grow in volumes and revenues, they risk taking on a corporate culture that tends to favor clinical productivity and cost control over innovation and research. This structure adheres closely to the norms of a clinical enterprise, allocating resources much as it allocates overhead, using the simplest formula that works across the entire clinical enterprise. And because of the complexities and subtleties of transplantation, one of the first unintended consequences is loss of the innovative spirit that bred the success of the program in the first place. In addition, transplant surgeons are often assigned administrative roles within the transplant centers, which cause them to inevitably adopt the corporate culture. Given that these surgeons/administrators are often part of the senior leadership of the institution, they often need to buy into the culture despite the fact that they fundamentally disagree with the restrictions imposed on truly innovative initiatives.

Physicians and surgeons, and their trainees, typically reside in departments within a medical school. A historical perspective of the evolution of Centers and Institutes, as well as the strong rationale for their development, is provided in a comprehensive paper by Braunwald [28]. In this paper, the authors also espouse the value of matrix organizations. But in their conclusions, they remind us that the administrative details of an organization, particularly in academic healthcare is only a means to an end, specifically that of carrying out the tripartite mission of education, research and patient care. Nonetheless, the author stressed the importance of galvanizing different specialties around common themes, and in the context of transplant centers, this theme or mission is to meet the needs of transplant patients. In a separate study, Mallon warns the reader about the risks of centers and institutes and suggests that although it is important to capitalize on the strengths of centers and institute, it is also essential to reward leaders who embrace a collaborative point of view while developing a culture that frowns on empire building. His main argument is that departments must maintain their place in academic institutions [29]. These arguments notwithstanding, it is clear that multi-specialty initiatives, which transplantation embodies, require organizational and administrative structures to transcend the traditional silos of the departmental structure of an academic environment. Braunwald accessed the evolution of cardiac centers around cardiac surgery. There are numerous parallels between this example and transplantation. Certainly, transplantation is significantly more diverse in its participant providers than cardiovascular disease. It is important that any structure that is created is used to facilitate the advancement of transplantation and that it does not become a bureaucratic hurdle driven by individual desires for independence and empire building. Enunciating the objectives (Table 126.2) of a center can serve as a set of guiding principles that will prevent the degeneration of the original intent. A list of such guiding principles for transplant centers should include the following:

1 foster interdependence among interdisciplinary members to fulfill the tripartite mission;
2 enhance, facilitate and remove disincentives to interdisciplinary collaboration;
3 overcome potentially restrictive organizational structures allowing for effective access to the necessary resources to support progress;
4 align interdepartmental objectives around transplantation;
5 provide a fiscal structure that supports the academic mission of the transplant program; and
6 foster pride of enterprise and a sense of shared responsibility and destiny among the various specialties engaged in transplantation.

In addition, transplant centers should *not*:

1 duplicate existing competencies otherwise available at the institution;
2 compete unfairly for available resources with other centers; and
3 strive to make departments obsolete.

If these principles are followed, centers and institutes should be viewed as positive developments by departments, rather than threatening competitors for power and resources. To this end, it should be clear from the onset that the stakeholders of the center or institute include department chairs, such as the departments of surgery and medicine. Finally, there should be an understanding that if the original objectives can be met without the need of the structure, it will be "sunset" in short order, or alternatively, that whatever functions the center or institute provides can be repatriated to departments if it makes more sense. One of the clear benefits of a center or institute that operates under these principles is the

Table 126.2. Two distinct but complementary sets of Guiding Principles for achieving Intrapreneurship in a highly reliable transplant center

Guiding Principles for a Transplant Center's Academic Mission	Guiding Principles for a Transplant Center's Clinical Mission
• Foster interdependence among interdisciplinary members (to fulfill the tripartite mission). • Enhance, facilitate, and remove any disincentives to interdisciplinary collaboration. • Overcome potentially restrictive organizational structures to allow effective access to the necessary resources to support progress. • Align interdepartmental objectives around transplantation. • Provide a fiscal structure that supports the academic mission of the transplant program. • Foster pride of enterprise and a sense of shared responsibility and destiny among the various disciplines engaged in transplantation.	• Deliver high quality care that achieves excellent clinical outcomes • Operate as efficiently (cost-effectively) as possible. • Maintain full regulatory compliance. • Assure team-based multidisciplinary care with a focus on communication. • Center care on the patient. • Strive for the largest possible local market share.

ability to focus recruitment of desirable faculty around areas of common interest.

Although it is relatively easy to build a center or institute around the research and education components of the tripartite mission, it becomes more difficult to reconcile its clinical components. This problem mainly revolves around physician compensation. Different departments may use varying scales to measure physician productivity which can be difficult to assess in the setting of transplantation for reasons described in a previous section. Therefore, as the hospital's role in supporting the infrastructure increases with the evolution and growth of a transplant program, these issues become less about money and more about authority and control, can cause significant friction, and have been known to result in implosion of very active centers as individuals become unhappy and leave for what seem to them as greener pastures. It is incumbent upon the leadership of the institution to also establish a set of guiding principles (Table 126.2) that can be used to regulate funds flow, as well as allocation of resources. In this capacity, the transplant center's clinical mission should be viewed to provide:

1 high quality (clinical outcomes);
2 high value (cost efficient);
3 regulation compliance;
4 team-based, coordinated; and
5 patient-centered care.
6 In addition, delivery of care should strive to capture a high percentage of market share in the local market.

Depending on the level of integration of the delivery system, physicians may be either employed by the hospital, or act fundamentally as contractors for transplant services. As such, the actual contractual agreements may differ, but in the end, the objectives remain the same. The reader should note the absence of "innovation" in the previous list, although one could argue that it is indirectly implied in some of the other terms, including high quality and market share, given that a program might not be competitive without growth through innovation. However, transplant programs could be first movers or late adopters of innovative product lines. The reality is that most large academic hospitals are not focused on being first movers, but only in being late adopters if there are margins to be had. Instead, these institutions are much more interested in the more corporate elements of care, clearly enunciated in the previous list. This is where the more academic partner, the academic physician, has to provide the balance. We submit that neither of these two sets of guiding principles can be used in isolation. Instead, we propose that a transplant program that is built-to-last will incorporate elements of both.

Although it is clear that many established programs within the transplant center can function under the principles of HROs, achieving efficiency, quality, and consistency in proven therapies and processes and the resilience to overcome failures, require that the transplant center maintain an intrapreneurial identity to be able to conduct research and build new programs. Previous authors have made the compelling case for centers and institutes that cut across corporate boundaries and empower the clinician-scientists who built transplantation [26]. Expanding on that idea, we suggest that institutions should foster transplant centers and institutes which can conduct cutting-edge research and build new programs modeled on the intrapreneurial groups discussed earlier. In establishing the Skunk Works at Lockheed Martin, Kelly Johnson laid out 14 "rules and practices" he considered essential for the group's success [30]. "Kelly's 14 Rules" have become a guide for successful intrapreneurial groups. Although these rules are specific to a defense contractor, and more easily generalized to engineering, we have developed a new set of rules based on the original list. Following these rules will ensure that the research and new program development components of a transplant center (or institute) can thrive (Table 126.3). These rules are intended to assure the highest possible level of flexibility and accountability such that an intrapreneurial transplant center can innovate and lead within a corporate environment that assures high reliability for programs once they are up and running. The rules are also consistent with the lessons of new ventures described earlier [24] and the lessons of previous attempts to develop integrated health centers [31].

Finally, one essential element of a successful transplant center is strong leadership. This is critical for the entire leadership team: physician leader, administrator(s), medical directors, and managers and team leads. Truly exceptional transplant centers consistently have a single visionary physician leader, typically a surgeon. In *Good to Great: Why Some Companies Make the Leap. . . and Others Don't*, Collins identified that every company that made the leap from being merely a good company to being a great one was led by what he called a "Level 5 Executive". He noted that many of the storied corporate leaders, such as Lee Iacocca, were "Level 4 Effective Leaders", whom he described as "Catalyzes commitment to and vigorous pursuit of a clear and compelling vision, stimulating higher performance standards". This description will no doubt ring true for anyone looking at the majority of the giants of transplantation, as well as the leaders of many large transplant centers today. Having a Level 4 Leader is likely a requirement for a successful transplant center. However, in identifying Level 5 Executives, Collins described a model of truly transformational leaders, capable of taking organizations to the very highest level of achievement. In describing the levels and models of organizational leadership, Collins identified that Level 4 Leaders are often "a genius with a thousand helpers" — strong-willed leaders with great vision and myriad interchangeable managers to execute one person's plan. Conversely, the best companies have a Level 5 Executive team, in which a strong-willed leader surrounds himself with the best people and works with them to find the best way to develop and advance

Table 126.3. Northwestern's 14 Rules for an Intrapreneurial Transplant Center (right hand column) based upon "Kelly's 14 Rules" for Skunk Works at Lockheed Martin. Reproduced from [30]

Kelly's 14 Rules [30]	Northwestern's 14 Rules for an Intrapreneurial Transplant Center
1 The Skunk Works manager must be delegated practically complete control of his program in all aspects. He should report to a division president or higher.	1 The Center Director must be delegated practically complete control of his program in all aspects. S/he should report to the dean of the medical school and CEO of the hospital.
2 Strong but small project offices must be provided both by the military and industry.	2 A small but strong and empowered core administrative staff must be provided by the institution to ensure the center is nimble and responsive.
3 The number of people having any connection with the project must be restricted in an almost vicious manner. Use a small number of good people (10% to 25% compared to the so-called normal systems).	3 Membership in the center should be limited to faculty actively engaged in transplant research and new initiatives and who are held by the center director to specific goals.
4 A very simple drawing and drawing release system with great flexibility for making changes must be provided.	4 Project plans should be straightforward and high-level, with great flexibility for making changes.
5 There must be a minimum number of reports required, but important work must be recorded thoroughly.	5 There must be a minimum number of reports required, but projects should have clear performance metrics.
6 There must be a monthly cost review covering not only what has been spent and committed but also projected costs to the conclusion of the program.	6 There must be a regular cost and performance review covering status of projects along projected path and any changes required.
7 The contractor must be delegated and must assume more than normal responsibility to get good vendor bids for subcontract on the project. Commercial bid procedures are very often better than military ones.	7 The center must be empowered and expected to develop collaborations with other specialties to create projects at the intersection of diverse disciplines.
8 The inspection system as currently used by the Skunk Works, which has been approved by both the Air Force and Navy, meets the intent of existing military requirements and should be used on new projects. Push more basic inspection responsibility back to subcontractors and vendors. Don't duplicate so much inspection.	8 Institutional leadership must commit to facilitating and not impeding, research projects and new initiatives.
9 The contractor must be delegated the authority to test his final product in flight. He can and must test it in the initial stages. If he doesn't, he rapidly loses his competency to design other vehicles.	9 The center must be supported in efforts to rapidly but safely translate research discoveries into new clinical programs.
10 The specifications applying to the hardware must be agreed to well in advance of contracting. The Skunk Works practice of having a specification section stating clearly which important military specification items will not knowingly be complied with and reasons therefore is highly recommended.	10 The metrics for success and failure for any project must be agreed to in advance, and failures to meet these metrics and milestones remediated immediately or projects terminated and unspent funds returned.
11 Funding a program must be timely so that the contractor doesn't have to keep running to the bank to support government projects.	11 Committed funding for new programs must be fully available until the program meets its goals for self-sustainability or is eliminated due to inability to achieve goals.
12 There must be mutual trust between the military project organization and the contractor, the very close cooperation and liaison on a day-to-day basis. This cuts down misunderstanding and correspondence to an absolute minimum.	12 There must be mutual trust between the transplant center, medical school, and hospital with full transparency on finances, goals, and changes in strategy.
13 Access by outsiders to the project and its personnel must be strictly controlled by appropriate security measures.	13 Center faculty and staff must be able to manage projects within budget and plan free from burdensome external review and approval.
14 Because only a few people will be used in engineering and most other areas, ways must be provided to reward good performance by pay not based on the number of personnel supervised.	14 Because center faculty will work toward goals beyond usual metrics of physician productivity, the compensation scheme must allow center faculty to be rewarded for achieving these goals.

a vision. Collins characterized these Level 5 Leaders as people who prefer not to take credit for themselves, giving it instead to members of their team, at the same time taking blame on themselves for mistakes. He further portrayed them as acting with quiet and calm determination, setting inspiring standards, putting their organizations before themselves [32]. Organizations seeking transplant leaders would do well to identify, not only physicians with a vision who will lead their programs to new heights, but also the much rarer selfless leadership that sets Level 5 Leaders apart.

Summary

The incremental implementation of the provisions of the Affordable Care Act (ACA) will undoubtedly result in evidence-based, team-based, coordinated, longitudinal, accountable, and value-driven care. The ongoing dialogue and debate over future delivery and payment reform underlines the many features and key success factors of the current models of both delivery and payment in transplantation. The direction that the rest of healthcare in the US seems to be taking emulates what transplantation has achieved over the past couple of decades. However, there has been no emphasis on innovation. Instead, the focus seems to be primarily on regulation, incentives for value, and models of care that reward coordinated and integrated care for patients. Although these are all laudable objectives, the biggest and mostly neglected victim is likely to be innovation and research with training and education being a distant second. Who will pay for innovation, and how will we make sure that regulations and the search for novel treatments are not mitigated by the dogged determination to bend the cost curve by replacing quantity with quality? Healthcare reform may foreshadow the demise of the entrepreneurial spirit, adding further to the challenge of transplant leaders and the need for intrapreneurial initiatives within organizational frameworks.

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CHAPTER 127

Transplant Quality Programs

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Introduction

As detailed in Chapters 103 through 110, the overall outcomes of all solid organ transplants have improved consistently over the past decades. According to international and national registries, more than half a million of kidneys and more than 100 000 liver and heart transplantations have been performed [1]. For kidney transplant recipients, patient and graft survival has improved continuously over the decades even though the co-morbidities of end-stage renal disease patients have increased, as has the age of the recipient at time of transplantation [2]. In non-renal organ transplantation patient and graft survival improved as well over the past decades. In liver transplantation patient and graft survival has improved despite a diminishing in the general condition of liver transplant candidates [3]. The success of heart transplantation is continuously increasing, despite an increase of donor age [4]. The estimated half-life for heart transplants over the time period of 1982 to 2009 is 11 years. The number of heart transplants is more than 3700 per year according to the International Society for Heart and Liver Transplant (ISHLT) registry [5]. Since organ transplantation is growing worldwide, the treatment modalities (immunosuppression, surgical technique) are manifold, and the expectations for outcomes are escalating, standards for quality assessment are increasingly required.

This chapter provides a listing of the major indices that should be prospectively tracked to maintain cognizance of the quality of a transplant program. The information technology evolving to assist with capturing and reporting these metrics is covered in Chapter 132. For the purposes of the present chapter, focus is given in an organ specific and chronologically-specific (relative to the time of transplant) manner to the major metrics impacting quality.

Transplant outcome quality: the role of registries

The transplant community has been a pioneer in periodic review of clinical outcomes to ensure the optimal use of limited donor organs [5]. Organ transplantation is constantly developing and is influenced by changing external and intrinsic parameters. These include surgical techniques, perioperative care, post-transplant follow-up, new immunosuppressive drugs and the combination of immunosuppressive medication. Without follow-up data analyzed by registries it is difficult to assess quality standards in this high-level treatment modality. Results from large registries influence the quality of organ transplantation and impact new developments. Although registries deal with retrospective analysis, the high number of patients and a high percentage of follow-up allow effective conclusions (also see Chapter 133 for registry data use). There are a number of national and international registries that accumulate and calculate patient and graft survivals. A large international registry is the Collaborative Transplant Study which follows more than 350 000 kidney, 60 000 liver, 11 000 pancreas, and 50 000 heart, transplant recipients worldwide [1]. In the past two to three decades the Collaborative Transplant Study produced several landmark papers on patient and graft outcome in comparison to a specific variable, such as HLA-system, type of immunosuppression and long-term care of transplant recipients. For example, one paper on blood pressure and its impact on graft survival [6] show that the quality of blood pressure control is strongly associated with the long-term outcome after kidney transplantation. The largest national registry is the United Network for Organ Sharing (UNOS), which follows the data of recipients with solid organs in the US. Another important national database is the United States Renal Data System (USRDS). The USRDS publications continuously impact the quality of outcome after kidney transplantation. A list of international and national registries and their websites is summarized in Table 127.1.

Guidelines

The primary role of globally accepted guidelines is to achieve a certain quality standard in organ transplantation despite geographic disparity. Many national and international transplant societies place significant emphasis on the improvement of transplant outcome quality. However, the suggested way to improve the quality of organ transplantation is naturally biased by local practice patterns, ethnic background, local medical peculiarities (e.g. endemic infections, cardiovascular comorbidity etc.), and the socio-economic conditions encountered.

An example of an international guideline collection is the Kidney Disease Improvement of Global Outcome (KDIGO) guidelines for kidney transplant recipients. This is the first initiative, initiated by the International Society of Nephrology and executed by the National Kidney Foundation, to establish globally applicable guidelines for the care of kidney transplant recipients. Two generalizations were made in the establishment of these guidelines: an evidence-based approach was to be taken, and guidelines were to

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 127.1. Registries that follow organ transplant recipients and publish their data on a regular basis in common transplant journals

International registries	Name	Web-site
Collaborative Transplant Study	More than 400 Transplant Centers worldwide	www.ctstransplant.org
European Liver Transplant Registry		http://www.eltr.org
Eurotransplant	Belgium, Germany, Luxemburg, Netherlands, Austria, Slovenia, Croatia	http://www.eurotransplant.nl
International Pancreas Transplant Registry (IPTR)		http://www.iptr.org
International Heart and Lung Transplant Registry (IHLTR)		http://www.ishlt.org
National Registries		
ANZDATA – Australia and New Zealand	Australia and New Zealand	www.anzdata.org.au
Organizacion Nacional des Trasplantes	Spain	http://www.msc.es/ont
Scandiatransplant	Denmark, Finland, Island, Sweden	http://www.scanditransplant.org
UK Transplant	UK and the Republic of Ireland	http://www.uktransplant.org
UNOS	United States	http://www.unos.org
USRDS	United States	

Table 127.2. Quality data for kidney transplantation

Primary quality data	patient- and graft survival
Secondary quality data	delayed graft function, glomerular filtration rate, surgical complications, rejection, complication of immunosuppression

be formulated such that they could be applied any place in the world [7]. What is the benefit of such a guideline initiative? In terms of quality assessment, guidelines give a basis for the standard of care. In addition, they are helpful when requesting the economic resources needed to establish a transplant program on a scientific based quality level. For non-renal transplantation there are guidelines from various national and international transplant societies available. Guidelines may also be downloaded from the national and international societies mentioned in Table 127.1.

Quality assessment of different organ transplants

Kidney transplantation

Kidney transplantation (summarized in Table 127.2) is the treatment of choice for end-stage renal disease patients. Although the perioperative risk is higher for transplanted patients, compared to age-matched dialysis patients, the long-term outcome of kidney transplant recipients is much better [8].

The outcome data for quality assessment on a national level is primarily restricted to patient and graft survival, as registry data are insufficiently granular to gain more in-depth quality measures. At a local level, however, much more granular data are available (as discussed in depth in Chapter 132). With the aid of local databases, it is also important to document primary function or the rate of delayed graft function, the quality of organ function (e.g. s-creatinine or glomerular filtration rate = GFR), the number and severity of rejection episodes, the number and type of surgical complications (e.g. lymphocele, vascular complications, ureteral leakage) and the immunosuppression associated morbidity and mortality. This specific assessment of graft function and patient morbidity has an important impact on the long-term graft survival.

Table 127.3. Quality data for pancreas transplantation

Primary quality data	patient- and graft survival, graft function (free of insulin)
Secondary quality data	rejection, surgical complications, blood glucose, HbA1c, regression of diabetes associated long-term complications

Table 127.4. Quality data for liver transplantation

Primary quality data	patient- and graft survival
Secondary quality data	30-day mortality, rejection, surgical and non-surgical complications, retransplantation, INR, bilirubin

Pancreas transplantation

In most cases (>90%) the pancreas is transplanted together with a kidney, a combined kidney-pancreas transplantation (Table 127.3). The quality standards for pancreas transplantation thus typically include the quality standards of kidney transplantation.

The outcome data for the kidney in combined kidney-pancreas transplantation are the same as mentioned above. Additional quality standards specific to the pancreas transplant include clinical and biochemical data. The clinical parameters contain the surgical technique (e.g. bowel drainage of the exocrine pancreas, arterial anastomosis, and the venous drainage either systemic or portal). Additional clinical information includes postoperative pancreatitis due to ischemia/reperfusion injury and pancreas fistula (e.g. due to explantation trauma, see Chapter 60 for major technical issues unique to the pancreas transplant). The number of rejections should be recorded, and in this context, a biopsy program to diagnose a rejection episode of the pancreas should be a quality feature of a pancreas transplant program. For long-term follow-up clinical quality data should include the progression of diabetic lesions such as retinopathy, angiopathy, and polyneuropathy. They are associated with the absence of insulin application (full endocrine function), the blood glucose levels, and oral glucose challenge and glycosylated hemoglobin.

Liver transplantation

The main causes for liver transplantation (Table 127.4) are end-stage liver disease, hepatocellular carcinoma, liver metastasis from neuroendocrine tumors, acute liver failure, and rarely, inborn errors of metabolism.

A liver transplant quality program should include the 1-year patient and graft survival, including the 30-day mortality and the rate of retransplantation. The 30-day mortality reflects the in-hospital mortality, and both are significantly influenced by recipient condition at the time of transplantation. This should be captured through the MELD score, with specific note of dialysis or ventilator dependence at the time of transplantation. The length of patient stay in the intensive care unit, the length of respirator

Table 127.5. Quality data on heart transplantation

Primary quality data	patient- and graft survival
Secondary quality data	1-year patient and 1-year graft survival, 30-day mortality, retransplantation, rejection

Table 127.6. Quality data on lung transplantation

Primary quality data	1-year patient survival and 30-day mortality
Secondary quality data	pO_2/FiO_2 at 6 hours post-transplantation, extra-corporal membrane oxygenation

treatment, and total duration of the hospital stay are also important metrics for consideration. The number of rejection episodes should be recorded, as should antirejection treatment. Biochemical parameters should reflect the excretory function of the liver (bilirubin) and marker for synthesis, e.g. INR, albumin.

Heart transplantation

Heart transplantation (Table 127.5) is a routine treatment for end-stage cardiac failure. It is of particular note that cardiac failure patients bear a specifically high risk when they go for transplantation and that they are at high risk for perioperative mortality.

The heart quality program should include 1-year patient and organ survival, including the 30-day mortality. An additional quality item is the number of retransplantations. Like for liver transplant recipients, the pretransplant general condition significantly impacts the outcome after transplantation, and should be captured by detailing specific co-morbidities including renal failure and ventilator dependence. The number of biopsy proven and treated rejections are an additional quality item.

Lung transplantation

Lung transplantation (Table 127.6) is a standardized treatment in specialized centers. Indications for lung transplantations are mucoviscidosis, pulmonary hypertension, primary lung fibrosis, and less commonly, emphsema.

The most important indicator in lung transplantation is the 1-year patient survival and the 30-day mortality. As with other life sustaining organs, recipient condition at transplantation significantly influences patient outcome, and in the case of lung transplantation, ventilator dependence and pulmonary infection/colonization are particularly telling indices. Additional quality factors are the duration of postoperative respirator treatment, the pO_2/FiO_2 co-efficient six hours post-transplantation, and the number of patients who required extra corporal membrane oxygenation (a marker of delayed graft function). In the long-term follow-up the appearance of broncho-obliterative syndrome is of critical importance.

The impact of pretransplant risk factors for the outcome in solid organ transplantation

Patient and graft survival are strongly impacted by the pretransplant condition of the transplant recipient [9]. This differs for the various organs in terms of alternative methods to bridge the organ failure (e.g. dialysis) or in terms of urgency for transplantation (e.g. heart and liver patients on the waiting list). For kidney patients, survival on the waiting list is normally guaranteed by the dialysis treatment; however, time on the waiting list has a specific impact on patient and graft survival and the number of rejection episodes [10]. Furthermore, almost every sixth patient on the kidney waiting list awaits a retransplant, which increases the risk for rejection (e.g. immunization) and early graft loss [11]. For liver and heart transplant patients on the waiting list, the waiting time is even more critical. Since there are not enough solid organ donors and living related donation is only done in a minority of liver patients, normally the most critically ill patients [12] have a chance to receive an allograft. These are important factors that influence the quality of a transplant program and this is summarized in Box 127.1.

Box 127.1. Pretransplant risk factors with significant proven influence on patient and graft survival in solid organ transplantation

- Age of the recipient (<16 and >65 years)
- Retransplantation
- Presensitization
- Time duration on the waiting list
- General health assessed by indices (e.g. Karnovsky etc.)
- Permanent hospitalization pretransplant (liver and heart candidates)

Access to organ transplantation

Access to transplantation varies for the different solid organs. In liver and heart transplantation the access to a transplant program is highly dependent on the current status of the patient. Due to the organ shortage only patients with advanced liver or heart failure are considered for transplantation. Quality programs should assure that patients who are not treated at hospitals with a concomitant organ transplant program should have the same access to a transplantation center. In this context it is important that a transplant center offers regular teaching opportunities for surrounding hospitals.

Scientific publications of the past 30 years document that many patients meeting the criteria for solid organ transplantation remain excluded from transplant waiting lists. For the kidney transplant waiting list barriers to listing include: low social status, a lesser degree of school and professional education, a history of non-adherence and many other variables. It is also thought that female patients have a lesser chance being wait-listed compared to male transplant candidates. Further barriers include the physicians prejudice, such as, not to wait-list an end-stage liver patient with a history of alcohol abuse. Therefore, transplant centers should provide information material on the different solid organ transplantations at their center. They should provide in-house meetings with referral centers to maximize the information for primary care-takers. Specific relationships with referring physicians is covered in Chapter 34. Primary care-takers should be encouraged to refer every potential solid organ recipient for individual counseling to a transplantation center, and indeed, the referral rate is a quality metric for nephrologists providing dialysis care. Information material such as flyers, booklets and videos are available from almost every national and international transplant society.

Organ procurement and organ quality programs

The number of patients on the waiting list is manifold higher than donor organs available for transplantation. The number of donors

per population varies widely between different countries and sometimes between different parts of a nation. The success in organ donation is related to evidence-based education. An example is the Transplant Procurement Management (TPM), an international project on organ donation and transplantation. The program started in 1991 under the auspices of the University of Barcelona and the National Spanish Transplant Organization. In 1994, TPM became international and was initiated in 1997 in Italy, and in 2006 in France. National training courses were organized adapting the same methodologies as the advanced international TPM courses. In 2002 the e-learning platform program was launched to facilitate the education of professionals. In 2005, an international master's degree was created at the University of Barcelona under the Life-long Learning Institute. In 2006, the courses were expanded to include pregraduate health science faculties with the International Project on Education and Research in Donation at the University of Barcelona. Finally in 2007, the European-funded European Training Program on Organ Donation project was started. Currently, TPM offers face-to-face, e-learning, and blended international courses. As of 2008, TPM has trained 6498 professionals in 89 countries on five continents [13]. Another example is the Southern California Organ Procurement Center. This program managed a significant increase of organ donors over the past years. They applied successfully educational strategies similar to the TPM. The number of deceased donor organs per year should regularly be recorded and published as a quality marker for the transplantation center.

Living organ donation — quality program for the donor

The perennial solid organ shortage has impelled a continuous increase in the use of living donors. Particularly in renal transplantation where the relative number living related transplants makes up 30–50% of transplants performed. Before a living related organ transplantation can be considered, all possible measures should be done to put the transplant candidate on the deceased donor waiting list. A standardized protocol is necessary to inform the potential donor on all short-and long-term risks (currently known from the scientific literature and annually updated) to guarantee informed consent. The information on risk and benefit should include the surgical (open vs. laparoscopic), the anesthesiological and the immediate postoperative risk. The evaluation of living donors for kidney and liver transplantation are covered in Chapters 23 and 24, respectively.

All solid organ donors should be included in a life-long follow-up program according to national or international guidelines. In some countries there are specific laws forcing the transplantation center to follow a living organ donor at least annually. These are followed to varying degrees worldwide, and are most problematic in countries without nationalized healthcare systems.

Living donation puts an enormous pressure on the donor and the family. Although the majority of donors are satisfied with their decision to donate an organ, a minority may have somatic complaints (incision site, medical problems, etc.). In addition, there are a number of donors who suffer from adaptive disorders. A predonation counseling as well as a postoperative counseling offer should be at hand. The quality program should have a predonation document referring to the result of the counseling process and this should compare to a similar psychological assessment after organ donation. Misinterpretations and a possible diversity in the estimation of the predonation psychological status are measurable and should impact the predonation work-up in the future

The donation of a solid organ (e.g. kidney) or a part of an organ (e.g. liver, lung) has an impact on the long-term function of the remaining organ. Quality standards are mandatory to avoid donors with occult dysfunction of the potential donor organ and/or exclude an underlying disease. This is of particular importance in patients with inherited diseases (e.g. polycystic kidneys or Alport syndrome in kidney transplantation).

Organ quality

It is a common experience that the donor age for solid organs has increased gradually, commensurate with a generally aging population in most countries [14]. For all solid organs, the age of the donor directly impacts the graft survival, and for heart and liver transplantation it also impacts patient survival [15]. The assessment of organ quality is therefore an important task; however this question is not completely solved at present. For example for renal transplantation the size of the kidney, scarring of the parenchyma, unexpected cysts and other possible malformations need care and attention. The more interesting question in this case is, however, are there microscopic tissue changes, such as interstitial fibrosis, arteriolar hyalinosis and global glomerular sclerosis? It is of great importance to have a representative biopsy size (number of glomeruli) to give an estimate of the quality of the donor organ. Similar questions are raised for a liver graft. The histological hallmarks are the macrovesicular steatosis, fibrosis and inflammatory changes, all of which are important quality criteria for a liver graft [16]. To assess the quality for either a kidney or a liver transplant a 24 hour pathology stand-by is required. There are also clinical and biochemical organ quality markers, diuresis in the past 24 hours, the course of the s-creatinine in kidney grafts, and serum sodium concentration, INR and bilirubin in liver allografts. Specific considerations on donor organ assessment are covered in depth in Chapter 53.

After the declaration of the brain death, organ procuring is the first of many steps toward successful organ transplantation. The quality of the organs procured from a deceased donor is of critical importance for the outcome of the transplants. The experience of the procurement team, and in particular, of the surgeon leading the team is critical. In the UK, a surgical procurement specialist has been created to assure a high quality standard of organ procurement [17]. In a study from Sweden the performance for liver and kidney procurement teams was reported. The most important findings related to missed information concerning organ abnormalities or organ damage from the procurement operation [18]. Damage to procured organs is naturally organ specific.

In the process of diagnosing brain death and the procurement surgery an experienced team is necessary to do all medical measures to stabilize the condition of the potential donor. Transplant centers should provide special trained physician and nurses who are able to deal with this specific situation. Blood pressure monitoring and a thorough volume balance are extremely important to avoid hypo or hypertensive insults to the donor. Quality assessment also includes the treatment of associated complications such as infections, which could significantly impact the potential donation (e.g. nephrotoxic antibiotics). Specific treatment of procurement methods is found in Chapters 21 and 22 for brain dead and heart beating cadavers, respectively.

Medical quality assessment post transplantation

Successful solid organ transplantation starts with an optimal surgical result; however, most of the challenges arise in the days and weeks following surgery. Thus, properly trained (see Chapter 130) transplant physicians should follow all transplant recipients in addition to the surgeon during the hospital stay and the immediate outpatient follow-up. This requires that transplant centers have access to a broad array of medical specialties to deliver quality care. Quality criteria for a physician trained for organ transplantation should include the diagnosis of rejection (including biopsy procedures), experience with immunosuppressive drugs and their interaction (either with other immunosuppressive medication or non-immunosuppressive medication) and the peculiarities with viral, bacterial and fungal infections of transplanted patients. Physicians should understand the potential of late surgical complications and their management, together with the surgical transplant team. The medical quality post transplantation critically depends on the availability of a team of transplant immunologists, particularly when sensitized patients are transplanted. Treatment options should be discussed with the immunological specialist. To assure a high quality for the diagnosis of rejection a specialist in transplant pathology (e.g. kidney, liver, and heart) is mandatory. Quality assessment includes regular biopsy conferences. A department of virology and microbiology with a 24 hour presence is required to treat infections early and with the appropriate antibiotic. Finally a clinical laboratory must be available at any time.

The above mentioned multidisciplinary approach is necessary to provide quality care and thus means of multidisciplinary staff communication are required to facilitate the exchange of knowledge. These include regular in-house teaching rounds or conferences, a written or electronic document where all the relevant process details are accessible to all members of the team, and morbidity and mortality conference for all disciplines involved. The disciplines include not only surgery and medicine, but should include specially trained pathologists and transplant-immunologists. As a quality goal in our unit, we measured the reduction of cold ischemia time. An optimized interaction between surgery, transplant-immunology and anesthesiology reduced the cold ischemia time by almost 20% [19]. Another part of this information chain is to provide the referring physician (e.g. nephrologist, hepatologist) with the present status of the patient. It is fairly common that the referring physician has additional information that makes the work of the transplant team easier.

Clinical pathways differ between the different types of solid organ transplantation. Though this is true, several pathways are similar for all types of organs. This comprises the laboratory control, including immunosuppressive trough levels, as well as bacteriology and virology. Laboratory results should be accessible to all physicians and nurses involved in the postoperative care of transplanted patients. Regular patho-anatomy conferences should be held to interact with the pathologist. These conferences exchange details difficult to relate in the written report, exposes the pathologist to clinically relevant information, and creates an environment to raise research questions.

The experience and the number of transplants for quality assessment

Quality programs for a number of surgical procedures show a reduction in mortality when more than 200 procedures per year are performed. For cardiac transplantation a learning curve has been readily documented: for the first five heart transplants the postoperative mortality is 20%, beyond this number the mortality rate decreases to 12%. It is of particular note that the surgical experience did not improve the chance of survival; it was the association of a transplant-experienced cardiologist in the team [20]. In another study, the number of liver transplants was compared and the authors differentiated between low volume (<20 cases/year) and high volume (>20 cases/year) centers. The mortality rate in low volume centers was almost 26% compared to 20% in high volume centers [21]. Another study from Europe (European Liver Transplant Registry) showed an 1-year survival of 74% in centers with less than 25 liver transplants per year and a 1-year survival of 79% in centers with more than 90 liver transplants per year [22]. A similar effect is also notable in kidney transplantation. Data form the UNOS registry has been used to compare graft survival data between the top 20 transplant centers and the bottom 20 as assessed by transplant volume. A comparison was made for HLA-matched and HLA-non-matched allografts. The graft half-life for unmatched grafts for the top 20 centers was 11.8 years versus 8.9 years for the bottom 20 centers. It is if particular interest that in the time period of 1994–1999, the graft half-life for unmatched allografts in top 20 centers was 15.7 years and in the bottom 20 centers 12 years for HLA-matched kidney recipients [23]. In kidney transplantation the center effect has an even higher impact on graft outcome than HLA-compatibility. Another important aspect is whether a transplant center offers a single transplant program (e.g. kidney) or a multi organ transplant program, which includes for example all visceral organs (liver, pancreas, kidney, if specialized also small bowel).

The quality of long-term follow-up following solid organ transplantation

Recipients of solid organs need a life-long follow-up to record the quality of graft function and the transplantation associated complications. Although the risks in the early months post-transplantation are higher (e.g. surgery, higher degree of immunosuppression, hospital readmission), it is of critical importance to follow the patient with the allograft over the many years. Quality assessment should include the organ specific measurements for the functional capacity and the potential loss of organ function over the follow-up period. Typical organ general and organ specific measurements are shown in Tables 127.7 and 127.8.

Immunosuppressive blood levels should be monitored, although the number of measurements will be less frequent beyond the first year post-transplantation. A specialized outpatient clinic should

Table 127.7. Summary of the surgical quality criteria of organ procuring

Liver	Portal vein length, aberrant arteries (in 15%). Avoiding the denudation of the bile duct. Sufficient cuff of donor caval vein. Avoiding parenchymal injuries
Kidney	Additional or aberrant arteries or veins. Particularly veins which vanish in the fat tissue of the hilus. No denudation of the ureter.
Pancreas	Very careful preparation of the pancreas (risk of pancreatic fistulas). Sufficient length of the splenic and the superior mesenteric artery for back-table reconstruction. Sufficient length of the portal vein. Avoiding injuries of the duodenal segment for the pancreatic drainage
Small bowel	Completeness of organ perfusion. Avoiding hematoma of the intestinal mesentery. Avoiding vascular injury

Table 127.8. Specific quality parameters which are required for the long-term follow-up of solid organ recipients.

Kidney	Glomerular filtration rate, blood pressure, diabetes, bone complications, infection and cancer rate
Liver	Laboratory work: AST, ALT, LDH, bilirubin, INR. Technical work: vascular duplex (hepatic artery)
Heart	Troponin, ventricular ejection fraction, protocol biopsy for occult rejection
Lung	FeV1 (forced expiratory volume in the first second)
Pancreas	Free of insulin, blood glucose, HbA1c

Table 127.9. General quality parameters which are required for the long-term follow-up of solid organ recipients

Blood levels of immunosuppressive medication
Blood pressure
Diabetes
Renal function (glomerular filtration rate)
Number of infections with hospitalization
Type and treatment of malignancy

council the patients in all matters referring to the transplant specific treatment (e.g. drug interactions with immunosuppressive medication, intercurrent illness or surgery, contraception and in female patients the possibility of pregnancy). Quality assessment includes also the number of infections requiring hospitalization and immunosuppressive associated malignancies.

In the context of long term follow-up, the quality assessment of adherence with particular emphasis on the transition period (adolescents) is included. Non-adherence of immunosuppressive mediation is a common cause of allograft loss (and is covered in depth in Chapter 120). The level of adherence affects clinical outcomes, and is associated with early and late allograft rejection. The suspicion to non-adherence should be raised, when missed appointments and fluctuating blood drug concentration occur in the renal transplant recipient. Non-adherence is, however, beyond the intake of immunosuppressive drugs. Additional areas of non-adherence that influence graft survival include exercise, tobacco, alcohol and drug abuse and the self-monitoring of vital signs. Non-adherence occurs early and/or late after transplantation. Non-adherence is particularly common during the transition from pediatrics to internal medicine (see Chapter 121 for a detailed discussion of this topic). How can the quality of adherence be measured in an individual patient? There are several measures which can be applied to assess the quality of adherence: self-reporting medication, collateral reporting by relatives or caretakers, patient diaries, laboratory tests (drug and metabolite levels), medical record review, prescription refills, monitoring pill counts, and electronic monitoring devices. There are several approaches to improve adherence they are summarized in the Kidney Disease: Improving Global Outcomes (KIDGO) guidelines [24].

The assessment of quality of life of patients after transplantation

Organ transplantation encompasses many aspects in the life of allograft recipient. Beside the need for surgery and the regular intake of immunosuppressive medication, there is often co-morbidity or treatment associated morbidity (e.g. infections, cancer). All these distresses taken together increase the psychological burden of an individual patient. Beside these sorrows, the organ transplant recipient knows that an organ from another person is within his or her body. Such patients understand that another person has died (in the case of a deceased organ donation) or that a relative or a friend has voluntarily donated an organ (in most cases a kidney). Therefore, it is important to offer psychological counseling for organ transplant recipients. A quality program of transplant center should actively ask patients about their psychological wellbeing. There are some questionnaires out to assess the psychological status of an organ recipient. Examples are the SF-36 Quality of Life Questionnaire or the Transplant Effects Questionnaire TxEQ-D. Other instruments, which are more organ specific comprise the Kidney Transplant Questionnaire (KTQ), The Heart Transplant Checklist, the End stage Renal Disease Symptom Checklist (ESRD-SCL) or Bone Marrow Transplantation Symptoms List.

Table 127.10. Important milestones to improve the quality of organ transplantation

Critical review of outcome results (registries)
Implementing the evidence based knowledge of guidelines
Optimization of process sequences
Assessment and enhancement of patient adherence
Organ quality measurement
The role of minimum quantity
Quality of organ procurement
Quality of life of organ transplant recipients

Summary

Solid organ transplantation is a complicated treatment for critically ill patients. The past decades have provided us with many new immunosuppressive drugs and a significant reduction in the acute rejection rate. Today tong-term survival with a transplanted organ is reality. Despite this success, the results for solid organ transplantation can be further improved. Transplant centers should understand that the treatment options (surgery, medication, diagnostic procedures) alone will not achieve this goal. They should measure the milestones (Table 127.10) and verify whether these milestones are considered in their transplantation center.

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CHAPTER 128

National and International Transplant Management and Oversight

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Introduction

Transplantation has, from its inception as a clinical therapy, challenged community attitudes, legal definitions of death and health system organizations. The field has driven the need for clarity on the definition of death; has required all governments to face decisions on how to operate organ donation programs; has sought to ensure that every individual has considered and made a decision about whether or not they wish to become a donor after death; and for families of individuals who have not made the decision, it has forced them into considerations that go far beyond absorbing the fact of the sudden death of their relative.

Far more than any other field of modern medicine, with the possible exception of human reproductive technologies, transplantation has demanded government policy attention and legislation in nearly all developed countries and emerging economies in the world. Figure 128.1 shows the countries that currently have active and regulated human organ donation and transplantation. Emanating from the 1980s in some countries and the 1990s in most of the rest, at least for the initial legislation, transplantation has been regulated and controlled within legal boundaries set by the national and international community through their legislators. In the last decade both international and governmental attention has been drawn to the problems that successful global spread of transplantation technology has created, specifically around human organ trafficking and illegal commercialization of the human body.

This chapter describes how international organizations and governments have responded around the world, specifically detailing the drivers and purposes of legislative and governmental control in the US, considering the impact of these policies on US transplant programs and practices. Although detailing US policy as an example, this chapter also covers the common issues that have required attention globally, and in broad terms, the means by which these issues have been regulated in geographic regions around the world. Also described are major international associations tending to global transplant practice, and burgeoning international agreements attempting to gain international consensus on aspects of transplant practice. The focus is on organ donation and transplantation rather than cell or tissue transplantation. Additional treatment of broad-reaching policy in transplantation can be found in Chapter 143.

Health and Human Services oversight of the US donation and transplantation system

The broad reach of transplantation is evident in the attention given to it by a vast number of governmental organizations and agencies, and by the numerous laws developed for its conduct. The US Department of Health and Human Services (HHS) is the principal agency for protecting the health of all Americans. This work is advanced through many of its agencies including the Centers for Medicare and Medicaid Services (CMS), the Health Resources and Services Administration (HRSA), the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA) and the Public Health Service (PHS). From the perspective of organ donation and transplantation, HHS' leading oversight agencies are CMS and HRSA.

In 1965 the US Social Security Act (Act) gave the HHS Secretary the authority to formulate and publish rules and regulations for delivering quality healthcare to Medicare beneficiaries [1]. Through this authority, all Americans benefit. CMS is responsible for establishing the minimum standards deemed necessary to protect patient health and safety and for implementing oversight mechanisms to ensure healthcare entities provide quality care to Medicare beneficiaries. Hospitals, transplant programs, organ procurement organizations (OPOs), dialysis centers and clinical laboratories are among the entities required to meet Medicare standards so they may receive financial reimbursement for services provided to Medicare and Medicaid-eligible recipients.

Section 1138 of the Act, passed in 1972, extended Medicare coverage to certain individuals with chronic renal disease. In 1978 the Act was further extended to provide for coverage under Medicare for end-stage renal disease (ESRD) patients to receive kidney transplantation services.

The HRSA's responsibilities for donation and transplantation resulted from the growing success of transplantation and the ever-increasing need for donor organs. In 1984 Congress passed the National Organ Transplant Act (NOTA) to, among other things, create the Organ Procurement and Transplantation Network (OPTN), the Scientific Registry of Transplant Recipients (SRTR), and an administrative unit within HHS to administer these activities, the Division of Transplantation (DOT) within HRSA [2].

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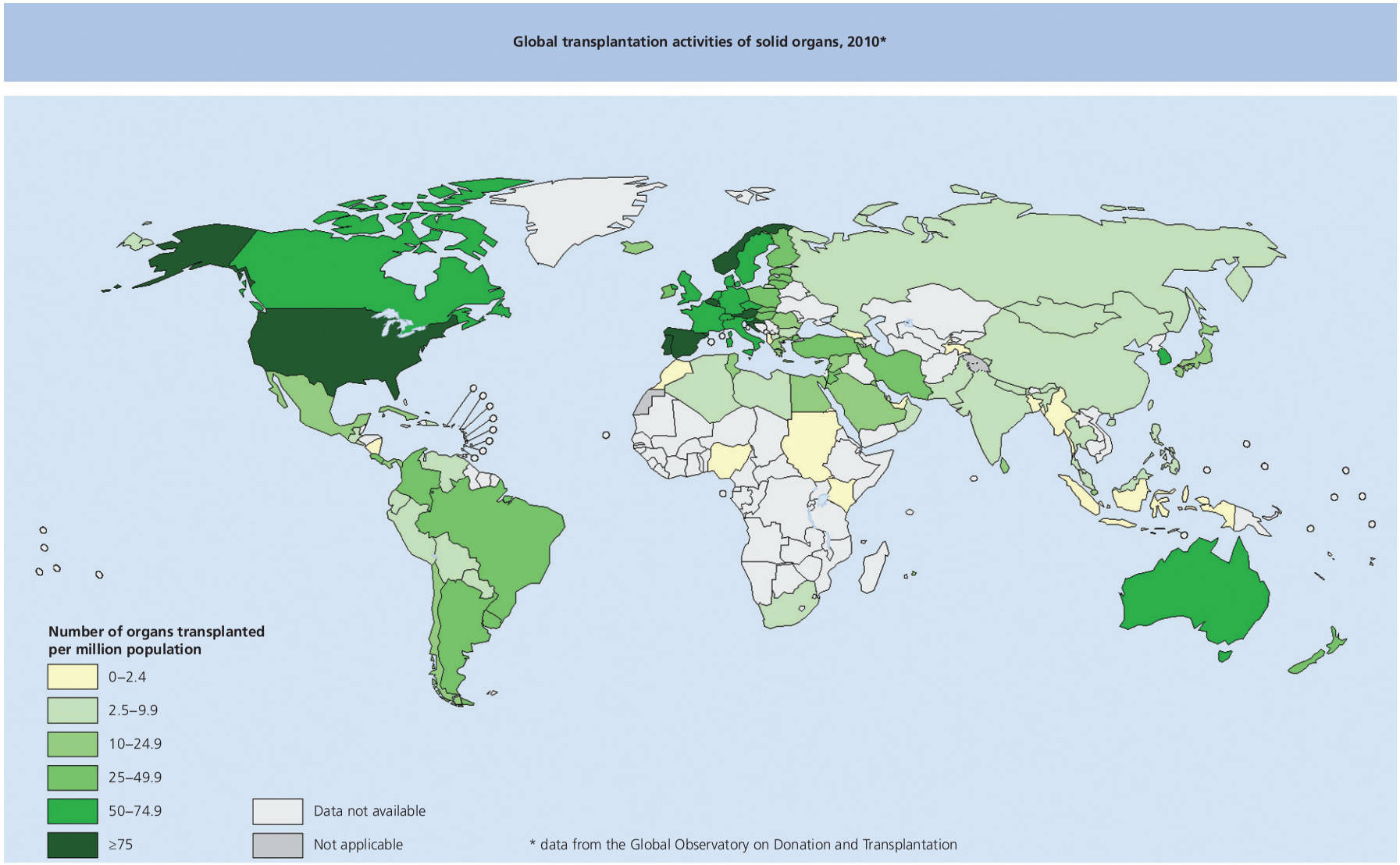

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: Global Observatory on Donation & Transplantation. Map Production: Public Health Information and Geographic Information Systems (GIS), World Health Organization

World Health Organization

Figure 128.1. National solid transplant organ donation activity in 2010. Reproduced from [12] with permission from the World Health Organization. Based on the Global Observatory on Donation and Transplantation (GODT) data, produced by the WHO-ONT collaboration.

Table 128.1. Department of Health and Human Services donation and transplantation regulations (1998–2007)

Year	Agency	Regulation
1998	HRSA	OPTN Final Rule (42 CFR, Part 121)
1998	CMS	Hospital CoPs for Organ, Tissue and Eye Donation; Final Rule (42 CFR, Part 482)
2006	CMS	Conditions of Coverage for OPOs; Final Rule (42 CFR, Parts 413, 441, et al.)
2007	CMS	Hospital CoPs and Requirements for Approval and Re-Approval of Transplant Centers to Perform Transplants; Final Rule (42 CFr, Parts 405, 482, 488 and 498)

Table 128.2. Centers for Medicare and Medicaid Services outcome measures for organ procurement organizations and transplant programs

Covered Entity	Outcome Measure
OPO	Donation rate of eligible donors as a percentage of eligible deaths is no more than 1.5 standard deviations (SD) below the mean national donation rate.
OPO	Observed donation rate is not significantly lower than the expected donation rate for 18 or more months of the 36 months of data used for recertification
OPO	At least two out of the three following measures are no more than 1 SD below the mean: organs transplanted per standard criteria donor; organs transplanted per expanded criteria donor; organs used per research per donor.
Transplant Prog.	Perform ten transplants per month in a 12-month period
Transplant Prog.	Observed patient- or graft-survival rate is lower than expected AND the following three thresholds are all crossed: **1** One sided *P* value is less than 0.05 **2** Observed minus expected events is greater than three **3** Observed divided by expected events is greater than 1.5

Passage of all of this formative legislation (with subsequent amendments) established the foundation by which the US Federal government oversees the organ donation and transplantation system. In the late 1990s, HHS activities built on this legislation in ways that would have important consequences on the future of donation and transplantation. From 1998 through 2007 HHS issued regulations (Table 128.1) spanning the continuum of donation and transplantation that included requirements for donor hospitals, OPOs, transplant programs and laboratories [3]. These regulations also impacted the operations of the OPTN and the SRTR, and the relationship between HHS and the organizations holding contracts to operate both entities.

The CMS published the first of three final rules, the Hospital Conditions of Participation for Organ and Tissue Donation on June 22, 1998 (42 CFR 482.45) with an effective date of August 21, 1998. This rule is better known as the "routine referral" rule requiring hospitals to identify and refer potential organ and tissue donors to an OPO or a third party designated by the OPO. Next, CMS published the OPO Conditions of Coverage on May 31, 2006 (42 CFR 496, Subpart G) in response to the Organ Procurement Organization Certification Act of 2000 that directed HHS to establish outcome and process performance measures based on empirical evidence. Finally, CMS published the transplant program final rule March 30, 2007 (42 CFR 482, Subpart E). Prior to the transplant rule, kidney transplant was governed through the ESRD regulations and the extra-renal organs were governed through the National Coverage Decisions published in the Federal Register. The implementation of these rules as a whole was unique and precedent setting for DHHS.

The Studer Group, a 2010 recipient of the Malcolm Baldrige Award for Quality, coaches healthcare organizations to achieve and sustain excellent performance outcomes. Studer recommends using organizational pillars to provide the foundation for setting goals and directing service [4]. Although regulations differ significantly from organizations, the concept of using pillars to align goals and focus behaviors can be applied to both. In the context of governance and oversight, four pillars that can be used to generate successful outcomes are: accountability, transparency, quality assurance and performance improvement (QAPI), and collaboration and co-operation.

Pillar 1: accountability (stewardship of resource and funds)

From an oversight perspective, accountability is the acknowledgment and assumption of responsibility for actions, decisions, and policies and being answerable for resulting consequences. In the US donation and transplantation system, there are two prime levels of accountability: individual organization performance and overall system performance. Both CMS and HRSA hold individual OPOs, transplant programs and hospitals accountable for performance outcomes. Congress and the US general public hold HRSA and CMS accountable for the overall performance of the national donation and transplantation system.

The CMS holds entities accountable for performance through the process by which they become entitled to receive Medicare money for services provided. This pathway to approval differs for donor hospitals, OPOs and transplant programs. For hospitals, initially, CMS must certify, or affirm, the organization is capable of providing the desired service. For OPOs, CMS designates the OPO (assigns the OPO a geographic service area), then certifies it is capable of service delivery. Transplant programs initially must be members of the OPTN, then be certified by CMS as capable of delivering effective transplantation services. The certification process consists of a review validating the organization's ability to perform donation and/or transplantation services. CMS reviews each applying organization's process and outcome measures to determine whether taxpayer money would be well spent by purchasing services from the applicant. Once certified, entities qualify to receive Medicare reimbursement for services delivered. No entity other than CMS, not even the OPTN, can designate, certify or in any way approve OPOs, donor hospitals or transplant programs to receive Federal funds.

CMS-certified institutions span the continuum of donation and transplantation. CMS has established Conditions of Participation (CoPs) for transplant hospitals regarding the evaluation, listing, in-hospital and postoperative care of transplant patients. Recognizing that the continuing success of transplantation relies on the supply of available organs, CMS established expectations for all US acute care and critical access hospitals to fully co-operate with OPOs in the identification, referral, management and recovery of organs from authorized donors. Finally, CMS has established conditions to be met by OPOs for the effective and efficient evaluation, management, recovery and transportation of transplantable organs from authorized donors.

For OPOs and transplant programs, CMS has established performance outcomes that must be met if the covered organization wishes to maintain its certification and/or designation to receive Medicare reimbursement (Table 128.2). Although CMS has not

specified organ donation performance objectives in regulations for donor hospitals, there are national performance goals established by HRSA that, if not met, could result in reviews by organizations such as The Joint Commission.

In fulfilment of its oversight of the national OPTN granted by NOTA, HRSA published a regulation (OPTN Final Rule, 42 CFR, Part 121) in 1999 describing its expectations of the OPTN's operations and objectives [2]. Among its requirements, HRSA charged the OPTN with developing and publishing allocation policies and performance goals; membership and designated transplant program requirements; review and evaluation processes; and practices by which OPTN members would be held accountable for policy compliance. As a result of the regulation not only did OPTN members become more accountable for their own compliance with OPTN policies, the OPTN itself became more accountable to HRSA and to the general public for providing quality care to deceased and living donors, transplant candidates and recipients.

HRSA, through the OPTN regulation, requires publication of center-specific performance outcomes for OPOs and transplant programs. Each OPTN member must submit donor, candidate and recipient data that are analyzed and published by the SRTR. Members performing at lower than expected levels must undergo the OPTN's peer review process and, when required, implement corrective action plans.

Similar to HRSA, CMS also tracks OPTN member performance to coincide with the biannual SRTR Center Specific Report publications. Similar to the OPTN, underperforming entities are notified and asked to submit an improvement plan that will ensure successful outcomes over the period allowing them to qualify for recertification. OPOs are recertified every four years while transplant programs are recertified every three years, which may be extended based on achieved performance measures. Although OPO and transplant program reviews by both CMS and the OPTN may seem duplicative, they serve to hold programs accountable to the federal entity charged with maximizing organ utilization (HRSA) and the entity charged with funding effective healthcare delivery (CMS). By both using the common set of data submitted to the OPTN, CMS and HRSA hope to minimize the regulatory burden on OPTN members.

Pillar 2: transparency

Transparency, in science, engineering, business and the humanities, generally implies openness, communication and accountability. The concept of transparency in organ donation and transplantation can apply equally to both the policy/regulatory process and to publication of performance outcomes.

All CMS donation and transplantation requirements are publicly available on the CMS website at www.cms.hhs.gov. Published information includes the regulations Conditions of Participation (CoP), interpretive guidelines (how compliance with the CoPs can be demonstrated) and the State Operations Manual (SOM) (how surveyors will conduct a performance review). Under the Freedom of Information Act (FOIA), CMS can disclose the results of any survey it performs to any interested party who requests them after the donor hospital, transplant program or OPO has submitted an acceptable plan to correct accessed deficiencies. CMS actions in response to non-compliance are governed by published regulations under Title 42 Code of Federal Regulations (CFR) sections 498 and the survey procedures are published at 42 CFR 488.

The proposed rule making process instructs CMS to consult industry experts and organizations while developing a regulation. After a proposed rule is published, CMS allows a comment period so the public can inform CMS of possible improvements to, implications for, or unintended consequences of its proposed rule. CMS will then consider the comments and publicly respond to them when it publishes the Final Rule and the date the Rule becomes effective. CMS responses to the public comments are always published in the preamble of the Final Rule. If public comments result in substantial changes to the proposed rule, CMS must publish another proposed rule with an additional comment period prior to publishing the revised Final Rule.

After the regulations are published in the form of Final Rules, CMS develops surveyor interpretive guidelines to be used during the survey process. The purpose of the survey is to establish whether the transplant program, OPO or donor hospital meets all of the regulations conditions (referred to as condition level compliance) for the purpose of certification or recertification. Many entities, including hospitals, are surveyed by accreditation organizations such as the Joint Commission, because these organizations have been granted "deeming authority" by CMS. Having deeming authority means an entity, such as the Joint Commission, can officially determine which facilities meet Medicare and Medicaid certification requirements and which do not [5]. CMS or its contractors do not routinely survey entities that are certified by an organization possessing deemed status. However, CMS does perform validation surveys of all accredited entities. Because there is no organization with deemed status for transplant programs and OPOs, CMS, state surveyors or CMS contractors must perform all certification surveys. Surveys are always conducted on an unannounced basis so covered entities are expected to deliver high quality care at all times and use quality management principles to be "ever ready" for unexpected visits.

In the event the results of a survey cause CMS to consider termination of a donor hospital, transplant program or OPO's Medicare eligibility, the entity has the opportunity to provide a plan of correction and if acceptable to CMS, an unannounced resurvey will be conducted to verify the plan of correction has been implemented. If the plan of correction is not acceptable, CMS continues with the termination process but the entity can request a re-consideration based on substantive grounds. If CMS denies the reconsideration, the termination process continues and the entity can file an appeal. All appeals are considered through the established appeal process (which is clearly outlined in the regulation) and the resulting decision is final.

The HRSA's process for developing and publishing regulations is identical to CMS'. Through its Final Rule, HRSA has established requirements for the OPTN's policy-making process, which is separate and distinct from that of the Federal government. According to the Final Rule, the OPTN must develop and publish policies within the mission of the OPTN such as equitable allocation of deceased organs, prevention of transmission of infectious diseases; reduction of inequities resulting from socio-economic status; training and experience of transplant surgeons and physicians in designated transplant programs; nominating officers and members of the Board of Directors; and other matters as the Secretary directs, such as living donation. A full description of the OPTN's policy development process can be found at www.unos.org.

The Rule further requires the OPTN Board of Directors to provide opportunity for the OPTN membership and other interested parties to comment on proposed policies and for OPTN committees to take the comments into account in developing and adopting policies for implementation by the OPTN. This process assures that any individual, organization or geographic region of

the US affected by a new or revised policy is able to comment during the development process.

All OPTN proposed and adopted policies and bylaws are available at www.optn.transplant.hrsa.gov. Any interested individual or organization, regardless of whether they are OPTN members, may review and comment on proposed policies in writing or via the OPTN's internet-based public comment system. OPTN policies continue to undergo plain language revisions so the intent and purpose of each policy is understandable to both professional and lay readers.

Both the OPTN and the SRTR are required by the OPTN Final Rule to "make available to the public timely and accurate program-specific information on the performance of organ procurement and transplant programs. This shall include free dissemination over the Internet, and shall be presented, explained, and organized as necessary to understand, interpret, and use the information accurately and efficiently". Any interested person or organization has access to program-specific, regional and national donation and transplantation data. Investigators, journalists or anyone conducting bona fide research or analysis, provided the requests are reasonable, may request OPTN data. The HHS Secretary also may direct the release of OPTN or SRTR data at his/her discretion. Complete transparency of OPTN data is one of the most important ways HRSA holds itself, the OPTN, and all donation and transplantation programs accountable for system performance.

When necessary, the OPTN and SRTR are permitted to recoup the costs of complying with data requests in the form of reasonable charges to the requester. This is done to minimize the financial burden of responding to data requests. If the Final Rule did not permit this, the cost of responding to data requests would be passed along to transplant programs and OPOs in the form of higher candidate registration fees. The Final Rule also permits the release of patient-identified data to researchers "showing a legitimate research designed (that) requires such data for matching or other purposes". In these cases the OPTN and SRTR require evidence of "appropriate confidentiality protections, including destruction of patient identifiers upon completion of matching."

Pillar 3: quality assurance and performance improvement (performance sustainability and patient safety)

The CMS defines Quality Assurance and Performance Improvement (QAPI) as a comprehensive data-driven program designed to monitor and evaluate the performance of all services, including services provided under contract or arrangement [6]. It is an ongoing evaluation of operating systems and clinical process that achieves measurable improvements in healthcare.

A review of CMS' donation and transplantation CoPs for donor hospitals, transplant programs and OPOs reveals the QAPI conditions are inter-related. In the interest of continuity of care, CMS has carefully ensured the QAPI rules are synchronized in such a way that optimum levels of care and successful outcomes can result when the QAPI conditions are diligently applied as intended. For example, in the donor hospital CoP there is a requirement to co-operate with the OPO to perform medical record reviews to assess the number of potential donors at the institution in a given time period. In the OPO rule, there is a requirement to provide data reports to hospitals and transplant hospitals of outcomes of medical record reviews to assess the hospital's organ donation effectiveness. In the transplant rule there is a requirement to establish outcome measures for organ donor recovery, which needs the co-operation of the OPO and the information obtained from the donor hospital during the medical record review

To ensure entities meet their performance standards, CMS requires all donor hospitals, OPOs and transplant centers to have robust QAPI programs that track and act on metrics that identify areas of underperformance. CMS expects all entities to maintain compliance with requirements and continuously strive to improve quality of care and patient and living donor safety in pursuit of optimal outcomes.

One example of a recently developed QAPI activity at the national level is the OPTN's ad hoc Disease Transmission Advisory Committee (DTAC). The Committee examines individual potential disease transmission cases reported to the OPTN in an effort to confirm transmissions where possible. DTAC also reviews aggregate data on all reported cases to assess the risk of donor disease transmission in organ transplantation in the US All OPOs and transplant programs are now required to identify a specific patient safety contact person and report all instances of proven or suspected transplant-related disease transmission to the OPTN Patient Safety System within 24 hours [7].The emergence of DTAC begins to close a long-existing knowledge gap about the true incidence of disease transmissions caused by transplantation of donor organs.

Prior to 2003, it was impossible to perform an effective QAPI analysis of the national organ donation system. Donation data related to potential donor referrals, eligible deaths, donation authorization rates and missed opportunities for donation were neither collected nor publicly available. In fall 2002, HRSA required OPOs to begin submitting these data to the OPTN and by the spring of 2003, the Nation was armed with information on which it could act to drive improvements in the number of organ donors and transplantable organs. The availability of these data altered the organ donation performance improvement culture from one of instinctive, anecdotal change process to a data driven process that could give rise to intelligent improvement. Organ donation took its first steps toward evidence-based practice.

To ensure both donation and transplantation remain on a data-driven improvement path, CMS' OPO and transplant regulations now mirror and reinforce the OPTN's existing data submission requirement of 95% of specified data due 90 days after the OPTN due date. Full compliance with the OPTN's data submission policies equates to full compliance with CMS' transplant program condition of participation. In this regard, the OPTN now plays a consultative role to transplant centers and OPOs in complying with Medicare requirements.

Pillar 4: collaboration and co-operation

Collaboration and co-operation are the means by which the components of a system work together to achieve a specific goal.

Everyone involved in donation and transplantation must be good stewards of a rare and medically valuable resource. Over the last five years, an average of 26 857 solid organ transplants was performed in the US from both deceased and living donors (Figure 128.2). In 2010, of the 14 508 organ donors, 53.4% were from deceased donors and 43.2% were from living donors. In 2000 living donation drew within 40 donors of deceased donation and surpassed it in 2001 continuing through 2003 (Figure 128.3) [8]. For the five years prior to 2003, the industry experienced a meagre 2.6% annual average increase in organ donation. In 2003, HHS launched the Nation's first Organ Donation Initiative with the vision of creating a "Donation Friendly America."

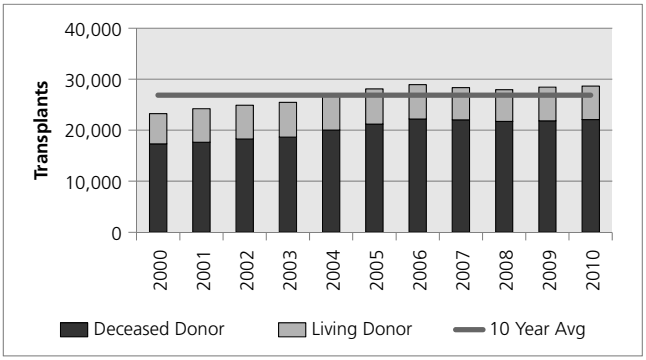

Figure 128.2. US annual organ transplants. Based on OPTN data as of January 11, 2013.

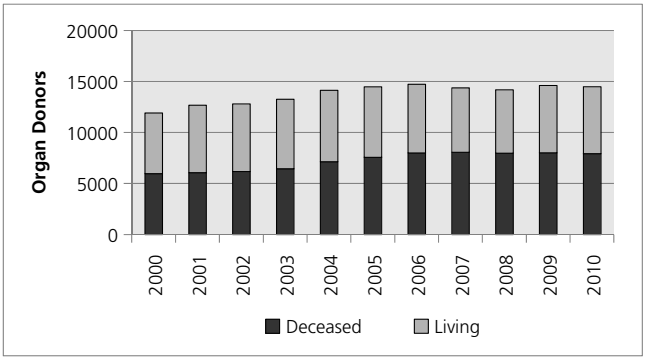

Figure 128.3. US deceased and living donors. Based on OPTN data as of January 11, 2013.

The HHS' premier example of donation and transplantation collaboration and co-operation is HRSA's Organ Donation Breakthrough Collaborative (ODBC) that was established to achieve significant increases in the number of US organ donors [9]. The collaborative's charge was to identify best practices in OPOs and hospitals leading to high donation (conversion) rates and spread those practices to all 58 donation service areas (DSAs) in the US. Through this initiative, the Nation experienced previously unimagined increases in deceased donation from 6190 organ donors in 2002 to 8019 in 2006 (29.5%). The ODBC evolved into the Organ Transplantation Breakthrough Collaborative (OTBC) intended to maximize the donation gift from every donor to increase the number of organ transplants. As with the ODBC, the increases were outstanding, with transplants increasing from 18 291 in 2002 to 22 208 in 2006 (21.4%).

The Collaborative translated the best practices identified in HRSA's two published reports by stressing the importance of using existing performance improvement methods, such as Plan, Do, Study, Act (PDSA) cycles to test and adapt changes for local use. Performance improvement progress was tracked at both the hospital and DSA levels. Collaborative teams (comprised of representatives from OPOs, donor hospitals and transplant programs) demonstrating rapid improvement were invited to share their progress and methods with similar teams from throughout the nation at joint learning sessions.

The original goal of the breakthrough collaborative was to increase the national conversion rate from 58 to 75% through the use of best practices and outcome oriented relationships between OPO and donor hospitals. The 75% conversion rate goal was achieved in 2010 and has subsequently been achieved each month through September 2011 [10]. In addition to increasing the national conversion rate, the Collaborative sought to maximize each donation opportunity by increasing the number of organs transplanted per donor (OTPD) from the existing 3.03 in July 2005 to 3.75. And although some individual OPOs have intermittently reached the national goal, most have not, so the national goal of 3.75 OTPD has yet to be realized. However, opportunities for improvement still exist. It is likely partnerships between critical care physicians and organ procurement professionals to maintain a high degree of organ viability from the time a potential donor enters a hospital until donation authorization is obtained could increase the number of healthy organs that could be procured. Such sophisticated partnerships do not yet exist in most areas of the country. It also is possible that closer involvement of certified QAPI professionals to review and evaluate OPO donor management processes and practices could shed light on previously unrecognized opportunities for improvement.

HRSA's Collaboratives have served as a catalyst to encourage ongoing co-operation among donor hospitals, transplant centers and OPOs at the local, regional and national level and to assure alignment of their QAPI activity to better serve the needs of donors, donor families, transplant candidates and recipients.

The Collaboratives also provided HRSA and CMS an opportunity to partner in pursuit of a common goal and with the purpose of establishing a viable platform based in the private sector that could maintain and sustain the work and momentum of the Collaborative into the future.

Transplant program perspectives

CMS Transplant Center Conditions of Participation (COP) went into effect on June 28, 2007 in an effort to improve oversight to transplant programs. This 84-page document published in the Federal Register required all previously approved transplant centers to submit a letter to CMS by December 26, 2007 requesting approval to continue to perform transplant procedures under the new CoP [6]. By virtue of this letter, transplant programs in the US were committing to conform to the regulatory requirements established by CMS. This meant CMS would survey each transplant program or the transplant program's State Department of Health every three years to ensure compliance with the new CoP's. Included in these regulations were volume and outcome requirements for solid organ transplantation. Directives and guidelines such as these had not been previously established and some programs and professionals questioned the need for such requirements. Transplant programs now were required to report any significant changes in their volumes or outcomes to CMS as well as any changes in key personnel such as a primary transplant surgeon or physician. To maintain clinical experience, volume requirements were established for each specific organ with heart, liver, intestines and lung programs requiring ten transplants each year and kidney programs requiring at least three transplants annually. No annual requirements were established for heart-lung combinations or pancreas transplants. Pediatric programs were also exempt from volume requirements.

Initially there were concerns from transplant professionals about the number of regulations being imposed from the OPTN and now CMS. Recognizing that the CoPs were tied to payment, transplant centers began reviewing their programs to ensure compliance.

Without approval by CMS to continue transplantation, programs would be at risk of losing private payors as well. Several professional transplant associations issued a crosswalk describing and comparing regulatory requirements from the OPTN and CMS. Both now would be surveying transplant centers. The OPTN provides a notice of surveys several weeks in advance with a list of patient records they will audit. CMS surveys are unannounced and occur every three years with requests for patient records upon their arrival. In 2011, The OPTN began specialized surveys of living donor programs separately from their regular chart audits of recipients and transplant candidates. Therefore, it is possible for a transplant program to have two OPTN surveys and a CMS survey in the same year. The OPTN surveys are usually 2–3 day audits whereas the CMS surveys may last a week or more depending on a transplant center's volume. When one considers the fact that transplant programs had not experienced such oversight from regulatory agencies prior to this, these surveys generated considerable concerns within the transplant community.

Data generates outcomes

Data requirements are also included in both the OPTN and CMS regulations. Each transplant program must submit data to the OPTN on a monthly basis. Transplant centers are expected to collect and submit 95% of required data within 90 days of the due date. This data is then transferred to the SRTR for analysis. Once analyzed the data is sent to the respective transplant programs for review prior to being released to the public. Transplant programs have approximately four weeks to review the data, make corrections and supply any missing data. Upon returning the data it is reanalyzed by the SRTR and released to the public on the website: www.srtr.org. Here the public has complete access to each transplant center's risk adjusted patient and graft survival rates at 30 days, 1 year, and 3 years. Other data such as deaths on the wait-list and a center's transplant rate are also reported. Both CMS and the OPTN monitor these outcomes. Expected one year graft and patient survivals are compared to one year observed patient and graft survivals using the following thresholds:

- observed minus expected >3
- observed divided by expected >1.5
- 1-sided P value <0.05

CMS considers a transplant center to be non-compliant with outcomes if the program crosses all three of the above thresholds in 2 out of the last 5 SRTR releases. SRTR reports one and three year outcomes that are based on cohorts within a 2.5 year period. Therefore following graft and patient survival in real time is important to allow programs to proactively intervene with problems identified and analyzed early rather than waiting for program specific reports issued by the SRTR.

Systems improvement agreements

Transplant centers that do not meet volume or outcome requirements may receive a Systems Improvement Agreement (SIA) from CMS. This document describes the program's requirements to remain active. Programs receiving an SIA are essentially considered on probation until their outcomes or volume improves. This requires that a letter is sent to every patient listed with the transplant program and to provide the patients with contact information for other transplant programs should they wish to transfer to another program as the center works to improve their volume and outcome requirements. This often is very costly for transplant programs and has been estimated by several programs to cost between $600 000 to over $1 000 000 to rebuild. Programs must pay for a peer review group to evaluate their program and make suggestions to CMS on what must be done for the transplant program to improve their outcomes and/or volume. This peer review group usually consists of a surgeon, physician, transplant coordinator, social worker, transplant administrator, and a quality consultant. The curriculum vitae of each professional must be sent to CMS for approval. Peer review members may not be selected from programs that are currently under an SIA themselves. The group surveys the transplant program for 2–3 days and submits a report to them based on their findings. This report provides suggestions for program improvements that the peer review group thinks will promote an improvement in outcomes. This report to the program is followed by a conference call with leadership and staff from the transplant program, the peer review team and staff from CMS. Recommendations from the peer review group are outlined during this call as the program begins the process of implementing changes.

Following the peer review group that consults with the transplant program under an SIA, a quality consultant must also be hired to work with the transplant program for eight days a month for approximately one year. This consultant remains on site with the transplant program for the allotted amount of time working with the program to improve their quality programs and to track data in real time to ensure the program is making progress in their outcomes. The quality consultant also helps update policies, protocols and monitoring of any adverse events. CMS requirements for an SIA may vary from program to program depending on the severity of the deficiencies.

It is interesting to note that a time when OPOs are being asked to increase organ donation rates, many transplant centers are becoming more risk averse. To improve outcomes some programs have taken less risk on DCD or ECD donors. Programs under an SIA are also being more cautious about patient and donor selection to ensure improvement in their outcomes. Determining the criteria for patient selection requires a process that must be taken seriously by transplant programs. Often the criteria is written into a protocol but may not be followed as written. CMS and the OPTN review patient selection criteria closely during surveys to determine if what is documented in a policy or procedure reflects current practice. According to CMS CoP, patient selection criteria used to accept or reject a patient for transplantation must be documented in the patient's medical records. Many programs have developed checklists that contain the selection criteria used to accept a patient for transplantation as well as absolute or relative contraindications used to deny transplantation. The checklist is completed, signed by a physician and coordinator and placed in the patient's chart. A letter is then sent to the patient with the team decision. This letter must be sent within ten days of the decision.

Quality assurance and performance improvement in transplant centers

All transplant programs now are required to have comprehensive quality assurance and performance improvement (QAPI) programs with performance measures that reflect regulatory requirements and program specific metrics that monitor outcomes in real time. These programs should demonstrate integration within the hospital's QAPI program in terms of reporting data on a quarterly or half yearly basis. This report is often shared with administration and the hospital's Board of Directors. A strong QAPI program requires a plan that describes a steering committee with oversight on the transplant program. If a transplant program has more than one

organ system being transplanted, a quality committee for each organ is needed to meet on a monthly or quarterly basis to review data. The program's QAPI plan and policy determine the frequency of meetings. Goals for a QAPI program plan should include:

- identification of opportunities for improvement based on real time metrics
- prioritization of performance improvement projects
- continuous tracking of patient care and organizational process outcomes
- monitoring regulatory requirements
- ensuring policies and procedures are evidence based, regularly reviewed and audited for compliance
- monitoring outcome data to ensure it is statistically aligned with national standards and expectations.

Changing practice to incorporate QAPI into the busy schedule of transplant professionals often requires a change in program culture, perspectives and priorities. Education is required to bring the various members of the multidisciplinary teams on board with this new era of transparency through QAPI and regulatory environments. Most have not been educated on the processes involved in QAPI. Thus, presenting programs on QAPI to explain its relation to transplantation in terms of structure, process and outcomes, helps teams understand the rationale for developing comprehensive data driven programs. Educating staff about SRTR outcomes is an important part of helping teams understand how CMS and UNOS are reviewing programs. Understanding how SRTR analyses the data each center submits, helps staff value the importance of reporting data accurately. A strong QAPI program also includes monthly meetings to address morbidity and mortality issues as well as adverse events. Staff must be included in these meetings to participate in discussions that address these issues in depth within a multidisciplinary team environment. Drilling down to assess and analyze the root cause of identified or potential problems allows programs to give serious consideration of changes that may be needed in the program's clinical practice or policies.

Global policies

The challenges set for the international community by the rapidly advancing field of transplantation, started in the 1960s as clinical transplantation became a clinical reality. Early experimental clinical programs had substantial practical difficulties with finding and using potential organ donors, operating as they were, within a legislative vacuum. What was needed for consent to donate after death? Who could provide consent? Who could over-ride consent in the face of disagreement? When was the donor dead? Heart transplantation took over from kidney transplantation to strike at the core of what death actually was for a general pubic attuned to the concept of death as a cold corpse resulting from cardiac standstill. The media role in promoting each new transplant as a miracle in each developed country of the world, raised the attention to a level that could not be ignored by governments and legislators.

Despite these uncertainties and the silence of most legislatures on organ donation, it was the medical profession that drove the clinical field forward and demanded legislation that defined death. The Harvard committee that, in 1976, developed criteria for death based on the function of the brain stem, opened the way to use of organs from heart beating donors to be accepted in most but not all countries. Japan, for example, prosecuted a surgeon undertaking the first heart transplant in that country, using these principles, demonstrating that public attitudes, opinion and standards impacted variably by country, community and religion. That event in Japan paved the way for more than 20 years of legislated stagnation, which has only very recently been relaxed to permit family consent to donation after death.

During the 1980s, with a number of countries legislating for the use of brain death criteria, it became evident that model legislation would be of assistance to national governments and that global consideration and agreement on a limited number of principles would also be of benefit. In particular the scientific progress achieved in human organ led to a trade for profit in human organs among living human being that contravened the Universal Declaration of Human Rights. Thus the World Health Organization (WHO) was tasked to bring to the World Health Assembly a considered and fully consulted set of guiding principles for Member States to use as a template for their individual national legal instrument to regulate transplantation activities.

World Health Organization and World Health Assembly

The World Health Organization (WHO) is the health arm of the United Nations, based in Geneva and in six global regional offices. It is governed by the World Health Assembly (WHA), which is itself the creature of the contributing Ministers of Health of its 194 Member States. See http://www.who.int/governance/en/.

The WHA, which guides and governs the international efforts to control disease and improve the human condition, directed the WHO to bring guiding principles forward for endorsement, which was first accomplished in 1991 (resolution WHA44.25). These principles provided the basis for development of modern transplantation practices and served the clinical programs well until the late 1990s. Two phenomena then drove the Assembly to request re-examination of the WHO Guiding Principles in 2004: the demonstrable failure of transplantation to meet clinical demand through inadequate donor numbers in developed countries; and the spread of technical expertise, with the subsequent rise of illegal commercial transplantation in emerging economies across Asia, Eastern Europe and Latin America.

Following an extensive consultation process, the revised WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation were endorsed by the 63rd World Health Assembly (WHA63.22) on 21 May 2010. See http://www.who.int/transplantation/en/ and Table 128.3.

WHO encourages collaboration between professionals and authorities from all region of the world and promotes a common global attitude to challenges arising from the success of transplantation. The WHO has taken on additional projects designed to assist national health authorities with the implementation of the revised guiding principles. These projects include: The Global Knowledge base on Transplantation and Global Observatory of Donation and Transplantation, conducted in partnership with the Spanish national agency Organización Nacional de Trasplantes (ONT), which documents global transplantation activity annually (Figures 128.4 and 128.5). The website provides a standardised international database of all organ transplantation activity and contains much useful data about different national legislations and acts as a repository of international documents [11,12].

The 4Ds programme, "Developing Donation From Deceased Donors" was initiated in 2006 in Kuwait and culminated with The Madrid Resolution resulting from the third global consultation on organ donation and transplantation [13] (for table of contents

Table 128.3. The 2010 World Health Organization (WHO) guiding principles on human cell, tissue and organ transplantation

1 Cells, tissues and organs may be removed from the bodies of deceased persons for the purpose of transplantation if:
(a) any consent required by law is obtained; and
(b) there is no reason to believe that the deceased person objected to such removal.

2 Physicians determining that a potential donor has died should not be directly involved in cell, tissue or organ removal from the donor or subsequent transplantation procedures; nor should they be responsible for the care of any intended recipient of such cells, tissues and organs.

3 Donation from deceased persons should be developed to its maximum therapeutic potential, but adult living persons may donate organs as permitted by domestic regulations. In general living donors should be genetically, legally or emotionally related to their recipients. Live donations are acceptable when the donor's informed and voluntary consent is obtained, when professional care of donors is ensured and follow-up is well organized, and when selection criteria for donors are scrupulously applied and monitored. Live donors should be informed of the probable risks, benefits and consequences of donation in a complete and understandable fashion; they should be legally competent and capable of weighing the information; and they should be acting willingly, free of any undue influence or coercion.

4 No cells, tissues or organs should be removed from the body of a living minor for the purpose of transplantation other than narrow exceptions allowed under national law. Specific measures should be in place to protect the minor and, wherever possible the minor's assent should be obtained before donation. What is applicable to minors also applies to any legally incompetent person.

5 Cells, tissues and organs should only be donated freely, without any monetary payment or other reward of monetary value. Purchasing, or offering to purchase, cells, tissues or organs for transplantation, or their sale by living persons or by the next of kin for deceased persons, should be banned. The prohibition on sale or purchase of cells, tissues and organs does not preclude reimbursing reasonable and verifiable expenses incurred by the donor, including loss of income, or paying the costs of recovering, processing, preserving and supplying human cells, tissues or organs for transplantation.

6 Promotion of altruistic donation of human cells, tissues or organs by means of advertisement or public appeal may be undertaken in accordance with domestic regulation. Advertising the need for or availability of cells, tissues or organs, with a view to offering or seeking payment to individuals for their cells, tissues or organs, or, to the next of kin, where the individual is deceased, should be prohibited. Brokering that involves payment to such individuals or to third parties should also be prohibited.

7 Physicians and other health professionals should not engage in transplantation procedures, and health insurers and other payers should not cover such procedures, if the cells, tissues or organs concerned have been obtained through exploitation or coercion of, or payment to, the donor or the next of kin of a deceased donor.

8 All health care facilities and professionals involved in cell, tissue or organ procurement and transplantation procedures should be prohibited from receiving any payment that exceeds the justifiable fee for the services rendered.

9 The allocation of organs, cells and tissues should be guided by clinical criteria and ethical norms, not financial or other considerations. Allocation rules, defined by appropriately constituted committees, should be equitable, externally justified, and transparent.

10 High-quality, safe and efficacious procedures are essential for donors and recipients alike. The long-term outcomes of cell, tissue and organ donation and transplantation should be assessed for the living donor as well as the recipient in order to document benefit and harm. The level of safety, efficacy and quality of human cells, tissues and organs for transplantation, as health products of an exceptional nature, must be maintained and optimized on an ongoing basis. This requires implementation of quality systems including traceability and vigilance, with adverse events and reactions reported, both nationally and for exported human products.

11 The organization and execution of donation and transplantation activities, as well as their clinical results, must be transparent and open to scrutiny, while ensuring that the personal anonymity and privacy of donors and recipients are always protected.

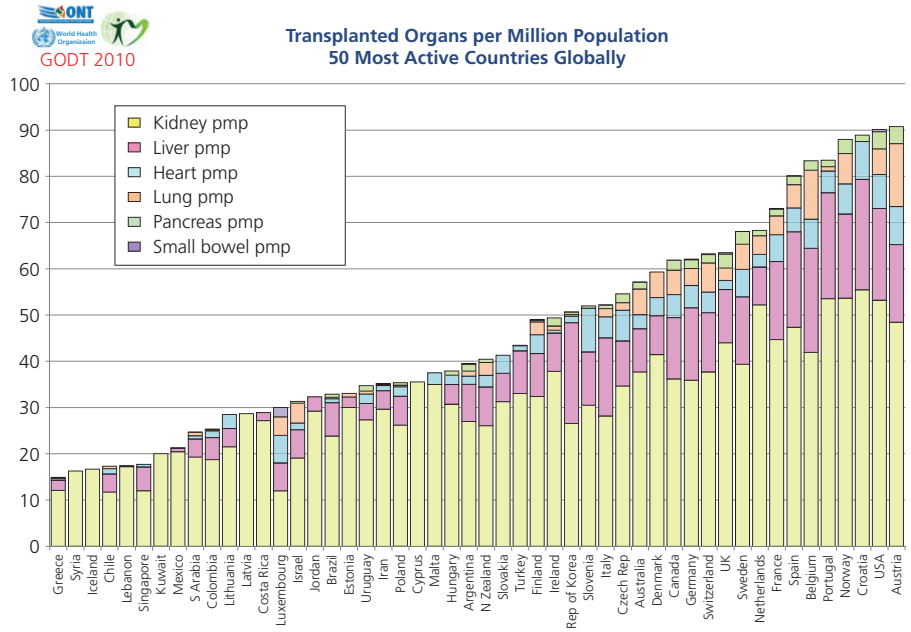

Figure 128.4. Organ transplantation by organ type per million population in 2009 in the 50 most active countries. Reproduced from [12] with permission from the World Health Organization. Based on the Global Observatory on Donation and Transplantation (GODT) data, produced by the WHO-ONT collaboration.

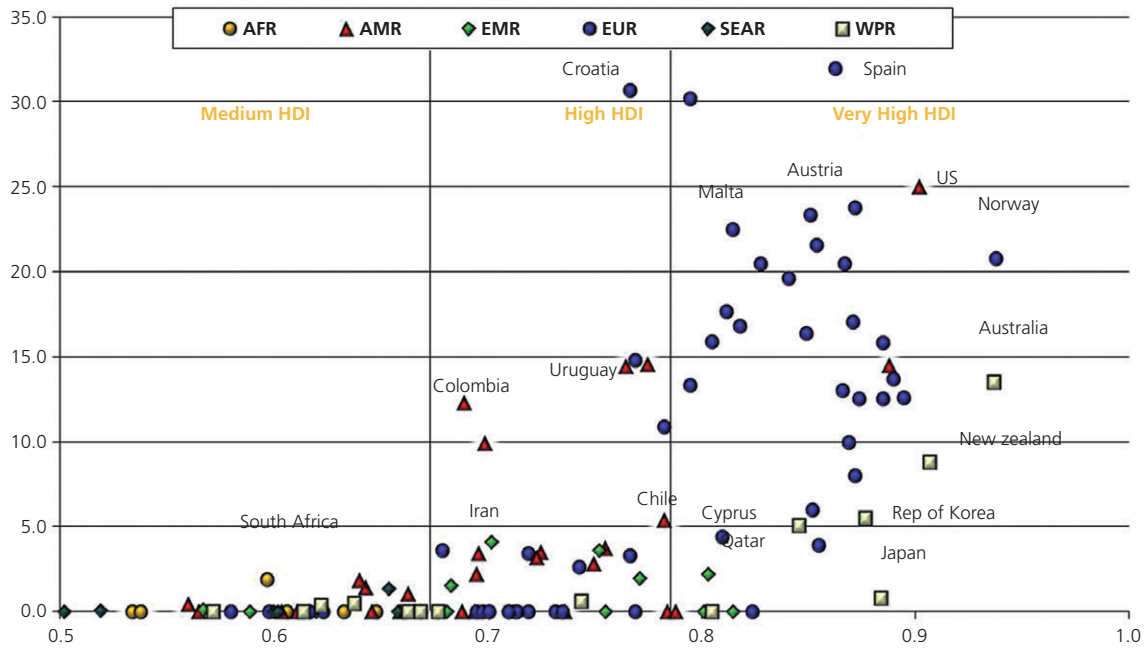

Figure 128.5. Organ donation per million population related to national human development index. Reproduced from [12] with permission from the World Health Organization. Based on the Global Observatory on Donation and Transplantation (GODT) data, produced by the WHO-ONT collaboration.

see: http://journals.lww.com/transplantjournal/toc/2011/06151). The Madrid Resolution stresses the responsibility of governments in striving toward self-sufficiency to fulfil the global needs in transplantation expressly by maximizing organ donations from deceased persons within their own national borders. It also promotes the complete evaluation of national responsibilities and accountabilities for diseases treatable by transplantation extending from educational and preventative measures to avoid disease, through bridging therapies such as dialysis, to donation, and transplantation safety and outcome measurement. The 4D programme exemplifies the complementarities between professionals and authorities and is currently on-going with the development of new global tools and national and sub-regional initiatives.

Project NOTIFY, conducted in partnership with the Italian national organization Centro Nazionale Trapianti (CNT) this project brings together the regulatory agencies and academic efforts to identify and reduce the transmission of disease from donors to recipients, but also to identify other adverse events and reactions occurring as a result of the donation, occurring in the recipient, that could jeopardize transplantation services [14].

Regional policies

European Union

The Council of Europe has, for more than two decades, acted to collate and generate collaboration in Europe over organ donation and transplantation. The Council of Europe's donation and transplantation committee has served to provide data and to stimulate interest in transplantation issues within Europe, especially around the ethical and practical donation related aspects of the field. The Council of Europe, based in Strasbourg, was founded in 1949 and is an inclusive organization of 47 European countries seeking to protect individuals, based on common and democratic principles through the European Convention on Human Rights, from which it derives its mandate in organ transplantation.

The European Union (EU), in distinction, is an economic and political partnership between 27 countries focused on economic co-operation as well as promotion of human rights and democracy. The EU parliament has regulatory powers across the member countries and has produced two main directives impacting clinical transplantation. The first directive covered cells and tissues which has impacted hematological stem cell transplantation and tissue banking, but the more recent European Legislative Framework on Quality and Safety aspects of Organ Donation and Transplantation has laid down a required organ donation and transplant framework for countries in the EU [15]. The binding nature of the directive requires national "competent authorities" to be established or designated — the various equivalents of the FDA — with responsibilities for a quality and safety structure to cover clinical donation and transplant activity. The EU Directive is accompanied by a fairly detailed and prescriptive Action Plan which must now be implemented (outline plan: Table 128.4) [16]. Work is now underway to establish a Europe wide standard safety reporting system for adverse events such as transmitted infections or malignancies, a Europe wide transplant outcomes registry and promotion of organ donation programs across the less developed economies of Europe.

South America

The Ibero Americana Council provides a structure for co-operation within South America across many fields of education, agriculture, science and technology, and health as well as projects designed to strengthen public institutions. In that context Spain and Portugal have engaged with the Ibero-Americana co-operative structure to support and enhance organ donation and transplantation services across the continent. In parallel and in co-operation with the

Table 128.4. European Union's ten point action plan for organ donation and transplantation [16]

Priority actions
1 Promote the role of transplant donor coordinators in every hospital where there is potential for organ donation.
2 Promote quality improvement programs in every hospital where there is potential for organ donation.
3 Exchange of best practices on living donation programs among EU member states: Support registers of living donors.
4 Improve the knowledge and communication skills of health professionals and patient support groups on organ transplantation.
5 Facilitate the identification of organ donors across Europe and cross-border donation in Europe.
6 Enhancing the organizational models of organ donation and transplantation in the EU member states.
7 Promote EU-wide agreements on aspects of transplantation medicine.
8 Facilitate the interchange of organs between national authorities.
9 Evaluation of post-transplant results.
10 Promote a common accreditation system for organ donation/procurement and transplantation programs.

Regional professional society: the Sociedad de Trasplante de America Latina y El Caribe (STALYC) this collaboration has ensured a dialogue between the countries in the region, some of which have well developed transplant programs like Argentina, Brazil, Chile, Uruguay, and some of which have more problems than solutions.

There has been a focus on Colombia and Peru during the past ten years, driven by problems with transplant commercialism and incoming transplant tourists. The government of Colombia has established a strong central organization designed to take control of the organ transplant programs in the three main cities and has been successful in reducing the transplantation of "foreigners for cash" from high levels in the years 2000–2005, to much smaller numbers — perhaps less than 20 per annum by the end of the decade. Israelis and Japanese transplant tourists constituted the bulk of the traffic but that has been reduced to a small trickle of Israelis, despite the action taken in Israel to ban funding by Israeli insurance companies of programs that would be illegal if conducted in Israel. Japan curtailed the trade through the same mechanism and highlighted that regulation of foreign transplantation by prosecution of patients returning after illegal transplants.

The difficulties in two countries cannot be allowed to overshadow the significant successes elsewhere in the continent, such as in Brazil — globally providing the third largest number of transplants each year to its population — and Chile, Uruguay, and Argentina undertaking excellent standards of transplantation for large numbers of their respective populations. The co-operation evident in the Ibero Americana Council will need to extend to regional capacity sharing if the smaller countries, especially in the isthmus, are to be able to meet the needs of their communities. Too small and too poor for sophisticated tertiary care facilities, some of these countries may not be in a position to support transplant programs without many decades of development.

China

China now performs the second largest number of transplants of any nation (after the USA) and has great technical expertise in a number of large centres, but has proved to be the most contentious transplant program in the world. China stands alone in using executed prisoners' organs and has also permitted — through the latter half of the 1990s and the first half of the first decade of this century — unfettered commercialism to govern use of those organs. The result was in 2005–2006 they were broadly accused of executing political prisoners from the "Falun Gong" movement and selling those organs to the wealthy-sick of the world. The annual number of transplants is hard to gauge accurately, but it is likely, based on a self-proclaimed number of 3500 deceased donor liver transplants, that totals of 8000–10000 total transplants from executed prisoners is a reasonable estimate.

The widespread expression of outrage at these practices and the looming 2008 Beijing Olympic games lead, in 2007, to legislation designed to control organ donation and transplantation, to prevent foreigners receiving Chinese organs and to ensure licensing of hospitals permitted to undertake transplantation. An allied decision was made to bring the final arbiter of the decision to execute an individual under the direct control of the courts in Beijing and not delegate it to the provincial level. The combined effect of these actions was positive in that the number of executions reduced, individuals were arrested for breaking the law (such as murderers selling organs surgically retrieved from their victims), 600 transplanting hospitals were reduced to around 167 licensed facilities and a few of those had their licenses revoked for breaking the law. The number of foreigners travelling to China for organs diminished and a number of countries, especially in the Middle East and Asia that had relied on Chinese venality to resolve their own incapacities in organ transplantation, focused instead on providing for their own citizens in their own country [17].

The current situation in 2014 is that control of commercialism remains a major policy concern. Foreigners have been transplanted in China more openly than in the year of the Olympics, no longer pretending to be Chinese through assumption of Chinese names. Extortion remains a concern, specifically that patients are kept waiting in China for an organ and charged enormous sums to advance their allocation of a kidney or liver, or pay for additional dialysis at high cost while waiting. Extension of organ donor sources to paid living kidney and liver donors is thought to have occurred, with a well publicized story in 2012 of a young man selling his kidney for an iPAD.

Concern for the stories of endemic corruption throughout the organ transplant trade have perhaps lead the new Chinese leadership to reinvigorate their control of organ sources and transplant programs. A new determination to turn to standard organ donors in intensive care has yielded more than 1000 transplants in the last year and many transplant programs are signing up to cease the use of executed prisoner organs [18].

The outside world is continuing to carefully observe the developments in China which, within the next decade, will probably surpass the United States as the country transplanting the most patients.

International associations

The Transplantation Society (TTS) has had, since its inception in 1966, a strong role in not only the scientific and educational aspects of the field but also in the legal, regulatory and ethical facets of this unique clinical practice. TTS and its membership have played a role in development of transplantation in almost every country of the world with a clinical transplant program. The International Society of Nephrology (ISN) has likewise had a major role in bringing education and capacity building to renal programs across the world and especially in emerging economies. Both societies have had a significant role in forging the professional view of the ethical

aspects of transplantation clinical practice, with ethics statements for membership and guidance documentation for their members.

TTS first developed a formal ethics statement on organ donation and aversion to the commercialization of the human body in the 1980s and has subsequently revised it on several occasions. The response to the problems presented by Chinese practices for example lead to a specific statement on interaction with Chinese transplantation programs promulgated in 2006 (see: http://www.tts.org/images/stories/pdfs/StatementMembs-ChineseTXProg.pdf).

TTS engaged with the World Health Organization as expert technical advisors, becoming a non-governmental organization in official relations with the WHO in 2006. The role of TTS was to assist the WHO in developing and consulting on the revision of the Guiding Principles. This was accomplished across all regions of the world and through three global consultations and resulted in the adoption of the principles as discussed above. This connection between professional associations and governmental and official international organizations, such as the WHO, is not seen as natural in official circles, since it is perceived that professional organizations usually have a conflict of interest, which prevents objectivity and their engagement simply enhances vested interests. That the WHO-TTS engagement was both relevant and important to the outcomes of the review of the WHO Guiding Principles, is a testament to the individuals on both sides of that collaboration.

Declaration of Istanbul

Creation of guidelines for professional clinical practices remain an important role for national, regional and international societies and there are many guidelines that are testament to that role in the field of organ donation and transplantation. It was out of this facet of work that TTS and ISN addressed the ethical and practical issues of organ donation and the rights of the organ donor, especially the living organ donor. A summit meeting of more than 150 individuals from 78 countries with backgrounds as diverse as philosophy, law, public administration and medicine, was convened in Istanbul in 2008. That meeting addressed a series of topics and created a declaration on organ trafficking and transplant tourism.

The resultant Declaration of Istanbul (DOI) [19,20] has been designed to stand beside the Declaration of Helsinki in guidance across both clinical practice and research. While the well-established Declaration of Helsinki protects the rights of human research subjects, the DOI protects the rights of human organ donors. The DOI contains four important definitions (Table 128.5) which provide guidance against which to evaluate specific instances or issues in question. The six principles and seven proposals contained in the DOI were designed to address the manner in which this world can escape from the denigration and actively destructive progression of transplant commercialism, the commoditization of the human body and the increasingly rampant preying by the rich on the poor and vulnerable communities of the world through the medium of healthcare.

The DOI has since been endorsed by more than 100 professional societies, national governmental and non-governmental organizations. A custodianship group has been formed to encourage implementation and taskforces of individuals from around the world focus on individual aspects of the DOI. The number of countries on active watch lists for indulging in transplant commercialism has reduced since 2008, the volume of illegal transplantation has decreased, but the driving force of long waiting lists and insufficient organs to meet the needs of the wealthy will continue to combine with poverty to create the deceptive but destructive illusion of a win-win solution which is in fact demonstrably and fatally delivers a lose-lose outcome.

Table 128.5. Definitions from the Declaration of Istanbul [19,20]

Organ trafficking	The recruitment, transport, transfer, harbouring or receipt of living or deceased persons or their organs by means of the threat or use of force or other forms of coercion, of abduction, of fraud, of deception, of the abuse of power or of a position of vulnerability, or of the giving to, or the receiving by, a third party of payments or benefits to achieve transfer of control over the potential donor, for the purposes of exploitation by the removal of organs for transplantation.
Transplant commercialism	A policy or practice in which an organ is treated as a commodity, including being bought or sold or used for material gain.
Travel for transplantation and transplant tourism	The movement of organs, donor, recipients or transplant professionals across jurisdictional borders for transplantation purposes. Travel for transplantation becomes **transplant tourism** if it involves organ trafficking and/or transplant commercialism or if the resources (organs, professionals and transplant centers) devoted to providing transplants to patients from outside a country undermine the country's ability to provide transplant services for its own population.

Table 128.6. A timeline for critical elements of global regulation of organ donation and transplantation

1976	USA — Harvard ad hoc committee on brain stem death
1984	USA — National organ transplant Act (NOTA)
1994	World Health Assembly WHA 44.25 — WHO guiding principles on organ donation and transplantation
1998	USA — CMS routine referral Rule
2000	USA — CMS OPO certification Act
2006	USA — CMS OPO conditions of coverage
2007	USA — CMS transplant program Final Rules
2008	Declaration of Istanbul
2008	EU — European action plan on organ donation and transplantation
2010	World Health Assembly WHA 63.22 — WHO revised guiding principles on organ donation and transplantation

Summary

Transplantation is, no matter how clinicians may view it, one of the areas of clinical practice deemed, probably rightly, to carry not only great benefits but also great risks and challenges to civil society. For this reason it is more heavily and specifically regulated than most clinical work (Table 128.6). Without that oversight and legislation organ donation from deceased donors would have remained in legal limbo, community values could not have been engaged to support removal of organs after death, safe practices would not have been developed and supported, investments in workforce development and hospital infrastructure would have been more piecemeal that they are. Transplantation has emerged from a phase of expensive experimentalism through contested clinical applications and approaches, towards a phase where transplantation becomes a normal and accepted part of standard clinical care throughout the world. Co-operation and collaboration of governmental and non-governmental agencies with professionals remains the cornerstone of that remaining transition.

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CHAPTER 129

Abdominal Solid Organ Transplantation Fellowship Training

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Introduction

Transplantation is, by its nature, a surgical endeavor; one that involves comparatively complex procedures on individuals with end-stage organ failure. This juxtaposition of surgical manipulation and grave illness has required that transplant surgeons exhibit exceptional technical skill and knowledge of underlying disease that arguably exceeds that of many surgical subspecialties. As transplantation has grown to become a standard treatment for most causes of end organ failure, the requirements for formalized training have become recognized and codified under the auspices of the American Society of Transplant Surgeons (ASTS). This chapter discusses the history of transplant fellowships, the process of fellowship selection, requirements for fellowship training in the US, innovation in education, and future prospects for transplant surgery trainees. Fellowship training for medical subspecialties is specifically addressed in Chapter 130.

History of transplant fellowships

In 1954, the first successful organ transplant was performed from an identical twin to his brother at the Peter Bent Brigham Hospital. Dr. Joseph E. Murray, a plastic surgeon, performed the recipient operation and Dr. J. Hartwell Harrison, a urologist, performed the donor operation. Over the following decades, transplantation continued to flourish as a specialty aided by the advent of potent immunosuppressants, particularly cyclosporine. It allowed for successful lung, pancreas, and heart transplants to occur during the 1980s. The availability of organs became and remains the limiting factor. In 1974, a group of surgeons, most without formalized transplantation training, set out to form the ASTS. They envisioned the ASTS would promote scientific scholarship, have a commitment to education, and recognize the welfare of transplant patients. The leadership of the ASTS initiated and, to this day, oversees surgical transplant fellowships in abdominal solid organ transplantation [1].

James Cerilli, president of the ASTS from 1980–1981, established the Education Committee as part of his vision to formalize the training certification process for transplantation. Dr. Cerilli initiated discussions with the American Board of Surgery, the American College of Surgeons, and the Residency Review Committee of the American Medical Association to establish and review transplantation training programs [2]. After much deliberation with these established administrative programs, it was decided that these established review processes were not the "right fit" for transplantation training programs and it was apparent that the ASTS would be the supervisory umbrella for postgraduate transplant education and would assume responsibility for the quality of transplantation fellowships. Dr. Cerilli notified programs interested in having a transplant surgery fellowship to submit an application to the Education Committee. Programs would then undergo a site visit by the Education Committee, which would make a formal report to the ASTS council to recommend approval of qualified programs. At that time, fellowship training focused on renal transplantation and candidates had to be certified by either the American Board of Surgery or the American Board of Urology. The duration of the program had to be at least 12 months with a volume of 35 new transplants per year. Alternatively, a program could be two years with a volume of 50 new transplants during that time.

In 1985, ASTS President A.P. Monaco formally extended the fellowship duration to a total of 24 months. He believed there should be an emphasis on basic science and immunology, and advocated for at least one year of investigational science within fellowship training. He also supported a review of each training program, conducted by the Education Committee, at least every two years. In 1987, Robert Corry, then ASTS president, established that all transplant centers have at least one transplant surgeon with at least one year in an ASTS approved fellowship or ASTS Accredited Program, which was essentially adopted as a requirement by United Network for Organ Sharing (UNOS). As liver, pancreas, and intestine transplants increased in volume, a minimum number of transplants performed to maintain certification was established.

From the 1980s to 2000s, programs trained transplant surgeons with relatively complete local control and little oversight from the ASTS on a national level. Eventually, Sandy Feng and others suggested to the then chair of the Education Committee, Mitchell L. Henry, that a match be established. The goal of the match was to provide assurance to the finishing resident that the program would

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

honor their commitments to train the resident and the resident would honor his/her commitment to participate in the program. With some controversy, the Education Committee recommended, and the ASTS Council subsequently approved, moving forward with an official matching program between the candidate fellows and the transplant programs to be administered by the National Resident Matching Program (NRMP).

Requirements for program certification

As per the NRMP, the objective of an Abdominal Transplant Surgery Fellowship Training Program is to develop proficiency in the surgical and medical management of patients with end-stage organ diseases amenable to transplantation. This objective should be achieved through a structured supplemental program for the study and treatment of these diseases in an accredited and properly supervised abdominal transplant surgery fellowship.

The recommended transplant volume guidelines for ASTS Accredited Programs require 75 transplant patients be available for each fellow in the program to serve as primary surgeon over the course of their training. Currently, a fellow must perform 30 kidney transplants, 45 liver transplants, 15 pancreas transplants, and ten intestine transplants to receive a certificate from the ASTS, as recognition that the fellow finished an approved fellowship program for that specific organ. Each fellow is responsible for submitting a case list with the minimum numbers in order to receive their certificate of training from an ASTS Accredited Program. UNOS requires that transplant surgeons complete essentially the identical number of cases outlined above to complete an ASTS-approved fellowship in order to be the director of an organ specific program. That is, to be a director of liver transplantation, an individual must have been trained in an ASTS-approved fellowship training program for livers and completed the requisite number established.

Philosophically, from the beginning, the leadership of the ASTS has felt strongly that the ASTS has the ability and responsibility to accredit the training programs. However, the individual leaders in the training programs are then responsible to ensure that the fellow has met all the criteria for completing the fellowship and that he or she is competent, safe, and ready to perform independently. As such, the ASTS does not supervise individual performance. Therefore, individuals are not certified by the ASTS to do particular solid organ transplants; individuals receive a Certificate of Completion from the ASTS that signifies they have successfully completed an approved training program. At present, 74 ASTS-approved transplant fellowship programs exist in the North America (Table 129.1).

Table 129.1. American society of transplant surgeons (ASTS) approved programs 2007–2012

Program	2007	2008	2009	2010	2011	2012
Kidney only	13	12	14	18	20	21
Liver only	3	3	2	2	2	2
Kidney/Liver	24	26	26	27	26	26
Kidney/Pancreas	4	4	5	6	5	5
Kidney/Liver/Pancreas	23	23	20	12	13	14
Kidney/Liver/Pancreas/Intestine	0	0	0	3	3	3
Kidney/Liver/Intestine	0	0	0	2	3	3
Total Programs	67	68	67	70	72	74

Matriculation into a solid organ transplant fellowship

In 2005, with overwhelming favor by programs, solid organ abdominal transplant fellowships became part of a match program. Qualified candidates for the fellowship must have completed a residency that satisfied the educational requirements for certification by the American Board of Surgery or the American Board of Urology, or foreign equivalency. The NRMP conducts the match program for the ASTS. The NRMP clearly specifies that the applicant is responsible for contacting the programs they are interested in, which can be found on the ASTS website. Program directors review the candidate's credentials and are responsible for conducting their own interviews. Fellowship applicants rank their program preferences accordingly in their Rank Order List (ROL) and programs rank their applicants accordingly. Each June, on the third Wednesday, the NRMP runs the match and announces the results. Unmatched applicants and programs often participate in the post-match scramble to fill vacant positions. The NRMP Match Statistics since its inception in 2006 are listed in Table 129.2 [3].

Future directions for abdominal transplant surgery fellowships

Work-hour restrictions

The current supervision of the abdominal transplant surgery fellowships is still under the direction of the ASTS Education Committee, now known as the Fellowship Training Committee. At this point there are no current plans to include these fellowships in the Accreditation Council for Graduate Medical Education (ACGME) or have them reviewed by the Resident Review Committee (RRC), nor is it felt that this would be a simple accomplishment should transplant surgery ask to have ACGME-approved status. There has been huge focus on ACGME resident work-hours and this has not gone unnoticed by the ASTS. Fellows' schedules for time off are ultimately at the discretion of programs, but workload practices were formally addressed in guidelines issued by the ASTS in January 2008.

The guidelines state that:

1 transplant fellowship training programs have a responsibility to ensure safe and responsible work habits;
2 such habits will promote a healthy state of mind in the fellow and lay the groundwork for routines that will form the foundation for a successful career as a transplant surgeon; and
3 working to the point of exhaustion is both unhealthy for the fellow and potentially unsafe for patients.

Furthermore, due to the non-standard timing of transplantation, the guidelines enumerate the following four principles that should lay the groundwork for fellow workload practices:

1 programs should be mindful of the workload they are placing on fellows with respect to all facets of their responsibilities (e.g., clinic, operating room, inpatient service, phone calls, etc.);
2 the fellow must feel comfortable saying that s/he needs to rest;
3 the program's faculty must recognize it may be necessary to tell the fellow to rest; and
4 the impact of activities which are neither educational for a fellow nor require their level of experience needs to be scrutinized on an ongoing basis.

While these principles provide guidance, the following structural elements are considered requirements of ASTS-approved fellowships:

Table 129.2. National resident matching program (NRMP) statistics

	2005 Match for 2006 Positions	2006 Match for 2007 Positions	2007 Match for 2008 Positions	2008 Match for 2009 Positions	2009 Match for 2010 Positions	2010 Match for 2011 Positions	2011 Match for 2012 Positions
Program Information							
Active Programs	45	51	48	61	56	66	59
Programs Filled (%)	67%	69%	58%	67%	67%	74%	69%
Slot Information							
Active Positions (slots)	60	67	65	78	71	79	72
Positions Filled (%)	65%	72%	65%	71%	74%	77%	69%
Applicant Information							
Active Applicants	52	69	64	86	96	94	80
Matched Applicants	75%	70%	66%	64%	55%	65%	63%
Applicant Breakdown							
Active US Grads	21	22	18	25	28	22	21
Matched US Grads	18	22	18	25	25	22	21
% Matched US Grads	86%	100%	100%	100%	89%	100%	100%
Active US Foreign	2	4	0	3	1	5	2
Matched US Foreign	2	4	0	2	0	5	1
% Matched US Foreign	100%	100%	N/A	67%	0%	100%	50%
Active Canadian	4	5	2	0	2	1	2
Matched Canadian	2	4	2	0	2	1	2
% Matched Canadian	50%	80%	100%	N/A	100%	100%	100%
Active Foreign	25	37	42	57	65	65	53
Matched Foreign	17	17	20	28	26	32	24
% Matched Foreign	68%	46%	48%	49%	40%	49%	45%

1 the training program should designate formal continuing medical education time for the fellows, including attendance at least one national meeting a year that does not count toward vacation time;
2 the fellow should be provided at least two weeks of vacation every year, and up to three weeks if only one week a year is designated for meeting time;
3 aggregate vacation and meeting time away from the training program should not exceed four weeks per year; and
4 the fellow should be off call and free from clinical responsibilities at least one weekend per month and at least two additional 24-hour periods every month, exclusive of vacation.

American society of transplant surgeons (ASTS) curriculum

Experiential learning based on organ transplant quotas for cases and the care of patients has been a requirement for trainees since the inception of postgraduate transplant training. It has been recognized that formal didactic teaching may be quite variable between programs. For this reason, the ASTS leadership charged the Curriculum Committee to develop a comprehensive, web-based curriculum, named the Academic Universe. The Academic Universe is composed of the following four features:

1 curriculum,
2 online surgical log,
3 credential storage, and
4 usage reports.

As per the ASTS, the curriculum is composed of discreet learning modules and defines the key areas of knowledge necessary for mastery of the field of transplantation surgery. Topics included in the Academic Universe include: immunobiology and transplantation research; pharmacology and immunosuppression; organ procurement; medical complications of transplantation; kidney transplantation; liver transplantation; pancreas transplantation; access for renal replacement therapy; public policy for organ allocation; ethics; and the economics of transplant. There are currently 13 units, containing more than 160 presentations that are approximately 20 minutes each. Each module includes a voice-narrated presentation; text summary; recommended references; self-assessment; and feedback mechanism. The ASTS now requires that fellows complete these online learning modules in addition to the established organ transplant quotas in order to receive their certificate of completion.

Additional certification possibilities

At many liver transplant programs, the liver transplant surgeons also perform hepatobiliary cases for a variety of malignant and benign indications. Fellows can garner significant experience by performing anatomic and non-anatomic hepatic resection, laparoscopic hepatic resections, pancreatic resections, etc. It has been suggested that future directions include a special certification for those individuals who complete an ASTS-certified program with significant experience in elective hepatobiliary surgery.

Since renal transplantation involves patients with end-stage renal disease (ESRD) and is a vascular surgery discipline, transplant surgeons have often performed vascular access for hemodialysis. Indeed with the growing number of patients with ESRD, vascular access procedures are rapidly approaching the second most commonly performed operation by general surgeons. Fellows may perform a large volume of vascular access cases during their transplant fellowship training, and it may be worthwhile to develop a program to signify advanced experience with vascular access for hemodialysis.

Critical care may also represent a significant portion of fellows' time during training, particularly the perioperative care of those patients with end-stage liver disease who subsequently undergo liver transplantation. A distinction in the certificate of these fellows for advance training in transplant critical care may also be explored.

Summary

Transplant surgery continues to grow in demand and success since the first transplants many decades ago. The ASTS has certainly been

an entity responsible for shaping transplant surgery, and can be credited with recognizing the need for formalization of transplant surgery fellowship training. Through many ASTS leaders' ideas and efforts, transplant surgery is a recognized subspecialty with a recognized and self-governing system for training transplant surgeons. Individual transplant fellowship programs and their leaders expend great effort to train future surgeons, researchers, and leaders in the field of transplantation.

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CHAPTER 130

Medical Solid Organ Transplant Fellowship Training

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Introduction

The expansion of solid organ transplantation over the past six decades has seen the field evolve from the early phase of a quasi-experimental clinical science targeting highly selected individuals, to a mature discipline that now impacts the lives of hundreds of thousands of patients and their families world-wide. From the very beginning, medical transplant subspecialists with specific organ system expertise have performed essential and complementary functions to their surgical counterparts. The primary responsibility of medical transplant subspecialists is to maintain optimal health of organ transplant candidates and recipients, including diagnosing and managing complications in this complex patient population. Transplant is an ever expanding field and the continued improvement in patient and graft outcomes has resulted in an increased demand for medical subspecialists with organ system-specific transplant training. This chapter discusses medical transplant fellowship training programs in terms of their history, current requirements and processes within North America, opportunities and future potential changes. Surgical training programs are discussed in Chapter 129.

History

The role of medical subspecialists in caring for organ transplant candidates and recipients dates back to 1954, when Peter Merrill, a nephrologist at the Peter Bent Brigham Hospital, and Harvard Medical School, was universally recognized as a critical member of the team that pioneered the first successful human kidney transplant. In the early days of transplantation, there was no formalized process by which physicians/trainees that were interested in becoming organ-specific medical transplant subspecialists could receive this training. Rather, trainees in the medical subspecialty fields of nephrology, gastroenterology, cardiology and pulmonology, in the course of fulfilling specialty-specific Accreditation Council for Graduate Medical Education (ACGME) requirements, received some basic instruction in transplantation medicine as part of their general subspecialty training. These ACGME requirements, by and large, remain in effect to this day in terms of general subspecialty training. In the case of General Nephrology training, for example, transplantation comprises approximately 10–15% of the overall two-year clinical experience needed to meet the eligibility requirements for subspecialty board certification. Historically, by completing the general nephrology fellowship training requirements, board-eligible or board-certified nephrologists would then be "qualified" to subsequently practice as transplant nephrologists. Such newly trained individuals would then typically acquire ongoing transplant experience under the mentorship and tutelage of more senior transplant clinicians.

In 1981, after their proposal to the incumbent American Society of Nephrology (ASN) leadership for an increased commitment to scientific and clinical transplant discourse was turned down, a group of transplant nephrologists decided that a separate society for transplant physicians should be formed. The decision by these charter members led to the founding of American Society of Transplantation (AST, formerly the American Society of Transplant Physicians, ASTP) in 1982. One of the major goals of the AST, now one of the largest of its kind in the world, was to promote and encourage education and research with respect to the science and clinical practice of organ and tissue transplantation and immunology. The first Annual Meeting was held in 1982 and subsequently a Fellows' symposium on Transplant Medicine was created in 1996. Two years later, the AST/ASN Renal Transplant Fellowship Training Program was created.

At the present time, transplant hepatology and advanced heart failure/transplant cardiology are the only ACGME-accredited medical transplant subspecialties for which (1) formal training requirements *must* be met in order to practice in the field, and (2) there is a formal board examination administered by the American Board of Internal Medicine (ABIM). The leaderships of most non-ACGME-accredited organ specific medical transplant subspecialties have recommended detailed requirements that *should* be completed in order for trainees to be considered to have been adequately trained in that subspecialty. Since these subspecialty training programs are not ACGME-accredited, this additional transplant-specific training is not enforceable for board-eligible and board-certified subspecialty physicians. However, United Network for Organ Sharing (UNOS) requires that these prerequisites be met in order for a physician to become the medical director of an organ specific transplant program [1]. Moreover, most transplant programs now preferentially look to recruit transplant physicians that have done additional organ-specific transplant training and have fulfilled the recommended requirements at a minimum, as a barometer of their experience and expertise. The requirements for each organ-specific medical transplant subspecialty are discussed in a later section.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

General principles of transplant fellowship training

The major purpose of Medical Transplant Fellowship Training is to provide trainees with an intense and expansive experience in Transplantation, focusing primarily on their subspecialty-specific organ. Trainees can therefore, in a structured setting, acquire the necessary exposure and skills required to become independent transplant clinicians and scientists and to develop expertise in the field. To this end, medical transplant fellowship training may encompass instruction and exposure in the areas of organ allocation, transplant ethics, organ donation and procurement, candidate evaluation and waitlist management, HLA laboratory management, transplant surgery for recipients and donors where relevant, early and late post-transplant complications and care. In some cases, there may a requirement for scholarly activity as well.

Current organ-specific training requirements

Kidney

In 1998, the ASN and AST jointly established the AST/ASN Renal Transplant Fellowship Accreditation Program, administered by the AST/ASN Renal Transplant Fellowship Accreditation Committee. This Committee is charged with accrediting institutions that have developed programs to provide specialty training in kidney transplantation. Accreditation is based on in-depth site visits, both at initiation as well as at periodic intervals thereafter, and submission of annual reports on the transplant training program. The goal of the AST/ASN Renal Transplant Fellowship Accreditation Program is to provide a basis for the standardization of transplant training and a method of uniform documentation of education for those who wish to lead renal transplant programs. As part of this standardization process, the AST/ASN Renal Transplant Fellowship Accreditation Program has recently developed a minimum list of topics that an accredited transplant center is expected to cover during fellowship training [2] (Table 130.1). Transplant nephrology training is only recognized if the fellowship program is associated with a UNOS-approved renal transplant program in good standing and that is also affiliated with an ACGME accredited general nephrology fellowship. At the present time, approximately 60 transplant programs, mainly in the US and Canada, have been accredited by the AST/ASN Renal Transplant Training Program Accreditation Committee.

Table 130.1. Renal transplant fellowship topics. Data from [2] AST/ASN renal transplant fellowship accreditation program: transplant fellowship topics. www.a-s-t.org

Transplant immunology
Immunosuppression
• induction
• maintenance
Medical complications of transplant
• infection (CMV, BK, EBV)
• hypertension
• hyperlipidemia
• post-transplant hyperglycemia
• transplant associated malignancy
Organ allocation
Recipient evaluation
Living donor evaluation
Graft dysfunction
• rejection
• recurrent and de novo disease
Transplant outcomes
Pediatric transplantation
Ethics
Pancreas transplantation
Kidney transplant in other solid organ recipients
ABO or crossmatch incompatible transplant
Paired kidney exchange
Transplant pathology

Transplant nephrology fellows are required to have matriculated in an ACGME accredited general renal fellowship training program, and must therefore be either board certified or board eligible nephrologists. The supporting center must have sufficient renal transplant volume to provide ten new kidney transplant patients for each first year general nephrology fellow as well as 30 new kidney transplant patients per renal transplant fellow. Transplant nephrology fellows should follow at least 30 inpatients during their initial hospitalization for transplantation as well as at least 30 kidney recipients in the outpatient setting during the first three post-transplant months. Clinical training should focus on the immediate and long term care of kidney transplant recipients, and patient management should be conducted in conjunction with the surgical team. Transplant nephrology fellow participation should primarily be to provide medical care/support in decision making in regards to dialysis, hypertension management, post-transplant hyperglycemia management and immunosuppressive regimens. Transplant fellows must observe at least three organ procurements and at least three kidney transplant procedures. Transplant fellows must perform at least ten kidney transplant biopsies and must be familiar with the indications for biopsy, the technique, potential complications/management of complications, and the interpretation of pathology findings.

At the time of writing, there is only one transplant nephrology fellowship track. This track consists of 12 continuous months of training, of which at least six months must comprise dedicated inpatient clinical training while the remaining time should be spent in other related activity (e.g. experience in an HLA laboratory or another organ transplant service) or research pursuits in the area of transplantation. In essence, this track emphasizes "clinical" transplant experience and most trainees that are certified through this program end up in clinical transplantation. A proposal to create an alternative track that caters more to research-oriented trainees has recently been approved by both the ASN and AST boards but has not yet been implemented. This alternative "research" track would enable fellows interested in pursuing clinical or basic scientific careers in transplantation the opportunity to meet minimal clinical requirements in the course of a more intensive research focus. Upon successful completion of the renal transplant fellowship, the program director must provide a letter to each fellow stating that the fellow has met all of the above criteria and is capable of being certified as a UNOS transplant physician. Copies of this letter along with a statement validating the transplant fellow's participation in the required didactic sessions and patient management experiences must be sent to the AST National Office and to the graduating fellow.

Pancreas

Fellows training in pancreas transplant can be trained in either endocrinology or more commonly, nephrology. The fellowship requires one year of transplant training, of which six months should be dedicated to clinical activity and six months can be research based. Fellows must manage at least eight pancreas transplant patients and follow at least eight pancreas transplant outpatients for at least three months. Three or more of the transplant patients

followed should be a combined organ recipient (simultaneous kidney pancreas or pancreas after kidney). Fellows should observe at least three organ procurements and at least three organ transplants. As pancreas transplant biopsy is not routine at most centers, there is currently no procedural requirement for pancreas biopsy associated with pancreas transplant fellowship training.

Liver

Transplant hepatology fellowship programs are overseen by the ABIM via the ACGME. The ABIM is an independent, non-profit organization that is charged with evaluation of medical professional. The ACGME is responsible for accrediting post-MD medical training programs, via a peer-review process that is based on established standards and guidelines. Liver transplant fellows are required to have completed an ACGME accredited Gastroenterology fellowship before pursuing additional training in transplant hepatology. The transplant hepatology fellowship consists of 12 months of liver transplant-focused training; in order to meet all of the programmatic requirements, most of the year is dedicated to clinical training, with limited opportunities for formal research activities. Training must be completed at an ACGME accredited program that is affiliated with a UNOS sponsored liver transplant program. The sponsoring center must have interventional radiology facility capable of performing transjugular intrahepatic portal system shunts and the center must perform at least 25 liver transplants per year [3]. The program must provide formal didactic instruction in hepatology and transplantation as outlined by the ACGME [3] (Table 130.2).

Fellows are expected to participate in the primary evaluation, presentation and discussion of at least ten liver transplant candidates. As per the ACGME requirements, fellows are required to care for at least 20 liver transplant recipients, and must follow at least 20 recipients as outpatients for a minimum of three months. Fellows must also manage at least 20 liver transplant recipients who have survived more than one year post-transplant. UNOS recommends that at least three recipients should have combined organ transplants that include the liver [1]. Fellows are required to maintain their own continuity clinic throughout the clinical year. Fellows must observe at least three liver procurements and at least three liver transplants. Trainees are required to perform at least 30 percutaneous liver biopsies, must be familiar with the indications for biopsy, the technique, and the potential complications/management of complications. In order to gain experience in pathology, they must interpret the pathology findings of at least 200 liver biopsies (native and/or transplant). At the end of their transplant training, fellows may sit for the certificate of added qualification boards, and a passing grade on the Transplant Hepatology Certification Exam is required for practice. In the past, established practitioners without formal fellowship training but reasonable practice experience could also qualify for the board exam via the "Practice Pathway" but this mechanism has since been eliminated.

Table 130.2. Liver transplant fellowship topics. Data from [3] ACGME program requirements for graduate medical education in transplant hepatology. *www.acgme.org*

Acute and chronic end stage liver disease (ESLD)
Evaluation and management of ESLD patients
Complications of ESLD
• refractory ascites
• hepatic hydrothorax
• hepatorenal syndrome
• portopulmonary syndrome
• portal hypertensive bleeding
Hepatocellular carcinoma and cholangiocarcinoma
Chronic viral hepatitis
Fulminant liver failure
Transplant immunology
Drug hepatoxocity
Nutritional support
Diagnosis and management of portal hypertension
Ethics

Heart

As of July 2012, heart transplant fellowships are also ACGME certified. This fellowship is unique in that the training encompasses all aspects of the heart failure patient management and is not limited to transplantation alone. Fellows are trained in heart failure, cardiac devices (ventricular assist devices, pacemakers, implantable cardioverter defibrillators) and transplant. Fellows must have previously completed an ACGME-certified fellowship in cardiovascular medicine. The 12 month fellowship training period must be conducted at a UNOS member cardiac transplant program, performing at least 20 heart transplants a year. Training focuses mainly on clinical transplantation with limited time allotted for formal research activities, fellows are expected to become familiar with the management of acute and chronic heart failure, the use of mechanical assist devices, recipient selection and postoperative immunosuppression management. The ACGME has outlined didactic topics that are to be taught in the training period [4] (Table 130.3) and a continuity clinic is again required.

Fellows should evaluate at least five patients for heart transplant or mechanical assist device placement. Fellows must follow at least 20 heart transplant recipients in both the inpatient and outpatient settings. Of these patients, UNOS recommends that at least three should be combined organ recipients [1] (heart-lung), but this is not required by the ACGME. Trainees must observe at least three organ procurements and three heart transplant operations. Fellows should follow at least 50 recipients of implantable cardioverter-defibrillators as well as at least 30 recipients of mechanical assist

Table 130.3. Cardiac transplant fellowship topics. Data from [4] ACGME Program Requirements for Graduate Medical Education in Advanced Heart Failure and Transplant Cardiology (Internal Medicine). *www.acgme.org*

Transplant immunology
Immunosuppression
Medical complications of transplant
• opportunistic infections
• hypertension
• renal insufficiency
• transplant associated malignancy
Organ allocation
Recipient evaluation (implantable devices, resynchronization, transplant)
Acute decompensation of chronic heart failure
Graft dysfunction
• rejection
• cardiac allograft vasculopathy
Infiltrative and inflammatory cardiomyopathies
Pulmonary hypertension
Inherited xardiomyopathies
Heart failure due to:
• chemotherapy
• congenital heart disease
• pregnancy
Heart failure in other solid organ recipients
Arrhythmias
Hypertrophic cardiomyopathies
Transplant pathology

devices in both the inpatient and outpatient setting. In order to demonstrate technical proficiency, the fellow should also perform at least 40 endomyocardial biopsies, be familiar with potential complications, and be able to interpret pathologic findings. The fellow must perform at least 100 interrogations of implanted devices, be able to interpret the results and adjust device settings. Performance and interpretation of cardiopulmonary stress tests in this specific patient population is also required. Upon the completion of the fellowship year, trainees may sit for the ABIM Advanced Heart Failure and Transplant Cardiology Certification exam. Established practitioners in the field who have not completed an ACGME transplant fellowship but have a minimum of three years practice experience will be permitted to take the certification exam via the "Practice Pathway" through 2014.

Lung

While there are over 45 UNOS certified lung transplant centers nationwide, very few of them are large volume centers able to support a formal fellowship training program. Lung transplant fellowships are not ACGME accredited at this time, nor are they overseen by the International Society for Heart and Lung Transplant; usually UNOS guidelines for training are applied. Lung transplant training can be completed as an additional year after a pulmonary or critical care medicine fellowship, or it can be incorporated into the standard three year pulmonary fellowship training. The fellowship is usually of 12 months duration if training is solely clinically based, but if there is a research component to the training period can be as long as 24 months.

Per the UNOS requirements [1], fellows should be involved in the primary management of at least 15 lung or heart-lung transplant recipients. Of these patients at least half must be single or double-lung recipients. Fellows must observe at least three lung procurements and at least three lung transplant operations. Fellows should be familiar with the management of acute and chronic lung failure patients, the use of cardiopulmonary bypass, ventilator management, in addition to donor and recipient selection. Fellows should perform endobronchial biopsies, be familiar with potential complications and be able to interpret pathologic findings.

Infectious disease

The transplant infectious disease fellowship is also often, but not necessarily, an extra year of training embarked upon after completion of an ACGME-approved Infectious Disease (ID) fellowship. ACGME requirements for general ID training take up a substantial proportion of available clinical time, but if the general ID fellowship is three years (or longer) it may be possible to incorporate a formal Transplant fellowship into the general training period. Transplant-specific training should be 12 months with at least six months clinical exposure, and include both inpatient and outpatient clinical care. The Transplant ID fellowship program should be affiliated with a multi-organ transplant program with sufficient patient volume to expose fellows to a variety of disease processes. Ideally this center should have an active allogeneic stem cell transplant program as well. If the training center does not perform lung transplants, ID fellows should complete an elective rotation at a center performing lung transplants during their training. Per the recommendations put forth by the AST Infectious Diseases Community of Practice Educational Initiates Working Group [5], training should emphasize the major post-transplant opportunistic infections as well as infectious diseases related to specific organ failure (e.g., dialysis access, spontaneous bacteria peritonitis, cystic fibrosis, ventricular assist devices). Education should also incorporate: donor derived infectious complications; postoperative infectious complications; hospital acquired infections; fever of unknown origin; and prophylaxis strategies. Laboratory or research exposure should emphasize laboratory testing techniques for common post-transplant pathogens. There is currently no supervising board or society for Transplant ID fellowship and no additional board certification exams are required.

Multidisciplinary training model

Transplant patients are complex patients, with multiple co-morbidities and are best cared for using a multidisciplinary approach. During the training period it is important for the transplant fellow to learn how to function as part of the multidisciplinary team and to co-manage patients with both their surgical colleagues and often, physicians from other medical transplant subspecialties as well. The fellow should understand the contributions of all members of the team, including but not limited to transplant pharmacy, social work, nutrition, physical therapy, and nursing. The medical transplant fellow should focus on the medical issues of transplant recipients, namely immunosuppression dosing, management of transplant related complications and antibiotic dosing for liver/renal function.

Educational opportunities

Since a major goal of medical transplant fellowships is to acquire knowledge and skills about transplantation through a process of immersion, structured educational opportunities are critically important adjuncts. There are several educational meetings during the course of a year that specifically target medical transplant fellows. The most well-known and widely attended event is the AST Annual Fellows Symposium, where in-depth basic and clinical transplant topics are presented by faculty with distinct expertise in these areas. This Symposium, held over 2–3 days, provides a unique learning opportunity and incorporates some content common to all organ systems as well as organ-specific breakout sessions. There were over 125 registrants from various medical and surgical subspecialties for the 2011 Symposium. There are also smaller scale, industry-sponsored Fellows Symposia, where fellows have opportunities to present their research.

The American Transplant Congress is the annual meeting of the American Society of Transplantation and the American Society of Transplant Surgeons. Fellows can apply for Young Investigator Travel Awards to offset the cost of participation in the American Transplant Congress. These awards are applied for during the abstract submission process and awardees are selected based upon the abstract's scientific merit. The Transplant Congress is an excellent opportunity for fellows to be exposed to cutting edge scientific research in transplantation and meet other transplant professionals.

The American Society of Transplantation has numerous "Communities of Practice". Communities of Practice are self-selected volunteer groups within the AST that work on member generated projects and advocate for issues important to their area of interest. Membership is open to everyone and provides the opportunity for involvement in the AST to members at every training level. The Trainee and Young Faculty Community of Practice is one such group, and was created by young transplant professionals to network, interact with senior faculty, and to provide educational programs. In recent years the Trainee and Young Faculty Commu-

nity of Practice initiatives have included an Early Career Development Symposium, networking sessions at the American Transplant Congress and the creation a directory for employment searches.

Multiorgan transplantation

Dual organ transplant is a small but growing proportion of transplant recipients, with special selection criteria and considerations. Ideally fellows should have experienced evaluating these patients as potential transplant recipients and following them post-transplant. Indication for simultaneous transplantation and recognition of roles/input of the various organ transplant teams should be emphasized.

Pathology

Transplant fellows should be familiar with the diagnostic histological criteria for acute/chronic T-cell mediated and antibody mediated rejection, as well as the diagnosis of organ-specific recurrent versus de novo diseases. Clinico-pathological biopsy conferences should be encouraged.

Pediatrics

Most transplant fellows are internal medicine graduates and trained in the care of adult patients. However in the course of many transplant fellowships, the transplant fellow may encounter former pediatric patients that have transitioned to adult care. The transition from pediatric to adult care is often a difficult one for patients, and exposure to this patient population during training, with attention paid to their specific issues (e.g., autonomy, adherence, burden of chronic illness) will enable physicians to better assist these patients as they make this challenging adjustment to their care.

Procedures

All transplant fellows should be familiar with the indication for allograft biopsy, the procedure itself, as well as the complications of biopsy and how to treat them. Fellows should perform the minimum required number of biopsies to be certified by the ABIM, but additional biopsies are suggested to achieve mastery.

Living donors

The selection and care of living donors is an important part of the renal, liver and occasionally lung transplant training process. Transplant fellows should have clinical experience in the evaluation of living donors and actively participate in donor selection meetings. They should learn the absolute and relative contraindications to living kidney and liver donation, with an emphasis on immediate and long term risks to donors. Fellows should be involved in the immediate postoperative care and be familiar with the most common surgical complications. Fellows should also follow donors at their routine postoperative outpatient visits.

Funding

Funding for trainees is a growing concern in medicine in general. Since most transplant fellowships are not ACGME certified and since this training is considered over and above general fellowship training requirements for most subspecialties, transplant fellows are not considered "true trainees" and are therefore not eligible for funding via Centers for Medicare (CMS). A variety of approaches has been used to support medical transplant fellowships. Unrestricted educational grants, once a major mechanism for supporting medical transplant fellows, are becoming obsolete in the era of increasingly stringent pharmaceutical industry guidelines, as well as the widespread economic downturn. A more common funding mechanism that has emerged to support medical transplant fellow training is the hiring of trainees into very junior faculty level positions (e.g. instructors). Since these individuals are usually board-eligible or board-certified, they can bill for the clinical services that they provide, structured in a manner that enables them to support their salary. In the future, when/if a research track for medical transplant fellows is formally implemented, the clinical component of the training could be covered within the restrictions of a developmental or training research grant. An additional avenue that may occasionally present itself is the use of philanthropic contributions to support medical education efforts.

Research

During the transplant fellowship, fellows are encouraged to participate in transplant related research. This may be original contributions to transplant science or may involve rotating in the HLA lab in order to familiarize them with the laboratory techniques used in clinical medicine. For those wishing to have an original research project there are three avenues of investigation open to them — clinical research, basic science research and translational research. While original research is ideal, fellows may not have the requisite time or scientific training to make this feasible during the training period. Fellows should be encouraged to write review articles, case series and single-center retrospective data reviews if more formal project are not possible, and writing exercises provide an invaluable opportunity for faculty mentorship of fellows.

Work hours regulations

Fellows who are part of an ACGME accredited transplant fellowship are required to adhere to ACGME work hours regulations (no more than 80 hours total per week, no more than 24 hours per shift and at least one day off per week). The majority of medical transplant fellowship programs are not ACGME accredited and are therefore not subject to these regulations. Sensitivity to the needs of medical transplant fellowship trainees is recommended and teaching programs are encouraged to follow ACGME guidelines.

Unaddressed needs

UNOS and ACGME training guidelines emphasize the clinical and research aspects of transplant fellow training, but practical issues, such as the transition to independent/attending practice and the search for employment are often left unaddressed. Transplant fellowship is an in-between state; fellows are board-certified or board-eligible in their general subspecialty but in the process of obtaining additional transplant related expertise. The transition to independent clinical practice can be challenging, and mentoring by more senior faculty is beneficial. The expectations and responsibilities of attending practice are enormous and senior faculty often serves as a model for interactions with colleagues, other trainees and patients.

Employment after fellowship concludes is of great concern to trainees. The path to an academic position is often obscure.

Senior faculty mentorship can be instrumental to guiding this process, as is networking through society memberships and meeting attendance.

Summary

Medical transplant subspecialists with specific organ system expertise are essential to a successful transplant program. As the field of solid organ transplantation has grown, training for transplant clinicians has evolved into a more structured and organized enterprise, with uniform training criteria and expectations. The ACGME has joined UNOS in its regulatory role. Fellowship training now reflects the more scientific and interdisciplinary nature of modern clinical transplantation.

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CHAPTER 131

Transplantation in General Medical Education

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Introduction

According to Organ Procurement and Transplantation Network (OPTN) data [1] from December 2011, approximately 528 000 patients have received a solid organ transplant between January 1988 and December 2011. In addition, each year adds another 28 000 new transplant recipients. These patients ultimately receive their care from a myriad of practitioners throughout their post-transplant lives. This includes transplant specialists, but also involves general internists and surgeons, family physicians, subspecialists, residents in medicine and surgery, nurses, pharmacists, midlevel practitioners, and medical students. With the growing number of solid organ transplant patients in the community, it is imperative that providers have a baseline understanding of the complexity of these patients, their medications, and their care.

Knowledge of the nature of generally disseminated transplant knowledge is useful to the transplant specialist in that it provides a reasonable expectation of what can perhaps be delegated outside of the transplant center. In this chapter we discuss the current general medical education in transplantation for several groups of medical providers, and then discuss further methods of education and training possibilities. The groups of medical providers that will be discussed include: general surgery residents, internists, subspecialists (including nephrologists, cardiologists, gastroenterologists/hepatologists, pulmonologists), nurses, pharmacists, and medical students. The training requirements for transplant surgical and medical specialists are covered in Chapters 129 and 130, respectively. The training requirements for specialized transplant nurse coordinators and transplant pharmacists are covered in Chapters 122 and 123, respectively.

Current education

General surgery residents

General surgery residents have several required rotations to complete prior to their matriculation as general surgeons. These include surgical procedures and management of the abdomen, breast, endocrine system, alimentary tract, skin and soft tissues, surgical critical care, head and neck surgery, trauma and non-operative trauma, pediatric, head and neck, vascular, and surgical oncology [2]. There are rotations that might exist at institutions that are not classified into general surgery residency requirements, including cardiac, neurologic, orthopedic, urologic, gynecologic, and burn rotations [2]. Currently, a rotation in solid organ transplantation is a requirement under the US Accreditation Council for Graduate Medical Education (ACGME) for general surgical residents [2]. The general expectations outlined for a rotation in transplantation include an understanding of immunosuppression and immunology as well as the general surgical management of transplant patients before, during, and after their allograft placement.

However in recent years, especially in the last decade with the implementation of resident work hour limitations, there have been concerns over the ability of residents to remain active members of the transplantation services. In coming years, a change might occur in the transplant service, with the previous ACGME requirement of a transplant rotation transitioning to a transplant experience [2]. The interpretation of this may differ among training centers. Training programs that perform large numbers of transplants might not adjust the number of residents on their transplant services, while other smaller centers might not require all their residents to rotate through the service. Other training programs that do not perform transplants might choose not to send their residents to other centers to rotate, as previously done.

In addition to this, resident feedback regarding the transplant experience has led to concerns over the need for changes to the rotation. Residents who commented on the rotation have identified several areas for improvement (Table 131.1). Among their major concerns are high service requirements and a low perceived educational benefit [3]. In addition, low numbers of operative procedures due to the difficulty obtaining cases from surgical transplant fellows can affect the education of residents [3]. In an effort to improve the general surgical resident experience on the rotation, the American Society of Transplant Surgeons (ASTS) has made recommendations to enhance the training experience for general surgery residents assigned to the rotation [3]. Several significant recommendations have been made and their implementation is underway to improve residents' training (Table 131.1).

First, the identification of a specific transplant attending for the team will help guide the education experience for the residents on rotation, including didactics, inpatient and outpatient clinics. Educating the transplant team members on expectations for the service will help define roles for each member. Including physician extenders to help with patient care will help decrease the service load of the residents, thus allowing them more operating room time.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Table 131.1. Transplant surgery rotation issues and recommended changes. Adapted from: [3] Fryer, JP and Magee, JC. Optimizing the surgical residents' educational experience on transplant surgery. *J Surg Educ.* 2009;66:196–200.

Rotation issues
1 High service requirements in the era of work hour limitations
2 Low perceived educational benefits
3 Competitiveness for cases between residents and surgical fellows
4 Low interaction with attending transplant surgeons
5 Residency training centers lacking transplant programs send residents to other centers
Recommended changes to improve rotation
1 Identifying a specific transplant surgeon as attending for the rotation to help guide learning
2 Maintain ongoing feedback system for resident and program improvement
3 Establish designated surgeries to optimize learning opportunities for residents and sustain educational experience
4 Physician extender addition to the team to assist with patient care
5 Educate all team members regarding expectations and the importance of teamwork

Establishing a designated minimum number of transplant surgeries for residents and fellows will help optimize learning opportunities during the rotation for both types of trainees [3]. There have been studies evaluating the learning curve for residents and their proficiency at performing procedures, as indicated by the decrease in operative time for these procedures. According to one study, which investigated performance of laparoscopic donor nephrectomies, residents had a statistically significant improvement in their surgical performance of this procedure after participating in 13 surgeries (assistant in the surgery) or six surgeries (as surgeon who performed the exposure and mobilization with ligation of the venous branches and ureter) [4]. In a specialty that has a history of variable numbers of cases, having an improved quality of teaching and a minimum achieved number of procedures will help enhance the learning experience of surgical residents.

The classic model for educating residents involves a multidisciplinary center at which the resident is exposed to a high volume of patients, and through immersion the skills necessary to independently care for future patients are learned. This model is changing with the new work hours and emphasis on standardized quality of education. There has even been some debate over whether organ specific centers, such as a hepatobiliary and liver transplant centers and other organ focused centers, would affect the training of post graduate surgical residents [5]. It is especially important for surgical fellows to focus their training in their respective field at a specialized center, as these centers provide focused care to patients. However, this is not necessarily the case for general surgical residents. Creating centers dedicated to a disease or organ would decrease the presence of these patients in hospitals where residents train, thus limiting or even eliminating their exposure to these complex patients [5]. This is true especially for rotations that are not required by ACGME. Though specialized centers do have benefits for fellow trainees and can provide specific care to their patients, the training of general surgery residents could suffer, and hence their ability to care for these complex patients in the community could also suffer. At least for now, the classic teaching institution for training residents remains the mainstay for most programs. Arguments for and against specialized centers continue, and the two competing models of training remain.

In this new era of surgery and surgical residency where new procedures and equipment are developing, the general surgical trainee requires education in multiple areas prior to their matriculation. The exposure of surgical residents to solid organ transplantation allows them experience in several areas important to their training as surgeons.

First, the transplant rotation provides surgical residents the opportunity to perform vascular procedures during each transplant surgery. The vascular anastomoses required for each organ provides residents the ability to perform open vascular anastomoses. In addition, general surgical procedures such as intra-abdominal and alimentary tract procedures are also performed on transplant patients after their initial transplant surgeries. Especially useful for resident training is the treatment and management of end-stage organ failure and their related surgical procedures, such as fistula creation for renal transplant candidates and hepatobiliary procedures for those with liver disease [6].

Second, the rotation provides ample opportunity for interdisciplinary interactions when treating these patients. As a field that provides care for very complex patients, transplantation virtually requires a multidisciplinary approach to care. This approach to patient management is important to the educational growth of a resident surgeon.

Third, residents develop an understanding of immunology, immunosuppressive drugs and the management of patients who are immunosuppressed [6].

Fourth, residents are able to perform surgeries while on the transplant service that they would not have the opportunity to perform otherwise, such as laparoscopic or open donor nephrectomies. These experiences help make them better prepared to care for the surgical complexities that accompany a patient with any solid organ transplant.

Internal medicine

Unlike their resident colleagues in general surgery, internal medicine residents do not currently have a rotation requirement for solid organ transplants. If required for service needs by the institution, the ACGME limits residents to a maximum of one month of service on transplantation over the course of their three years of residency [2]. Some residency programs might have inpatient services dedicated to specific solid organ transplants, and so might have residents rotate through. Other residency programs might not have dedicated services for transplant patients, and so the residents might only obtain experience of caring for transplant patients through individual transplant patients who are admitted to their team.

Some residency training programs have dedicated organ medical teams, such as gastroenterology with hepatology, renal, pulmonary, and of course cardiology. This is in addition to their general medicine teams. On these subspecialty teams, a variety of patients with organ specific diseases are admitted to the service, including patients with solid organ transplants. On these services, residents can have a significant portion of their census composed of transplant patients. They would be able to focus their attention on the organ transplant patients to improve their knowledge of the pathophysiology, infectious complications, and medication interactions for this population of patients.

For residency programs that divide all patients between general medical teams, residents may have fewer clinical interactions with transplant patients. The lack of exposure may lead to internal medicine graduates who are less comfortable managing these complex patients in their careers. This is not an ideal situation given that the numbers of solid organ transplant patients is increasing, making the likelihood higher for these patients to present as inpatients or

outpatients. This can lead to higher risks for over or under dosing of medications, possible interactions between newly prescribed medications and immunosuppressants that can lead to adverse outcomes in these transplant patients.

There are virtually no formal studies that have investigated the education of internal medicine residents in the care of solid organ transplants specifically. Much of their education on transplantation is likely in the form of didactics presented by transplant faculty. Residents may supplement their education by reading relevant texts and articles presented during the didactics. Internal medicine residents' education on these topics is also dependent on their attendance to scheduled didactics. There has been a previous study evaluating the attendance of residents to didactics and the association with scores on the internal medicine board exam [7]. This single center study found that scheduled mandatory didactics were only attended by an average of 34% of their residents, and improved whenever lunch was provided [7]. However, attendance had no correlation with residents' scores on the American board of internal medicine certification examination [7]. This study underscores the need for improvement in our presentations of didactics, or modification of didactics into a more interactive presentation, similar to a problem-based learning method.

Subspecialists

The ACGME does not mandate transplant rotations for gastroenterology/hepatology, pulmonology, or cardiology fellows [2]. However, each subspecialty does require formal education in their respective fields. This could be though continuity clinics, in-patient consults, or even didactics if the training facility does not perform transplants. Each of these specialties offer dedicated transplant fellowships to train individuals interested in gaining expertise in transplantation for their respective organs. Table 131.2 lists the different subspecialties with required clinical training service months for transplant and the presence of specialized training in transplant.

There has been a movement among subspecialties toward requiring specific transplant certification for physicians seeking special training in transplantation. For instance, after seven years of preparation, the board initiated the transplant hepatology certification examination in 2006 [8,9]. Between 2006–2010, examinees who had prior experience with transplants and liver diseases could take the exam without enrolling in a transplant fellowship. However, beginning in 2011, all applicants for the certification exam must have completed a transplant hepatology fellowship [8,9]. This could potentially lead to some gastroenterologists/hepatologists in the community feeling less comfortable with caring for complex hepatology or transplant cases. This would potentially have the effect of the community gastroenterologists referring all cases to hepatology specialists, which would overload the hepatologists' referral clinics.

Table 131.2. Subspecialties and required transplant rotations and specialized training

Subspecialty	Required transplant rotation during general fellowship training	Specialized fellowship training in transplant
Gastroenterology/hepatology	–	X
Cardiology	–	X
Pulmonology	–	X
Nephrology	X	X

There was a recent task force formed of representatives from several gastroenterology societies who provided recommendations on the training of gastroenterology fellows. They specifically advocated that the general fellowship remain a gastroenterology/hepatology fellowship [10]. They also specifically recommended a modification to certification in transplant, where a fellow would normally complete the general fellowship in three years, then have another three years of practice focusing on transplant alongside a transplant mentor. The first maintenance of certification would be after the three years of transplant work where an individual could re-certify for gastroenterology and have what they define as a focused recognition of transplant hepatology [10].

Cardiology is another subspecialty that has just initiated a new certification examination, with the plan similar to gastroenterology/hepatology. The first examination was just administered in 2010. In 2012 training for advanced heart failure and transplantation at ACGME accredited facilities began. [2]. This training will add another twelve months of training to the already required 36 months of training for those who wish to pursue a career in advanced heart failure and transplantation [2]. A committee was formed and defined the training and expected knowledge and skills of an advanced heart failure and heart transplant specialist. A heart failure/transplant specialist would be able to assist other practitioners who care for patients with less severe heart failure, assist with the advanced care, and cardiac devices that some of these patients require, and assist in the evaluation of patients for candidacy of heart transplantation [11].

The exception to the subspecialties is nephrology, which outlines specific ACGME requirements for transplantation training during a nephrology fellowship. First, ACGME requires that fellows have at least two months of transplant clinical experience on an active transplant service, as well as incorporate procedures such as transplant biopsies into the rotation [2]. This includes the evaluation of both pretransplant patients and living donors. In addition, fellows should have the opportunity to follow at minimum ten new post-operative transplant patients. This provides them the experience of managing their patients' immunosuppressive medications. Fellows should also have at least twenty post-transplant patients who they follow for at least three months in a continuity clinic [2]. Besides the required clinical months of transplantation during general nephrology fellowship training, there is another twelve months of focused clinical transplantation training for those who wish to gain additional instruction.

The rigorous and well defined expectations for nephrology fellows in transplant training are understandable. Kidney transplantation has the longest history, thus renal transplant patients are more abundant in the community, and nephrologists are much more likely to encounter kidney transplant patients in their facilities than are other subspecialists to find transplant patients in theirs. In addition, the general nephrologist is the physician who usually has the longest continuity with their patients, and can follow these patients from their diagnosis of chronic kidney disease, to initiation of dialysis, to transplantation, and at times, back to dialysis in the event of kidney transplant failure. Unlike other specialties whose organ is a life-saving organ, renal patients can survive without kidneys by undergoing dialysis. Thus, it is now becoming more common to have patients who have had more than one kidney transplant in the past. The increasing numbers of transplant patients highlights the need for practitioners with the knowledge and skills necessary to care for these complex patients. It is understandable that subspecialties are moving toward requiring further training for

advanced care for their transplant patient population. Transplant fellowship education is discussed separately.

Nurses

Little data exist regarding the general education of nurses in transplantation. Much of the nursing management for transplant patients occurs during the pre and post-transplant period, where nurses play an integral part in the management of these patients. In addition, the care for these patients immediately postoperatively requires intensive care units in most cases, with the exception of perhaps kidney transplant patients. Several years ago, significant changes to nursing education regarding transplantation and donation were made during a five year long program [12]. Nursing students were educated through the use of pamphlets, booklets, and direct interaction with transplant nurses and other team members. In addition, nursing schools have implemented nursing rotations in transplantation for centers that perform transplants, and included transplant topics on the nursing certifying examination [12].

Pharmacists

Over the last several decades, it has become increasingly evident that specialists, including pharmacists dealing with transplant patients require training beyond the typical training period. Prior to the 1990s, pharmacists who wished to gain further education on transplantation pharmacology usually learned through experience while working [13]. Over time there has been a transition to increased formal training to attain transplantation specialization through a year of general pharmacy residency followed by another year of transplant focused residency [13]. This transition is likely due to the requirement in 2004 by both the United Network for Organ Sharing (UNOS) and Center for Medicare Services (CMS) to have an identified pharmacist within the institution with defined roles and responsibilities as a member of the transplant multidisciplinary team [13].

The importance of transplant pharmacists in the care of transplant patients has been studied previously. Their education and expertise in medications and pharmacotherapy is exceedingly helpful in the inpatient and outpatient setting. Transplant pharmacists are invaluable in patient and family teaching about medications as well as with medication compliance prior to and after discharge [14,15]. In addition, pharmacists provide important recommendations for medication dosing and information on drug-drug interactions to help prevent potential adverse events [15,16].

Medical students

There has been some research evaluating the education of medical students about transplantation. Many of these studies have been surveys on medical students and their knowledge and exposure to transplantation [17,18]. One study conducted in the UK used a questionnaire to evaluate fourth year medical students' knowledge of transplantation. Approximately 54% of the students completed the survey, and of those students, only 42% ever rotated through one of the hospitals that performed transplants [17]. Of the total number of students, only 14% had ever witnessed a transplant surgery [17]. Only 46% of students ever examined a patient with a transplant, while 43% could identify the correct incision and placement of the allografts [17]. Two-thirds of students understood that several types of donors were available for transplanted organs [17]. Only 53% of students could identify at most one immunosuppressant [17].

A similar study was conducted among three different medical schools in Ohio, administered to a total of 537 first and second year students [18]. The authors used a previously validated questionnaire and had a high response rate of 93%. Only 11% of students received any formal education on transplantation and donation prior to medical school, and only 22% ever received education up to that point in medical school training. Of those who completed the survey only 8% had ever cared for a solid organ transplant patient [18].

Another cross-sectional study conducted in Germany evaluated levels of knowledge regarding transplantation and donation among 1645 medical students in their early and later years of education, and among physicians from multiple fields [19]. This study had a response rate of 67% and found that knowledge on transplantation and donation increased with an increasing level of education. In addition, those with a higher education level tended to be more comfortable with approaching families for organ donation. This number, however was only 8% of the total population studied, and among the physicians, only 14% perceived themselves trained adequately to approach families [19].

These studies show that the target stage for introducing the key concepts for transplantation to physicians is throughout the early years of medical school. In addition, the reinforcement of these key concepts throughout residency, and/or fellowship training will help solidify the knowledge and confidence of practitioners in the care of solid organ transplants. As the majority of providers are not transplant specialists, all providers should possess a baseline knowledge and understanding of transplantation to provide the appropriate care or refer to subspecialists who can assist with the care of transplant patients. The following section provides suggestions for education throughout medical training to forge a lasting knowledge of the management of solid organ transplant patients.

Further methods for education and training

This section describes the ways in which transplant concepts can be incorporated into general medical education at several levels of training. Included are education and training in medical school, residency, subspecialties, and continuing medical education. These are suggestions, and are not necessarily implemented throughout institutions that teach individuals who care for transplant patients.

Medical school

The proper integration of solid organ transplant training into general medical education should begin in medical school. Medical students are most curious and eager to learn, thus making these years the most impressionable. There are currently two methods of teaching students during the first two years, either lecture based or the newer problem based learning. Core transplantation concepts as listed in Table 131.3 can be introduced into both systems of learning.

If a school maintains lecture based learning through the major core courses of anatomy, physiology, biochemistry, immunology, pharmacology, ethics, microbiology and pathology, transplant related topics are easily integrated into these courses. For instance, discussions on the locations of allografts can be discussed during anatomy. Pathology and Physiology are the ideal courses to discuss the diseases and end-stage appearance and function of transplantable organs. Longer term outcomes related to malignancies and cardiovascular disease can also be introduced during the pathology and physiology component. The importance and central ideas

Table 131.3. Suggested core transplantation topics for introduction to medical students

Transplant statistics – waiting list and recipient lists
Indications for transplantation
Improvement in quantity and quality of life after transplantation
Recipient evaluation, selection, contraindications for transplant (some organs, donor evaluation)
Donor-recipient allocation
Human leukocyte antigens and histocompatibility matching
Transplant surgical procedure and surgical complications related to transplanted organ
Immunology, types of rejection, pathological diagnosis
Immunosuppressive medication and side-effects
Significant drug-drug interactions with immunosuppressive medications
Infectious complications after transplantation months to years post-transplant (viral, bacterial, fungal)
Long term risks for malignancy and cardiovascular disease in transplant patients
Recurrence rates of disease in the transplanted organ

behind organ transplant immunology, histocompatibility and matching, and rejection should be introduced during the core Immunology course. Subsequently, the concepts behind immunosuppressive medications and their mechanisms of action and side effects can then be discussed in depth in the Pharmacology course. The Microbiology course could be used to introduce the students to concepts in infectious diseases, both common and opportunistic. Lastly, due to the significant ethical issues that arise with organ donation, students can have engaging discussions regarding the ethics surrounding transplantation and tissue donation.

When students attend medical schools that have adopted a problem based curriculum, students can also be effectively introduced to and instructed in solid organ transplantation. An example of how transplant concepts can be incorporated into a problem based learning system, is a case of an end-stage renal disease patient on hemodialysis who comes in to receive a renal transplant. The case can discuss the anatomical position of both the native kidneys as well as the allograft. Following this, the case can discuss the immunology surrounding recognition of self and non-self antigens, as well as types of rejection. The pharmacology, mechanisms of action and significant drug interactions of the various anti-rejection medications can then be discussed. If one wanted to extend the case further, it could continue with the patient presenting several weeks or months later with an infection. The students could then discuss the differential for types of infections, both common and opportunistic, as well as medications and potential interactions with immunosuppressive drugs.

Upon students' introduction to the wards, multiple different services could help educate them in caring for solid organ transplant patients. Whether the students are on a surgery or medical service, or even on the subspecialty services, they can gain further experience in treating and managing solid organ transplant patients. The various aspects of the care for transplant patients can be learned through the management of several patients by the student. The student will be able to read about the history, medications and pathophysiology regarding the transplanted organ and assist with formulating an appropriate plan for managing the patient with the background information that they have learned previously in the first two years of their medical school instruction.

Some clinical service months for students can provide better exposure to transplant patients than others. For instance, the infectious disease consultative service can be regularly requested to evaluate transplant patients with infections to provide guidance on antibiotics. The gastroenterology/hepatology, nephrology, pulmonary, and cardiology services may also have ample consultations on transplant patients. In addition, the dedicated transplant clinics for these subspecialties allow for immersion of students into the care for these patients. Adequate exposure of medical students to solid organ transplant patients will improve their recognition of the need for medication reconciliation and close follow-up, as well as increased vigilance for infection prevention and health maintenance.

Internal medicine

The lack of data regarding the education of internal medicine residents in transplantation topics has already been discussed. Also previously presented is the low numbers of residents attending formal didactics, where much of the formal education is usually presented. Methods for improving didactic attendance, or changes to didactic style can be done to improve the attendance of residents. Usually, formal didactics are presented by faculty in each clinical division, where relevant topics, including transplant didactics, can be discussed. The didactics covering transplantation should provide information on the indications and contraindications for transplantation, advantages of transplantation, basic immunology, human leukocyte antigen (HLA) matching and histocompatibility, immunosuppressive medications, transplant rejection, infectious complications, long term outcomes and management, similar to that presented to medical students, but more in-depth than provided to the students.

The above topics are complex enough to subsume hours of didactic teaching. However, given the time constraints provided for these sessions, focused presentations should be given on the more clinically relevant topics that residents will require to care for solid organ transplant patients. For instance, focused lectures on immunosuppressants and their common interactions and the need for dose adjustments can provide valuable information. In addition, another didactic discussing infectious complications and the differential diagnosis for transplant organ dysfunction, including rejection, should also be discussed.

Several previously identified factors can adversely affect resident attendance. A previous article has provided methods of diagnosing and classifying the etiologies of lectures that are unable to adequately deliver their material to their audiences [20]. Some of these factors include poor judgment in didactic topic, poor choice of lecturer, overestimation or underestimation of audience baseline knowledge, poor organization of the didactic, and poor delivery of the lecture material [20]. Residents continue training for three years, making some didactics repetitive. However, as much of learning tends to be through repetition, a lecturer who presents the same material that has difficulty engaging their audience will eventually have a waning attendance. Given this, another shortcoming that some lecturers and presenters may have is the inability to obtain and adjust their presentations according to audience feedback [20]. Those who give didactics to residents should regularly solicit feedback with the intention of improving the presentation for the following year.

There are emerging methods for improving resident attendance through active participation. An example is to incorporate a mechanism of participation with the use of anonymous audience responses. This allows the residents to absorb information and review their knowledge within the presentation thus reinforcing what was just presented. Residents may also find that this system allows them more freedom to express their thoughts without being

the focus of attention [21]. These response systems can also lead to further questions and discussions by residents who will then become more curious and request clarification or further information [21]. One study that evaluated the utilization of an audience response system found that their audience was much more engaged in the discussion than previously. The audience also had the perception that they comprehended the material more when using the response system [21]. There are caveats to designing questions using the interactive response system. Recommendations for formulating questions are reviewed in several articles [21,22]. Some recommendations include creating questions that are brief and easy to read without overly complex answers, and to use questions for emphasis and discourse [21,22]. One important recommendation is to design the presentation and questions with time enough for discussions throughout the didactic [21,22]. A group in the UK has provided a guide to lecturers who are preparing presentations [23]. Their suggestions include improvements in methods of lecturing, styles of presentations, the skills necessary to present a clear didactic, and uses of audiovisual material and handouts [23].

Educators should also recognize that others may have different preferences for learning. Some residents may learn better through repetitive self-study than through didactics. In these cases, lecturers can post their didactics onto an on-line resource site or folder accessible to residents. These lectures would require updating each time the presentation has occurred to remain current. Others could post recordings of their lectures along with a copy of the slides as an on-line resource. A folder of seminal articles for transplantation can be created to provide residents with additional resources to review independently. On-line interactive tutorials have recently become more common as education tools.

General surgery

General surgery residents' education in transplantation is undergoing fluctuations with the potential changes in ACGME requirements. A rotation in transplant may no longer be required and some residents might not have an opportunity to rotate through a transplant service. Other residents may have an abridged experience on the transplant service rather than the typical several week rotations.

There have been concerns over the education of general surgery residents. More procedures that were previously thought to be under the auspices of general surgery residency training are slowly becoming part of the fellowship training realm. For instance, some general surgeries such as cholecystectomies and appendectomies are becoming surgeries that fellows in a minimally invasive gastrointestinal surgery fellowship increasingly perform. This is unfortunately a result of several factors. Many additional fellowships that are based in general surgery (e.g. minimally invasive gastrointestinal surgery, breast surgery, surgical oncology, transplantation, trauma) have become increasingly common [24]. Due to an increased number of fellows performing surgeries, more residents feel that they require specialized training. In fact approximately 70% of residents seek subspecialty training in surgery after completion of their general surgery residencies [24].

The number of logged procedures for general surgery residents has been closely followed. One study looked at the number of procedures that were completed by graduating residents that program directors believed were most important [25]. In this survey of 254 residency program directors, the response rate was 45%. The authors found that of the 121 surgical procedures considered core procedures requiring competency by the end of their training, residents performed 68% of them less than five times during their residency [25]. Due to concerns over the education and training of surgery residents, a committee composed of several key stakeholders was formed in 2002 to address concerns and offer solutions [26]. After this, they formed a consortium called the Surgical Council on Resident Education (SCORE) whose purpose is to continue to improve residency education in general surgery [26,27]. SCORE members include representatives from the American Board of Surgery, American College of Surgeons, American Surgical Association, Association of Program Directors in Surgery, Association for Surgical Education, the Residency Review Committee for Surgery of the ACGME, and the Society of American Gastrointestinal and Endoscopic Surgeons [27]. Since their formation, SCORE has been making recommendations to improve the education of surgery residents through the creation of a national curriculum for educating trainees [27].

Key recommendations by SCORE regarding transplant education for general surgery residents include an understanding of the indications for solid organ transplantation, determination of brain death, organ preservation and donor selection, immunosuppressive medications, post-transplant surgical and medical complications including malignancies and infections [27]. The operations associated with transplantation including donor procurement, donor nephrectomies, and combined organ transplants are not required for residents to be proficient, though an exposure to these procedures is recommended [27]. As changes are occurring, key learning points should be maintained throughout the programs. The recommended learning goals presented by SCORE are reasonable to maintain for all programs, as many can be presented through didactics that all surgery residents attend. Other goals such as the surgical rotation/experience should be provided for all residents prior to the completion of residency. If a full rotation cannot be achieved, an abridged rotation could be developed for trainees. It would be preferable to have residents also experience a minimal number of transplant surgeries (procurement and implantation) prior to their graduation. However, a minimum quota may not be achievable in all instances given the unpredictability of transplant donor availability. Possible avenues to help residents learn about transplant-related surgeries are through simulators that can provide a similar scenario for a transplant surgery. An understanding of the anatomical position and possible surgical complications will be important educational points about which residents should be aware. As more patients undergo transplantation surgeries, it becomes more likely that these patients will present to hospitals where resident graduates are employed. The recognition and understanding of both the surgical and medical complications of transplantation will allow these residents to provide timely and appropriate care to transplant patients.

Subspecialists

Subspecialists are in a unique position to care for patients with transplants. They are provided with the specialized education in their respective specialties, and in many cases may be the first consultant contacted regarding transplant patients who present to primary care offices or urgent/emergency care facilities. Thus, subspecialists should have baseline knowledge of transplantation, complications, and be able to provide recommendations. All subspecialists require an education component to their fellowships regarding transplantation. Didactics can be minimal (few lectures) to extensive (several weeks) depending on the training program. Similar to the student presentations (Table 131.3), the lecture series

Table 131.4. Numbers of subspecialty fellowships and transplant fellowships and transplant patients. Data regarding fellowships were obtained from the Accreditation Council for Graduate Medical Education (ACGME) and American Society of Transplantation (AST) websites [2,28]

Subspecialty	General training fellowship	Transplant fellowship	Transplanted patients^
Cardiology	168	Undergoing accreditation	52 658
Gastroenterology	157	51 (36 ACGME certified)	113 962
Nephrology	146	53 (AST accredited)	331 765
Pulmonary/critical care	133	N/A	24 035

^Numbers of patients transplanted since 1988 per OPTN data [1].

given to subspecialists should cover all relevant topics with one exception. This syllabus should be covered in-depth compared to what medical students receive. Each topic should be presented separately to allow the lecturer time for extensive discussion, providing the most information and data. The reason for expanding on each topic presented is illustrated in Table 131.4. The table lists various subspecialties, the number of general training fellowships, transplant fellowship, and transplanted patients for each organ since 1988. Transplanted patients increase by approximately 28 000 per year [1]. Similar to the situation in which transplant surgeons are limited compared to general surgeons, the number of medicine subspecialists trained in transplantation is not substantial, while the number of transplant patients is substantial. Subspecialists need the education and knowledge required to make preliminary recommendations and refer patients to transplant centers if needed.

If possible, general subspecialty medicine fellows should be provided at least some exposure to transplanted patients through their fellowship. This could be through continuity clinics or inpatient consultations. Although lectures are extremely informative, providing care directly to transplant patients is an invaluable experience. Through direct patient interactions, fellows can systematically go through the various items necessary to care for transplant patients including transplant function, immunosuppressive medications and interactions, inquiring about infectious and cardiovascular problems, and reminders on health maintenance.

Continuing medical education

Care providers of solid organ transplant recipients require continued education to maintain and update their knowledge of transplant patient management. Continuing medical education in transplantation for subspecialists in gastroenterology, pulmonology, nephrology, and cardiology is a regularly occurring event at yearly national meetings. These sessions are presented by transplant specialists within the field who provide updated information for their fellow subspecialists. Some transplant groups also provide information sessions to their colleagues in the community through regional meetings and clinic outreach programs.

The continuing medical education of general internal medicine physicians and hospitalists is not as robust at national meetings. Thus it becomes the responsibility of the internal medicine physician to independently seek updated information regarding transplantation and the management of solid organ transplant patients. Some physicians can obtain continued education through their respective hospitals where transplant specialists present at grand rounds. Others may seek to attend specialized conferences given by other national societies. Transplant specialists can offer to provide a review and update to general internists within their hospitals and at national meetings.

Summary

Solid organ transplantation is a field in which the patient population is growing steadily. Advances in immunosuppressants and management post-transplant have led to increasing survival among transplant recipients. The care providers for these patients require a baseline amount of knowledge and experience with patients to adequately care for this patient group. Education for the care of transplant patients begins in medical school, and should continue throughout the training of general surgery residents, internal medicine residents and subspecialty fellows. The use of new methods of teaching including interactive didactics and audience response systems, as well as simulators will help further educate care providers. Transplantation is a field that continuously evolves over time, requiring those who provide medical care to solid organ transplant patients to also maintain their knowledge through regular updates in education and national meetings. Transplant patients will benefit the most when all their care providers continue their education in transplant management.

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SECTION 10

Clinical Trials and Data Management

CHAPTER 132

Building and Sustaining a Local Transplant Data Management Program

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"Without data, you are just another person with an opinion."
Anonymous

Introduction

Few fields of medicine are as data driven as transplantation. This extends beyond the reporting requirements imposed by federal regulations to include acquiring highly granular data relevant to an individual patient's day-to-day needs, and the centers ability to deliver them. Accordingly, a sound local data management program is instrumental in the success of any transplant program. Indeed, an integrative and sustainable one can be transformative. Through the application of local data management strategies and practices, a transplant program can:

- Track and perform trending analysis on cohorts of patients on their post-transplant graft and survival rates, drawing comparisons with national baseline and outcome data.
- Interrogate an integrated collection of outcome, research, clinical, national, and reference data. Through data mining and analysis techniques, patient characteristics and interventions can be investigated, modeled, and analyzed to improve standard care practices and facilitate discovery opportunities.
- Establish an early warning notification system to consistently monitor trends and when the trends move in an undesired direction, facilitate proactive interventions. This system could operate at both an individual patient level or across the entire transplant program.
- Track and present outcome data to practitioners to identify variations in standard of care practices and reinforce positive outcomes.
- Measure and evaluate key performance indicators (KPIs) with stoplight (red, yellow, green) symbols to quickly ascertain how the transplant program is performing based on its objectives and goals. These KPIs can range from tracking patient waiting times to meeting fiscal goals to trends in laboratory values.
- Deliver tracking reports and checklists highly customized to facilitate decision-making and enable a standard set of practices for clinical care and scientific inquiries. Drawn from the local data store, these reports can be formatted to prioritize specific data points and dynamically generated with logic to calculate data based on if-then scenarios (e.g., if creatinine levels in renal post-transplant recipients are 115% above baseline, then the measurement is considered functionally significant and may indicate acute rejection of the transplanted organ [1]).
- Perform population counts based on a wide range of variables to determine potential subject pool and evaluate feasibility for clinical trials recruitment opportunities.

For this discussion, local data management is defined as the strategies, principles, and ongoing procedures necessary for a transplant program to manage the various data requirements and sources in a unified approach. It is distinct from, but at the same time integral to, national data which is typically held in centralized registries. National data management will be discussed in Chapter 133. The successful local data management program delivers a framework that facilitates access to the appropriate data at the appropriate time to make informed and effective decisions about the clinical care, research, education, quality outcomes, personnel, investments and operations of the Transplant program.

This chapter introduces the Transplant practitioner to key concepts associated with local data management programs. It describes a process for building and maintaining a data management program as well as guidance and recommendations on important data management principles.

Case study

The following case study contextualizes the value of a local data management program. Performed at Emory University's Transplant Center, weekly tracking conferences and post-transplant monitoring reports were implemented to improve adherence to treatment pathways for renal transplant recipients and to identify patients at risk. The weekly tracking conferences took place in the outpatient transplant clinic, during which time post-transplant coordinators and physicians reviewed patient charts at set intervals, including one, three, six, nine and twelve months post-transplant. The reports were created to provide a centralized view of patient data for use during weekly tracking conferences.

Prior to creation of the post-transplant tracking reports, the follow-up review of patients was complex and labor intensive. The volume of new patients tracked was approximately 160 per year. Data acquisition from the Organ Transplant Tracking Record

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

(OTTR), Emory's system of record for transplant patient care, took approximately two hours per patient, leading to approximately four hours of weekly overtime per transplant coordinator. With the data decentralized, eight patients could be reviewed per hour during conference. In an effort to increase the number of patients reviewed during conference, a tracking worksheet was implemented, completed by the transplant coordinators, and used for patient review. The time required by coordinators to gather and record the data in the worksheet was further extended, but it served as a template for the tracking reports.

While the tracking worksheets organized patient data in a readily reviewable format, a more efficient methodology for assembling and presenting data was required to increase throughput for patient reviews. This led to the development of an automated data reporting tool, a collection of post-transplant tracking reports customized for the one, three, six, nine and twelve month review periods. The reports pulled data from the OTTR system and could be run at any time by any approved practitioner from the OTTR user interface. The reports expanded the focus of the tracking conferences to include adherence to pathways, identifying patients at risk and referring physician communication. They reduced labor required for data acquisition from two hours to ten minutes per patient, and coordinator overtime was eliminated. The post-transplant tracking reports provided a centralized view of all of the data points and could be run on demand for specific cohorts of patients. Conference capacity increased from eight to twenty patients per hour.

A key benefit of the reports was better protocol adherence, which, in turn, led to a statistically significant improvement in graft survival rates among renal transplant recipients within the transplant center. Other observed benefits included: BK virus protocol testing adherence increased from 50% to 90%; compliance with protocol immunosuppressive target levels was facilitated; and referral physician letter communication improved. The project was approximately six months in duration and required a single data analyst working with multiple subject matter experts. Leadership concluded the investment was well worth the effort and initiated several additional local data management initiatives.

Challenges and scope

Transplant local data management programs face many challenges. Within a transplant program, critical clinical data can reside in a variety of systems across multiple geographic locations and organizations, including:

- Complex infrastructure of healthcare systems that are used to manage a range of clinical information related to imaging, prescriptions, laboratory, surgery, scheduling, billing and others.
- Distinct, and non-connected healthcare information systems managed by multiple institutions in which a transplant program may operate (e.g., Children's Hospital, Geriatrics Hospital, Veteran's Affairs, etc.).

Add the complexity of operating a large clinical and basic science research enterprise and another layer of systems data sources and management challenges are introduced, including:

- Numerous and sundry disease- and study-specific databases created in different, sometime incompatible, technologies and formats using varying terminologies, values, and units of measurements based on the sponsor or investigator's direction.
- Instrument-specific consoles in the research laboratory that are highly customized to a specific vendor solution and may require proprietary programming to extract the data in an automated fashion.
- Spreadsheets and documents stored on local file servers and on workstations in personal directories organized by individual preference and retrieved through one's memory of where the latest version is stored.
- Handwritten notes meticulously documented sequentially in notebooks and stored on shelves or in file cabinets.

At a high level, the challenge becomes bringing the right data together from this landscape of systems and records to inform decision-making. The process, subject matter expertise, technologies, and the time and effort to integrate data systems is significant and resource intensive. It involves lobbying for changes in institutional systems to enable the collection of and access to specific sets of data, reconciling differences in values in data fields across multiple data sources or systems, and, almost certainly, introducing changes to the processes and policies within the transplant program itself. As such, establishing and sustaining an integrative local data management program should be approached through a methodical process guided by multiple levels of leadership. Done correctly, the data management program will be a continuous process of evolution, growing as the transplant program itself grows, looking at new ways of measuring its own performance, exploring new research for discovery, and evaluating new pathways for improving care.

There are no prefabricated or "out-of-the-box" solutions for a local data management program that can easily be bought and installed. The local data management program is heavily dependent on the principles and objectives of the transplant program as well as the culture and technology environment of the institution. However, there are core components, principles, and concepts that, though they may be implemented with different technologies and techniques, are applicable for most institutions.

Figure 132.1 visualizes the high-level concept of a transplant local data management solution. On the left side, there are multiple data sources of interest. They could include local databases created by investigators conducting clinical trials or laboratory tissue banks; institutional systems, such as the electronic health record that tracks and manages the care for all hospital inpatients, including transplant patients; and national data sources, such as the United States' UNet, an online database system storing and analyzing all Organ Procurement and Transplantation Network (OTPN) data related to waiting list, organ matching and transplantation [2].

Subsets or the entire set of the data can be integrated into a transplant program specific repository to provide a consolidated source for reporting and analysis. There are various ways to accomplish this. The repository might be a physical database managed and operated by the transplant program or it could leverage existing data infrastructure at the institution-level. The data might be physically moved to a separate repository, linked to an existing repository, federated through middleware, or some combination thereof. The final solution will depend on the information technology direction and infrastructure. However, there must be a location, either physical or virtual, where the transplant program can access an integrated set of data designed and optimized to meet the transplant program needs.

Utilizing data from the transplant program repository, reports, dashboards, analytics, and other tools facilitate the display and analysis of data to facilitate decision-making.

Supporting and shaping this solution are the management processes and structures that ensure the solution is meeting the needs of the transplant program, guide the day-to-day operations of the

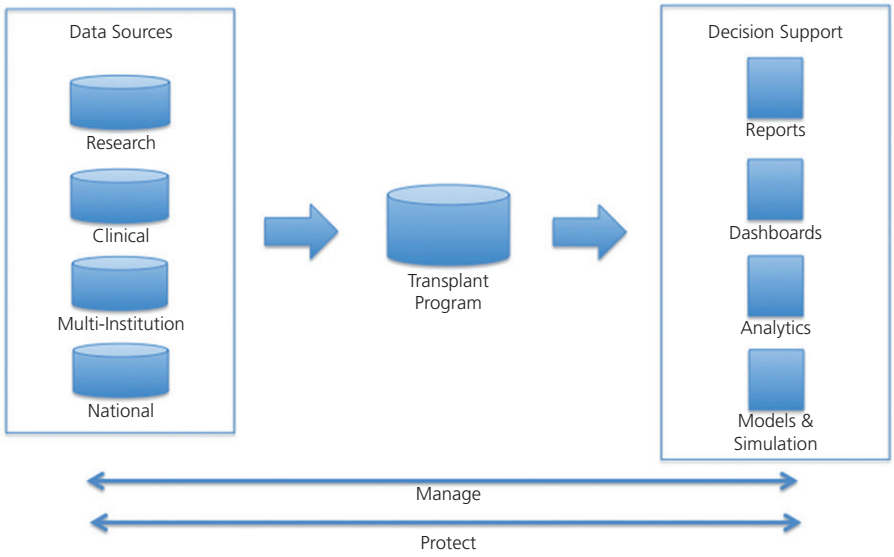

Figure 132.1. High-level data management solution.

program, and map out and continuously refine a strategic direction. Finally, there are controls in place to protect the confidentiality, integrity, and availability of the data.

Process

It is rare that a program has the opportunity to start its data management program from a blank slate. Rather, there is a dependency on institutional systems, national data sets, and a variety of existing local data repositories. Moreover, there are business processes and functional needs that are already in place and met, in one fashion or another, through the use of data. One can employ strategies to leverage, expand, and improve upon these existing infrastructure, technology and practices to develop a local data management program that remains flexible for future growth. This section describes the process that can be used to evaluate existing data management practices and plan strategically for a more effective data management program. The local data management program should support the organization by moving it closer to achieving its goals. In turn, the organizational goals will define the objectives for the data management program.

The steps involved in developing a local data management program are:

- Plan. Understand the current state of the program, specifically eliciting input from the clinical team at all levels, determine the future state, and prioritize the path and sequence to move from current to future state.
- Design. Based on the priorities and sequence defined in the plan, the design will usually include multiple phases of implementation allowing an iterative approach along the path from current state to desired future state. Iterative approaches provide more flexibility as implementation proceeds and allow for changes as new needs are discovered or priorities are adjusted. In general the design phase will collect requirements, analyze different options, and set forth the necessary specifications to create a solution.
- Deploy. Implement the design as specified, ensuring appropriate testing is conducted and incorporation into the support structure. From the local data management perspective, data governance and active engagement with the Transplant community should continue.
- Evaluate. Assess the program by evaluating it based on metrics, aligned with the Transplant Program's goals and objectives. Any gaps should be incorporated into the next phase of planning.

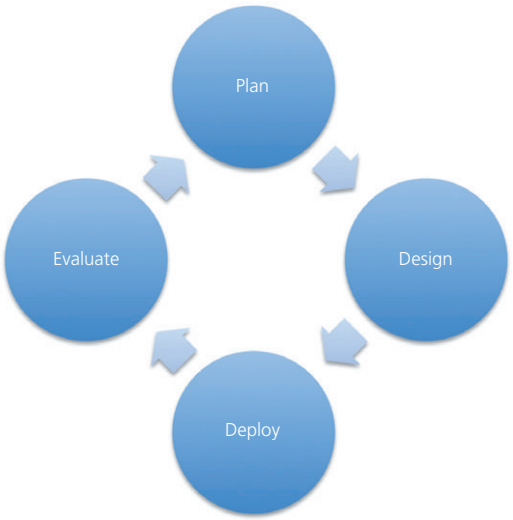

Figure 132.2. Local data management process.

The local data management process is cyclical, as depicted in Figure 132.2. Importantly, it cannot be created without buy in from the clinical (and if applicable, research) staff, the institution, and a dedicated data management design team actively collaborating with one another.

Plan

The planning stage involves evaluating the current state of the environment, building a vision for the future state, and prioritizing the activities that need to take place in moving from current to future

state. The mission, objectives, and key principles must drive the future state of the transplant program. For example, a key principle that "every patient is a potential research subject, and every research subject is a potential patient" would drive a series of fundamental design principles within the core local data management program. While this concept may seem simple on the surface, the complexity introduced by regulatory constraints on the use of patient clinical data for research and integration of these data with data sources designated as research-only makes the task complex. The creation of a common core data set for all patients for quality metrics and outcomes management that also serves as the core research record for patients can facilitate management of information across both research and clinical domains. Use of this core record with extensions for specific clinical specialties, clinical trials and research studies could be an approach for fulfilling the design requirement based on the key principle above. In addition to these key principles, understanding how the transplant program will evaluate its own progress should, in turn, focus the local data management program on the associated data variables and sources to facilitate the analysis and predictive modeling of the metrics.

Together with key principles and performance metrics, the future state should be driven by the business (or practice), not by the technology. To facilitate this process, there are a number of methodologies for documenting current state and sustaining the development of successive future state iterations. One overarching methodology is Enterprise Architecture. An enterprise architecture is an overarching description of the goals, business entities, processes, relationships, information, and systems of an organization or business. There are many enterprise architecture strategies and frameworks, and in many cases, sub-methodologies from multiple frameworks may be used depending on the scope and focus of the project. Emory University has implemented a business architecture modeling methodology in lieu of a full enterprise architecture approach. Our business architecture modeling approach focuses on business processes and the relationship of those processes to systems and data.

In a nutshell, the business architecture model process documents the current operating environment through a series of diagrams, workflow analyses, business scenarios, and system diagrams. Business functions are mapped to their supporting business workflows, which, in turn, are mapped to their supporting information systems and data repositories. Through analysis work, the future state can be derived by understanding business functions and ensuring alignment at the top levels first, then optimizing the business processes and evaluating the supporting technology and data. This approach attempts to minimize the practice of building a data management program in isolation of the business it should be supporting and avoids building the program on inefficient business processes and practices.

Success with this particular methodology depends heavily on working closely with subject matter experts and leadership. As the current state is being defined, a number of broad and detailed diagrams are created. A business function, such as research, is documented with details about the key roles, major processes that enable the function, technology domains, and types of data. For an example, please see Figure 132.3.

Working from this diagram, a deeper level of analysis is performed on the current state processes, documenting what role performs which major step in what sequence and what data types are stored in what system. A frequent way to represent these activities is through a swim lane diagram, an example of which is Figure 132.4. Based on the complexity or issues associated with a particular process, additional levels of detail might be documented. The workflow documents should be simple and clear. In conjunction with the workflow documents, supporting narrative in a form of a business scenario can help to provide more context and details to

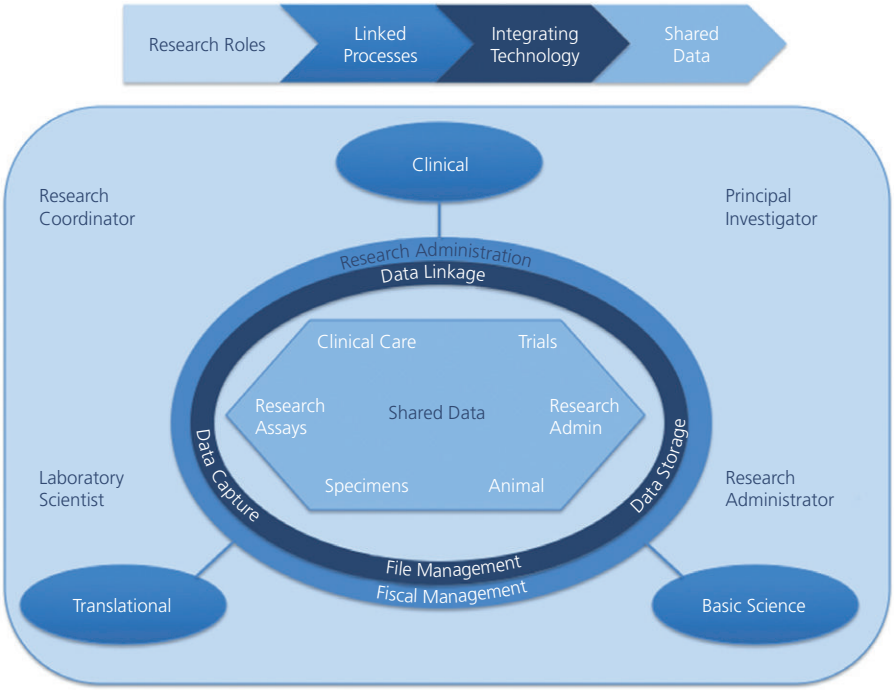

Figure 132.3. Example of a business function diagram (Emory's Transplant Center).

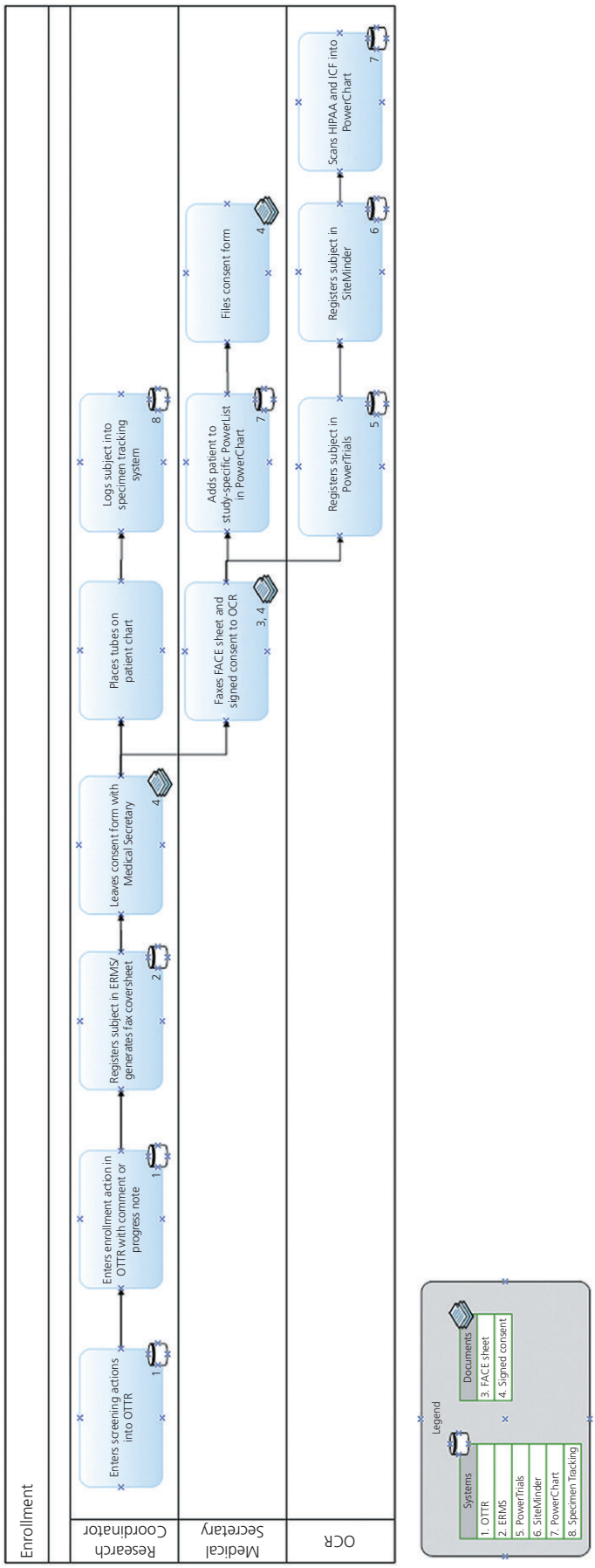

Figure 132.4. Example of a swim lane diagram (current state enrollment process).

the workflows. This current state documentation provides opportunities to understand and analyze how work is currently being conducted in addition to pain points expressed from the subject matter experts and the individuals who perform the work on a daily basis.

From the analysis work on the current state, one can start to build the picture for the future state. By looking at opportunities to optimize processes, reducing the number of duplicative systems storing similar data, developing solutions to resolve the expressed problems, and determining gaps in capacities or functions, the future state will start to emerge. To assist in the analysis, there are business architecture process applications that will model the impact changes in the current process could have on resources, time to completion, and other metrics; however, the analysis can be performed without such an application. The end goal of a future state document is defining goals and capacities for the data management program with the understanding that the future state will continue to evolve and be refined.

With an understanding of the current environment and the target goals of the program, the next step is to develop a transition plan, which defines intermediate steps for achieving the future state. Once the current state analysis is complete, there will probably be a number of quick wins that are self-evident and easy to achieve. As these analyses can take some time and effort, showing progress by completing quick wins can help keep the momentum going and build support for the overall effort. The next step is to develop a list of projects that need to be accomplished, and for each project, establish an estimated level of effort, resource requirements, and any dependencies (i.e., a specific project needs to be accomplished before another project can commence). The list can then be taken to the appropriate governance body (see discussion of governance in the Deploy section) for prioritization and leadership for ultimate approval.

In addition to the planning process, there are a number of planning principles that the transplant program should consider:

- Major authoritative sources of data, such as a clinical data warehouse, should be an early target for integration. These institutional level sources typically have specific policies and required controls that influence the overall design and operations of the data management program. In general, it is usually more cost effective to build these into the program earlier than retrofitting them after major components have been built. In addition, data warehouses are systems built specifically for integrating data across multiple systems. As such, with the appropriate permissions, access into a data warehouse might provide additional data from multiple systems, and a path to gain more data as they are integrated into the institution's data warehouse.
- As soon as possible, establish an identifier strategy for your data management program. As data is integrated from different sources, it is critical that each distinct entity (i.e., person, specimen, etc.) has an identifier that is consistently and persistently unique. It is common for multiple systems across clinical care enterprise to store different medical record numbers or other identifiers. In many cases there is a master patient index that binds all system identifiers to an enterprise-wide identifier. The master patient index number can be used to link data from multiple clinical care systems together for data analysis. However, linking data across research data sources is a much larger challenge as these data sources may be a local study spreadsheet developed by an investigator or a stand-alone database with study or function-specific identifiers. With research data sources it is important to develop a strategy for linking a research subject across multiple sources early in the design of the research systems and the overall data management plan. In addition to linking across research sources, a link must be maintained to clinical records to enable the fusion of data sourced from research and clinical care systems. A sound identifier strategy enables the linkage of data across these multiple data sets so that a person's clinical data stored in an electronic health record can be paired with their specimen data stored in a separate and distinct laboratory information management system.
- As feasible, establish standards for information systems and their supporting data repositories. Through standardization, templates can often be built to facilitate consistency in terminology and values as well as controls for data quality assurance and privacy.
- Be certain to leverage existing institutional resources whenever possible. As mentioned in the first bullet, access to a data warehouse will provide a wealth of data. Partnering with these institutional teams and resources will also provide different perspectives, assist with the strategic planning, and present opportunities to reuse infrastructure and tools.

Design

Whether it is for establishing a core component of the data management program or adding a new data set, there are certain data management concepts and principles that should be investigated. These principles include: evaluating the data sources, capturing data discretely, preparing data for integration, establishing quality controls, assuring the data use complies with regulations and policies, and protecting the data.

Scoping data management solutions

While planning may yield broad strategic data management objectives, there may be constraints with limited funding, resources or infrastructure. As such, there may be the need for an interim solution while waiting for the constraints to be removed so that the longer-term solution may be achieved.

Though not always ideal, interim solutions have some advantages. They provide short-term value and quick return on investment, especially when implementations of more strategic, longer-term based solutions are held up due to infrastructure requirements or policy issues. Interim solutions often provide a basis for gathering requirements for long-term strategies. For example, an interim solution may be an isolated database with a limited dataset and a small number of reports that support a very specific scenario or research question. This prototype database may contain information that is applicable to other scenarios and may facilitate the design of a data model that is flexible and scalable for broader use. Finally, interim solutions may help establish building blocks, design strategies or satisfy contingencies for implementation of more complex systems. However, interim solutions do have a tendency to become permanent solutions without the appropriate governance and willpower to make changes later in the future. So, when selecting whether to move forward with a "less than optimal" solution, be certain to weigh the benefits and limitations of each approach.

Regardless of scope, many of the design principles described in the sections that follow are applicable to data management implemented on any scale.

Evaluating data sources

Effective local data management will make use of a variety of data sources yet each data source may not have equal value to the transplant program. Origin, purpose, and format of data are significant factors when considering the value of a data set. Origin and purpose are usually considered together when evaluating a data source as these factors will determine the context in which the data was captured and if it requires some transformation, abstraction or interpretation to be most useful. The most important data formatting considerations include whether the data is encoded as discrete fields with explicit or unambiguous meaning or consists of mostly blocks of narrative text that must be abstracted.

Capturing data discretely

In an ideal world, all information collected during clinical care could be discretely captured and easily analyzed. In actuality, clinical care is still very narrative based (e.g. physician notes) and the corresponding data reflects that lack of structure. As such, strategies must be taken to either: (1) capture additional discrete elements during clinical care, or (2) manually or programmatically abstract discrete information from the narrative text.

Medical record abstraction

A common, often used method for capturing clinical data is through manual medical record abstraction. Abstraction allows for the synthesis of the complete record in order to answer the specific question at hand for a researcher or clinician. Medical record abstraction is often considered the gold standard when comparing other methods of data extraction from medical records. Electronic data collection tools [3] can be used to increase the efficiency and integrity of clinically abstracted data, but the process of medical record abstraction is often an expensive and time-consuming endeavor. In addition, medical record abstraction has the same risks as any secondary-use data capture when the abstracter must interpret notes and variables captured in a setting not within their control or knowledge.

Combining medical record abstraction with extraction of data from sources with discrete elements can significantly enhance manual abstraction and minimize data entry burden. An analyst can pull discrete variables that are easily extracted from the medical records, such as vital signs and lab values, allowing the abstracter to focus on collecting variables that are not readily available through extraction. This hybrid approach proves valuable when collecting large sets of data where data extraction cannot provide all variables needed for a data set.

Clinical workflow data capture

Based on the guiding principles of the program, such as "every patient is also considered a potential research subject", a local data management program can shape its data acquisition strategy to capture data for both clinical care and research purposes. Using the normal clinical care workflow, variables intended for research can be collected within the Medical Record using tools such as nursing flow sheets and physician templated notes. The variables should have either distinct choices or discrete measurements. Free text fields should be avoided.

It is important to minimize the burden of data collection while maximizing compliance of data entry when determining what variables can be collected during clinical care and where in the workflow they can be collected. Questions that should be considered are:

- How accessible is the variable during the stage of the workflow? Variables such as cold ischemia time should be collected during or soon after surgery, not during the patient discharge.
- Is the information inherently discrete but currently collected in non-discrete form? Variables that can be easily made discrete are good candidates for data capture.
- Is the variable novel to a particular study or does it have application to multiple questions or projects? The number of variables collected may quickly become unmanageable if care is not given to general usefulness of variables.

Natural language processing

An additional strategy for discrete data capture worth mentioning is the practice of natural language processing (NLP). NLP attempts to extract meaning from narrative texts by processing the text through a computer system of complex algorithms. In the context of medical text, NLP systems will often map the text with one or multiple medical terminologies [4]. Interest in NLP is on the rise due to the improving precision of NLP systems [5]. Because much of EMR information exists in non-discrete narrative form, NLP's future as a more concise, accurate information source is promising in both the clinical and research arenas.

Data sources

For the purposes of this text, data sources that might be used in a local data management program will be divided into two categories, secondary and primary. Data for which the principal use is for direct clinical care or administrative objectives, but could be reused for non-clinical care purposes are described as a secondary data source. Data collected specifically for non-clinical care purposes are primary data sources. The distinction between secondary and primary use is an important one. Data collected in the clinical setting are readily available but may be inaccurate or have meaning not useful in the healthcare quality measurement and research settings. Data collected specifically for a specific purpose such as quality metrics or research are generally more accurate and potentially useful because, in most cases, specific data collection systems or tools are created to suit the purpose. However, collecting data in a clinical setting with tools or systems that are not integrated into the clinical system workflow can be cost and time prohibitive. An effective data management program will make use of both sources of data.

Secondary data sources

There is a wealth of information to be found in administrative and clinical data collected during the normal care of a patient. These data will often be loaded into a data warehouse or other data repositories for easier extraction and analysis.

Administrative data

Administrative data from hospital and provider billing systems are by far the most widely available. Health system payers rely on standardized diagnosis and procedure codes to process billing reimbursement, so as a result, administrative data can be compared across institutions. ICD codes in particular are used in one form or another by most clinical care facilities throughout the world. In addition, billing codes have been used for decades and thus provide

a large longitudinal resource. Unfortunately, research has found that administrative data are limited in providing accurate clinical information due to coding errors [6] and limited diagnostic and prognostic detail of the codes used [7].

Re-purposing administrative data is particularly problematic when attempting to infer a result from missing administrative information. Administrative codes are usually recorded for the purpose of billing reimbursement. A clinically relevant condition may not be included because it is unnecessary for justifying a procedure or visit. For example, a patient with Stage Four kidney disease may never have a Stage Four ICD code recorded for a visit if that code is not needed for administrative purposes. While the limitations of administrative data must be considered when it is used in analysis, this large, accessible body of coded data can be very valuable. The most effective uses of administrative data are in instances when absolute accuracy is not important, such as summary data or in initial identification of a patient population. When a transplant program extrapolates detailed clinical meaning such as co-morbidities, disease severity and complications, it is best to use other data sources.

Electronic medical record data

Data captured for clinical care from an Electronic Medical Record System (EMR) provides a wide array of both discrete and narrative elements. The EMR's primary purpose is to collect data around clinical care and is therefore an ideal source for local data management needs. Modules of an EMR of interest include orders, nursing documentation, medication information, lab and pathology information, computer-assisted physician documentation and results from medical devices. Availability and format of these sources will vary by institution, but recognition of the value of these data is driving increased investment in these areas. A transplant program has much to glean from data captured for clinical care, but as with administrative data, re-purposing clinical data will require careful attention to the original meaning of the variables and purpose of collection. A PRN medication order, for example, may only indicate that a medication was available to a patient, but will not indicate whether that medication was administered. Because of these potential misconstructions, it is important to always include a subject matter expert when interpreting EMR information.

Primary sources of data

Capturing data directly for local data management is a strategy for compiling accurate information without some of the complications associated with re-purposed clinical data. Data may be captured in a variety of settings but differs from secondary-use datasets in that one of the intended uses of the dataset is for research and quality reporting. Primary use data capture has a clear advantage over secondary use data capture because additional control over the quality, meaning and context of the variables is typically implemented. For instance, a variable such as "history of smoking" may be available in medical records, but:

- the precision — was the question asked the same way each time?
- the meaning — smoked in the last year? smoked more than a pack a week?
- or the context — was the question asked when family members were present?

of the variable captured may be unknown or unclear. With primary use data capture, specific protocols can be defined around the collection, definition and reporting of variables.

Local databases

A common method of managing pertinent data for a transplant program is to create and maintain a database separate from a data warehouse or other data repository. This practice is very popular among investigators because it can be directly managed and requires little infrastructure. The data is often stored in a Microsoft Excel spreadsheet or Access database. There are multiple ways a local database can be populated, but often the data is manually abstracted or periodically extracted from a larger data repository such as a data warehouse. A local database can befall an unpleasant demise if not managed vigilantly. An investigator managing a local database needs to pay attention to data integrity issues such as:

- accuracy — is the variable coded exactly as the definition warrants?
- data validity — is the value correct and verifiable?
- and, consistency — is a value for the variable recorded the same way each time?

The investigator should also avoid free-text fields when possible. Other issues to consider are:

- versioning — which version of the database is current?
- temporal changes — how do you manage changes in demographics and medical history?
- changes in variables — will a modification in a variable definition orphan historical data?

A local database will require substantial effort on the part of the investigator to create and maintain but if information technology infrastructure is limited, a local database with the appropriate security controls in place may be an effective method of local data management.

Multi-institution registries

There are multiple transplant registries currently in existence. Participation in national registries may be mandated by government entities or could be driven by projects in a transplant program. Data maintained in registries are often strictly defined and curated. Information from registries is valuable to transplant programs because it allows for comparison of key performance indicators and practices to peer programs. Also, because of the reliability of the data collected in a registry, transplant programs can often get detailed information on their internal populations. In the US, some key registries that will provide data to investigators are the Scientific Registry of Transplant Recipients (SRTR), the United Network of Organ Sharing (UNOS) and the United States Renal Data System (USRDS).

Electronic case report forms

Clinical trial information is often captured through a Case Report Form (CRF). Information collected in CRFs consists of observations collected during clinical trials, which, in many cases, may be transcribed or abstracted from clinical care systems. Historically, CRFs have been paper-based, but with the proliferation of web-based electronic data capture tools, CRF data are increasingly being captured directly or indirectly through electronic CRFs (eCRFs). The use of web-based data collection tools over paper-based collection provides multiple benefits to the researcher in cost, efficiency, data integrity and security [8].

Biorepository data systems

Biorepositories are used to store specimens and their associated clinical information for a specific population that will be used in future research studies. A Laboratory Information Management System (LIMS) supports biorepository operations through

management of information related to specimen collection, processing, storage, retrieval, and distribution. Most LIMS solutions also provide audit tracking, electronic signature, and barcode labeling and scanning as well as workflow automation and enable a more efficient and secure means of tracking specimen information than log books and spreadsheets. LIMS functionality is optimized for management of specimen data, thus most LIMS solutions are not designed for collection of patient clinical annotations. Rather, a biorepository providing a mechanism to link the specimen data in a LIMS to the clinicopathological profile of the specimen from one or more other data sources is a more scalable approach.

Research laboratory data

Laboratory assays such as flow cytometry, image analysis, and gene expression profiling produce large amounts of data. A data management program that attempts to use the results from these instruments must consider how to effectively store, analyze, and archive these data. In addition, specimens and assay results are less meaningful without also understanding the clinical profiles of the patients from which the specimens are collected. Furthermore, for most longitudinal specimen collections, the clinical information linked to a specimen is a snapshot at the time of collection and specimens must be matched temporally to clinical information. Keeping this link between specimen and patient while maintaining anonymity when appropriate requires a well-designed workflow, incorporating clinical care, specimen collection and specimen analysis.

Preparing the data for integration

Data integration involves the retrieval and combining of data from multiple sources. When integrating heterogeneous data sources or joining small data sets with a larger data source, controls should be in place to ensure that the data is valid, consistent, accurate and complete. Data quality problems are often present in data sources, such as files or databases due to inaccurate data entry, missing information or invalid data. When integrating data from multiple data sources, the need for establishing quality controls increases because the sources may contain duplicate data, different representations, and inconsistent identifiers [9]. There are several principles that can be applied when integrating data sets to ensure high quality data.

- data validation when collecting data through electronic data capture
- consistency in data definitions
- cataloging common variables
- understanding derived data elements (categorically, quantitatively and temporally derived data)
- understanding the use of coded concepts
- identifier strategies for joining data across systems.

Data validation

Data is frequently collected via electronic data capture through the use of desktop applications or web-based online forms. There are several design strategies that can be employed when creating the interface for data collection that will promote the capture of high quality data:

- use drop-downs and pick lists to limit input options to a defined set of terms or concepts
- limit the use of free text fields
- limit or eliminate "comments" fields
- when appropriate, make fields required so that the user cannot proceed without providing a value
- when possible, limit any calculations the user has to perform manually.

Many form-based electronic data capture platforms enable validation logic to be programmed into the forms. Form validation enables the assignment of rules to a specific field to limit data entry to valid values. Examples of constraints that may be placed on data entry fields include:

- specifically formatted text
- specific data type, such as numeric
- numbers within a set range.

Consistent data definitions

When retrieving data from clinical or other systems for quality or research purposes, there should be consistency in definitions of common data elements, including variables and their sources, methods for extraction, standard interpretations and consistent formulas for calculating derived data. For example, a standard lab value, such as serum creatinine, should have a definitive data source and retrieved the same way every time. In another example, interpretation of serology results for transplant recipients is frequently based on specific laboratory test results, but may also be recorded in a separate location by program staff, as in a call center. In cases where the data may have more than one source, a standard source of truth and definition should be established and used for the same variables every time they are retrieved.

Cataloging common data elements

To promote consistent reuse of data, common variables should be cataloged to provide a point of reference for a core set of data elements and in some cases to provide the mappings for an automated process to extract, transform, and load the variables into a target database. The catalog should contain metadata or "data about the data". At the most basic level, the catalog may only provide documentation for a core set of attributes and list the following:

- variable name
- description
- domain or subject area (e.g., demographics, labs, medications, procedures)
- data source, identifying the source system or database
- data type (e.g., text, numeric, date)
- business rules (identifying derived data elements and logic or formulas for calculating results).

For a transplant program specific data repository, which may be a shared resource, the catalog should also include information on how to access the data in the repository by specifying the table name and column where the variable is located.

For automated processes, the metadata is stored in a metadata repository, in which all of the information about the source, target, transformation, mapping, workflows, sessions and other information required to automatically extract, transform and load the data is stored [10].

Derived data elements

Raw data obtained from clinical data sources, including clinical information systems and clinical data warehouses, are not always informative, complete, or structured in a format that is useful for analysis [11]. Clinical data sources include any information systems used for patient care, and a clinical data warehouse is a place to gain

access to clinical data gathered in the patient care process [12]. The data may be organized in different tables or structured differently and from different sources. As a result, the raw data must be transformed into a structure that is more useful for analysis [13]. Many of the concepts valuable for analysis are more complex than single values, and may be derived from a combination of many data points or some inference on one or more variables captured at event-specific time points.

Derived data consists of alternative representations based on rules applied to one or more native elements to create new data. The elements can be categorically, quantitatively or temporally derived. The source fields may be native to the source systems or derived elements themselves. While derived data is not written back to the data sources, it may be displayed as the result of a query performed on those sources or stored in a local data repository.

Categorically derived values are represented as a text result or symbol based upon logic applied to one or more fields. Categorically derived data may capture a binary value or status (e.g., "yes" or "no", "positive" or "negative", "+" or "−", "1" or "0"). In other cases, the result may indicate one of several states (e.g., 1 = male, 2 = female, and 3 = unspecified.). In a clinical research database, a status field could represent a diagnosis, lab result, or medication status at a specific time point with a "yes" if the diagnosis, lab, or medication is indicated at the time point or "no" if it is not.

Quantitatively derived data is generated by performing numeric calculations on one or more fields and storing the resulting value. Examples of quantitative results include descriptive statistics and predefined or user defined formulas applied to any combination of numerical fields. Variables used in quantitatively derived data may be constrained by temporal elements, such as maximum value of a specific variable over a fixed interval.

Temporally derived data analysis applies to data that occurs at a specific time point relative to an anchor event. The data may be constrained or calculated by the time point specified. In a clinical research setting, anchor events may describe diagnosis date, procedure date, encounter date, discharge date, date and time medication was administered, or date and time a lab specimen was collected. Temporal data describing data captured at specific time points is usually constrained by intervals of time relative to the anchor event or may be calculated over the set interval. For example, a time point representing three months post-transplant may be calculated as an interval of days from the day of surgery and may be used to constrain the selection of data, such as lab values, medications or adverse events recorded around this time point. Temporal data may also be used to calculate postoperative days for the purpose of reporting patient or graft survival.

Coded concepts

Coded concepts apply to any data element that uses an encoded value in lieu of a textual representation for the value supplied for the data element. Any data element used in a data set or system represents some concept (e.g., resting heart rate, systolic blood pressure value or gender). The concept represented by a data element may require a text or numeric value as with resting heart rate and systolic blood pressure or it may require a set of predefined values as with gender. These predefined values are coded concepts which can either be some locally defined set of values or code sets (e.g., 1 equals male and 2 equals female) or could be sourced from a standard terminology (e.g., SNOMED or ICD). It is important to note that a given data element used in different contexts might represent a different concept and thus be bound to a different code set. Therefore it is important to understand the context of usage of data element and any code sets bound to it to appropriately interpret the meaning of the element.

In addition to providing encoded value lists for data elements in local data sets, code sets are used for other purposes including:

- defined for computable representation
- compliance with data standards required by some governing body
- integration with other data sources with the same data elements and coded values
- concept not part of standard vocabulary.

Assuring data compliance

Data compliance ensures that data adheres to the appropriate guidelines and specifications, whether they are legal regulations, institutional policies, or contractual obligations. The scope of these requirements may include protections to ensure the integrity of the data, stipulations on how long the data must be kept, or even assurances of how the data must be cited. Data compliance requirements can often be complex and multi-layered with multiple guidelines driven by international, national, state, or local laws, contractual requirements or guidelines. In addition, the same compliance domain/area may vary by geography. One major compliance area, for example, is the protection of personal privacy. Although varying in specificity and scope, most countries have some regulation around the protection of personal privacy in electronic communications. In the US, there is the Health Insurance Portability and Accountability Act, Title II (HIPAA); Canada has the Personal Information Protection and electronic Documents Act (PIPEDA) [14]; the European Union has the Data Protection Directive, also known as Directive 95/46/EC [15]; and South Africa has the Electronic Communications and Transactions Act, 2002 [16]. Due to the variety, complexity, and nuances associated with data compliance in general, it is very important to have someone with expertise in compliance review and provide guidance to the local data management program.

One key challenge in data compliance is the assurance that the use or disclosure of data is consistent with the parameters to which the individual consented at the time he/she provided his/her data to the institution. Data provided for clinical care, for example, should not be utilized for research purposes without following the appropriate compliance regulations and attaining appropriate authorization. It is therefore of especial importance that the local data management program institute a process by which the consent is tracked and managed. Research data is particularly challenging as all nuances associated with the informed consent and any associated information use authorization must be understood and complied with before data is disclosed or used. For clinical data, the data management team must understand under what circumstances it is acceptable to use or release the data, especially if that data will contain personally identifiable information. Also, data might undergo one use, followed by a later second use outside the scope of the original intended use. One example of this scenario arises when data and analysis used for a clinical care quality review (a use typically defined as a part of healthcare operations) is subsequently proposed for use in research or publication in a research journal, a secondary use that would require additional compliance steps. Due to these complexities, it is important to have clear, documented standard operating procedures for the local data management program describing permitted circumstances and required processes for such data use. Not only will this assure compliance in

general, it also will help protect the individuals whose data was released to the transplant program.

The following represents a small sampling of national and potential local regulations that a transplant program should be aware of. These are specific to the US, though other countries may have similar type of regulations.

Health insurance portability and accountability

The Health Insurance Portability and Accountability Act of 1996 (HIPAA) consists of multiple rules and sections. Of particular note for the local data management program are the following rules: Privacy Rule, Security Rule, the Patient Safety Rule, and the Breach Notification Rule as part of the Health Information Technology for Economic and Clinical Health (HITECH) Act. Overall, these rules outline a series of national standards to protect the health information of individuals. For most institutions, HIPAA provides the baseline to design their information security and privacy policies, procedures, and supporting infrastructure.

The Privacy Rule dictates the type of data that is protected by HIPAA, as well as the circumstances under which institutions can use and disclose protected health information. The Security Rule covers the standards necessary to protect electronic protected health information (ePHI). The Breach Notification Rule details under what conditions and to what extent institutions and their business associates must notify affected individuals following a confirmed breach of unsecured data.

These rules in HIPAA apply to institutions that are part of a covered entity, which is defined by HIPAA as "a health care provider that conducts certain transactions in electronic form; a health care clearinghouse; or a health plan" [17] or one of their business associates. More information on HIPAA can be found at: http://www.hhs.gov/ocr [17].

21 CFR Part 11 compliance

The 21 CFR Part 11 establishes a series of regulations "under which the [FDA] considers electronic records, electronic signatures, and handwritten signatures executed to electronic records to be trustworthy, reliable, and generally equivalent to paper records and handwritten signatures executed on paper" [18]. Part 11 focuses on approaches to assure the reliability and integrity of the data, with particular focus on electronic signatures, audit trails, and access control.

This regulation applies to institutions that work in or collaborate with FDA-regulated industries, and encompasses clinical trials. More information on Part 11 compliance can be found at: http://www.accessdata.fda.gov [18].

Federal information systems management

Federal Information Systems Management Act (FISMA) of 2002 is a federal law that mandates that all federal agencies establish an agency-wide information security program "for the information and information systems that support the operations and assets of the agency, including those provided or managed by another agency, contractor, or other source" [19]. The law identifies a framework maintained by NIST to build, monitor, and continuously improve upon. Unlike other regulations, such as HIPAA and Part 11, there are very specific processes, procedures, and security controls detailed at a granular level that must be adopted for FISMA compliancy.

This law applies to federal agencies as well as institutions wanting to contract with a federal agency. As government contracts are a viable vehicle for transplant programs, it is important for the program to review and understand the effort and cost it takes to be FISMA compliant. More information on FISMA can be found at: http://csrc.nist.gov [19].

Patient safety and quality improvement

This Patient Safety and Quality Improvement Act 2005 (PSQI) was established with the intent to improve patient safety within healthcare institutions in the US. As summarized by Cartwright-Smith et al. PSQI "was intended to prompt providers to report patient safety and other health care quality information by using the legal tool known as 'privilege' to encourage robust examination of quality and safety without fear that the results of such efforts would be 'discoverable' by a plaintiff during a liability trial" [20]. Of particular interest to a local data management program is understanding what information is considered patient safety work products (PSWP), and as such, are considered protected by this Act. More information on this Act can be found at: http://www.iso.org/ [20].

Record management policies

Each institution should have its own set of record management policies that stipulate how records, both electronic and paper-based, should be handled through their lifecycle of acquisition to eventual destruction. Typical record management programs categorize records into distinct classes, such as clinical research records or healthcare administration records, and ascribe different policies for each class of records, such record retention periods. At times the sponsor may dictate specific rules that can influence how records are managed, such as data dissemination requirements.

Record management policies will apply to each local data management program and vary by institution. More information on Records Management can be found at: http://www.iso.org [21].

Protecting the data

Whether it is to protect patient privacy, safeguard intellectual property, or comply with any of the various local, state, national, or international regulations or policies, an information security and privacy program must strike an appropriate balance between protecting the institution from risk and providing access to the data for programmatic use. For a transplant program, there are a number of security controls that the program should ensure are in place. The implementation of these controls will vary based on the expertise of the staff and the available resources and funding, but, at the very least, the program should understand and authorize how they have been implemented. The following is a small sampling of controls and guidance on how to think through their implementations.

Data recovery

This process addresses the risk of data being irrevocably destroyed through a hardware failure, disaster, computer virus, or other means. To mitigate this risk, the program should determine how frequent they would like the data backed up. This generally is determined by how much data they can afford to lose. If the data is critical, back ups can occur almost instantaneously, though at relatively larger expense, while if the repository is an aggregation of multiple authoritative sources, the back up strategy might be less frequent (e.g., once a week).

In general, many institutions back up their data on a daily basis and store snapshots of it offsite every month. Large data, such as

genetic sequence data, might have a different backup strategy due to the sheer cost of backing up such a large volume of data.

In addition to the frequency of the back up, the program should determine what data should be backed up. One maxim is any data that you cannot recreate in a timely manner should be backed up. For example, original data acquired through interviews with a research subject or specialized tests performed on a tissue sample might merit a full back up plan. Due to the costs and time to back up, some programs may choose to not back up all variations of a data set, but rather, simply keep a backup of the raw data and notes on what algorithms were run against it to produce the analyzed set.

Other items to consider include:

- If data is backed up to an external device (e.g., tape or external hard drive) and it contains sensitive information, it is important to encrypt the external device or the data on the encrypted device. This prevents a breach if the external device is lost or stolen.
- If the data must comply with HIPAA regulations and is stored offsite, there must be a business associate agreement in place with the offsite hosting vendor and the institution.
- The integrity of the backup should be tested by establishing a process to restore the entire data repository at least once a year.

Separate the identifiers from the data

Assuming individually identifiable information (i.e., name, medical record number) are necessary for research, it is often best to separate the identifiers from the core data set and store them in a separate data repository with limited access. Instead of the individually identifiable information, substitute a local identifier that is mapped to the individually identifiable information through a translation key to enable de-duplication of records and the ability to integrate data from additional sources. In most situations, the individually identifiable information, such as electronic medical records, names, and addresses, is not necessary for most research, education, and outcomes usage. In addition, dates can be rolled backwards or forwards a consistent number of days to obfuscate them while retaining their pertinent to certain types of analysis. Without the individually identifiable information, the confidentiality risks significantly decrease.

Train and set expectations

Many security controls depend on individuals of widely divergent skill sets knowing the right thing to do. Whether it be not disclosing their passwords to anyone or not clicking on links embedded in emails, there is a set of expectations and standard operating procedures that a person should know before they have access to any data, especially sensitive data.

Although most institutions have an information security course that faculty, residents, post doctorates, students, and staff can take, the course might not address specific procedures and processes that the transplant program has created. Establishing a course specific to transplant can provide a greater level of specificity and relevance to their day-to-day jobs. Moreover, it can provide them with the knowledge and tactics to protect critical data.

In addition to training, visual cues and rules of behavior can be implemented within each electronic system to help set expectations on how sensitive the data is and how it should be utilized. For example, login screens of each application can be modified to insert language to remind individuals accessing the system of the sensitivity level of the data. Links to additional information can also be included in order to remind them what are the "rules of behavior" associated with the system, for example, the data cannot be downloaded and distributed to anyone outside the protocol team.

Conduct a security assessment by a third party

An assessment by an objective third party provides a method of testing the security of the system. These tests are often called "application security assessments". Working with a reputable vendor with excellent references, a set of objectives should be established and the exact systems for testing should be documented. The vendor then conducts a series of tests to identify and quantify any vulnerability associated with the system. Based on the objectives, these tests may be conducted from inside or outside the transplant program's network. If the application is developed internally, the vendor might have access to the actual code to conduct a more detailed review. Though engagements may differ, the transplant program should receive a list of vulnerabilities, an explanation of the vulnerability, and some rating to help quantify the risk the vulnerability has introduced. Based on how the contract is established, there may be an option to re-run the assessment after the program has a chance to resolve the vulnerabilities.

Due to the sensitivity of these types of engagements, it is important to ensure the contract appropriately protects the institution from intentional or unintentional disclosures of information. Also, since it is important to test actual production systems that might contain sensitive data, the contract should specify any and all constraints with how this data should be accessed. Finally, before any test is conducted, all appropriate parties, including the institution's security and information technology teams, should be brought into the loop and sign off on the engagement.

Deploy

In the Deploy stage, the local data management program constructs a solution based on the design and incorporates it into the production environment. Based on the type of solution, the deployment activities will vary. However, whether the solution is a new or a modification to an existing data source, report, or functionality, there needs to be a level of assurance that adequate testing has been conducted and that the new solution is incorporated into the support structure.

In addition to deploying a specific solution, activities that support and advance the overall management and operations of the local data management program should continue. Key areas of data governance and engagement of the transplant community are of particular importance to the transplant program.

Role types in the data management program

In describing the governance structure and operation support, it is helpful to have a description of the type of roles within the data management program. Of course, some individuals might be part of multiple role types based on their expertise and position in the organization.

- **Stakeholders.** Individuals with a level of ownership of a particular program, process, or area within the transplant program. They help guide strategy, prioritize resource investment, and review project objectives to make sure they are aligned with organizational objectives. Stakeholders may represent different organizations within the institution and may include physicians, principal investigators, administrators, technical directors and other members of the transplant program who provide input on data management activities.

- **Subject matter experts (SMEs).** Recognized experts in a particular subject domain (e.g., liver transplants). In general, the data management program will seek out these individuals to help address specific questions about the data associated with that domain or to lend their knowledge in the design of complex reports or data structures to assist with decision making.
- **Data producers and data consumers.** Interact directly with the data, each from different sides of the data. Data producers typically own the major systems that acquire and source data, such as clinical information systems, laboratory information systems and study management systems. Data consumers utilize the data for analysis, decision making, and research. Both groups of users play a critical role in communicating how data is used and how processes can be improved [22]. Data producers are on the front-line interacting with the data and can provide feedback from a process perspective on data collection and when it is most appropriate to record specific data points as part of their workflow. Meanwhile, data consumers provide feedback to the data management team on the applications, utilities and mechanisms for delivering data — what works and what does not.
- The **data management team** consists of several roles and is ultimately responsible for data extraction, collection, analysis, delivery, security, management, handling release of the data in compliance with information management policies, and maintaining ownership of the metadata. A data management team may consist of multiple roles, including:
  - **team lead** role that is responsible for overseeing and managing the program in general, assuring that requests and projects are delivered on time;
  - **business analyst** role that gathers and documents business requirements;
  - **data analyst** role that translates business requirements into data acquisition and delivery processes;
  - **biostatistician** role that provides expertise in study design and statistical analysis;
  - **data steward** role that reviews the release request and ensures the appropriate steps have been taken before authorization the release of the data; and
  - **honest broker** role that ensures that released data is appropriately de-identified before released to an individual investigator.

Depending on available resources, many of the roles described above may be held by a single or small number of analysts.

Testing

As data is used to support decision making, new solutions should not be released without conducting formal testing. Before conducting a test, a test plan should be created that enumerates in granular detail the steps each tester will be taking. This activity provides consistency across all testers and also enables any errors to be traced back to a specific step. The test plans should incorporate a number of scenarios, including a "blue sky" scenario, which involves the steps a user would take assuming everything worked perfect; an unconventional scenario in which the tester attempts to perform tasks that purposefully divert from the blue sky approach; and a security scenario in which the tester attempts to access information they should not be able to access. In addition to the formal testing, requesting actual users to test drive the new solution and provide feedback is always highly informative. It is important to not only test to ensure the new solution functions as designed, but also to test for accuracy and usability of the data as well as assure that the new solution does not introduce any unanticipated issues with the current data management system.

Support structure

Without a solid support structure, a local data management program cannot be sustainable. The program would depend on informal communications to carry its messages, it would lack common documents and processes to provide consistency in how the program operates, and the ability to remediate issues would depend on the goodwill of volunteers. Overall, the support structure, though not as innovative as the planning and design portions of local data management program, is still a critical factor for success.

Support structures should have individuals who are customer friendly, active participants in the program and knowledgeable in subject matter, highly analytical in their ability to think through problems, and strong communicators in articulating the root cause of problems and their associated solutions. The individuals who provide support might be the same individuals who help plan, design, and deploy solutions. In addition to the personnel, it is important to have the right documents and processes continuously updated. As solutions transition from design into production, the processes and documentation for supporting the local data management program should be updated to reflect any new changes. Standard operating procedures should have detailed instructions on how to handle and process requests and data. The data dictionary, or metadata repository, should be consistently updated to reflect the latest fields and values. Training material and support documentation should be posted on internal web sites to help provide self-independence and expand the support structure beyond the local data management team.

Finally, it is critical for all members of the local data management team, in particular the support team, to understand the rules and standard operating procedures for the disclosure and use of each type of data. As mentioned in the Data Compliance section, different steps and authorizations may be required based on the consent associated with the data and intended use of the data.

Data governance

Data governance provides the opportunity to bring together individuals representing multiple roles and subject matter expertise to discuss and recommend strategic directions, policies, and priorities associated with the data management program. For the local data management program, the governance body provides a sounding board to discuss challenges and validate intent. Generally, individuals who agree to serve on the data governance board are committed to the success of the program and become strong and collaborative advocates. By incorporating data governance into the process, the local data management program abdicates some of its authority to make decisions, but gains a broader insight into how data is utilized, provides a greater level of transparency in the decision making process, and incorporates a shared sense of accountability and responsibility for the program as a whole.

In forming and operating a data governance process, the local data management program should consider:

- Data governance should govern, not manage. Governance bodies should be focused on guiding principles and policies that, in turn, influence how the data management program operates. Governance inserted at a lower, tactical level can often disrupt or delay the day-to-day operations of the data management program.

As an example, the governance body should review and make recommendations on the standard operating procedure for disclosing data; however, it should not be the approving body for each data disclosure request.

- Governance should be linked into the Transplant Leadership structure and have the authority to make a specific class of decisions, as articulated by the governance charter. Having a member of the Transplant Leadership team chair or co-chair the data governance board will facilitate alignment with leadership.
- Key areas for a data governance body to deliberate on include: standard operating procedures; opportunities to standardize on data capture forms, data values, and terminologies; security and privacy policies; prioritization of data sources to incorporate into the data management program; and methods and metrics to evaluate the success of the program.

Engagement

The stronger the engagement with the community, the stronger the partnership between the data management program and the population it serves. As such, it is important to reach out to all levels and all roles in the Transplant Program. From these engagements, the local data management program should receive help from the community with the identification of issues and challenges, new opportunities to expand the program whether through new ideas or funding opportunities, and positive and critical feedback on the specifics components of the program.

A successful data management program is fluid in its approach and needs constant realignment to ensure that its principles and priorities are consistent with the goals of the transplant program. As such, the local data management group should ensure there is a formal, regularly scheduled meeting with the Transplant Leadership team. In these meetings, the team should report out on key performance metrics, project status, issues and risks encountered, and finally, success stories.

Evaluate

In this final phase, the local data management program, working with leadership and the governance structure, puts into place a series of key performance indicators, evaluates its performance against them, and based on the evaluation, incorporates changes into the planning phase to improve the program. Though many evaluations are performed informally, having a formal evaluation of the program at specific intervals will help quantify the overall performance of the local data management program.

Although each program will have its own distinct set of metrics, the following are some examples of areas to consider:

- Efficiency. Reduction in the number of systems, steps or time required to querying or gathering information. Examples of metrics supporting this include reduced staff hours spent gathering data for reporting or querying a single repository for data rather than multiple source systems. This has significant impact in cases where staff can move from a manual process of gathering and recording information for reporting toward an automated process, such as running a report.
- Process improvement. Standardization and optimization of processes within the Transplant Program due to automation of workflow and supporting systems. Examples of metrics or evidence include ability to track and improve upon protocol adherence, reduction in paperwork, or reduction in number of systems or steps required for data entry.
- Decision support. Number of reports and data artifacts that are used to inform members of the Transplant Program in key decisions.
- Data quality. Number of records that are identified through data assurance scripts and techniques that have incorrect values associated with them.
- Mission support. Use of the data management program by investigators and quality programs to conduct research, quality improvement projects, public health surveillance, and other activities that support the Transplant Program's mission and objectives.
- Cost-benefit analysis. Analysis of the total cost of ownership versus the value of the benefits received. Important evaluation component to determine whether the program is worth the investment.

Summary

An active and integrated data management program can be a valuable asset for any Transplant program. However, as with any critical component, the local data management program needs nurturing and refinement, and a continuous process of plan, design, deploy, and evaluate can help shape the program to meet the ever-changing needs of a Transplant Program. In the end, an effective data management program will provide a framework to deliver appropriate data at the appropriate time to enable evidence-based decision-making related to care for defined populations of patients, support opportunities for continuous quality and outcomes measurement and interventions, provide for data intensive clinical research, support comparisons of local populations to national or international data sets, and aid in fulfilling reporting requirements for quality and outcomes.

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CHAPTER 133

National Transplant Data Registries and Population Studies

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Introduction

Transplantation remains a growing field, with rapidly evolving treatment protocols and ongoing policy changes. Flexible, up-to-date scientific inferences are critical to advancing knowledge in our field, informing policy and designing optimal treatment protocols for patients.

Properly conducted randomized controlled trials (RCT) have long been acknowledged as the gold standard for evidence in scientific research, and the specific factors making up a well-designed RCT are covered in depth in Chapter 134. However, RCTs suffer from several disadvantages:

- RCTs require long follow-up time. The most clinically relevant outcomes in organ transplantation are patient survival and graft survival which may require years to measure properly. Long follow-up times not only make a trial expensive to conduct, they also delay any scientific benefit until follow-up is complete.
- RCTs are expensive. Most affordable clinical trials are powered only to detect large differences. In order to perform an RCT of a relatively rare outcome (such as post-transplant mortality) with adequate power, hundreds or thousands of patients may be required, often rendering the trial unaffordable.
- RCTs require strict selection criteria to draw inference about a given population. Even if funds were available, the number of participants required for a properly powered RCT may exceed the total number of eligible patients transplanted in the US in a given study period.
- An RCT may be impossible, or unethical, to conduct. For example, the question of whether diabetes worsens transplant outcomes cannot be answered through an RCT because the exposure (i.e. diabetes) is not modifiable. The question of whether cigarette smoking worsens transplant outcomes cannot be answered through an RCT because it would not be ethical to randomize patients to cigarette smoking, even if patients were willing to participate in such a trial.

Long-term, prospective, observational studies — in which participants are followed with no experimental intervention — avoid some of the feasibility and ethical problems potentially posed by clinical trials, but otherwise suffer from many of the same drawbacks, such as cost, recruitment, and long gaps between exposures studied and outcomes of interest. Retrospective data collection addresses (to some extent) cost and follow-up gaps, but suffers from recall bias, survivor bias, and other biases which may distort inference.

However, studies of registry data, which are collected prospectively and analyzed retrospectively, marry the advantages of prospective data collection (avoiding recall and survivor bias) with those of retrospective analysis (cost and long follow-up). Furthermore, mandated reporting in some countries makes registry data readily available and free from any selection bias. For this reason, the transplant community relies on registry-based data for many of its important inferences. It is important to understand techniques for performing studies of registry-based data, as well as the advantages and shortcomings of a population-based approach.

The goals of this chapter are to describe the various registries available to researchers, to review the advantages and limitations of registry-based analyses, and to provide an overview of analytical approaches and study design. Although the focus of this chapter is analysis of data from organ transplant registries, many of the principles elucidated will be relevant to any observational, population-based research.

Advantages of registry-based and population-based research

The field of organ transplantation is well-suited to the creation of registries, since the maintenance of a deceased-donor waiting list and the mechanics of deceased-donor organ allocation lend themselves to a centralized database with detailed, up-to-date information on the health and characteristics of transplant candidates as well as deceased organ donors. Moreover, the number of treatment providers is relatively small. As of July 2011, there were 265 transplant centers performing kidney transplantation in the US, and smaller numbers of centers performing extrarenal organ transplants [1]. Consequently, a relatively high degree of standardization in data collection and recording is attainable.

Registries typically include all relevant patients within a country or region, meaning that the complete population of transplant candidates or recipients over a given time frame can be included in a registry-based study. The large numbers available through registry studies make robust statistical inference possible. Moreover, the population is more diverse than that attainable through single-center or even multicenter studies, which tend to over-represent

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

large-volume academic centers. Registries also allow for the analysis of multilevel effects (e.g. center-level or Organ Procurement Organization [OPO]-level effects). Since patients are followed for the duration of their interaction with their treatment provider, long-term follow-up is possible. Lastly, transplant registries can potentially be linked with other registries (e.g. the Social Security Master Death File, administrative claims databases, or cancer registries).

Limitations of registry-based and population-based research

It is important to understand several shortcomings inherent to registry-based studies. Although data are submitted to registries in a standardized format, the data are collected by hundreds of healthcare workers, with varying degrees of training and expertise. In most national registries, there is no mechanism for centralized quality control of data collection (although some auditing does occur in some registries). Missing data are common for some measurements in registries in the US; some of the missingness is random, and some is systematic, and the distinction is critically important. Data collection errors or recording errors can lead to flaws in the data that may be difficult to detect; particularly in cases where these errors are systematic, significant bias can arise.

Additionally, registry-based studies suffer from two major drawbacks common to all observational research. First is the problem of *unobserved (latent) confounders*. Much important information about transplant patients is not captured in registries. For example, an investigator may wish to study the biological relationship between age and risk of graft loss in pediatric patients. Teenagers are less likely than younger children to comply with post-transplant immunosuppression regimens, and non-compliance is a major cause of graft loss [2]. Consequently, the investigator would want to adjust for medication compliance in the analysis. However, no data on medication compliance are available in organ transplant registries in the US. Therefore, a registry-based analysis of the biological association between age and risk of graft loss will be *confounded* by compliance (technically, in epidemiologic terms, compliance would be a *mediator*, which is analytically treated like a confounder for most intents and purposes).

A related concept is *residual confounding*. For example, a researcher may wish to study the relationship between age in deceased donors and post-transplant outcomes, independent of recipient diabetes (in other words, adjusting for any confounding effect of diabetes). If data are available on which patients have diabetes, this variable can be included in a statistical model to adjust for diabetes status. However, without data on diabetes severity (e.g. duration of disease, glycemic control, insulin therapy history), patients with mild diabetes and severe diabetes will be treated the same by the model. Therefore, the relationship between age and mortality will continue to be partly confounded by severity of diabetes.

Another drawback of registry-based studies is *confounding by indication*, also known as *treatment selection bias*. Confounding by indication occurs when sicker patients are more likely to receive a treatment than healthier patients [3]. Patients who receive the treatment may have poorer outcomes, not because the treatment is inferior, but because of the severity of disease which led to the treatment. Confounding by indication is of particular concern in studies of pharmacological therapies and other treatment interventions [4].

Examples of national and regional transplant registries

The Organ Procurement and Transplantation Network (OPTN) transplant registry

Since 1986, the United Network for Organ Sharing (UNOS) has operated the Organ Procurement and Transplantation Network (OPTN), under contract with the US Department of Health and Human Services [5]. All deceased donor organs in the US are allocated through the OPTN; moreover, participation in the OPTN has been a requirement for transplant programs wishing to participate in Medicare/Medicaid since 1987 [6]. The same year, the OPTN began collecting medical data on organ donors and transplant recipients [7]. Since 1999, data on transplant candidates, living and deceased donors, and transplant recipients have been stored in UNOS's integrated database known as UNet^{SM}, with data entered electronically by transplant centers via a secure online interface [7]. These data are made available, in de-identified form, to researchers.

As of June 2012, the main online forms collected by the OPTN include [8]:

- The Transplant Candidate Registration (TCR) Form, required at the time a patient is registered for an organ waiting list, containing patient demographics, health history, clinical information, and organ-specific information.
- The Transplant Recipient Registration (TRR) Form, required for all transplant recipients at the time of hospital discharge or death, containing updated patient demographics and health history, information about the transplant procedure, and other treatment information (e.g. immunosuppression during the initial transplant hospitalization).
- The Transplant Recipient Follow-up (TRF) Form, requested at six months after transplant and at every one-year anniversary of the transplant for recipients who remain alive with a functioning graft, containing information on current patient status and interim treatment.
- The Deceased Donor Registration (DDR) Form and Living Donor Registration (LDR) Form, containing donor demographics, health history, and details of recovery (deceased donors) or post-operative clinical information (living donors).

Other OPTN forms include the Post Transplant Malignancy Form, the Liver Recipient Histocompatibility Form, Donor Histocompatibility Form, Recipient Histocompatibility Form, and Living Donor Follow-up Form. All OPTN forms are available online at http://www.unos.org/. Additionally, the registry contains wait list updates reported by transplant centers (such as changes in Model for End-Stage Liver Disease (MELD) score for deceased donor liver candidates) and administrative data generated by the OPTN (such as deceased donor organ offers). OPTN data can be obtained for analysis from UNOS; these analytical files are linked to the Social Security Death Master File (SSDMF) to allow more accurate ascertainment of death, since many deaths are not captured by OPTN reporting mechanisms.

The Scientific Registry for Transplant Recipients (SRTR) standard analysis files

The Scientific Registry for Transplant Recipients (SRTR) also produces Standard Analysis Files (SAFs) for research of transplant outcomes in the US. Like the UNOS datasets, the SRTR datasets are based on OPTN data supplemented with a link to SSDMF data. However, unlike the UNOS datasets, the SRTR datasets also link to data from the Center for Medicare and Medicaid Services (CMS),

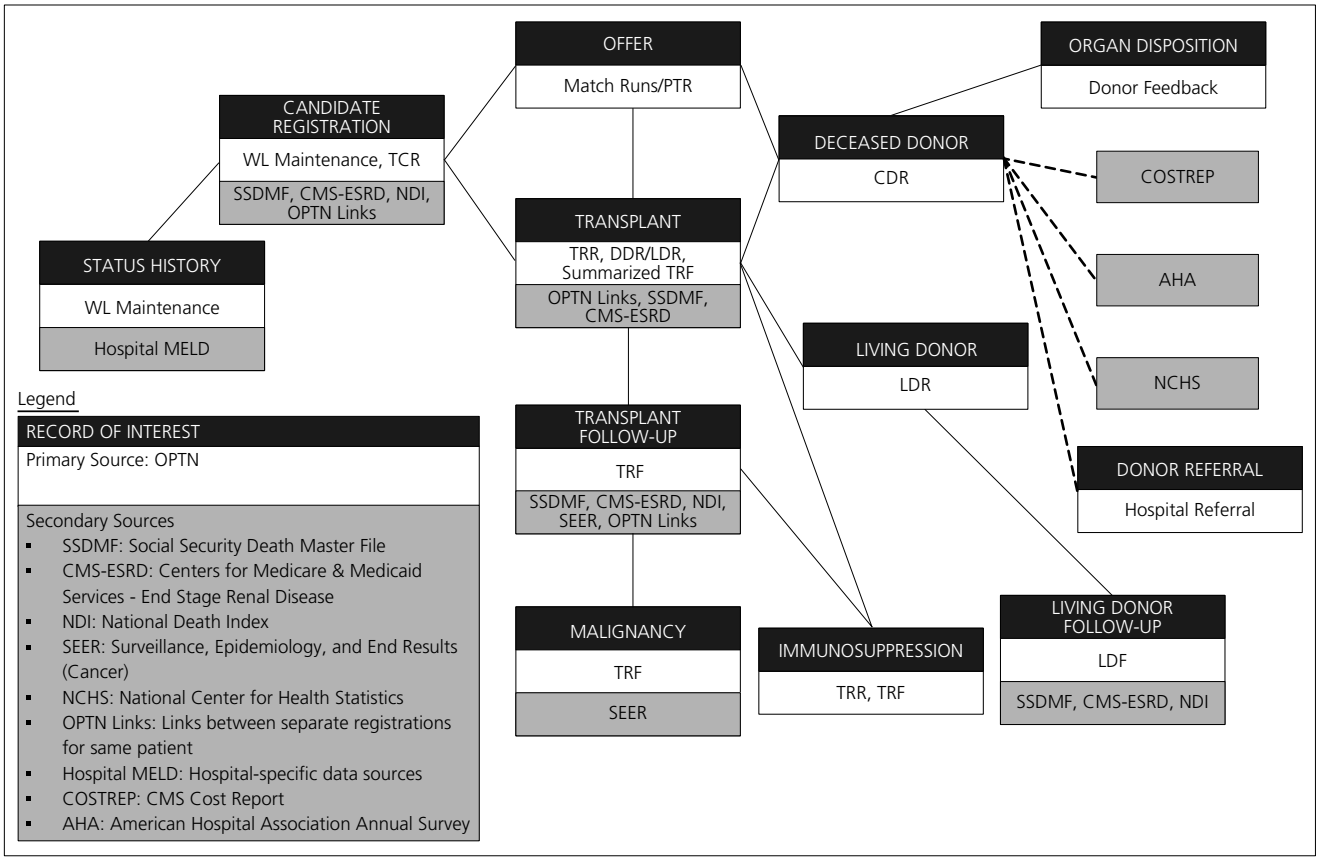

Figure 133.1. Schematic diagram of data sources and organization of Standard Analysis Files (SAFs) made available by the Scientific Registry for Transplant Recipients (SRTR). Modified from [9] Levine G et al. *American Journal of Transplantation* 2006; **6**(5p2):1228–1242, with permission from Wiley.

allowing for more accurate ascertainment of kidney graft failure in surviving patients, since many graft losses are not detected by OPTN reporting mechanisms [9]. The CMS data draw from the CMS Medical Evidence Report as well as from administrative claims data. The Medical Evidence Report (also known as Form CMS-2728) collects demographic and diagnostic data and is completed for all patients diagnosed with end-stage renal disease (ESRD) in the US [10]. A schematic of the data organization of SRTR SAFs appears in Figure 133.1. The UNOS and SRTR datasets organize OPTN data in different ways and follow different variable naming conventions. Details of the organization of the SRTR database can be found at http://www.srtr.org and in various descriptions in the literature [9].

Other registries

Registries outside the US

Data on organ transplantation and ESRD in Canada are captured in the Canadian Organ Replacement Register (CORR) [11,12]. The Eurotransplant registry contains data on organ transplantation in Germany, the Netherlands, Belgium, Croatia, Luxembourg, Austria and Slovenia [13,14]. National registries exist in Australia/New Zealand [15,16], Sweden [17], Japan [18], Malaysia [19], Brazil [20], and elsewhere. Each of these registries have data collected that is similar to that found in the US registries, and can serve to facilitate similarly useful analyses with similar caveats. However, the standardization of the data collection methods can vary, and in those countries with socialized healthcare systems, the overall healthcare system can facilitate standardized data reporting to include elements beyond those directly related to transplantation.

The role of registry and population-based research

Descriptive statistics

Registries (especially those with mandatory data reporting that capture all patients) are often the best tools for obtaining descriptive statistics about a population. In transplantation, questions answered with descriptive statistics may include:

- How many transplants of a given type are performed per year?
- How many patients join the waiting list?
- What is the demographic breakdown of patients who receive a transplant?
- How do these numbers change over time?

The SRTR publishes a yearly report which provides descriptive statistics relevant to transplantation [21]. In addition, many published papers have focused on providing descriptive statistics that give an overview of the state of the field of transplantation, or describe one aspect of transplantation in detail [22–28].

Inferential statistics

Inferential research involves estimating an association between an exposure and an outcome; for example, finding that transplantation with extended donor criteria (ECD) kidneys is associated with decreased graft survival relative to standard donor criteria (SCD)

kidneys [29]. The large numbers of patients included in registries, and the rich variety of clinical variables available, makes registries well suited for inferential research. Registry-based inferential studies have examined the role that patient demographics [30–32], deceased donor characteristics [29,33–35], policy [36–40], and many other factors play in organ transplantation. Although carefully performed inferential analyses can minimize the risk of mistaken inference, statistically significant associations must be interpreted with caution, since an association does not demonstrate causality.

Prediction models

A history of donor cigarette smoking is associated with slightly increased risk of graft loss after deceased donor kidney transplantation (hazard ratio = 1.05 in one study, $P < 0.05$) [41]. Yet knowing only a donor's smoking history conveys very little information about the risk of accepting an organ for transplant, since many other qualities (e.g. donor age, history of hypertension, cause of death, terminal serum creatinine) have much stronger associations with risk of graft loss [29]. Zero-mismatch deceased donor kidney transplants are associated with much lower risk of graft loss (relative risk = 0.65 in one study, $P < 0.001$) [42], but, again, knowing zero-mismatch status conveys limited information about the risk of graft loss since only about 15% of deceased-donor transplants are zero-mismatch [43], and since a zero-mismatched kidney from a 72-year-old with hypertension and a terminal creatinine of 2.4 is likely far worse than a 6-antigen mismatched kidney from a 25-year-old otherwise healthy donor. The goal of a *prediction model* is to draw inference about the risk to an individual patient, based on many factors. Often, composite scores are used. Registry-based studies have been used to create composite risk scores for kidney graft loss [44–48], mortality in patients with end-stage liver disease [49], liver graft loss [50] and kidney discard or delayed transplant [51].

Simulation-based research

Simulation-based studies are useful for estimating the effect of an experimental intervention when the experiment would be impractical or unethical to conduct in practice. Simulation studies are often used to predict the effects of changes in organ allocation policy. To test the effect of a policy change experimentally would require extensive administrative changes and months to years of follow-up, but a simulation can compare the effects of many different strategies on virtual patient outcomes. Simulations have been used to model the effects of policy changes in allocation of deceased-donor kidneys [52–54], livers [55,56], hearts [57,58], and lungs [58]. Additionally, simulations have been used to compare strategies for matching patients to compatible living donors for kidney-paired donation (KPD) [59–61].

Simulations are only successful if their representation of actual patients and policies is accurate. Registry data are useful for populating simulations with a case mix that mirrors actual patient populations and for constructing time-to-event models to estimate the probability of key events (e.g. organ offers, receipt of a transplant, death on the waiting list, graft failure after transplant).

Study design considerations in registry-based research

Prospective studies require detailed planning before enrollment begins. Funding agencies and institutional review boards will not support studies with poor design or small chances of success. Before a prospective study can launch, the researchers must show that the research is feasible. The population of interest must be identified. A plan must be made for collecting and preparing data. An appropriate method of statistical analysis must be chosen, and contingency plans must be made for problems that may arise once the study is underway.

Preparation for a retrospective study is simpler and less burdensome from a regulatory standpoint, but involves many of the same considerations. Advance planning before beginning a retrospective study will make data analysis easier and minimize the risk of mistaken inference. The actual analysis should reflect the execution of a well-chosen study design.

Hypothesis

Since clinical trials are expensive and time-consuming, it is reasonable to expect that they are driven by a well-defined, biologically plausible hypothesis for which the investigators believe there is equipoise. In the case of registry-based studies, hundreds of variables are at a researcher's fingertips, and a cursory investigation of correlation or association between two variables may take only seconds. The chance that a single statistical test will be statistically significant due to chance is low: by definition, it is the statistical α, by convention usually set at 5%. But in any national transplant registry, hundreds or thousands of spurious correlations certainly exist. Conditions are ideal for publication bias [62], in which these spurious associations are identified by investigators on "fishing expeditions" in the data, tested, found to be significant, and published. To avoid publication bias and type I error, investigators should take care to only perform analyses that are driven by a carefully considered, biologically plausible hypothesis.

Population selection

A well-defined research question is targeted to a particular population. For example, when investigating the association between cause of liver failure and survival after liver transplant, the population of interest is liver transplant recipients. Atypical patients (e.g. multiorgan recipients, patients with a history of prior transplant, or pediatric patients) are often excluded in order to provide accurate inference for a specific category of patients (e.g. adult first-time liver-only transplant recipients), depending on the research question.

There are three populations to consider in planning a study:

- the *target population* (the population about which we wish to draw valid inference);
- the *source population* (an enumerable list of potential participants in the study, representative of the target population);
- and the *study population* (the individuals whose data are actually included in analysis, drawn directly from the source population).

The research question should drive the definition of the target population, which in turn, along with feasibility and data availability, should drive the definition of the source and study populations. In prospective studies, the study population may be smaller than the source population because it is infeasible to enroll all eligible individuals, or because some individuals refuse consent. In a registry-based study, the source population and study population are often the same. However, the study population may be a subset of the source population when using certain analytical techniques (e.g. in a matched cohort analysis in which the study population is drawn from a pool of eligible matches) [63,64].

The precise inclusion criteria for a registry-based study vary depending on the research question. However, typical inclusion criteria may include a date range (e.g. all transplants performed in the years 2005), an age range (e.g. adult patients), and an organ/procedure type (e.g. lung wait list registrants, live donor kidney recipients) to ensure some degree of homogeneity.

Data integrity/exploratory data analysis

Transplant registries typically make use of data that were originally gathered for a purpose other than research. The primary purposes of OPTN data collection efforts are to manage deceased donor waiting lists, to facilitate organ distribution, and to provide data for the SRTR program-specific reports. Data are of variable quality. Missing data and erroneous data are common, particularly for fields which do not address the primary purposes of the OPTN. Proper exploratory data analysis is essential to avoid bias in a population-based analysis.

Missing data

Data may be missing by design. For example, collection of the international normalized ratio (INR) of prothrombin time, a measure of liver disease severity, was added to the OPTN database in 2002 [65]. Therefore, this information is missing for all liver wait list registrants before that date. Alternatively, information may be missing because it was not submitted by treatment providers. For example, cold ischemia time (CIT) was not reported in 30.3% of live donor kidney transplants between 1990 and 2005 [66].

Missing data may be *missing completely at random* (MCAR), when any record is equally likely to have missing data; *missing at random* (MAR), when there is a systematic mechanism of missingness that is predictable from another covariate (e.g. missingness is less likely for more recent records but otherwise random); or *missing not at random* (MNAR), when there is a systematic mechanism of missingness, but this mechanism cannot be predicted from other available data (e.g. if CIT is less likely to be recorded for smaller CIT values) [67]. Any missingness that is MAR or MNAR may lead to biased inference if not properly handled in analysis. Exploratory data analysis should include careful consideration of missing data and a search for patterns in missingness (e.g. more often missing in a given era or for a given category of patient). Common strategies for handling missingness include deletion of missing records, creation of a missingness indicator variable, and advanced techniques such as imputation [67,68].

Errors in measurement and data recording

Even when not missing, data in a registry come with no guarantee of accuracy. There are several reasons why a measured value might be inaccurate. There may be an error in the measurement process (e.g. a flawed lab result). Patients may not remember their health history accurately. The wrong measurement units may be used (e.g. recording patient height in inches instead of centimeters). Health care providers may make typing errors when entering data into a registry. A *sentinel value* may be used as a placeholder or to indicate missing information (e.g. an age of 99 if age is unknown) and later mistakenly interpreted as data. The probability of any one of these events is low, but even a handful of truly erroneous values can bias an otherwise robust analysis. Furthermore, any registry that contains hundreds of measurements on thousands of patients is likely to contain a large number of errors.

In considering the problem of measurement and data-recording errors, investigators should bear in mind how reliable measurement of a particular variable is likely to be. Transplant centers have a high incentive to accurately report some variables. For example, allocation of deceased donor livers is largely dependent on the MELD score [55]. If MELD scores are misreported, transplant centers run the risk of denying patients a chance to obtain a liver transplant. They are consequently likely to take great care in measuring and reporting MELD. As another example, the SRTR uses certain patient co-morbidities for case mix adjustment in the preparation of program-specific reports (PSRs) [69]; centers with poor adjusted outcomes in the PSRs run the risk of losing patients to competing centers, or even losing eligibility for Medicare reimbursement. Consequently, centers are likely to report those patient co-morbidities included in the PSRs as accurately and completely as possible. Conversely, variables for which non-reporting or erroneous reporting have no consequences for a center (e.g. immunosuppressive regimens, history of malignancies), especially those that are difficult to collect properly (e.g. follow-up data), are more likely to be missing or erroneous.

Erroneous data points might be *outliers*, falling outside the typical range of a variable. For example, if a patient's true height is 170 cm, but the height is entered as 70 cm, that height may be the shortest recorded height among adult patients. Outlier values may be *influential*, meaning that their presence or absence in a dataset may change a co-efficient estimate. Even a small number of high-influence points can affect regression co-efficients; if the high influence comes from measurement errors or data-recording errors, these values can lead to mistaken inference [70]. A variety of techniques exist for detecting and handling high-influence points [70,71]. At a minimum, investigators need to explore the distribution of variables used for analysis, and carefully consider the treatment of values that seem outside the normal range for a variable.

Statistical approaches to registry-based studies

The main statistical methods used in analysis of registry-based studies are techniques that are widely applied in the health sciences. A detailed description of all available statistical methods is beyond the scope of this chapter, and statistical issues related to transplantation studies are covered in depth in Chapter 134. Nevertheless, this section provides an overview of techniques that are common to population-based studies and other large-sample studies in the field of transplantation. More detailed reviews can be found in transplant-specific articles [72] or general textbooks of biostatistics and epidemiology [70,73].

Survival models

A *survival model* is any statistical model in which individuals are followed over time, and at any time are at risk of having an event of interest. Most survival models also allow for *incomplete follow-up*, which is a natural component of observational studies in transplantation (since not all patients undergo transplant on the same day, so at end-of-study there are different follow-up times for each patient). The event of interest may be, for example, mortality on the waiting list, receipt of a transplant while on the waiting list, graft failure, or mortality after transplant. Survival models require a *time origin* which may be, for example, date of wait listing or date of transplant (birth is also a possible time origin, though infrequently used). At the end of follow-up (which can vary, as above), an individual will either have the event of interest (often referred to as a

failure) or will no longer be followed (often referred to as *censorship*). Note that, despite the emotional valence of the terms "survival" and "failure", the event of interest may be a desirable outcome (e.g. an organ transplant is technically a "failure" in a model assessing time to transplantation). A survival analysis requires data to be in a specific format, depending on the statistical software used; typically, data management will be required to transform registry data into a format suitable for survival analysis. The data management and analysis techniques for survival analysis are described in reference manuals for several common statistical packages [71,74,75].

The most basic statistical technique for survival analysis is the *Kaplan-Meier estimator* [36,76,77]. The Kaplan-Meier estimator is a *non-parametric* technique, meaning that it does not incorporate any prior assumptions about the distribution of survival. Kaplan-Meier curves can only be produced for unadjusted data, and allow only basic comparisons between different categories of patients. Another common statistical technique for survival analysis in organ transplantation, as in other fields, is the *Cox proportional hazards model* [24,29,32,34,66,76,78,79]. Cox models are *semi-parametric* models which make no assumption about the shape of the hazard curve (distribution of risk over time) for any one subject, but assume that the hazard curve for one subject is proportional to that of any other subject. Other survival models include parametric models such as the *Weibull model* [56] and the *generalized gamma model* [80], which assume a specific shape for the hazard curve, and *competing-risks regression* [81]. It is important for researchers to understand that every statistical model relies on certain assumptions about the distribution of the data; these assumptions should be thoroughly checked as part of any study. Many excellent references exist for survival analyses [70,71,75].

Binary outcomes

The most common technique for modeling binary outcomes is *logistic regression* [40,82–85]. Logistic regression models the association between an exposure and odds of a binary outcome (odds = probability/[1-probability]) [70]. Logistic regression therefore implicitly incorporates the *rare disease assumption*: that a positive outcome is rare. If the rare disease assumption is violated, then the odds ratios (OR) returned by logistic regression will be artificially large compared to the relative risk (RR), causing a bias in the apparent effect size of a variable [38]. In other words, in a cohort study it is inappropriate to interpret an odds ratio of 2.0 as a "twice the risk" but rather as a "2-fold higher odds of risk." A well-developed set of diagnostics exists to check the fit of logistic regression models [86]. An alternative to logistic regression, with a more recognizable interpretation of the co-efficients (namely as a relative risk), is modified Poisson regression with a robust variance estimator [87].

Considerations for prediction models

Prediction models require *validation* to assess their predictive accuracy. For example, a well-constructed model to predict risk of delayed graft function (DGF) after kidney transplant should on average assign a high probability of DGF to transplant recipients in whom DGF is observed, and a low probability to patients in whom DGF is not observed. Predictive accuracy can be broken into two components:

1 Among all patients predicted to have an X% probability of DGF, is DGF in fact observed in about X% of them? and
2 Do patients predicted to have a high probability of DGF in fact have DGF more often than those predicted to have a low probability of DGF?

The first component is known as *calibration* and the second is known as *discrimination* [88]. Calibration can be assessed using the Hosmer-Lemeshow test [89]. Discrimination in binary or survival models can be assessed using the c statistic [89]. In the context of binary outcomes, the c statistic is also referred to as the area under the receiver operating characteristic (ROC) curve, or simply the AUC [90]. The predictive accuracy of two models can be compared using the methods of Delong et al. [91]. The net reclassification index (NRI) and the integrated discrimination index (IDI) [92] are alternative techniques for comparing two statistical models, based on clinically relevant metrics.

Predictive models run the risk of being overfitted to the data used to generate them, in general, this occurs when the model is overly complex, and as such, describes random variations in the data rather than meaningful trends. For this reason, a model should be validated against a population not specifically used to construct the model. *Internal validation* involves validation from other points in the same dataset; techniques include cross-validation [93] and the bootstrapping technique of Steyerberg et al. [94]. *Temporal validation* involves constructing a model on data from one time period, and validating it on data from a different time period [88]. Other kinds of validation (e.g. independent validation, in which a study is repeated at a different site to account for differences in case mix) are of less concern in population-based studies, although case mix may differ from one country or region to another. These techniques have all been used in the transplant literature [46,51].

Some pitfalls in registry-based studies

Identifier issues

Registry data are typically provided to researchers in a de-identified format, with a unique, arbitrary numeric/alphanumeric identifier (ID) assigned to each record. One patient may have several individual IDs. In the OPTN dataset, patients may have or be associated with the following IDs:

- wl_id: an ID assigned to each registration on a deceased donor waiting list
- trr_id: an ID assigned to each recipient of a transplant
- donor_id: an ID assigned to each organ donor
- multiorg_id: for multiorgan recipients, an ID to link two records corresponding to the same transplant event (e.g. to link a kidney record and a liver record for a kidney-liver transplant)
- pt_code: an idea assigned to each person, possibly spanning multiple wl_ids or trr_ids

For all of the above, each listing generates a separate ID. So a single patient who lists at two different centers, or who receives more than one transplant, may be represented with several different values for wl_id or trr_id. Similarly, a patient who receives two transplants separated by some amount of time will have a different trr_id for each transplant. Care must be taken to manage ID variables properly, particularly when merging information from several datasets (e.g. waitlist, donor, and transplant).

Informative censoring

Most statistical models used for survival analysis assume *non-informative censoring*; that is, among patients who are lost to follow-up or administratively censored, their subsequent risk of

having the outcome of interest (had they not been censored) would have been equivalent to the observed risk among patients with subsequent follow-up. For example, in a study of post-transplant survival in patients who received a heart transplant between 2005 and 2010, with follow-up through 2011, patients who received a transplant in 2010 can be followed for at most one year. The assumption of non-informative censoring states that these patients' risk of mortality in the (unobserved) second year after transplant will follow the same distribution as the observed risk of mortality in the second year for patients who received a transplant between 2005 and 2009. Censoring is *informative* if this assumption is violated. For example, liver transplant candidates believed to be at the highest risk of short-term mortality receive the highest priority in liver allocation [39,40,55]. Therefore, a study of wait list mortality must account for the fact that patients who are censored for transplant would have been more likely to die (had they not received a transplant) than most other patients followed for a similar amount of time [40]. Informative censoring can create substantial bias, but is unfortunately often disregarded in registry studies in transplantation.

Competing risks

Patients on deceased donor waiting lists may have several outcomes. They may receive a transplant; they may die on the waiting list; they may be removed for deteriorating condition; they may remain on the waiting list. These outcomes represent *competing risks* [95]; in other words, once a patient dies, they are no longer at risk for receiving a transplant, and, similarly, once they receive a transplant, they are no longer at risk of dying while on the waiting list. As an illustration, there is evidence that patients with MELD exceptions due to hepatocellular carcinoma have a lower risk of death than patients with the equivalent allocation priority due to a laboratory-derived MELD score [39,40]. The lower risk of death does not affect hepatocellular carcinoma (HCC) patients' *hazard* of transplant; the chance that a patient on the waiting list will receive a liver transplant on any given day is, in principle, the same for patients with and without HCC exceptions. However, since patients without exceptions are more likely to die before they receive a transplant, the *cumulative incidence* of liver transplant is higher in patients with HCC exceptions; a patient entering the waiting list today with an HCC exception is more likely to receive a liver transplant than a patient with equivalent allocation priority but no exception (because they are less likely to experience the competing event of dying) [40]. Conversely, factors that affect the time to transplant — for example, increased wait times as the wait list grows — affect cumulative incidence of death, but not hazard of death [95]. Death after transplant and death-censored graft failure represent another example of competing risks in the field of transplantation [15].

Investigators wishing to study determinants of survival time or time to transplant must decide whether to study hazard or cumulative incidence. A hazard analysis considers the outcome of interest as if competing risks were not present (e.g. patterns of mortality in the absence of transplantation); a cumulative incidence analysis accounts for how competing risks affect the event of interest. Traditional survival analysis techniques (e.g. Cox regression, generalized gamma) model hazard, while specialized techniques specific to competing risks must be used to model cumulative incidence in a survival framework [96]; unfortunately, often the choice of analytical technique is not consistent with the study design (in which competing risks may naively have been disregarded).

Non-proportional hazards

Cox models incorporate the *proportional hazards assumption*: that the ratio of any two individuals' hazard of an outcome is constant over time. For example, if diabetic patients on the deceased donor kidney waiting list have 40% higher risk of mortality than equivalent non-diabetic patients at the time of listing, then they have 40% higher risk of mortality three months after listing and also 40% higher risk of mortality three years after listing. Many medical interventions — including, notably, surgery — violate this assumption. The day that a patient receives a deceased donor kidney transplant, the risk of mortality is higher than if they had remained on dialysis; however, among patients who survive the perioperative period, mortality risk decreases to a risk lower than that for patients who remain on dialysis [31]. Cox models and related techniques are not appropriate when the proportional hazards assumption is violated, unless techniques such as stratification [97] or time-dependent covariates with time-varying effects [98] are used. Other techniques for modeling non-proportional hazards include Poisson regression [99], the generalized gamma distribution [80], and Markov models [100].

Effect modification

On average, black patients on dialysis have lower mortality than white patients with similar risk factors; however, among younger patients, black patients have higher mortality than white patients with similar risk factors [32]. Age is therefore an *effect modifier* for the relationship between race and mortality in this population (and, conversely, race is an effect modifier for age). This relationship can also be described as an *interaction* between race and age.

Standard regression techniques assume that the relationship between an exposure and an outcome is the same for all subgroups of patients: for example, that race affects mortality on the waiting list equally in young patients and in older patients. Overlooking interactions can lead to mistaken inference about subgroups of patients. Investigators should test interactions between key variables through the use of interaction terms in regression models [101].

Summary

This chapter has provided an overview of the uses of registry-based studies in the field of organ transplantation, and common techniques for analysis of registry-based data. Large, rich datasets and fast microprocessors put a wide array of tools at the researcher's fingertips. When used without care, these tools may yield biased or mistaken results. When used properly, these tools can yield powerful, novel insights that advance the field of transplantation and improve patient care and clinical outcomes.

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CHAPTER 134

Clinical Research Methods and Analysis in Organ Transplantation

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Introduction

Clinical transplantation has developed in close association with many companion sciences and has been the initial testing ground for numerous novel techniques, medications and practice patterns. This close association with medical advancement has required an almost continuous relationship between clinical transplantation and clinical research. As such, a working knowledge of research methods is necessary for even general transplant practice, and is an important means of guiding rational treatment decisions. Indeed, in order to generate appropriate data summaries and inferences about any population, it is necessary to utilize the appropriate statistical methods and analyses. In this chapter, we describe fundamental methodological principles and common analyses utilized with examples from the transplant literature. Regardless of the size, study design, or scope, an important guiding principle for all forms of research is the proper application of the scientific method throughout the course of study (Figure 134.1). Without this, the validity and reliability of study findings, even with the use of appropriate study design and analyses described in this chapter, may be unclear. Importantly, when a relevant, important, and novel research question is posed, the goal would be to pursue research that furthers science, regardless of whether the results of the study are "positive" or "negative."

Clinical research, as compared to basic science research, focuses on the outcomes of patients in response to specific interventions or risk factors, for example exposure. In order to examine these relationships and quantify the impact of a given exposure on an outcome of interest, a study must be thoughtfully designed so that valid inferences can be drawn from its results. The first section of this chapter will outline the major issues in the conduct of clinical research as they relate to study design while the second part will focus on study analysis.

Major types of study designs

A useful classification system for the different types of clinical research study designs is depicted in Figure 134.2 [1]. If the study investigator assigns the exposure or intervention, the study is considered *experimental* in design. If the assignment of exposure or intervention is not under the control of the study investigator, that is not for the purposes of the study protocol per se, then the study is *observational* in design.

Experimental study designs

Once a study has been deemed experimental in design, the next step is to decide on the mechanism by which the intervention or exposure will be allocated. If it involves a process whereby every patient recruited into the study has a fixed probability of receiving the intervention or the comparator, then the study is called a *randomized controlled trial* (RCT). If a non-random mechanism (e.g. alternation) is used for allocation, then the study is called a *non-randomized controlled trial*. The latter study design is uncommon in the current era of clinical research since randomization offers considerable advantages with respect to study validity (as described further on). As a result, non-randomized controlled trials will not be discussed further.

The term *random* in statistics is not synonymous with haphazard. Instead, it refers to a probability distribution that defines the likelihood of some event occurring. In the context of a classic one-to-one, 2 arm, parallel-group RCT, this probability distribution would have two possible values, intervention and comparator, and the probability of each is 50% (i.e. a coin flip). Similarly, in a two-to-one, 2 arm, parallel-group RCT, the probability of intervention would be 67% and the comparator would be 33%. In a 3 arm RCT with equal sized groups, the probability of receiving any of the three treatments would be 33%. An extension of this principle may be applied to a 3 arm RCT with unequal sized groups.

One of the most important features of randomization is that every patient who enters the study has the same probability of receiving the intervention. Effectively, this reduces the likelihood that prognostic characteristics of the study patients will be unequally distributed between the intervention and comparator arms. As the sample size increases, the probability that important factors will be imbalanced across treatment groups will decrease. Moreover, this uncoupling of the link between treatment allocation and patient prognosis ensures that, on average, both *known* and *unknown* baseline characteristics of study patients will be balanced across treatment groups. This balancing property of randomization for known and unknown factors is the key feature that elevates randomized controlled trials to its "gold standard" status among all study designs.

An example of a well-conducted RCT in kidney transplantation is the ALERT study [2]. This study evaluated the efficacy of fluvastatin versus placebo in reducing cardiovascular events in a

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

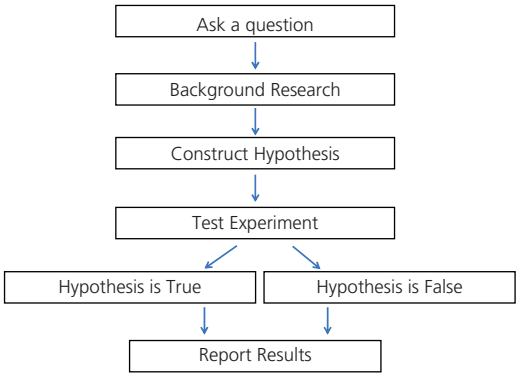


Figure 134.1. The scientific method.

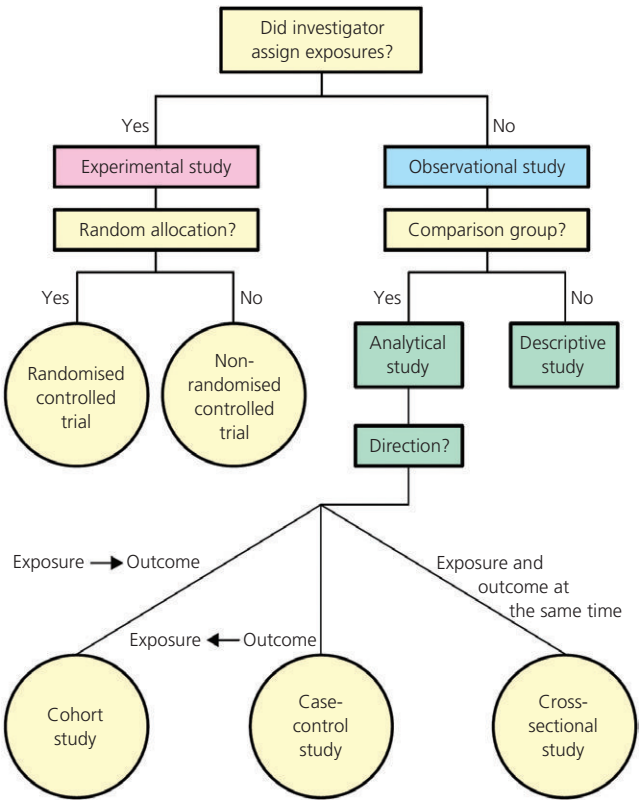


Figure 134.2. Classification of clinical research study designs. Reproduced from [1] *Lancet* 359. Grimes DA, and Schulz KF. An overview of clinical research: the lay of the land:57–61, 2002. Copyright 2002, by permission of Elsevier.

Table 134.1. Baseline characteristics of kidney transplant recipients randomized to fluvastatin or placebo in the ALERT study. Reproduced from [2] *Lancet* 361. Holdaas H, Fellstrom B, Jardine, AG, et al. Effect of fluvastatin on cardiac outcomes in renal transplant recipients: a multicentre, randomised, placebo-controlled trial. 2024–2031, 2003. Copyright 2003, with permission from Elsevier

	Fluvastatin (n = 1050)	Placebo (n = 1052)
Demographic and clinical characteristics		
Mean (SD) age (years)	49.5 (10.9)	50.0 (11.0)
Male	701 (66.8%)	686 (65.2%)
Mean (SD) diastolic blood pressure (mm Hg)	85.6 (10.1)	85.6 (10.0)
Mean (SD) systolic blood pressure (mm Hg)	143.8 (18.7)	144.0 (19.1)
Mean (SD) BMI (kg/m^2)	25.8 (4.4)	25.8 (4.6)
Mean (SD) total cholesterol (mmol/L)	6.4 (1.1)	6.5 (1.1)
Mean (SD) LDL cholesterol (mmol/L)	4.1 (1.0)	4.1 (1.0)
Mean (SD) HDL cholesterol (mmol/L)	1.3 (0.5)	1.4 (0.4)
Mean (SD) triglycerides (mmol/L)	2.2 (1.2)	2.2 (1.5)
Primary cause of renal failure		
Glomerulonephritis	387 (36.9%)	364 (34.6%)
Polycystic disease	138 (13.1%)	183 (17.4%)
Diabetic nephropathy	135 (12.9%)	138 (13.1%)
Pyelonephritis or interstitial nephritis	124 (11.8%)	135 (12.8%)
Hypertensive nephrosclerosis	59 (5.6%)	46 (4.4%)
Systemic lupus erythematosus or vasculitis	30 (2.9%)	22 (2.2%)
Unknown	58 (5.5%)	53 (5.0%)
Other	157 (15.0%)	134 (12.7%)
Transplant characteristics		
First transplantation	894 (85.1%)	900 (85.6%)
Mean (SD) time taking renal replacement therapy (months)	88.5 (56.5)	88.8 (58.3)
Type of last transplant		
Live donor	240 (22.9%)	229 (21.8%)
Cadaveric donor	809 (77.0%)	822 (78.1%)
Mean (SD) serum creatinine (μmol/L)	147 (54.4)	143 (51.0)
Concomitant immunosuppressive therapy*		
Azathioprine	684 (65.1%)	680 (64.6%)
Prednisolone	851 (81.0%)	848 (80.6%)
Cyclophosphamide	9 (0.9%)	10 (1.0%)
Mycophenolate mofetil	167 (15.9%)	159 (15.1%)
Other	198 (18.9%)	224 (21.3%)
Cardiovascular risk factors		
History of angina pectoris	71 (6.8%)	77 (7.3%)
Previous MI	32 (3.0%)	34 (3.2%)
Diabetes	197 (18.8%)	199 (18.9%)
Hypertension	798 (76.0%)	777 (73.9%)
History of cerebrovascular disease	62 (5.9%)	60 (5.7%)
History of PVD	80 (7.6%)	78 (7.4%)
Current smoker	204 (19.4%)	185 (17.6%)
Known family history of CHD	91 (8.7%)	124 (11.8%)
Concomitant cardiovascular medications*		
Any cardiovascular drug	1001 (95.3%)	999 (95.0%)
Aspirin	371 (35.3%)	353 (33.6%)
Dipyridamole	21 (2.0%)	26 (2.5%)
Coumarin or warfarin	90 (8.6%)	94 (8.9%)
β blockers	649 (61.8%)	627 (59.6%)
Calcium antagonists	728 (69.3%)	738 (70.2%)
ACE inhibitor or AIIRA	520 (49.5%)	529 (50.3%)
Diuretics	590 (56.2%)	573 (54.5%)
α blockers	176 (16.8%)	170 (16.2%)
Other	316 (30.1%)	373 (35.5%)

population of kidney transplant recipients. The table from the original paper outlining the baseline characteristics across treatment groups is reproduced (Table 134.1) to highlight the balance achieved between groups on all known (and measured) baseline prognostic factors. As mentioned above, the balance achieved in known prognostic factors provides assurance that unknown prognostic factors are also likely balanced since a random allocation scheme (with 50% probability of receiving the intervention) was used to determine who received fluvastatin versus placebo.

Observational study designs

If allocation of the intervention or exposure was not in the control of the study investigator, then the study is considered *observational*. The presence or absence of a comparator group must then be ascertained. The specific use of registries in observational studies is covered in depth in Chapter 133.

Descriptive observational studies

An observational study with no comparison group, for example patient(s) exposed to a novel treatment, is considered a *descriptive*

study. This is the domain of case reports and case series where the outcomes of patients on a novel treatment may be described. Alternatively, the course of patient(s) with a rare disease or an unusual presentation of a common disease may be presented. Trends in disease incidence over time or utilization of health care resources are also examples of descriptive observational studies. Although these studies may be helpful in highlighting findings of clinical interest, therapeutic importance, or health policy relevance, the absence of a comparator group prevents investigators from quantifying the association of a novel treatment, risk factor, or policy with an outcome of interest. The latter can only be achieved in a study with at least two groups for comparison (i.e. an analytical study).

Despite their shortcomings, descriptive observational studies can provide the basis for more rigorous, hypothesis-driven, analytical studies to examine the effect of a novel intervention or risk factor on clinically important end-points. An example of a descriptive study that has led to the design of more definitive analytical studies is the case report by Locke et al. [3]. This report described a patient with refractory acute antibody-mediated rejection who, upon treatment with eculizumab, had an improvement in kidney function and histology, both of which returned to baseline within two months of treatment. This report was followed by an analytical observational study by Stegall et al. that has subsequently formed the basis for an RCT evaluating eculizumab in the prevention of acute antibody-mediated rejection in high-risk kidney transplant recipients [4].

Analytical observational studies

There are three major types of analytical observational study designs in clinical research. They are described below.

Cohort studies

Figure 134.3 displays the structure of a cohort study. In a cohort study, patients from a population are recruited into the study using clearly defined and prespecified inclusion/exclusion criteria. At the time of recruitment, an exposure or risk factor, such as delayed graft function or current smoking, is measured in each patient and the development of the outcome or disease in the "exposed" group is examined against a comparable group of patients who are "unexposed". Exposures can be grouped into categories (as shown) or may be measured on a continuous scale (e.g. systolic blood pressure). Continuous exposures can also be categorized into clinically meaningful groups (e.g. systolic blood pressure <140 versus ≥ 140 mmHg).

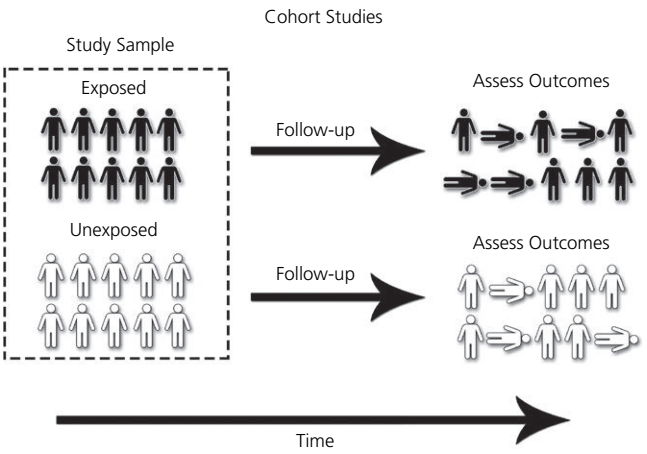


Figure 134.3. The structure of a cohort study.

Since all patients entering a cohort study are free of the outcome or disease of interest at the time of study recruitment, the *incidence* or new case rate of a disease over follow-up can be estimated from the cohort. The time from study recruitment , that is the time when patients come under observation, to the time of the outcome of interest can be measured in cohort studies, therefore this is the only study design that permits survival analysis. Importantly, only patients at risk for the outcome of interest should be included in a cohort study. For example, a study of risk factors for recurrent acute rejection should only include patients who have already had one episode of acute rejection. Patients with no history of acute rejection would not be eligible to have a second acute rejection until they have already had their initial episode.

Cohorts of patients may be assembled in the present and followed into the future for the event(s) of interest. This is known as a *prospective* cohort study. Alternatively, existing datasets may be used to assemble cohorts from the past and then track the occurrence of the outcome over time. This is known as a *retrospective* cohort study. Both designs are fundamentally cohort studies since they assemble patients at some clearly defined time point (either in the present or past) and then follow them forward in time to measure the outcome of interest. An alternate way of expressing this concept is that a cohort study ascertains exposure status at the time patients enter the study and then outcomes are tracked in each exposure group over time. Retrospective cohort studies are often assumed to be inherently inferior to prospective cohort studies in terms of study validity. This is not necessarily so. The key issue is the quality and breadth of data collection and use of the basic principles of the scientific method. An existing dataset may not have been meant to answer a specific scientific question, but if the relevant data have been rigorously captured, the rigor and quality of a retrospective cohort study can rival any prospective cohort study.

Sometimes, instead of entering exposed and unexposed patients into the cohort over a similar time period, unexposed patients from the more distant past (also known as historical controls) may be compared to exposed patients from the more recent past or the present. This study design is known as a *non-concurrent* (as opposed to *concurrent*) cohort study. Non-concurrent cohort studies are typically performed when a new treatment has been widely adopted and the outcomes under the new treatment are compared to the outcomes under an older treatment that was more commonly used in the past. For example, the aforementioned study by Stegall et al. compared the acute antibody-mediated rejection rates of consecutive living donor kidney transplant recipients receiving prophylactic eculizumab post-transplant with historical patients receiving prophylactic intravenous immunoglobulin and plasmapheresis post-transplant [4]. The major shortcoming of this design is that changes in the general medical management of patients and other secular changes cannot be effectively accounted for since most (if not all) of the more recent patients will have been treated differently from past patients. This may be less problematic if the time period during which exposed and unexposed patients are recruited is relatively short.

A cohort study is the preferred study design in the setting of a rare exposure. For example, the long-term implications of acute radiation exposure at the end of World War II have been studied in

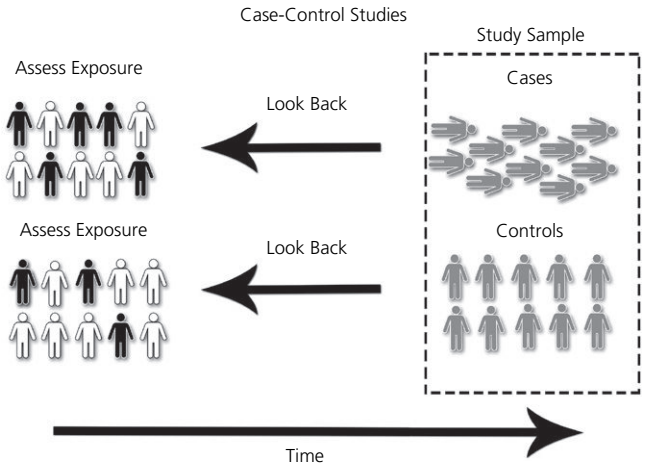


Figure 134.4. Structure of a case-control study.

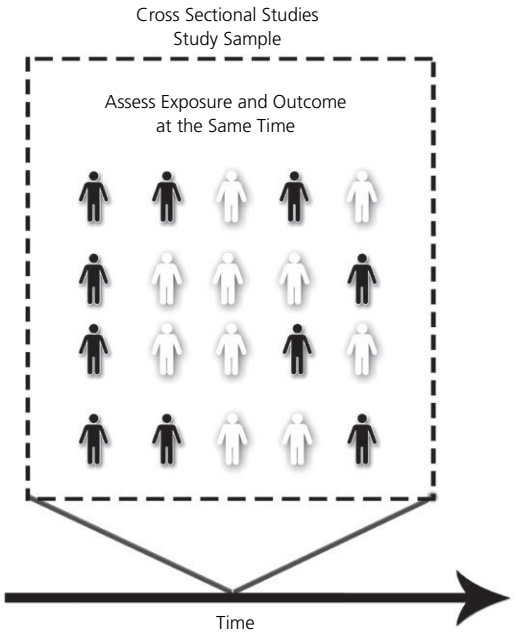


Figure 134.5. Structure of a cross-sectional study.

a cohort of atomic bomb survivors from Japan [5]. This cohort is special in that the exposure of interest is, thankfully, uncommon but provides a unique opportunity to study its health effects over many years of follow-up.

Cohort studies are considered the central study design in epidemiology. All other study designs are ultimately derived from a cohort. In fact, an RCT is also a cohort study except the treatment, intervention, or exposure is randomly allocated in the context of the study protocol. In contrast, exposures measured in observational cohort studies are typically allocated in non-random ways by the patients themselves (e.g. smoking) or their caregivers.

Case-control studies

Case-control studies are less common in the transplant literature but are widely used in other areas such as genetic epidemiology. The structure of a case-control study is depicted in Figure 134.4. Case-control studies initially assemble patients on the basis of their outcome, such as diseased versus non-diseased patients. Subsequently, the exposure status of diseased patients at some time point in the past is ascertained and compared to the exposure status among non-diseased patients. Inherently, case-control studies are retrospective in the sense that both the exposure and outcome have already occurred by the time the study is conceived. Note that the case-control study is still an analytical observational study since the investigator did not assign the exposure or risk factor and there are at least two comparison groups (Figure 134.2).

The case-control study is the most efficient design for evaluating rare diseases or outcomes that take many years to develop since it takes advantage of existing datasets with exposures and outcomes that have already been captured. Moreover, if there is additional effort needed to measure the exposure, for example through chart review, patient interviews, or testing of stored blood samples, a case-control study will allow investigators to estimate the association between exposure and disease in a more timely and economical way than in a traditional cohort study.

One of the major drawbacks of case-control studies is that the incidence of disease cannot be estimated since the proportion of patients with the disease of interest is determined by the number of cases and controls that are sampled by the investigator and not by the disease frequency in the target population. Also, there may be a greater tendency for biased results (versus a cohort study) if non-diseased controls are selected inappropriately (see *Major types of bias in clinical research* further on in this chapter). In general, controls should be selected from the pool of patients from which the diseased cases in the study are derived. This pool of patients is also known as the *study base* or *source population* (since it is the source of the cases). However, this population may be difficult to determine in advance and thus the approach to control selection requires careful thought and execution.

A recent case-control study examined the association of betapapillomavirus (betaPV) and squamous cell carcinoma (SCC) among organ transplant recipients [6]. A total of 210 cases of SCC and 394 controls were recruited from dermatology and transplant clinics at five European medical centers. Human papillomavirus DNA genotyping and betaPV serology were performed on eyebrow hairs and blood respectively. To improve comparability between cases and controls, the investigators matched patients based on age, sex, and time since transplantation. Using a logistic regression model, the investigators found significant relationships between several betaPV serotypes and SCC in this study population. Note that the study question was well suited for the case-control design since the occurrence of SCC requires long-term follow-up and is a relatively uncommon occurrence. Moreover, the measurement of specific biomarkers was efficiently performed on a sample of cases and controls instead of all organ transplant recipients at the five study centers.

Cross-sectional studies

Cross-sectional studies sample patients at one point in time or over a short duration and both exposure and outcome are assessed simultaneously without additional follow-up. The structure of a cross-sectional study is shown in Figure 134.5. Since patients can be categorized into exposure groups, and the proportion of patients with the outcome of interest can be calculated among exposed and unexposed patients, this design qualifies as an analytical observational study. Surveys performed on a population of patients where

information on exposures (e.g. smoking status) and outcomes (e.g. history of myocardial infarction) are assessed at the same time are the most common examples of cross-sectional studies.

Cross-sectional studies can be performed quickly, since no follow-up is necessary, and at little expense. It can also provide clues to scientifically interesting associations that may be later confirmed in cohort studies or an RCT. As a result, cross-sectional analyses have been typically referred to as *hypothesis-generating* studies. Furthermore, since there is no follow-up of patients, the new case rate or incidence cannot be calculated (as in a cohort study). Instead, the *prevalence*, or the proportion of patients with the outcome or disease at a given time, is the main metric of interest while the prevalence of disease in the exposed versus non-exposed is the main comparison of interest.

The major weakness of cross-sectional studies, however, is that the temporal relation between exposure and outcome may not be clearly delineated and thus associations derived from these studies may be susceptible to *reverse causality*. The latter refers to situations where the outcome has affected the exposure such that the association measured in a cross-sectional study may be biased. For example, a survey of patients' current smoking habits and a history of lung cancer may erroneously suggest that smoking is protective against lung cancer. However, the development of lung cancer may have altered the taste for cigarettes leading to a reduction in smoking frequency versus the non-cancer group.

Experimental versus observational study designs

The RCT has become the gold standard study design for evaluating treatments or health care technologies since random allocation of the intervention improves the validity of causal inferences about the effect of intervention on the outcome(s) of interest. This primarily results from the balancing property of randomization for both known and unknown prognostic factors. However, there are settings in which the RCT is infeasible or unethical. Exposures or risk factors that cannot be readily allocated by the investigator, such as delayed graft function or hyperhomocysteinemia, must be studied in an observational setting. In addition, potentially harmful exposures such as smoking cannot be ethically allocated to patients, and thus can only be evaluated using observational study designs.

Typically, only one or two hypotheses can be formally tested in an RCT with sufficient statistical power whereas multiple hypotheses can be examined in observational studies. In transplantation, a significant challenge of RCTs is also the relatively small eligible population for enrolling a sufficient number of patients to test research hypotheses. Often, RCTs must utilize surrogate endpoints rather than traditional hard endpoints (such as death or graft loss) due to resource demands or lack of long term follow-up. While these surrogates can be important indicators of mechanisms of effects of interventions, they also can detract from inferring the broad impact of a given treatment [7]. Moreover, RCTs tend to include a relatively homogeneous, well-defined, population of patients treated under ideal conditions. This may limit the generalizability of the results. In fact, transplant patients that participate in research are systematically different from patients that do not participate and have significantly different outcomes [8]. Thus, investigations of research questions with observational studies often serve as important complements to RCTs, which can examine the impact of interventions in practical application and typically with more statistical power. The non-trivial tradeoff of observational studies is the potential for bias associated with non-randomized designs.

Random and systematic error

The major types of errors in clinical research can be categorized into random and systematic.

Random error

Random error refers to variability in measurement that may impact the reproducibility or reliability of study findings. Sources of variability include:

- the observer — that is the person making the measurements;
- the instrument — a sphygmomanometer for blood pressure readings;
- the subject — the individual on which measurements are being made.

A reproducible or reliable measure is said to have good *precision*. Therefore, random error and precision are inversely related; less random error implies greater precision (and vice versa).

In general, studies with larger sample sizes improve the precision of summary measures (e.g. hazard ratio) derived from the study subjects (Figure 134.6). Moreover, standardizing approaches to measurement, properly training research personnel, and calibrating instruments are other ways to improve precision. The implication is that the greatest gains in precision are made during the *design and conduct* phases of a study. Although there are statistical methods that can improve precision in the analysis phase, the gains are relatively minor in comparison.

Systematic error

Systematic error refers to a fixed deviation from the true value of the measurement of interest. Unlike random error, it is not improved or reduced by increasing the sample size of the study (Figure 134.6). Another term for systematic error is *bias* (which will be further explored in the next section). Sources of systematic error can be classified in a similar fashion to random error:

- the observer — how patients are selected to be included in the study
- the instrument — measurement of key variables
- the subject — tendency to possess other prognostically important risk factors along with the exposure of interest.

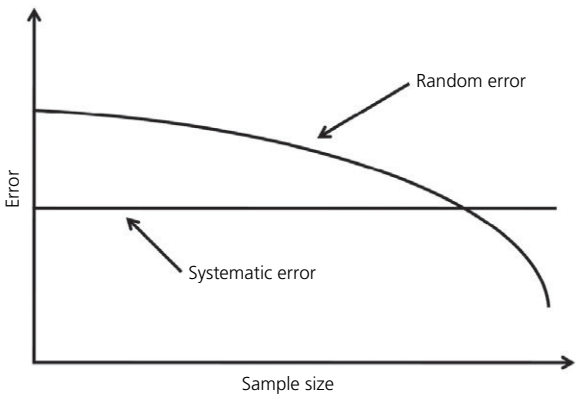


Figure 134.6. Random and systematic error as a function of sample size. Adapted from Rothman KJ. *Epidemiology: An Introduction*. Oxford University Press. 2002 [53].

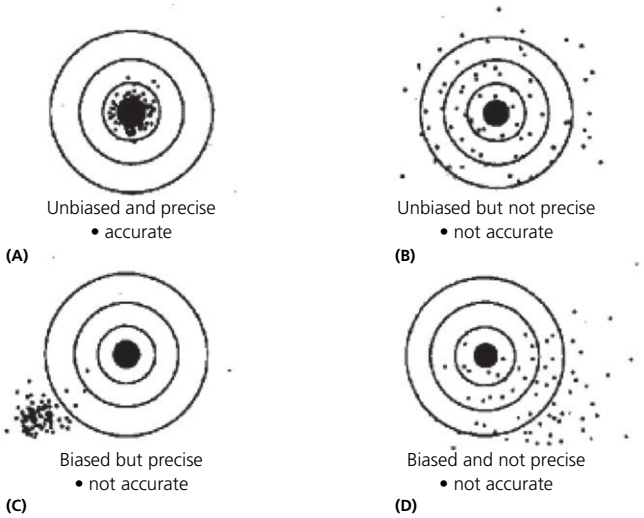


Figure 134.7. Bias versus precision.

Note that precision is to random error as *validity* is to systematic error. Thus, greater bias means less validity. Approaches to reducing systematic error will improve the validity of measurements. Furthermore, akin to random error, these approaches are most effective when implemented during the design and conduct phases of the study. Statistical adjustment is most helpful in the setting of confounding bias (see further on) but requires that the relevant variables are considered and measured during the earlier phases of the study.

Relationship between precision and bias

The goal of every clinical researcher is to have his/her study provide both a precise and unbiased/accurate measure of association for the exposure-outcome relationship of interest. This is reflected in Figure 134.7a. However, the results of a study may be highly precise but biased (Figure 134.7c). This is typical of large population-based observational studies of therapy where the measure of association is precisely quantified but may be erroneous due to biases that have not been taken into account. Alternatively, some studies may provide a valid measure of the relation between exposure and outcome but it may be imprecise (Figure 134.7b). This can be observed in the setting of a well-conducted randomized clinical trial where a valid measure of the treatment effect can be obtained but the sample size is insufficient to allow a precise calculation of this effect.

Internal versus external validity

As previously mentioned validity is inversely related to bias, a study with less bias is more valid and vice versa. A valid study provides an estimate of the exposure-outcome relationship that approximates the true value of interest. However, the "true" value may depend on which population one is referring to when making epidemiologic inferences.

A study that sampled an appropriately representative group of patients (i.e. no selection bias), made accurate measurements of all relevant variables (i.e. no information bias), and controlled for factors that can confound the relation between exposure and outcome (i.e. no confounding bias), is said to have *internal validity*.

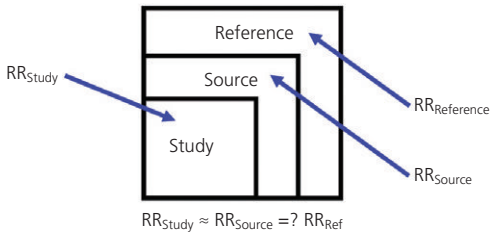


Figure 134.8. Generalizability of study findings (internal vs. external validity).

Effectively, this means that the measure of association (such as the risk ratio (RR)) calculated for an exposure-outcome relationship from the study population is a good estimate of the RR that would have been calculated from the source population (Figure 134.8).

Note in Figure 134.8 that the study population is nested within the source population, which in turn, is nested within the *target* or *reference* population. As mentioned above, internal validity relates to the RR in the study being a good estimate of the RR in the source population. Moreover, if the latter is also a good estimate of the RR in the reference population, then the study result is said to have good *external validity*. An example of study, source, and reference populations include a sample of kidney transplant recipients from a given center, all kidney transplant recipients from that center, and all kidney transplant recipients from similar centers throughout the world, respectively. Similarly, US nurses recruited into a study cohort, all nurses in the US, and all women in the US may constitute the study, source, and reference populations for the Nurses' Health Study [9].

Based on the definitions outlined above, internal validity is required before external validity can be established. Most times, internal or external validity cannot be established independent of the data from the study itself. One must rely on the characteristics of the study design, conduct, and analysis to infer that the results are likely internally valid. External validity may be partially assessed by the characteristics of the study participants and their similarity to a reference population of interest. However, there may be other unmeasured or unknown factors that differ between study subjects and the reference population. It is also important to note that the source and reference populations to which the results of a study may apply are specific to a given study's objectives and design. There is no absolute source and reference populations, only those that are relevant to a given study.

Major types of bias in clinical research

There are three major types of study bias in clinical research that encompasses all the ways that the results of a study can be misleading or incorrect (apart from the play of chance).

Selection bias

Selection bias is the distortions that can be observed in the exposure-outcome relationship due to the way patients are recruited into a study or the way they are lost over follow-up. The factors that determine participation in a study may also be related to health status such that those who are included in a study may be more or less healthy than those who were theoretically eligible to be studied (but were not included). Note that the selection of specific patient subgroups into a study is common but bias only occurs when the exposure-disease relationship is altered as a result of this selection

process. In this setting, the measure of association calculated in the study population is different from the relation in the source population.

A recent example of selection bias can be seen in the observational studies relating intake of hormone replacement therapy (HRT) to the development of cardiovascular disease among a cohort of US nurses. Analysis of the Nurses Health Study (a large prospective cohort study of US nurses followed over several decades) showed that HRT significantly reduced the risk of cardiovascular events [9,10]. However, a large clinical trial of HRT subsequently showed that the risk of cardiovascular disease is not reduced, and in fact, may be increased in the early period after initiating treatment [11]. This suggested that nurses who participated in the study may be different from the general population of women with respect to their health status and these differences were difficult to measure and adjust for in the original analysis.

Although one of the most effective ways to eliminate selection bias during study recruitment is to randomize the exposure or intervention of interest, this is not feasible in many settings. Selection bias may occur during recruitment of patients into a cohort study or as a result of inappropriate selection of controls in a case-control study. Furthermore, the potential for selection bias exists over follow-up in both clinical trials and cohort studies. If exposed (or unexposed) study subjects with the best (or worst) prognosis are preferentially lost to follow-up, the remaining cohort may provide a biased estimate of the association between the exposure and disease.

The control of selection bias is critically dependent on the design phase of the study. Adjustment for selection bias during the analysis phase is difficult, if not impossible. Careful recruitment of study subjects who are representative of the source population should be undertaken. Moreover, strategies to optimize retention in clinical trials and cohort studies will be necessary to reduce the risk of selection bias developing over follow-up.

Information bias

Information bias results from errors in the ascertainment of exposure, outcome, and/or confounders leading to invalid assessments of the association between exposure or treatment and the outcome of interest. This bias is also referred to as measurement error or misclassification (for categorical variables). Errors of this kind are ubiquitous in clinical research and are often underappreciated. In most cases, the degree of bias caused by measurement errors is difficult to predict in advance. If the errors are consistent across binary exposure or disease categories (e.g. the rate of misclassifying hypertensives versus non-hypertensives is the same regardless of the future risk of cardiovascular disease), then the association will tend to be attenuated towards the null effect. This is also referred to as non-differential misclassification. In the setting where the misclassification is differential (i.e. error rates vary as a function of exposure AND disease), then the direction and magnitude of the bias is difficult to predict.

Reducing the risk of information bias involves the proper training of research personnel, calibration of measuring instruments, and appropriate codification of all data elements to be collected. In addition, if the exposure, outcome, and/or confounders were captured using a surrogate measure (versus the gold standard), it would be useful, when feasible, to perform a sub-study where a smaller group of subjects undergo measurements by both surrogate and gold standard approaches. This will provide a means to assess the degree of measurement error associated with the former. For example, a food frequency questionnaire that collects data on dietary patterns based on recall over an extended period of time should be validated against a daily food diary performed in a sub-set of patients. Validation work of this kind can also be used to support future research that uses this questionnaire.

Confounding

Fundamentally, confounding refers to a confusion of effects. Confounding of a measured relation between exposure and disease occurs when this relation may be fully or partially explained by the influence of an extraneous third factor.

In order to cause confounding, the third factor must have the following three properties:

1 It is an independent risk factor for the outcome (in the unexposed).
2 It is related to the exposure (in the source population).
3 It is not affected by exposure or disease.

The first property implies that the third factor is causally related to the outcome. This relationship should exist independent of the exposure (i.e, a relation between the confounder and the outcome should be observed among the unexposed). The second property highlights the need for the third factor to be related to the exposure among those who have not yet developed the disease, such as the source population, since the exposure and third factor will tend to be related among the diseased, regardless of their relation in the source population. The last property includes settings in which a third factor may mediate the effect of an exposure on the outcome (i.e. an intermediate in the causal pathway). For example, the impact of statin therapy on cardiovascular disease is, at least partially, mediated by its influence on LDL cholesterol levels. In this setting, LDL cholesterol does not confound the relation between statin therapy and the occurrence of cardiovascular disease.

An extraneous third factor that is responsible for differences in disease frequency among exposed and unexposed individuals is called a *confounder*. Moreover, related variables that can act as surrogates for these extraneous factors may also be considered confounders. These factors are *extraneous* in the sense that they are not part of the causal pathway from exposure to disease (as noted in the third property above).

Control of confounding is dependent on measuring the factors that confound the relation between exposure and disease and then accounting for them in the study design or analysis phase. Study design approaches to control for confounding including randomization, matching, and restriction (study analysis approaches will be discussed in the second part of this chapter). Although randomization is the most effective way to control for both known and unknown baseline confounders, it is infeasible or impractical in many settings (e.g. if the exposure is a biomarker in the blood).

Matching refers to pairing exposed and unexposed subjects at the same level of the potential confounder(s) of interest. For example, matching on gender will ensure that it no longer acts as a confounder in the exposure-outcome relationship since the frequency of males (and females) will be comparable in exposed and unexposed individuals. Multiple confounders can be matched simultaneously but this tends to reduce the number of matched pairs that can be created. Moreover, once matched, these confounders cannot be assessed in terms of their relation to the outcome.

Restriction involves limiting the study population to only a certain level of a given confounder to eliminate its influence on the

exposure-disease relationship. For example, if gender is a potential confounder, then studying only females will eliminate confounding by gender. Although it can be a powerful way to control for confounding, there are some disadvantages to restriction. This method tends to reduce the sample size and thus adversely impact on the power. As a result, one needs a large dataset in order to employ restriction effectively. In addition, if the association between exposure and disease differs in males versus females, then restriction to only females will prevent the investigator from examining the potential for effect modification by gender. Finally, the generalizability of the results may be diminished since the study population has been altered from the original one that was being evaluated.

Power and sample size issues

In designing a clinical trial or observational study, one of the main goals is to show a difference in the effect of the intervention or exposure on the outcome of interest, if it truly exists. The target sample size needed to show this difference depends on several factors. These include:

- the hypothesized effect size;
- the baseline rate of the outcome in the control or unexposed population;
- an estimate of the variability around the outcome;
- the type 1 error rate, such as the probability of finding a difference when there is really no difference (typically set at 0.05);
- the power or one minus the type 2 error rate, such as the probability of finding a difference when there really is a difference (typically set at $\geq$0.80);
- subject attrition rate (or lost to follow-up);
- the recruitment period; and
- the follow-up period [12].

The specific elements that are relevant to a given situation depend primarily on the type of outcome being evaluated (i.e. continuous, categorical, rate, or time-to-event).

Sometimes, the sample size for a study may be relatively fixed and thus a calculation of power for various effect estimates is necessary. Regardless of which quantity is desired, sample size and/or power calculations are necessarily artificial since one needs to make various assumptions about the values of the parameters mentioned above. These assumptions may be guided by existing data in other populations or theoretical estimates but the true values are not known in advance of conducting the study but can have a marked effect on the actual sample size required to conduct a study [12]. Appropriate sample size/power calculations are not only important to increase the likelihood that there will be adequate precision in the estimated treatment or exposure effect, it provides study subjects the assurance that their participation in the study will yield useful information to clinicians and future patients.

A number of proprietary statistical software packages have built in sample size/power calculators (e.g. Stata or SPSS). There are also excellent sample size/power calculators that can be freely downloaded from the web (e.g. http://biostat.mc.vanderbilt.edu/PowerSampleSize).

Data sources: strengths and weaknesses

The opportunity and quality of research is often dependent on the data source. Without careful evaluation of the quality of data, findings may be erroneous or misleading or do little to guide prospective practices. It is also important to consider that data derived from different sources can also be utilized for unique purposes and have a different variety of strengths and limitations. Researchers and consumers of research must be cognizant of these in order to best design studies and interpret and implement findings into practice. Data sources can be broadly categorized as deriving from prospective trials or observational studies. A further distinction for observational datasets is those derived from single- or multi-center studies and those from national registries. The general strengths and weaknesses of these data sources are considered below.

Randomized controlled trials

As discussed previously, well-designed RCTs provide the best evidence for a research hypothesis while mitigating the presence of selection and confounding biases that are endemic to observational studies. Despite the advantages of RCTs, it is important to recognize that the design, conduct, and reporting of the trial remain critical to the interpretation of results.

The primary limitations of clinical trials relate to significant resource constraints, inability to test for rare events, or address study questions that may be considered unethical to test (e.g. testing the efficacy dialysis versus kidney transplantation will likely never be validated in a clinical trial). In addition, there is substantial literature to suggest that clinical trials are both designed and conducted with varying degrees of quality [13]. The CONSORT group has published numerous articles in an attempt to standardize trials based on best practices [14,15]. Consumers of this research should be cognizant of potential pitfalls or sources of potential bias that may accompany results of clinical trials.

Clinical trials are typically designed and statistically powered to answer one research question based on a primary endpoint. One common misconception is that failure to detect significance for secondary endpoints suggests non-significant differences. Secondary endpoints are rarely powered to show differences, and as such, lack of statistically significant findings cannot be interpreted in the same manner as primary endpoints. A source of contention about clinical trials is the use of subgroup and post-hoc analyses [16]. While these analyses may provide additional information about the effects of an intervention, they cannot be assessed in the same manner as the primary research hypothesis for which a trial was designed. Most prominently, secondary analyses often do not maintain the benefits of randomization and each test may not be sufficiently powered. In addition, it should be clear whether hypotheses were tested a priori and whether adjustment for multiple testing was incorporated into the analysis.

An important consideration for the interpretation of clinical trials includes whether results are applicable in practice. One must consider the potential for "study effects" in which, based on the conditions of the trial (e.g. reduced medication prices, enhanced follow-up protocols, or selected populations), results may not always be applicable outside of the study setting. More recently, trials in transplantation have been designed with non-inferiority endpoints. The attraction of these designs is reduced resource requirements and potentially more rapid findings. However, there are also significant notes of caution about non-inferiority trials including the use of a somewhat arbitrary effect sizes and the choice of control groups [17]. Finally, due to resource constraints, trials often utilize composite endpoints (for kidney transplantation these may include acute rejection, renal function, serious adverse events along with death and graft loss). An important consideration for these trials is how to reconcile results in which each component of a composite endpoint may have different clinical

ramifications and may occur with different frequency between study arms [18].

Single-center observational studies

One of the advantages of research deriving from single centers is acquisition of data with granular information that is specifically related to a given research hypothesis. That is, rather than relying on existing data sources, research from single center studies have an opportunity to collect specific information pertinent to a study question. This type of specificity may yield novel findings that are difficult to replicate across different settings. Another advantage of these studies is that findings often reflect observations with a homogenous environment and care protocols. Based on this, the variability that may be attributed to different models of care at different institutions is not salient for the interpretation of study findings.

A primary limitation of single-center studies is potential lack of generalizability to other contexts. As an example, a given center may utilize protocol biopsies as a standard of care and report rejection events at different rates than centers that only perform biopsies for cause. As such, validation of findings from single centers is often critical towards understanding the implications of study findings. Single-center studies may also lack statistical power to test hypotheses for "hard endpoints" such as patient death unless aggregated over a broad era. Moreover, acquiring data over a broad era may lead to potential bias related to secular trends. These aspects should be considered for both the design and interpretation of studies from single centers.

Multi-center observational studies

The general principles of observational studies derived from a single institution apply to multi-center studies, often referred to as collaboratives. The advantages of combining data across institutions include (a) an increase in sample size and (b) validation of findings between settings with potentially diverse populations and care practices. As compared to single-center studies, findings derived from multiple institutions may be considered more externally valid and more likely applicable in novel settings. However, a limitation of multi-center studies is often a lack of data consistency, which may compromise the ability to pool data in a standardized form. As such, prospective planning of these studies prior to any data collection is often critical to study completion.

National registries

The field of transplantation is fortunate to have a mandatory data collection process such that information on virtually the entire census of patients in the US is available [19]. Data collection on transplant candidates, recipients, and donors is administrated by the United Network for Organ Sharing (UNOS). This agency oversees the collection of forms and compiles data for research files and study reports. In addition, the US Renal Data System (USRDS) and the Scientific Registry of Transplant Recipients (SRTR) are contracted agencies that also utilize these data to produce research files. The SRTR also contains data on all non-kidney solid organ transplant recipients and donors. National registries have been utilized extensively for research and present a rare opportunity in medicine to evaluate a complete cohort of patients. However, as with smaller observational studies or randomized trials, national registries are also associated with various strengths and weaknesses. More details regarding population studies and transplant registries are discussed in Chapter 133.

Exploratory data analysis

One of most important and perhaps underappreciated aspects of any research study is a comprehensive evaluation of the applicable data elements. Exploration of the pattern of these data can be important to identify outliers and influential observations, determine if there are linear or non-linear patterns of continuous data, describe the shape of data elements, and examine bivariate relationships between variables. This process can also be critical to avoid utilizing data with miscoded information or for variables that may have been used in different forms over time (e.g. UNOS forms collected the same data element using a different scale) as well as verifying the data are in an appropriate format. In general, investigators should be comfortable with the structure of data prior to conducting more complex analyses.

Univariable analyses

Univariable analyses typically involve depicting the distribution or shape of a variable, estimate measures of central tendency and variability or dispersion. The applicable statistics that reflect these characteristics depend on the type of individual variables. As such, identifying the type of variable within a study is critical to inform the descriptive statistics and further modeling of these variables for subsequent analyses. There are multiple purposes for univariable analyses including: investigating the presence of outliers and influential observations, assessing the validity of distributional assumptions of variables, and initially examining whether a given sample compares to standards in a known or broader population. For data that does not meet desirable distributional properties for a given analysis, univariable analyses can also be utilized to examine appropriate transformations of variables on different scales.

Multivariable analyses

For most research studies, the primary hypotheses examine the association of two or more variables. Bivariate associations are common analyses applicable to both categorical and continuous variables in contexts in which one variable represents a response, and another an explanatory variable, or when variables do not have such a relationship. Examples of bivariate analyses include Spearman's correlation Fisher's Exact test, Student's t-test, Wilcoxon signed-rank test and Kappa statistics among many others. For each type of bivariate analyses, there are different assumptions concerning the type, distribution and size of the variables that are important for the appropriate application and interpretation of the findings. Failure to utilize the appropriate analyses for a given set of data can significantly impact the interpretations and tests of significance. One of the limitations of bivariate analyses in the context of observational studies is to determine whether any observed associations between variables are strongly influenced by additional factors. Thus, while it is often interesting and important to evaluate the relationship between two variables, models incorporating additional factors are also typically useful in practice.

One of the most common frameworks for statistical models is the least squares (or linear) regression model. Basic assumptions of this model include that errors are additive, normally distributed, and independent with a common variance. Violation of these assumptions can variably impact the point estimates and conclusions of a hypothesis test. However, it is not uncommon for some data to have departures from these assumptions and the impact of these departures depends on (a) the magnitude of violation(s) and

(b) which assumption(s) is violated, and (c) the sample size. Least squares analyses (such as common t-tests and F-tests) assume that the residuals of the model are normally distributed. Failure of this assumption impacts the conclusion of hypothesis testing and confidence intervals of parameters. One potential remedy for variables that do not meet these assumptions is to transform the data (e.g. logarithmic or square root). In addition, other modeling strategies such as non-parametric models or models that have different distributional requirements (e.g. Poisson, Survival or Logistic models) may describe the relationship between variables more appropriately.

Another common violation of statistical assumptions for models is the independence of observations. Specifically, many standard models assume that the errors are uncorrelated between observations. The impact of correlated errors leads to loss in precision of estimates. Data that represents multiple observations for single patients or observations that can be clustered within a broad characteristic should be modeled to account for this dependence. In the context of transplantation, this may arise when patients are matched (e.g. paired donor kidney studies), when study populations are treated within larger units such as centers or individual physicians or when longitudinal data is collected for a given patient over time.

Survival analysis

One of the most common approaches for analysis of outcomes among the transplant population is the use of survival analyses. Survival models (otherwise known as time-to-event models) are appropriate for examining the time to specified events (such as patient death or graft loss) in a cohort of patients from a specified initiation time. These models allow investigators to evaluate the relationship of an explanatory factor and an endpoint of interest while considering that patients may have differing amounts of time at risk for the endpoint to occur. This allows models to incorporate patients with highly variable follow-up periods into a single analysis.

The most common examples of these are the Kaplan-Meier plots and the Cox proportional hazard models. Kaplan-Meier plots demonstrate the cumulative incidence of a given endpoint without adjustment for subject covariates while the Cox models can be developed with or without adjustment for covariates. One of the important principles of these models is the concept of censoring. Models are censored at time points that represent the last at-risk period (right censoring) for subjects that is not defined as an event. For example, most survival plots are censored at the time of last patient follow-up or known status, such as the time point when patients are no longer at risk for an event to occur. Of note, the censor time can be different for each patient in a given model but the models assume that the censor mechanism is not related to event of interest. As such, if models are censored at time points that are not distributed randomly, the estimates deriving from the models can be biased. For example, a common endpoint in transplantation is death-censored graft loss, which treats deaths as a "non-informative" censoring event. This implies that patients who die are no more or less likely to experience graft loss if they had remained alive versus those that actually remain at risk for graft loss. In many contexts, this can be an egregious violation of the assumption of a random censoring. One approach to handling non-random censoring is the use of competing risks analysis [20]. These analyses allow for estimates of cumulative incidence for events that are correlated over time and can vary significantly from the standard product-limit (Kaplan-Meier) methodology.

Diagnostic testing

A common research question relates to whether a specific parameter can serve as an accurate diagnostic test for a particular outcome. This may be in the context of a diagnostic parameter for identifying the presence of a disease state or as a prognostic evaluation of a subsequent event of interest. For example, there may be interest in understanding whether a given biopsy reading from a deceased donor has prognostic value for transplant outcomes. In a similar fashion, we use screening tests to rule out donors for disease transmission based on criteria from the Centers for Disease Control and Prevention [21,22]. We may also be interested in understanding the predictive value of surrogate makers, such as renal function as a surrogate for graft loss [19,23]. For these examples and others, the salient question may be how well a diagnostic parameter predicts a given outcome. We can quantify that in several ways. In the context of a "2 × 2" table with a binary outcome and predictor, we can calculate:

- the sensitivity — probability of a positive test when an outcome is present
- specificity — probability of a negative test when an outcome is absent
- the positive predictive value — probability of an outcome present when a test is positive, and
- the negative predictive value — probability of an outcome absent when a test is negative.

Each of these statistics can be helpful for quantifying the predictive accuracy of a given diagnostic parameter but the value of each statistic may vary depending on the clinical context. In certain circumstances, it may be more helpful to understand the sensitivity and positive predictive value (evaluating how well positive cases are identified), while in others the specificity (evaluating how well negative cases are identified) may be more salient. These data can also be utilized to determine a cut-off value or a threshold at which a diagnostic parameter can best be utilized for prognostic application.

Importantly, the criteria for a diagnostic parameter to truly be predictive are different and typically more rigorous than the criteria for a parameter to have an association with a given outcome [24]. There are many examples of parameters that have well known associations with clinical outcomes that have poor discriminatory power which would be characteristic of a highly predictive diagnostic parameter. As such, in research, it is important to distinguish those parameters that are highly predictive and to utilize appropriate statistical methodology to demonstrate the predictive capacity of a diagnostic parameter.

Risk adjustment

In order to generate inferences about a broader population (e.g. all transplant recipients in the US) from a sample or to compare characteristics or outcomes between two study groups, it is desirable to minimize the effects of characteristics other than the exposure(s) of interest (Figure 134.9). For any non-randomized clinical research study, there is almost certain variation of donor, recipient or other transplant-related factors between exposure groups. As such, in order to evaluate the independent effect of exposure on a particular outcome, statistical methods to adjust for differences beyond the

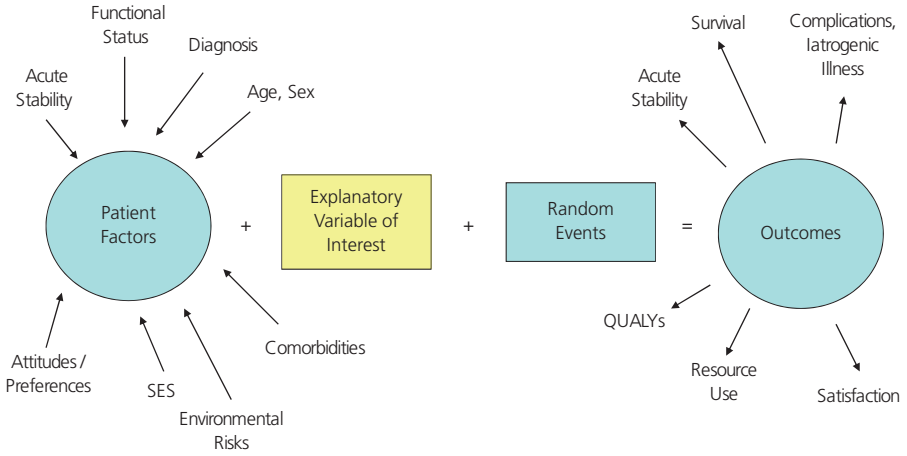


Figure 134.9. Risk adjustment in clinical research. * Adapted from [25] Iezzoni LI. *Risk Adjustment for Measuring Healthcare Outcomes*, 3rd edition. Chicago, IL: Health Administration Press, 2003, (Chapter One, p.5, Reasons for Risk Adjustment).

exposure groups are critical. However, it is important to recognize that "risk adjustment" is not developed through uniform or cookbook methodologies. Exactly how to account for variation in risk factors depends on several conditions including: (a) the outcome of interest; (b) the temporal relationship between the exposure and outcome; (c) the purpose of the study; and (d) the population(s) evaluated [25]. In this sense, investigators and consumers should be cognizant of the particular nuances of each study and not necessarily apply the same risk adjustment techniques to every analysis.

In the US, we are fortunate that we have a wealth of data collected on donor, recipient and transplant characteristics on a mandatory basis for every solid organ transplant candidate, recipient, and donor by the Organ Procurement and Transplantation Network (OPTN). As such, there are opportunities for standardized and comprehensive risk adjustment using statistical models across and between transplant centers. However, it is important to recognize that even with risk adjustment for these clinical parameters, the discriminating power of models is relatively modest [26]. In fact, compared to many other high profile medical contexts, this predictive capacity is substantially lower suggesting there are non-codified factors that substantially explain outcomes and/or there is a relatively high degree of random variation for explaining events in these patients. This does imply that, in many transplant studies, there may be a serious threat of confounding from factors not consistently documented and the potential role of these factors should be considered in the context of a study and the appropriate interpretation and cautions acknowledged.

Subgroup analyses and effect modification

An important, and often interesting, component of any statistical analysis assessing any study population is to evaluate whether findings are consistent for all groups of subjects within the population. An interaction (or effect modification) exists when the relationship between two variables differs with respect to the level of a third variable. For example the association of an immunosuppressive regimen on the risk of acute rejection may be modified by patients' age or immunological status. The effects of exposure on the outcome in different subgroups can be visualized in a Forrest plot with the tests of interaction included to indicate whether there is a significant difference of the main effect in each subgroup (Table 134.2). Analyses of interaction can have important clinical implications, especially when they are adequately powered, namely whether a given finding is likely to be generalized across a population or might be more or less applicable for certain strata of patients.

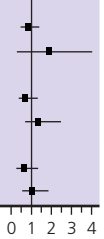
Interpretation of models

For any non-randomized study, it is rare that causation can be strongly inferred from documented statistical associations. This is primarily due to the potential for confounding and the impact of intervening factors not ascribed to study group assignment that may explain the observed associations. The argument that an explanatory variable is likely in the causal pathway of a given response can vary based on the strength of the association, the biological plausibility of the effect, whether the effect is observed in different settings and among different populations and in some contexts if there is a dose-response effect. However, as there are often a variety of pathways in which explanatory factors may be related to outcomes acknowledgment of the hypothetical role of unmeasured factors is important for the interpretation of results.

Another important but often overlooked aspect of the interpretation of statistical results is the probability of detecting a difference given a true effect (i.e. the statistical power of a model). In general, the capacity to demonstrate statistically significant findings is highly dependent on sample size, variability of the effects and study design. Importantly, failures to demonstrate an effect do not necessarily indicate that an association does not exist. This is particularly salient in the context of smaller samples in which the probability of finding a statistically significant effect is limited by the observations and variability of the effects. The corollary to the failure to detect effects in smaller observational studies, is the potential result of studies based on large registries (e.g. UNOS data). For these latter studies, it is not uncommon that statistically differences can represent very minor, potentially clinically irrelevant, differences. However, it is important to understand that even small differences can be important to document since they can still reflect an important association even if such differences may not represent an effect that is useful in day-to-day clinical decision-making. The interpretation of study findings should distinguish between results that are

Table 134.2. Forrest plot for displaying potential interaction or effect modification. Reproduced from Tsagkaropoulos S, Belmans A, Verleden GM, et al. Single-lung transplantation: does side matter? *European Journal of Cardio-thoracic Surgery*, 2011:40(2);e83–e92. Copyright 2011, with permission from Oxford University Press [54]

	L-SLTx		R-SLTx				L-SLTx Better	Better Better
Subgroup	n/N	3-Year Surv, (95% CI)	n/N	3-Year Surv. (95% CI)	HR (95% CI)	P-value		
Sex (Interaction: p = 0.7302)								
Male	29/48	56.8% (45.0%; 71.9%)	27/39	48.2% (35.5%; 65.3%)	0.77 (0.46; 1.31)	0.3373		
Female	8/24	75.2% (60.4%; 93.8%)	13/31	73.5% (60.2%; 89.7%)	0.93 (0.38; 2.24)	0.8645		
Gender Mismatch (Interaction: p = 0.5470)								
No	32/64	64.2% (53.9%; 76.4%)	36/66	61.9% (51.8%; 74.0%)	0.93 (0.57; 1.49)	0.7510		
Yes	5/8	43.5% (19.3%; 98.1%)	4/4	24.9% (5.3%; 100%)	0.60 (0.16; 2.28)	0.4515		
Perfusion Match (Interaction: p = 0.3166)								
<50%	31/55	54.3% (42.5%; 69.3%)	21/37	53.1% (39.7%; 71.0%)	0.96 (0.55; 1.68)	0.8957		
>=50%	6/17	81.6% (67,9%; 97.9%)	19/33	69.3% (55.9%; 86.0%)	0.56 (0.22; 1.39)	0.2107		
BOS (Interaction: p = 0.9511)								
Absence	24/52	67.3% (57.2%; 79.2%)	27/51	64.9% (54.6%; 77.0%)	0.92 (0.53; 1.60)	0.7563		
Presence	13/20	7.5% (1.1%; 50.6%)	13/19	6.4% (1.0%; 40.2%)	0.94 (0.43; 2.08)	0.8865		
Arrhythmias (Interaction; P = 0.4109)								
No	34/66	61.8% (51.5%; 74.1%)	38/62	57.6% (47.1%; 70.4%)	0.87 (0.55; 1.39)	0.5624		
Yes	3/6	61.9% (34.2%; 100%)	2/8	77.7% (53.8%; 100%)	1.90 (0.32; 11.41)	0.4842		
ICU Stay (Interaction: p = 0.1496)								
<=6 days	20/44	71.8% (60.0%; 86.0%)	16/29	60.2% (46.0%; 78.8%)	0.65 (0.33; 1.29)	0.2178		
>6 days	17/28	48.9% (33.8%; 70.7%)	24/41	57.3% (44.2%; 74.4%)	1.29 (0.69; 2.41)	0.4304		
Hospital Stay (Interaction: p = 0.3013)								
<=30 days	11/33	78.0% (66.0%; 92.2%)	19/36	67.3% (54.5%; 83.3%)	0.63 (0.30; 1.32)	0.2202		
>30 days	26/39	49.8% (36.7%; 67.7%)	21/34	50.9% (37.1%; 69.9%)	1.03 (0.58; 1.85)	0.9117		



N = total number of subjects; n = deaths; 3-Year Surv = Estimated 3-year survival probability (%); HR = Hazard ratio (L-SLTx/R-SLTx); 95% CI = 95% confidence interval.

clinically useful and statistically significant versus results representing an important mechanism but with modest clinical relevance.

Missing data

A common occurrence in clinical research is to encounter records with missing data. This may include data missing for response or explanatory variables and can present significant challenges both for analysis and for the interpretation of study results. Importantly, for many standard analyses, missing values may lead to the exclusion of observations (i.e. complete case analysis) which can reduce the statistical power of models, limit the external validity of findings, and potentially create biased comparisons. Thus, it is important to: (a) document the presence of missing data; (b) understand why the data are missing (e.g. randomly missing, missing due to loss of follow-up, high acuity cases likely to have missing data, etc.); and (c) consider different analytic strategies for handling missing data. Options for treating missing data include ignoring these cases, which may lead to exclusion of observations from models, simple imputation for missing cases (e.g. mean or modal levels) or multiple imputation [27]. In general, deleting observations due to missing data elements or simple imputation methods have been shown to lead to biased results. More sophisticated methods in which assumptions of the data hold can improve model performance and estimation [28].

Endpoints in transplantation

Acute rejection

Acute rejection has been one of the primary endpoints in transplantation for observational studies and randomized controlled trials. One of the challenges for use of acute rejection as an endpoint in research is the lack of a uniform definition. Acute rejection may be based on treatment for the condition (i.e. a clinically relevant need for care), biopsy proven rejection (including histological grade), or based on other clinical indicators (e.g. decline in renal function in the context of kidney transplantation). The variability in definition may lead to wide variations in the estimated effect of acute rejection on graft loss or death. Other considerations for the use of acute rejection as an endpoint include the timing of acute rejection events and rejection episodes that do not lead to permanent reductions in renal function (which may have no significant impact on graft survival) [29,30]. Finally, some patients may experience multiple acute rejections episodes following transplantation. These repeated events require different analytic approaches as well as some consideration for whether each episode is clearly a distinct event or a product of ongoing processes.

Delayed graft function

Delayed graft function (DGF) is a primary endpoint following the kidney transplant surgery. DGF is a form of acute kidney injury, which is most commonly defined as the recipient's need for dialysis within the first week following transplantation. One of the analytical challenges for assessing the impact of DGF on outcomes is distinguishing the event from other surgical events (i.e. technical failures) that may lead to early dialysis treatment among recipients. Center practice patterns may also impact the treatment of patients with dialysis and thus the incidence of DGF [31]. Thus, despite a relatively uniform definition of DGF that is conventionally used for research, there may be different causal factors that are important for the interpretation of study findings.

Infections

Transplant recipients are susceptible to various types of infections due to immunosuppressive therapy, the surgical procedure, onset of other complications and other co-morbid factors [32–34].

Common types of post-transplant infections evaluated in clinical research include cytomegalovirus (CMV), BK-virus, Epstein-Barr virus and various other types of bacterial and fungal forms [35–37]. Some infections may be asymptomatic or poorly documented and each varies substantially in severity, which may impact systematic documentation of incidence rates. For certain types of infections, prophylaxis can strongly mitigate the likelihood of developing clinical infections and may need to be considered in the analysis. Moreover, the particular inception and ending dates of infections is difficult to quantify in a systematic manner yet important to guide analyses. Cumulatively, infections are common sequela of transplantation, but vary widely in incidence and severity, are not always clearly documented, and may be strongly correlated with treatment protocols.

Graft loss

Graft loss is a primary "hard" endpoint for clinical research investigation. Graft loss is generally defined as either a graft failure requiring a return to dialysis or retransplantation (in the context of kidney transplantation) or patient death (this composite endpoint is also known as overall graft loss). Patient deaths are typically included in this outcome based on the assumption that many deaths are related to organ decline, and as such, it is difficult to separate the mechanisms of these endpoints from each other in all cases. At the very least, it is important to evaluate the individual contribution of death and graft loss for any comparison of study groups. A challenge for clinical investigation is that graft failure rates are relatively low in the immediate post-transplant periods. As such, either longer follow-up periods or large sample sizes are required in order to test research hypotheses with sufficient statistical power. As a result, there have also been extensive efforts to develop and understand good surrogate markers for long-term graft loss that can be utilized to assess treatment interventions with limited follow-up accrual periods [7,29,38].

Patient death

The primary limitation of death as a study endpoint is a lack of statistical power for comparing study groups. Most trials cannot be designed for patient survival, particularly in the field of transplantation. For research and study design, we often assume that patients who are more likely to experience acute rejection, infections, or DGF are also more likely to subsequently lose their graft and ultimately more likely to die. Despite this, patient survival should at least be considered a secondary endpoint. Studies designed for other complications should incorporate a separate analysis including mortality or composite endpoint to ensure that the findings are not misleading based on differential mortality rates between study groups.

Costs and resource utilization

An important endpoint for research and the application of most scientific inquiry is costs. Cost-effectiveness of a given intervention is commonly related to patient characteristics, therapeutic strategies, type of complications, and can have important practical and policy implications [39–42]. Principles of health economics can also effectively guide optimal utilization of scarce resources, which is particularly salient in the context of organ transplantation [43]. Cost-effectiveness analyses are clearly important for comparing interventions but they should also be used to study care that is effective for some individuals but results in inefficient use of resources and may lead to diminished care for other patients. Econometric methods often have unique considerations such as discounting of costs over time, modeling behavior, provider and patient decision-making, and quantifying life-years. Although it is often difficult to place economic analyses in the context of clinical efficacy studies, they are a pivotal component of the delivery of healthcare in the context of organ transplantation.

Provider quality of care

There are numerous studies evaluating the impact of provider practice and outcomes [44,45] In the US, transplant centers are currently evaluated for performance by the SRTR. The SRTR reports risk adjusted graft and patient survival on a publicly available website [46]. These data include wait list and post-transplant outcomes and demographic characteristics of the population. One of the primary metrics by which transplant centers are evaluated is the Standardized Mortality Ratio (SMR). The SMR is a commonly applied statistical metric that can be utilized to assess risk-adjusted outcomes at a provider or regional level based on indirect standardization of results to a normalized reference group (e.g. the US transplant population). SMRs are utilized to assess performance of transplant centers comparing the observed number of events (graft losses or deaths) with the expected number of events after adjustment for donor, recipient, and transplant covariates over a given follow-up period and using the national experience over a contemporaneous time period as a reference group. SMRs equal to one indicate that centers have outcomes equivalent to what is expected based on the acuity level of their transplant population while higher SMRs are indicative of poor performance relative to the number of expected events in the population. One limitation of the SMR as currently utilized in transplantation is that larger centers are more likely to receive statistically significant differences when compared to smaller centers due to increased statistical power. SMRs are also a metric by which quality of care is evaluated by CMS and other insurance companies in order to gauge transplant centers [47].

Patient satisfaction and quality of life

Perhaps one of the most overlooked but important endpoint in healthcare is patient satisfaction. Patient satisfaction is often difficult to assess objectively but is not always a primary focus of research. Proxies for quality of life in transplantation include complication rates, rehospitalizations, and graft survival. However, clinical endpoints alone clearly do not fully characterize patient satisfaction and quality of life. Prospective studies designed to capture patient satisfaction in a uniform manner are important in transplantation and further application of these data for the clinical application of interventions, allocation policy, and decision-making are needed.

Special topics

Beyond the many standard forms of analyses in the field of statistics and epidemiology are refinements to modeling strategies and novel approaches that have been developed and incorporated more broadly into clinical research. Often, these new modeling strategies are embedded into statistical packages but may also require more sophisticated programming and understanding of the underpinnings of the methodology. As with any model, it is critical for analysts to be cognizant of the assumptions of these approaches as well as the proper interpretation of the results. Some of the more sophisticated modeling strategies utilized in contemporary medical research are briefly discussed here.

Propensity score analyses are aimed at achieving a pseudo-randomized study design. The concept of this approach is generally to "recreate" a randomized experimental design comparing interventions by identifying factors that are related to the initial allocation of treatment assignment. For example, it may be challenging to compare recipients directly that received two different immunosuppressive agents given that many codified and non-codified factors were considered for treatment selection for a patient that could be related to their prognosis [48]. However, in a context in which the factors that relate to treatment assignment are known and risk factors have a strong relationship with outcomes, propensity score analysis is considered a method to minimize potential confounding [49].

Another attractive modeling strategy is the use of instrumental variables. Unlike standard multivariable analyses and propensity score analyses, an instrumental variable can be used to estimate the effect of an intervention accounting for the impact of *unmeasured factors* [50]. This unique opportunity to account for latent variables however comes with two strong assumptions: (a) the instrumental variable is associated with the likelihood of receiving the intervention, and (b) the instrumental variable is not directly associated with the outcome of interest. The latter assumption is often very difficult to identify in a standard clinical setting and limits the application of this form of analysis. However, when utilized correctly, instrumental variable analysis can serve as a powerful tool in clinical research by minimizing sources of unmeasured confounding.

As discussed previously, one of the common limitations of observational research is the inability to draw inferences regarding the causal mechanisms between exposure variables and responses of interest. In more recent years, there has been tremendous attention on analyses that can be utilized to draw causal conclusions, even in the context of non-randomized observational data. One of these analytical tools utilized to infer causality are marginal structural models. These models in essence simulate a randomized setting by empirically evaluating the probability of being treated over time [51,52]. While there are a number of complex analytics that are needed to develop these models, the value of advancing statistical analyses beyond associations has great appeal in both the theoretical and applied sciences.

Summary

One of the core misperceptions about clinical research is that models used for analysis involve little more than implementing a common algorithm on a new set of patients or new endpoints using standard statistical packages to generate results. For those that have not actively participated in research, there may be limited understanding of the many underlying processes that evolve from the conception of an idea to a finished clinical research project. At the same time, the individual steps and processes required to conduct rigorous and thorough analyses are critical to the validity and rigor of a study. Understanding these processes may evolve from intuition, training and experience and allow researchers to make sense of what is typically a compilation of complex information. Scientific rigor involves not only adherence to statistical methods and analysis but appropriate interpretation of results and transparency about how research is conducted. As both a consumer and conductor of clinical research, understanding and use of these methods are fundamental to moving science forward.

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CHAPTER 135

Comparative Effectiveness in Transplantation

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Introduction

Comparative effectiveness research (CER) is, to some extent, an American repackaging of the evidence-based medicine (EBM) developed by the Scottish epidemiologist Archie Cochrane, and is a conceptual framework that shifts the orientation of medical research from de novo discovery to clinical applications that facilitate both the conveyors and consumers of health care to better utilize research results and improved medical decision-making. CER became formalized into American medical research efforts with the passage of the American Recovery and Reinvestment Act of 2009 (Pub.L 111-5), which allocated $1.1 billion for CER, and created the Federal Coordinating Council for CER [1,2]. These monies are to fund CER in three departments of the federal government (Agency for Healthcare Research and Quality, National Institutes of Health, and Office of the Secretary of Health and Human Services) that will foster the [1] conduct, support, or synthesize research that compares the clinical outcomes, effectiveness, and appropriateness of items, services, and procedures that are used to prevent, diagnose, or treat diseases, disorders, and other health conditions; and [2] encourage the development and use of clinical registries, clinical data networks, and other forms of electronic health data that can be used to generate or obtain outcomes data (Title VIII).

The Institute of Medicine (IOM) promulgated the definition of CER as follows:

> Comparative effectiveness research is the generation and synthesis of evidence that compares the benefits and harms of alternative methods to prevent, diagnose, treat, and monitor a clinical condition or to improve the delivery of care. The purpose of CER is to assist consumers, clinicians, purchasers, and policy makers to make informed decisions that will improve health care at both the individual and population levels [3].

The Congressional Budget Office (CBO) adds the proviso that CER "analysis may focus only on the relative medical benefits and risks of each option or it may also weight both the costs and benefits of these options [4]. Clearly the operative word is effectiveness versus the more narrowly focused clinical efficacy standard that dominates the typical Phase III clinical trial. The efficacy model is singularly focused on what intervention will work for the so-called "average patient." The conceptual shift with CER is to enhance clinician and patient knowledge of how effective a therapy is for particular types of patients, creating a more patient-centered outcomes approach [5]. With the passage of the Patient Protection and Affordable Care Act of 2010, the Patient-Centered Outcomes Research Institute (PCORI) was created with the mission of fostering research that achieves high-integrity, evidence-based information that includes the values and interests of patients to achieve improved health care. PCORI is charged with developing a patient-centered translation framework tool that will guide patient-centered effectiveness research [6]. Inherent in any robust data-driven research is access to reliable data. Specific discussions regarding the acquisition and analysis of local and national data sets can be found in Chapters 132 and 133, respectively.

Limitations of random clinical trials for CER

Random clinical trials (RCT) have long been considered the "gold standard" of clinical research and as such are uniquely appropriate for establishing drug, device, and procedure efficacy in a homogeneous patient population. This singular focus of RCT is its strength, for determining efficacy, and its weakness, for determining effectiveness. First, all RCT protocols specify strict inclusion and exclusion criteria for patients who may or may not qualify to participate in the trial. Those patients meeting inclusion criteria tend not to approximate the typical clinical practice patient by being less sick, having fewer co-morbidities, higher treatment expectations, and greater adherence ambitions. Inclusion criteria functions as a sampling sieve to populate the trial arms with a population of patients as homogenous as possible, while clinical practice is populated by the truly sick and in need of reparative therapy. Secondly, any RCT that has a placebo arm inherently lacks an effective comparator since the entire basis of CER is that two viable therapies are being compared to establish the therapy with the greatest good, most tolerability, lowest cost, and fewest adverse side effects. The placebo is a void treatment arm, and without a real CER the practicing physicians are left with doubt as to which therapy is the most proficient for each particular patient [7]. Transplant RCTs are typically designed without a placebo, yet the emphasis is still on determining the relative efficacy between or among comparators.

The central strength of the RCT is to establish causal efficacy by means of a narrowly focused group of subjects, yet this deductive method renders internal validity primarily for those chosen types in the study, and as such the results have limited external validity [8,9]. Manifest external validity is enhanced by random selection of subjects from the subpopulation into the study population, yet this is rarely undertaken. Even if this were entertained, there is still no guarantee that the factors of interest would be represented in the

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subjects randomly sampled. The RCT is based on the deductive notion of probabilistic causality; such that X intervention causes Y outcome in some homogeneous portion of the experimental subpopulation, such as. X causes Y in Θ if probability of Y >P(Y|-X). Thus, the RCT results establish probabilistically that the intervention X worked in some subjects, yet this is of limited utility for policy formulators and decision-makers trying to determine if the X will work in their context [9].

Proponents of RCT in CER point to the intention to treat (ITT) as a strategy to better approximate clinical effectiveness. ITT is employed to enable salvaging a RCT when patients do not receive the intervention they were supposed to get based on their study arm assignment. Take an example of a three-arm RCT; arm 1 is standard therapy, arm 2 is new therapy low dose, and arm 3 is new therapy high dose. Often RCT are multi-center based, and multi-country based, and inevitably patients end up being placed in a different study arm than they were randomized to. In a three-arm study design there are six possible mistakes for any three randomizations; patient 1 is randomized to arm 1, and is placed in arm 2 or 3, patient 1 is randomized to arm 2 and ends up in arm 1 or 3, and patient 1 randomized to arm 3 ends up in arm 1 or 2 (n permutation k: 3p2 = 6). The ITT strategy is a stated protocol commitment to analyze the final data as per the arm allocation, such as there will be no artificial re-assignment of patients back to the arm with the intervention they actually received. ITT has the effect of diluting efficacy by analyzing the patient data inconsistent with the intervention they actually received. This is because randomization is presumed to equally disperse unmeasured confounding factors (i.e. patient heterogeneity) among the arms of the study. Proponents of ITT argue that it is precisely this dilution of efficacy that permits characterization of RCT as achieving greater real-world context by better approximating clinical practice where patient compliance is never 100% perfect [10,11].

ITT is not necessarily a means to improving the clinical relevancy of a RCT. Diluting efficacy does not assure an increase in effectiveness. Efficacy is establishing therapeutic plausibility in a tightly controlled circumstance, while effectiveness addresses the question of how well the therapy works in the clinical setting [12]. By reducing efficacy ITT diminishes internal validity of the results, and not necessarily the results' correspondence to clinical practice. Patients may favor or disfavor taking the regimen prescribed in the RCT protocol, resulting in reduced or increased adherence in an unpredictable manner. In other words, efficacy can be underestimated or overestimated by ITT given the indeterminate nature of patient adherence [13]. Secondly, misallocation to study arms introduces selection bias of unknown patient characteristics, and is not equivalent to clinical practice. Clinical practice renders therapy in a patient-specific manner by continuous monitoring of efficacy and adaptation of therapy-based ongoing measurement outcomes. RCTs are designed to minimize data noise; the randomization tries to neutralized potential confounding factors that the patients bring to the study but remain unknown, while the exclusion criteria excludes patients with confounders that are of crucial interest to clinical practice [14]. Finally, there is little evidence that increasing RCT within-trail variability of patient's characteristics or clinician practices will augment external generalizability [15].

Given the limitations of RCT to manifest clinical effectiveness, such as generalizability of results to a diverse heterogeneous patient population, a spate of recent articles have proposed extensive modification to RCT designs and even entirely new research design models. These new trial designs are referred to as explanatory or pragmatic clinical trials that focus on answering the question: Does this therapy work under normal treatment conditions [16]? Inclusion and exclusion criteria are relaxed, if not eliminated, to permit heterogeneity in the patient sample to approximate the diversity of patient characteristics encountered in clinical practice. A hybrid design of pragmatic randomized control trial design is the cohort multiple randomized control design. This CER design involves creating a large cohort of patients with the disease of interest, where some patients are then randomized to the option of an intervention trial and are subsequently compared to patients not randomized [17]. Clearly RCTs have been granted an exaggerated level of faith in the evidence-based medicine community of researchers, to the point that all other study design results are conveyed with an implicit apology and generate the requisite nod that future RCTs are necessary before definitive conclusions can be made [18].

The use of N-of-1 study design in CER

Given the limitations of the RCT design to advance CER, it would seem counter-intuitive to contend that a trial with a sample size of one has any relevancy or utility for advancing clinical effectiveness. Most clinicians have a bias against small sample size studies, often considering them exploratory, yet powering a study and conducting it to meet the stated sample size regardless of any practical constraints or ignoring the declining marginal utility of each additional subject enrolled into the study is equally misguided, particularly in the context of innovative research [19].

To begin, an N-of-1 trial can easily be seen intuitively as an approximation of the therapeutic trial that the clinician conducts on a day-to-day basis with each patient constituting a subgroup of the patient population [20]. The N-of-1 experimentation comes famously from a tea party attended by one R.A. Fisher and Dr. Muriel Bristol in 1925 who politely declined a cup of tea that had tea poured into the cup first. Dr. Bristol insisted that tea tasted far superior when tea was added to a cup with the milk already present, rather than the reverse, and that she could tell the difference [21]. Fisher quickly designed an experiment to test this hypothesis, preparing eight cups of tea, four with milk first and four with tea poured first into the cups. Fisher determined that there were 70 ways the eight cups of tea could be divided into two groups (8!/4!4!). Fisher then randomly presented the cups to Dr. Bristol, and used an alpha significance of .01428 (1/70), reflecting the probability of correctly choosing all eight cups correctly by chance. How did Dr. Bristol do? According to an account in a biography of Fisher by his daughter (Joan Fisher Box), Dr. Bristol was successful in correctly discerning the type of all eight tea and milk combinations.

The standard design of an N-of-1 trial involves randomly assigning times to a single patient of two different treatments (one of which could be a placebo). Suppose we had two treatments A and B, and eight times where the patient is exposed to each treatment four times. There are a total of 70 possible permutations (8!/4! 4!), and one of possible schedule could be AABABABB. For N-of-1 designs to be useful the disease condition needs to be chronic, since acute conditions would have excessive symptomatic fluctuation to attribute their resolution to the intervention. Likewise, the therapy itself needs to have an on-off pharmacokinetics of a known timeframe to enable the investigation to avoid carry-over effects [22]. Carry-over effects are a particular concern given the potential for some drugs to remain potentiated in vivo, and hence distort subsequent effects in additional times of intervention [23]. Therefore, the need is evident to have precision in the washout period for both

pharmaceutical and behavior interventions, the latter of which can manifest a fatiguing or learning effect. In addition, it is necessary to have an intervention that achieves therapeutic dose in a rapid manner in the prescribed time interval between time blocks [24]. The N-of-1 also needs an appropriate baseline period at the outset of the trial, a wash in period, and follow strict double blinding of patient and clinician to minimize the placebo effect. The central objective of the N-of-1 trial is establishing personalized efficacy between two repeated therapies [25].

Recently Scufham et al. used N-of-1 trials to identify optimal drug regiment to reduce health care costs of patients with osteoarthritis, chronic neuropathic pain, and attention-deficit hyperactivity disorder (ADHD) [26]. The study design involved utilizing results from several N-of-1 trials. In all N-of-1 trials a large variability of patient response within the individual poses a threat to internal validity, and the addition of more treatment times would manifest more useful information. Likewise, when within-subject variability is modest, then adding additional times of measurement does not generate more useful knowledge [27]. Thus, in N-of-1 trials it is useful to minimize the patient burden for participating in the trial to diminish the dropout rate, since their loss to follow-up reduces validity and effectiveness of therapy in clinical practice.

N-of-1 trials certainly have potential to enhance the research efforts in CER and patient-centered medicine. The future use of wireless monitoring devices will dramatically augment the data collection and enhance patient's willingness to participate in N-of-1 trial. The deployment of multi-center coordination N-of-1 trials, meta-analysis N-of-1 studies, and electronic medical records all will serve to enhance the adoption and use of this research design.

Observational Data and Meta-Analyses in CER

Observational data can be generated retrospectively or prospectively. The former typically involves using existing databases or chart reviews. The latter involves following a cohort forward in time, and measuring factors and events of interest, for example the Nurses' Health Study (Cohort I, II, and III), a longitudinal cohort study. Another prospective design is referred to as quasi-experimental, with two or more study arms that form comparison groups (rather than control groups), because subjects choose their preferred arm. Quasi-experimental designs include the interrupted-time-series that has a sequence of preintervention measures and a sequence of postintervention measures. When there is more than one intervention to be tested, a quasi-experimental design used is called counter-balanced or Latin squares. The order of patients receiving the intervention is rotated across time, hence safeguards against carry-over effects need to be established [28].

The central argument in favor of observational designs is that contrary to RCT they permit, if not necessitate, strong external validity (i.e. generalizability) because the data are gathered that more closely approximate the heterogeneity of patients found in the clinical setting. Observational designs are completed in a timelier manner than RCTs, hence reducing expenditures per unit of analysis; and typically benefit by amassing a large data set for analysis. However, the extent of internal or external validity in either RCT or observational designs is a function of how the study is put together. A poorly executed RCT may achieve less internal validity than a well-structured observational design [29]. Advocates of observational research point to their utility for rare disease, since in case-control designs the subjects are selected on the basis of their disease status. Observation studies can investigate multiple therapies simultaneously, and capture additional patient-centered outcomes, like therapy preferences, and are particularly useful when RCT are ethically, temporally, politically or financially infeasible [30,31].

The pivotal concern with observation study designs is systematic bias, which tends to limit causality conclusiveness [32]. For example; sicker patients may be shunted into more aggressive therapies while the more frail patients are relegated to less invasive (i.e. non-surgical interventions) depending upon their need or ability to tolerate the treatment. This creates a disproportionate heterogeneity among the comparison groups, such that potentially confounding factors (e.g. patient frailty) unduly influences the outcomes of interest. Careful designs, large numbers, and innovative analysis (e.g. propensity scoring regression, randomization statistics), can minimize the effects of bias and confounding to a large extent [31]. However, when it comes to confounders, what is not measured or measured incompletely cannot be accounted for in the analysis, and can lead to partial estimates of the effect size associated with the treatment modality [33].

There is ongoing debate as to the epistemological nature of medical research models in generating knowledge. The standard perspective is that the observational designs are so-called hypothesis-generating studies that will be later confirmed by a rigorous RCT [34]. This view contends that science, and medical science in particular, is an open-ended never-ending system that generates testable hypotheses that spawn unanswered questions which is the genesis for further testing of new hypotheses, and the RCT constitutes the gold standard of distinguishing the factual from the artifact as a deductive model. As such, observational research can play a pivotal role in the ongoing process of medical knowledge acquisition, in part, by generating newly testable hypotheses. This common conceptualization of the scientific process has been challenged in light of the pressing needs of CER to more efficiently advance medical care [20]. The new emphasis placed on patient-centered research invites a new theoretical framework, of hypothetical-deductive empiricism. As Figure 135.1

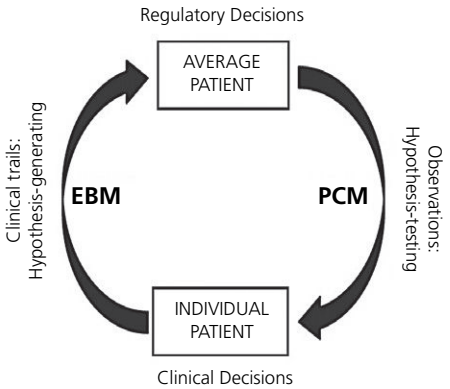

Figure 135.1. PCT and RCT: The directional flow of scientific reasoning, from the aggregate based average patient, and hypothesis testing in the domain of patient-centered medicine to improving individual based clinical decision-making, back to hypothesis generating RCT. Reproduced from [20] Sacristan JA. Exploratory trials, confirmatory observations: a new reasoning model in the era of patient-centered medicine. BMC medical research methodology. 2011;11:57. Epub 2011/04/27 [Open Access].

conveys, RCT would serve to generate hypotheses regarding the average patient, while patient-centered CER would engage observational studies to test hypotheses for the individual patient [20].

Observational research can also utilize large national data base registries. With improved electronic medical record keeping, and integrated IT systems data mining can function to investigate research questions. However, since the data bases were constructed to be repositories of patient-specific information, any abstraction of data for use in a study should realize that their utilization is inherently a secondary level analysis. Currently there are many nationally based data bases that lend themselves to data mining investigations. National Surgical Quality Improvement Program (NSQIP) is a risk-adjusted data set that focuses on clinical outcome data, with 69 clinical measures, and 30+ procedure-coded variables. There is the National Trauma Data Bank (NTDB) maintained by the American College of Surgeons, which is a national convenience sample data set, based on trauma data voluntarily submitted by participating hospitals. The US Organ Procurement and Transplantation Network (OPTN) and the Scientific Registry of Transplant Recipients (SRTR) maintain the largest data set in organ transplantation and publish an annual report on all organ transplants, graft-survival analyses, and wait-list statistics. SRTR processes all requests for organ transplant data for secondary research projects.

Large registry databases do have limitations, chief among them is the so called "Z-bias" and "M-bias" [35]. These registries may measure a plethora of variables that are related to the exposures considered harmful, yet miss variables related to outcomes. Z-bias occurs when z-variables are included in the statistical model. These z-variables are impacting the outcome by unmeasured confounders, thus adding them to the model actually increases bias, which may seem peculiar since these variables are only related to the exposure. M-bias or collider-stratification bias results when adjustments to the model are made for a non-confounding variable (i.e. the collider) that is not related to the exposure or the outcome of interest, but sits at the crossroads of variables related to the outcome of interest. This M-bias is a kind of over-adjustment for a variable that is a descendent of the exposure, yet is not really in the causal pathway [36,37]. Additional discussions of national datasets are found in Chapter 133.

Despite the limitations of observational research, and particularly the potential for selection bias that can occur from the inclusion criteria use for national data bases, there are critical instances when a disease process is virulent and the treatment options are limited, and as such decisions must be made with less than perfect data. Observational data are particularly useful for CER studies; for example when diseases have a low prevalence and therapeutic interventions manifest large differences in patient response. Large studies are able to locate rare diseases, and allow comparison of simultaneous therapies. Observational studies are useful when patient adherence rates differ, when providers vary greatly in their level of training and expertise, and when investigating off-label drug side effects.

Systematic reviews, more commonly known as meta-analysis when the review includes a statistical combination of several studies that have similar research hypotheses being researched, have long been given a prominent evidentiary status in the pantheon of research techniques. Meta-analysis in essence is a process of compiling the results from a group of related studies that focus more or less on the same working hypothesis to enable ascertaining a summary result. The key is establishing a priori precise inclusion criteria for the studies to use in the final quantitative synthesis of results. All meta-analyses involve retrospective data collection; hence this form of research is relatively inexpensive and quick. Secondly, most important aspect to a meta-analysis is determining the effect measure that is to be summarized for comparative purposes. Examples of effect typically include odds ratio (OR), relative risk ratio (RR), or hazard ratios (HR). Effect size is the estimation of the size of differences between the treatment group and the non-treatment (or lesser treated) group. Individual results are not being pooled into one big study; rather each study's effect is being pooled.

The primary concern of meta-analysis is the extent of heterogeneity among the studies, which can undermine the validity of the results: the so-called "apples versus pears" problem. Study heterogeneity can be from stochastic random differences between studies, or individuals in each study may actually be from different populations. Assessing for true heterogeneity is done using Cochran's Q test [38]. The Q test is computed by summing the squared deviations of each study's effect estimate from the overall effect estimate, weighting the contribution of each study by its inverse variance. Under the hypothesis of homogeneity among the effect sizes, the Q statistic follows a chi-square distribution with k – 1 degrees of freedom, k being the number of studies. Not rejecting the homogeneity hypothesis usually leads the meta-analyst to adopt a fixed-effects model because it is assumed that the estimated effect sizes only differ by sampling error. In contrast, rejecting the homogeneity assumption leads to applying a random-effects model that includes both within-studies variability (from sampling error) and between-studies variability (lots and lots of differences in study characteristics, design quality). The typical P-value cut point of a significant Q statistic is .20. The newer technique to assess heterogeneity is called the I^2 Index, which is similar to the intra-class correlation and is calculated as follows:

$I^2 = [Q - (k - 1)/Q] \times 100 =$ and k is number of studies.

The I^2 Index is essentially a measure of inconsistency among the studies, and if greater than 50% is considered to be an unacceptable level, or lacking in sufficient intra-study similarity [38].

Meta-analyses have long been known to have the ripe-strawberry problem, that of publication bias, in that journal editors and reviewers tend to favor positive studies that exhibit statistically significant results over negative studies (unless the authors pledge to have their study "properly power" the next time around). In this bias, meta-analysts who comb through the published literature tend to find positive outcome studies rather than retain null hypothesis studies [39].

Another thorny issue is whether to combine the results from RCT and observational studies [40]. This is because observational studies are inherently more prone to confounding by unmeasured confounders. Yet there seems to have emerged a general consensus that non-experimental and quasi-experimental study design should be included because they are "data rich" sources of information with large sample sizes and can have utility to assess potential therapy harms. A more recently identified problem has been named "meta-bias" [41], which delineates a bias introduced by single-center studies versus multi-center based studies. The single-center studies demonstrated an elevation in effect size by as much as 26% [42]. What the researchers did was to examine the odds ratio effect sizes comparing single-site versus multi-site studies among 48 meta-analyses totaling 421 RCT, with 233 conducted at a single site, and 198 that were multi-center based. Dechartres and colleagues found that single-site RCT's had a much larger intervention effect compared to RCT's that were multi-centered. Goodman [41] speculates

that metabias may simply reflect another variation of publication bias that favors multi-center studies, or that single-center studies have fewer people involved and as such there is reduced strictness and control over the study operations, allowing the researchers to achieve their desired results.

A no less intriguing bias was demonstrated by a meta-analysis by Danaei and colleagues [43] involving the use of statins and prevention of cardiovascular diseases. What they found was that when the RCT compared statin use between current users and non-users (secondary prevention) the hazard ratio was .84 (a 16% reduction in CVD mortality in patients with history of CVD), while observational studies that compared statin use between new users versus non-users (primary prevention) had a hazard ratio of .54 (a 48% reduction in CVD mortality in patients with no history of CVD). The authors comment that, given the considerable discrepancy found between RCT and observational study effect measurements, future research needs to control potential bias by structuring the inclusion criteria for observational designs that more closely match those of RCTs, and to take into consideration incident (new) versus prevalent (existing) users of medications.

A fairly new and innovative variation of pair-wise meta-analyses is termed "network meta-analysis" which involves meta-analytic comparisons across multiple interventions or treatments. Network meta-analysis takes direct evidence from RCTs (e.g. drug A vs. B, and drug B vs. A), and combines it with indirect evidence obtained across RCTs (e.g. drug A vs. C). This technique yields pooled effect estimates for several outcomes of interest, rather than just one effect in standard meta-analysis [44,45].

Meta-analyses do hold substantial promise for CER, by permitting greater latitude in study heterogeneity, by combining study estimates of costs, and scaling adverse drug reactions, which all contributes to the driving question as to the most effective course of treatment. Proper consideration of potential bias is simply incumbent upon any research project and necessitates engaging those with sufficient expertise to undertake meaningful research. Meta-analyses provide the needed synthesis and unification across a plethora of disparate studies, and thus creating a more comprehensive portrayal of the answers to critical clinical questions [46]. Research analysis imparts empirical results, while meta-analyses provide coalescence; and it is this mutual interaction of results and synthesis that advances clinical understanding [47].

Decision analysis and cost utility effectiveness in CER

Perhaps no other fields of analysis are more appropriate for CER than decision analysis (DA) and cost-effectiveness (CE). DA is a highly sophisticated and structured analytical technique that came out of game theory and economic behavior to aid in decision making. DA is highly useful when there are competing alternative components of medical decisions, which involve costs, risks, and probabilities of outcomes. DA is best used when the focus is on an individual's decision, yet DA can also assist and inform the development of consensus guidelines in clinical practice [48]. The heart of DA is the construction of a Decision Tree see Figure 135.2. In an example by Best, et al.[49], regarding smallpox inoculation in 1736, to inoculate or not is the first decision node, and the circles represent the chance nodes. Notice in the first tree branch of Inoculate, the results are Live or Die, and below each of those branches are the probabilities of that event; staying alive probability is .9790 and dying probability is .021. At the end nodes (triangle) are the utilities of that outcome which range from 0 (dead) to 1.0 (perfect health).

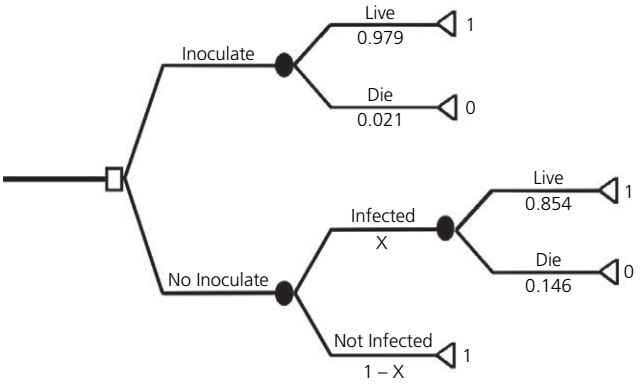

Figure 135.2. Decision Tree: Decision tree of mutually exclusive decisions to inoculate or not to inoculate, leading to the probability-based consequences of living or dying after inoculation or getting smallpox infection and then living or dying, or not getting infection of small pox. Reproduced from [49] Best M, Katamba A, Neuhauser D. Quality & safety in health care. 2007;16(6):478–480 with permission from BMJ Publishing Group Ltd.
Root node/decision node (square); event (chance) node (circle); and end points (triangle)

The Decision Tree diagram, is a simple example of DA; a more thorough analysis would include the costs associated with each tree branch, to enable obtaining the expected value for an uncertain alternative (calculated by multiplying each possible outcome by its probability and then summing the results). With the inclusion of costs, we can determine the expected value decision criterion, such as selecting the option that manifests the best expected value. This is the essence of CE: determining which optional decision leads to the highest monetary outcome.

A special type of CE is termed cost-utility-analysis, in which the health benefits obtained are quantified into measurement units called quality-adjusted-life years (QALYs) (Figure 135.3). QALYs are the preferred unit of time, usually expressed in years, which has been adjusted by a human-derived preference weight termed "utility." A utility is value a person gives to a preferred outcome measured under conditions of uncertainty, which typically ranges from zero (defined as death) to 1.0 (defined as a state of perfect health). In the Basic QALY Diagram a person is expected to live until they are 75 years old, and currently they are age 25. In the linear QALY model we assume their less-than-perfect health utility weight is .75, and we can calculate their remaining QALYs as follows:

$$\text{QALYs} = (75 - 25) * (.75) = 37.5$$

We subtracted their current age from their expected life expectancy of 75 to get the number of years remaining, and then multiplied this by the utility weight of .75 to get 37.5 QALYs to represent the number of years living that are "adjusted" by the perceived quality of those years.A QALY then can easily be understood as a quality-adjusted-life year, which combines a person's years of life and the perceived quality of that time.

Take a simple example: we can estimate a person's remaining years of life, and they would be questioned as to how many years they would give up to attain perfect health given a condition of non-perfect health. We then can calculate as follows:

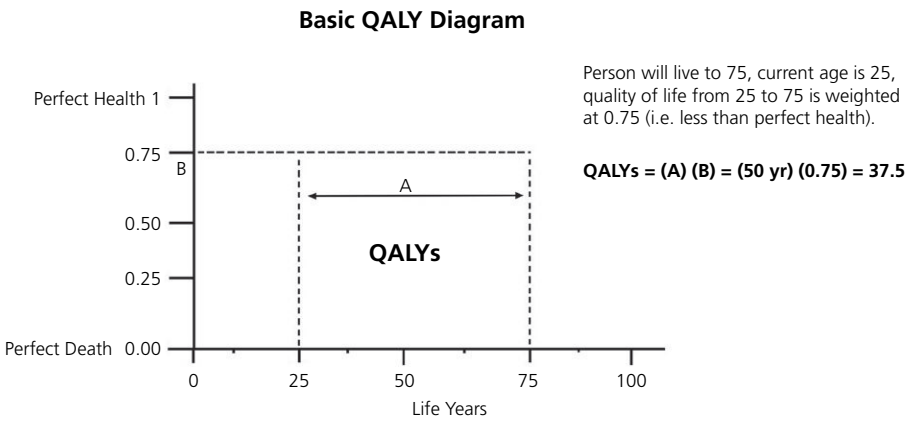

Figure 135.3. Basic QALY: The vertical axis displays the range of utilities: from zero of death to +1 of perfect health, with expected life-years on the horizontal axis from birth to death. Notice the example of a person expected to live to age 75, with current age of 25, and a linear utility of .75. Thus, QALYs are calculated as the product of A and B; (50 years) * (.75 utilities) = 37.5 quality-adjusted-life-years.

$$\text{Utility} = (\text{NYEL} - \text{NYWTT})/\text{NYEL} = ?$$

NYEL is the number of years expected to live, and NYWTT, are the number of years willing to trade. This calculation is often more formally written as; $U(H_i) = x/t$, where x is the number of years in perfect health (i.e. expected years of life minus years willing to trade), and t is the number of years in non-perfect health, H_i, that are remaining. Taking a published example regarding imagined hand amputation [50], a hand transplant patient is expected to live 45 years as an amputee, and is willing to give up four years of life for the transplantation ,so the utility can be calculated as follows:

$$U(H_1) = (45-4)/45 = .9100$$

The QALY is used extensively in cost-utility analysis because a QALY captures a person's preference for a health status in a single index measure [51–54]. A QALY is derived from the captured utility as follows: QALY = Utility * Years of Life Remaining [48]. Thus, a QALY is the product of expected remaining life and the value attributed to that time (i.e. the utility weight). Let us consider two patients receiving a kidney transplant that has a utility of .70; Patient A expects 30 years of life, and Patient B expects 25 years of life.

$$\text{QALY}_{\text{Patient A}} = .70 * 30 = 21$$

$$\text{QALY}_{\text{Patient B}} = .70 * 25 = 17.5$$

Clearly Patient A is driving more quality life years measured in QALYs than Patient B. We can also use QALYs to compare the difference between two treatment options, deriving the difference net benefit. A recent study obtained 9.456 QALYs for combined liver and kidney transplantation to the same patient versus splitting the organs to different patients of 8.650 QALYs, for a net difference benefit of .806 QALYs, demonstrating that combined organ allocation was the better approach when end-stage-renal disease (ESRD) was certain [55].

The question arises, where exactly do the weighted utility factors come from? Utility weights can be generated from two basic methods:

1 direct valuation techniques such as; time-trade-off (TTO) questionnaire, visual analogue scales (VAS), and standard gamble (SG); or
2 indirectly using a standardized questionnaire-based health utilities index, such as SF-36, EQ-5D, or HUI-3 [56–59].

TTO technique poses questions in a survey that present the respondent with a choice between X years in a poor health state and assesses the number of years they are willing to trade measured in Y years to return to a perfect health state. The Basic TTO Diagram (Figure 135.4) demonstrates the willingness to trade off years of diminished health utility to gain perfect health utility.

In this hypothetical example the patient has 25 years to live (75-50=), but at a reduced quality of life of .75 utilities. They are willing to trade five years of expected life to move up to perfect quality of life. The gain in QALYs can be calculated as follows:

$$\text{Current State: QALYs} = (75-50)*(.75) = 18.75$$

$$\text{TTO State: QALYs} = [(75-50)-(75-70)]*(1) = 20$$

The patient is willing to trade five years of life to gain 1.25 QALYs ($\Delta_{\text{QALYs}} = 20 - 18.75$).

The TTO technique assumes a property called constant proportional time tradeoffs (CPTO) which is that utilities elicited are unaffected by the time the respondent imagines they are in that health state. For example, if I am willing to trade two years for ten years of perfect health, then I should be willing to trade two months for ten months of perfect health. Attema, and Brouwer reviewed the TTO literature and found that CPTO was commonly violated, such that with shorter durations there was less willingness-to-trade for perfect health state [60]. In other words, a person's preferences for health are influenced by the time they obtain in that health state. In a follow-up study by Attema and Brouwer [61] the authors utilized the TTO risk free (direct method) technique using a health state questionnaire based on full health and less than perfect health exhibited by chronic back pain to capture discounted weights. The authors distinguish ordinary CPTO (oCPTO) from generalized CPTO (gCPTO): the former — the years a person is willing to trade to get a full health state — is not influenced by the absolute years of remaining life; the latter posits that a person's remaining utility of life years that they are willing to trade for perfect health are not affected by the absolute number of remaining utility years. This distinction is important, because a violation of gCPTO indicates that the general QALY model is proven false, while violation of the oCPTO means that only a special subset of the QALY model is

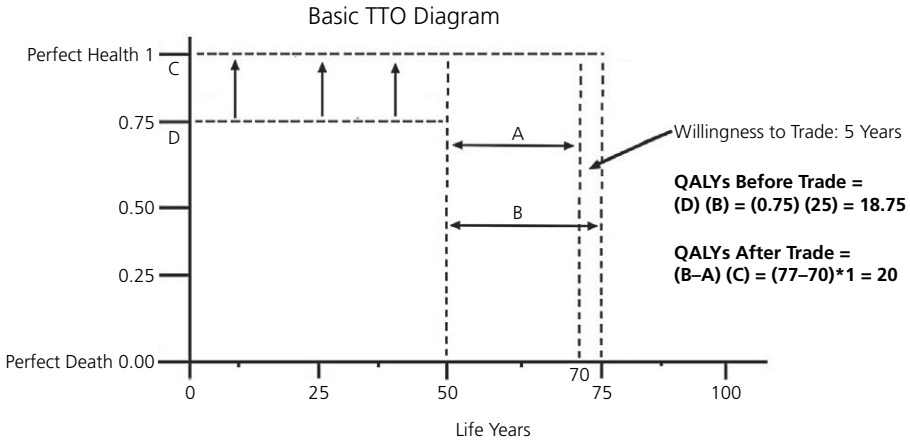

Figure 135.4. Basic TTO: The vertical axis displays the range of utilities from zero of death to +1 of perfect health, with expected life-years on the horizontal axis from birth to death. The patient is willing to trade 5 (B − A) years to return to perfect health (C) from less than perfect health (D) .75. The QALYs before tradeoff is: D * B = .75 * 25 = 18.75, and the QALYs after tradeoff is B − A * C, or 75 − 70 * 1 = 20. Hence, the increase in QALYs for trading five years was 1.25 (20 − 18.75).

proven false, that of the power family. Their results provided evidence for violation of both gCPTO and oCPTO assumptions, casting doubt on the TTO deriving of QALYs. Hence, it appears as though humans link the quality of life and the expected duration of their life.

The SG technique involves respondents having to make a choice between a certain health state and two possible outcomes: Perfect health versus death, and is based directly upon the axioms of von Neumann-Morgenstern utility theory (i.e. expected utility theory) (Figure 135.5). The von Neumann-Morgenstern utility model is $U(Q,T) = p^*$, where Q represents present health state, for a period of T years, and p^* is the indifference probability between choosing to gamble or not gamble [62]. Each alternative choice presented to the respondent has a prescribed probability of occurring, and the probabilities are varied until the respondent does not favor one over the other; called the point of indifference [63,64]. Bala et al. give an example involving chronic pain in which respondents imagined a current health condition of severe pain for the next 20 years, and they could choose to gamble on a therapy that manifested normal (pain free) life for 20 years with a probability of 95%, and a 5% chance that therapy would incur immediate death [65]. Thus, SG gives us the respondent's preferred score for an improved health state over time.

Recently there has been considerable debate regarding the generation of health states. TTO, SG, and EQ-VAS are considered direct valuation measures of health, while a variety of indirect questionnaire-based methods have gained new prominence both in the USA and in Europe. These include the HUI-3 Health Utilities Index, the EQ-5D five-dimensional questionnaire, the 15-D measure, and the six-dimensional health state SF-6d [66]. Given all these different methods of generating health-state utilities, the question looms as to how comparable their results are. Not too surprisingly, a recent comparative analysis of HUI-3, EQ-5D, TTO, and EQ-VAS by Hientz et al. [66] for the generation of QALY weights for diabetic retinopathy found significant differences among the four techniques in the derived utility weights. They demonstrated that the measurement instruments did not follow the course of the disease worsening, except for HUI-3, which was able to detect changes is the severity of the diabetic retinopathy

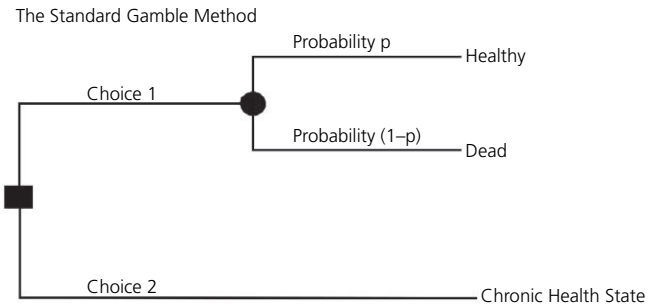

Figure 135.5. Standard Gamble: The decision tree plot displays the choices a person makes: Choice 1 takes on the gamble of some form of therapy with subsequent probability of returning to healthy state or death. *P* is the probability from 0 to 1, and 1-*P* is the remaining probability of death.

(the decrement for blindness was actually two times larger than the other methods). Their conclusion is inescapable: the quality-adjusted life year technique that is chosen will affect the assessment of cost-effectiveness of a treatment or intervention [66]. HUI-3 also demonstrated construct, convergent, and discriminative validity in a study of indexing health utilities in lung transplantation patients [67].

One of the basic concerns is who should be scaling health values: patients involved in the health condition, non-patients in the general population or people with expertise in health? Some economists have argued that patients and non-patients in general would render very diverse utilities because they start from a divergent perspective: that the non-patients will tend to over-estimate the losses attributable to a given health state, while patients will underestimate adaptation to the non-perfect health state [68,69]. However, recent research found no significant difference in health-state values comparing patients to healthy people using the VAS technique [70]. In that study TTO technique did manifest considerable differences between patients and healthy people; this may be due to that fact that TTO method contradicts measurement theory as a one-dimensional measurement since it is trying to measure health

state and temporal longevity at the same time. Thus, the differences observed in health-state utilities may be more a function of the measurement instrument tool employed than a reference bias.

In addition, there is a basic economic presumption that humans value present health higher than future health. When we make this assumption and apply a high discount rate (promoted by advocates of opportunity-cost theory) the result favors a therapy that augments quality of life over longevity (in contrast to a therapy that adds to survival but bears little fruit for quality of life) [71]. Others question the whole business of health states, that is as non-temporal measures, that are valued without taking into consideration the time spent in the health period or the health states before and after the measured health state is captured [68]. The notion that individuals calculate their health-state utilities as risk-neutral and are valued the same over time seems excessive [72].

A more complex, and perhaps more critical, concern is how the scaling of utilities by these quality of health-state techniques is undertaken. Most economists and policy analysts still maintain that SG and TTO methods are the most defensible techniques for obtaining health-related quality of life preferences. The typical TTO approach involves making a choice between a set number of years in a particular health state that is better than being dead, against a lessor chunk of time in perfect health. When the participant becomes stuck, offers no further trading of bad health years to get good health times, this is referred to as the indifference point, and a utility ratio is calculated x/y. With SG good health certainty is compared to uncertain gamble between demise and perfect health, and when the indifference point is reached it is taken to be the utility of the preferred health state. There are two major concerns with this technique (1) it does not capture the utility of bad-health states that are worse than death, and (2) the scaling of utility between 0 and +1, or −1 to +1 is not a statistical distribution that permits valid use of means, medians, modes, and ratio statistical analysis. These two concerns are different dimensions of the same problem: scaling of the utilities as is currently done is essentially arbitrary and in need of complete revision. Lamers [73] initiated the need for refinement by arguing that health states can be assessed as better, equal to, or even worse than death; and the scaling of utility from 0 (= death) to +1 (perfect health) fails to capture the reality of human health preferences. If the utility of death is zero, what about health states that are worst than death? When a health state is assessed to be worse than death, utility can be less than 0, going from 0 to negative infinity. There is considerable debate as to whether to terminate a negative utility at −1, and even discussion on how to unbound a +1 utility [74].

Pullenayegum et al.[75]. acknowledged that there are health states that are worse than death, and as such utilities need to go below 0 and be measured on a negative scale, and Sullivan [74] contends that utilities should not be bounded by +1 since there are utility states that are far above full health as designated by one. The obvious rationale for bounding utilities between 0 and 1 is that they are used as the weights to derive QALYs, and if a utility weight is less than 0, how are negative QALYs to be interpreted? This is particularly troubling when doing an incremental cost-effective utility analysis (ICUR) as follows:

$$\text{ICUR} = \mu_{\text{cost(new Rx)}} - \mu_{\text{cost(standard Rx)}} / \mu_{\text{outcomeQALY(newRx)}} - \mu_{\text{outcomeQALY(standard Rx)}}.$$

It becomes obvious that whenever we have negative outcome interpreting a negative dollars per QALY it is problematic and may

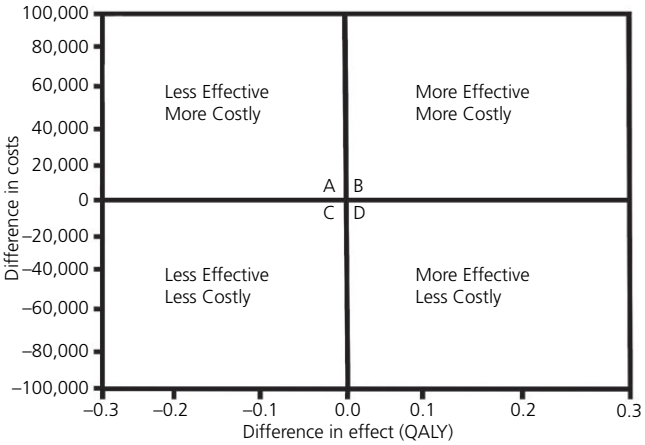

Figure 135.6. ICUR possible results: The diagram has the difference in costs between two competing therapies on the vertical axis, and the difference in QALYs between the same two competing therapies on the horizontal axis, with the possible results of the ICUR displayed in four quadrants. Quadrant A is ICUR that is more costly and less effective, Quadrant B is more costly and more effective, Quadrant C is less costly and less effective, and Quadrant D is less costly and more effective. Reproduced from [113] Kontodimopoulos N, Niakas D. An estimate of lifelong costs and QALYs in renal replacement therapy based on patients' life expectancy. Health Policy. 2008; 86(1):85–96. Copyright © 2008, with permission from Elsevier.

lack a fruitful interpretation [76]. What exactly are we supposed to make of an ICUR of — $80 000 per QALY? Larsen and colleagues have proposed a solution. If we divide the four possible results of an ICUR into quadrants, by plotting the difference in incremental costs by the difference in incremental gains, the ICUR can be interpreted as indicated in Figure 135.6 [77].

In Figure 135.6 Quadrant A, the new therapy costs exceed existing therapy, yet the new therapy's QALYs are less than the existing therapy and hence renders a negative effectiveness. The best ICUR is in Quadrant D, where new therapy costs are less than existing, and new therapy QALYs exceed existing therapy QALYs, hence the new therapy is less costly and more effective.

A plethora of more technical solutions have been proposed: such as rescaling quality of life values into discrete (binominal) measures [78], using negative scores in the health-state questionnaire and simply converting them all to zero [79], converting all scores below −1 to a negative one [80], using monotonic transformation by arbitrarily dividing WTD (worse than death) TTO responses by 39 to increase QALY estimates [73,81,82], or by converting to a scale ranging from −1 to +1 [83,84]. In an elegant mathematical proof, Flyn et al. demonstrated that utilities from 0 to +1 were impossible; the idea being that the probability of at least one person with a quality of life score below 0 cannot be zero [78]. As such, the health value must extend to a negative infinity below zero and positive infinity above zero. Edlin and collegues have proposed distinguishing theoretical from empirical health quality values, suggesting that health values below zero represent "pits" and above +1 are "supra-states" that capture the latent quality of life measures [85]. This is critical, since there can be instances when the majority of cases may register quality of life values that are latent rather than empirical. Perhaps the most intriguing solution to the health-value scaling problem has been put forward by Craig et al.[86]. The problem is

that all the health-value measures use ratio statistics, which are not appropriate because x and y are not interchangeable, for example $\mu(x, y)$ is not equal to the inverse of $\mu(x, y)$.

The ratio of y/x, where y = x — years willing to trade, and x is the expected number of years to live — thus if someone is willing to trade ten years to live two years in perfect health; $\mu = 8 - 10 - 8 = -.2500$. What Craig proposed is to use directional statistics in the use of utilities by converting every y and x into Euclidian space and map them as a set of polar coordinates, delineated by an angle and radius [87]. Each y/x ratio is the tangent of angle Θ ($\Theta_i = \arctan(y/x) + \varepsilon_i$, and the radius is equal to the size of the participant's trade off time [87]. Thus, Craig proposes substituting the estimated tangent of the mean angle for the ratio statistic as the value estimator, referred to as aRUM (angular random utility model) [87]. Angular means are more robust, and as such would be less distorted by large negative ratios than arithmetic means. Finally, Craig and Busschbach propose their own random utility model which is based on probit modeling, called eRUM (episodic random utility modeling) [88]. The eRUM method derives the health-state estimator as:

$$\beta_h = \Sigma x_i y_i / (\Sigma x_i^2), \text{and then a QALY}_i = (\beta_h)(\text{years}) + \varepsilon_i$$

The β_h is a coefficient that approximates the ratio of means [84].

In their comparative analysis of EQ-5D state in the USA, Craig and Busschbach conclude that either eRUM or aRUM are better models and less subjective than simply dividing the lower values of TTO ratios by 39. eRUM has itself come under increased scrutiny and appears to lack monotonicity. Non-monotonicity is a bad property because it means that as some respondents increase their health valuation for a specific state, the aggregated values many actually decrease [89]. Supposing an individual values WTD infinitely, resulting in a negative infinity ($-\infty$), in the eRUM approach that value would exercise no downward pull on the least squares fitted regression line [89].

The validity of the QALY model — which has become pervasive in medical decision-making, health policy and, in the context of CER, useful for patient-centered decision-making — has always been employed with the presumption that in the simple linear model we get the QALY as the product of derived utility of health state and time (in years) that the person is in that health state. In other words utility and time are independent of each other. This independence has been brought into serious question recently, and its violation has implications for all the recommendations made using the linear QALY model, since it may not necessarily be representative of a person's true desires. A recent study by Atemma and Brouwer [90] found evidence in support of this independence property for health state that were better-than-death, yet not for health states that were worse-than-death (WTD). WTD states exist because there are health states that exceed the maximum endurable time a person can tolerate such a state. Attema and Brouwer's [90] results indicate that utility may then depend upon how much time a person is in a health state that is WTD and, as such, additional modifications to QALY modeling need to be considered to better capture human preferences.

As we have reviewed, health-related utilities can be derived from a variety of methods, both direct and indirect. Advanced methodological models are attempts to resolve problems and limitations that have been identified in determining the utility of a given health state; of particular vexation is the boundary scaling of utility matter. Although there are many enticing techniques currently available, such as competing models with various assets and liabilities, there is yet to be a final consensus as to which approach constitutes the ideal rendering of utilities and, hence, QALYs.

CER in renal organ transplantation

The renal organ transplant literature is littered with a plethora of high quality RCTs that primarily focus on the post-transplant immunosuppression therapies to establish superiority or equivalency among competing drug regimens [77,91–95]. There has also been a considerable amount of quality of life research in ESRD patients and related utilization of dialysis [96–104].

Morel and colleagues at University of Minnesota Hospital and Clinic published one of the first studies addressing long-term quality of life after renal transplantation [105]. The study population consisted of 51 pediatric patients who were contacted to participate in the study, who were between 11 and 27 years post-transplantation. Their results found that 90% viewed their life-as-a-whole satisfied or completely satisfied, 91% rated their health as good to excellent, and 65% rated their life as normal with no complaints. Russel [106]and colleagues at the Saint Joseph's Hospital in Ontario undertook a more sophisticated design capturing prospective TTO utilities pre and post-transplantation (n = 27). With TTO technique patients were queried as to how much time they would be willing to trade for perfect health. Patient baseline utility score was a mean of .41, and post-transplant was .74, representing a 33% increase in quality of life.

Laupacis and colleagues undertook the first formal cost-utility analysis study to assess the quality of life and the cost-utility of renal transplantation in Canada [107]. Laupacis used several instruments to capture quality of life: the Hemodialysis Questionnaire (KDQ), the Kidney Transplant Questionnaire (KTQ)-25, the Sickness Impact Profile (SIP), and TTO, with death scored at zero and perfect health scored at 1. Follow-up took place at the following months: 1 (n = 146), 3 (n = 131), 6 (n = 137), 12 (n = 132), 18 (n = 107), and 24 (n = 76). All the health questionnaires demonstrated substantial improvement, as did the TTO utilities. The authors calculated the ICUR at one year and at two years as follows:

$$\begin{aligned}\text{ICUR}_{1\text{year}} &= \$66\,540 - \$73\,659/.65\text{QALY} - .53\text{QALY} \\ &= -\$59\,325/\text{QALY}\end{aligned}$$

$$\begin{aligned}\text{ICUR}_{2\text{year}} &= \$27\,474 - \$70\,869/.62\text{QALY} - .51\text{QALY} \\ &= -\$394\,500/\text{QALY}\end{aligned}$$

These negative costs per QALY were interpreted as meaning that at both one year and two years the renal transplantation rendered more quality of life years and was substantially less expensive compared to dialysis. Not all patients made it to their visits to record the TTO utilities, and the study estimated 224 utilities (24%) of the total potential patient visits (note that all patients too sick to complete the TTO measure were given a score of .20). The Laupacis study (whatever its limitations), at the Vancouver General Hospital, does represent pioneering work to try to empirically quantify the cost associated with quality of life gained for renal transplant patients.

The Franke et al. [108]. study at the University of Essen (Germany) marks a milestone by the development of the ESRD-SCL (End Stage Renal Disease Symptom Check List) that was specifically designed to assess the physical and psychological quality of life among renal transplant patients. The ESRD-SCL consists of

79 quality-of-life measures that are measured by a five point Likert scaling. Construct validity was assessed by correlational analysis with the SF-36, and hierarchical multiple regression revealed six reliable subscales with Cronbach's alpha of .76 to .85. Ziegelman et al. [109]. developed the TxEQ (transplant effects questionnaire) for assessing multiple dimensions related to the outcomes of transplantation in patients with ESRD. The specific domains in TxEQ were as follows:

- worry about transplant
- guilt regarding donor
- disclosure of transplantation
- adherence to anti-rejection medications
- taking responsibility to value the transplant.

The TxEQ proved to have good psychometric properties, and may be useful for a variety of organ transplant studies. Recently Chisholm-Burns and colleagues undertook an analysis of the concurrent validity of the KTQ-25 among USA renal transplant recipients [110]. Concurrent validity is comparing a new measurement instrument with a supposed "gold standard" measurement instrument to see how well the results of the new tool concur with the older and validated tool [111]. In this study, KTQ-25 should have results similar to the SF-12v2 when administered simultaneously if KTQ-25 is measuring the same underlying construct that the SF-12v2 is. KTQ-25 is a 25-item questionnaire, with five domains: physical signs, fatigue or tiredness, uncertainty and fear, appearance, and emotional content. The SF-12 is a much-used shorter version of the well-established SF-36 general health survey instrument, with eight domains. Chisholm-Burns et al. found supportive evidence of concurrent validity; with Cronbach's alpha exceeding .75 for most domains, and thus KTQ-25 can be an important addition to the health quality of life instrumentations used in renal transplantation [110].

Although there is currently a paucity of formal CER studies in renal transplantation given the newness of its emphasis, there are a few studies that approximate some of the key components of CER. One of the very first renal transplant studies to be undertaken using a decision analysis methodology in the elderly patient population was done by Jassal and colleagues in Toronto [112]. They used previously published utilities of .49 for dialysis and .78 for transplantation. For patients age 65 obtaining a cadaveric donor with a two year wait-list baseline the transplant ICUR was $67 779.00, with a gain of 1.1 QALY years, while for those with no wait-list the transplant ICUR was $14 910.00, with a gain of 2.0 QALY years. When wait listing periods were expanded to four years the ICUR at age 60 was $175 107.00, and at age 85 the ICUR plummeted to $14 585 442. The authors conclude that ICURs remain reasonable for patients aged $\leq$70 with baseline wait times not exceeding two years.

Kontodimopoulos and Niakas, at the Hellenic Open University, undertook the first cost-effective and quality of life study comparing hemodialysis (HD), peritoneal dialysis (PD), and renal transplantation (RT) in Europe [113]. Utilities were estimated using the SF-6D, a subset of the SF-36 General Health Survey, and QALYs were calculated assuming the linear QALY model based on projected individual life expectancy, with zero and five percentage present value discounting assumed. SF-6D derived utilities were .639 for HD, .599 for PD, and .716 for RT. QALYs by treatment modality were as follows: HD 5% discounted 3.67, and 4.37 0% discounted; PD 5% discounted 3.38, and 3.94 0% discounted; and RT 5% discounted 9.58, and 16.11 0% discounted. ICURs were calculated for each treatment, with renal transplantation demonstrating the lowest rate of cost per quality of life-year gained particularly at the three year and life-time projections (Figure 135.7). Since their cost estimates were gathered in Greece, caution must be exercised in extrapolating them to a for-profit health care context (e.g. USA).

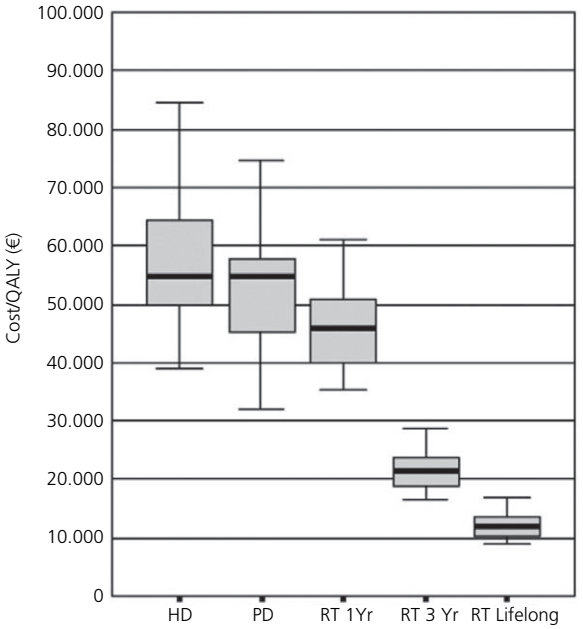

Figure 135.7. Cost per QALYs: The cost/QALY in euros (€) is plotted on the vertical axis, and the specific ESRD therapy is plotted on the horizontal axis. HD is hemodialysis, PD is peritoneal dialysis, and RT is renal transplant. The median cost/QALY is similar for one year RT to HD and PD, and the cost/QALY decreases substantially at RT three years, and RT lifelong.

The Kontodimopoulos and Niakas study raises an important point in cost-utility analysis, that of whether or not to discount future health gains along with future costs [54,113]. The authors disclose that they chose to discount QALYs at 5% rate to achieve a net present value, and used discount rates of 3% to 10% for costs. Some economists contend that since the value of health effects increases over time (presumably because good health causes income to increase) then they should be discounted but at a lower rate than costs [114]. This is referred to as differential discounting of costs and health where different rates of discounting are applied to health values and costs [115]. Equivalent versus differential discounting is a contentious issue among economists, and both approaches are severely criticized. The assumption for both approaches that a QALY is stable or constant over time is itself questionable. Van Hout has argued in favor of hybrid differential discounting adjusted by the economic growth rate and by modeling the growth of health [116].

Recently, Haller and colleagues undertook a cost-effectiveness analysis of kidney transplantation at the Medical Center of Vienna [117]. Quality of life utilities were measured using the EQ-5D, SD, and TTO techniques, while costs and QALYs were equivalently discounted at 3%. Results favored renal transplant and peritoneal dialysis over hemodialysis with a gain of 839 QALYs when 20% of patients received PD, and a gain of 2242 QALYs with 10% of patients receiving transplantation over a projected ten-year period of time.

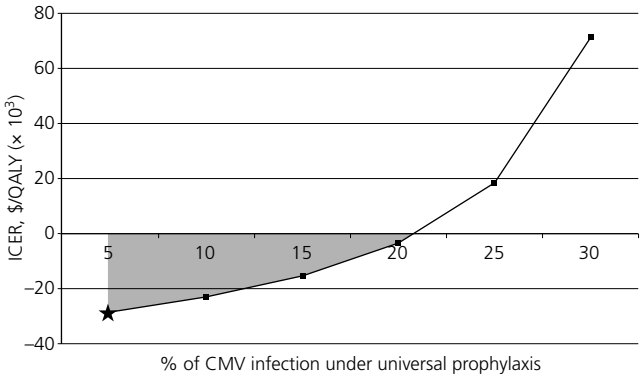

Figure 135.8. CMV infection rates and ICER: the ICER (incremental cost-effectiveness ratio) is plotted on the vertical axis, and the percentage of CMV infection under universal prophylaxis is on the horizontal axis. The figure represents the effect on ICER at various levels of universal CMV prophylaxis. The figure shows that at CMV universal prophylaxis less than 20% the ICER has greater cost-effectiveness ($ per QALY) in the shaded area over the preemptive strategy.

Figure 135.9. Utilities for lung transplantation: utilities are plotted on the vertical axis, and bar graphs are plotted along the horizontal axis for wait-listed versus years after transplantation. All year durations from one year out to five years show greater utility for lung transplantation versus being wait-listed.

In another recent study they utilized data from the Dutch End-Stage Renal Disease Registry (n = 15435), and took previously published utilities (HD .5560, PD .5817, and RT .8077) to simulate the benefit in terms of life-years and QALYs based on a living donor assumption. Early RT manifested improved quality of life-year gains of 6.7 to 8.8 QALYs for patients 40 years old, and a gain of 4.3 to 6.0 QALYs for patients 70 years old [118].

Recently Luan and colleagues completed an interesting quality-effectiveness study comparing the ICUR of universal versus pre-emptive treatments for cytomegalovirus in renal transplant patients [119]. Universal prophylaxis involved treatment with valganciclovir for 90 days for all CMV positive kidney recipients. The study used a pre-existing renal transplant utility of .73, and .57 for dialysis maintenance. The ICUR was −$27967/QALY with universal prophylaxis, indicating a cost savings of $27967 for each QALY gained over a ten year period. Their one-way sensitivity analysis showed a favorable cost saving ICER by implementing universal prophylaxis up to the point of approximately a 22% CMV infection rate (Figure 135.8).

The two-way sensitivity analysis demonstrated a less than $100000 per QALY gained with universal prophylaxis when varying the universal prophylaxis CMV infection rate 5% to 30%, and varying preemptive therapy CMV infection rate between 35% and 60%. Finally, a quality of adjusted-life study was done in Chile recently comparing renal cadaveric transplant with dialysis using established utilities of .80 for transplantation and .50 for dialysis. The study found a positive cost savings began at two years post-transplant, with dialysis costs approximating $134000, and the expected transplant costs of $106000 and a gain in QALYs of 7.30 [120].

Applying CER to organ transplantation

Transplantation is a unique subspecialty of medical practice in that its primary intervention, the organ transplant, is not readily available and is dependent on a national procurement system with donor organs supplying substantially less than patient's demand. CER seeks to determine the most effective therapy, and to derive the lowest risks, lowest costs per gain, and highest gain in quality of life per adjusted years. Clinicians and researchers in the domain of organ transplantation need to incorporate the analytic methodology of CER as we have articulated. For new fields of transplantation, such as CTA (composite tissue allograft), CER may prove useful in the process of advancing CTA as a standard of care. Liver, lung, and heart solid organ transplantation is essentially life-saving and, as such, comparisons can be done for wait listing versus organ recipient. In a Canadian study by Vasiliadis and colleague they compared lung transplant versus patient wait-listing and found a significant variability in derived utilities using standard gamble methodology [121]. The QALYs gained was a modest .62 for the full cohort, and only by making a ten-year projection did the QALYs gained reach 2.19. Figure 135.9 shows the mean utility registered at each time interval following lung transplantation.

How is a lung transplant patient wait-listing able to use these results in their decision making? We can conjecture the following: they should expect, overall, a good improvement in perceived usefulness of their life, yet over the years there is considerable variability to these gains, and the overall quality of life is modest and improves gradually over time.

In a CER study in Finland investigating the quality-adjusted life years following liver transplant, the researchers found a median increase in one year QALYs of .895, and at five years of 3.960. Median cost per QALYs gained (expressed in €) was: (141769/.895 =) 158400/QALY at one year, and at five years post- transplant (177618/3.96 =), 44854/QALY [122]. This study demonstrates two dimensions of informative use; for the patient-centered perspective the increase in quality-adjusted life-years gained, and for medical policy decision-makers the costs associated with each QALY gained. The latter obviously has use for determining society's willingness to pay assessments.

Grauhan and colleagues investigated the effect of the degree of coronary atherosclerosis (CA) on quality of life adjusted-life years (QALYs) for patients undergoing heart transplantation in Germany [123]. They used the SF-36 to measure health status for deriving QALY's among three comparison groups: single artery CA, multiple-artery CA, and those hearts with no discernible CA.

The mean SF-36 scores were very similar across the three groups: single CA 64.6, multiple CA 61.5, and no discernible CA 62.4; however the QALYs were significantly different, with single CA 8.5 QALYs, multiple CA 2.2 QALYs, and no discernible CA 8.0 QALYs. Thus, undertaking a program of pretransplant screening angiography is indicated as a reasonable investment given the gain in QALYs (5.8 to 6.3).

Methodological concerns of CER

CER is not without limitations and challenges as it undertakes the principal goals of demonstrating effectiveness, cost-utility, and favorable incremental utility, all while focusing more on the realities of clinical practice and patient-centered care. The initial broadly stated concern is that comparative effectiveness that focuses exclusively on gain in QALYs comparing new treatment to an established treatment does little to control costs and can actually lead to allocation inefficiencies if strictly adhered to. Secondly, cost-benefit or incremental cost utility analysis — both of which incorporate a comparison to costs and quality of adjusted-life years gained — renders comparison inclusive of their relative merits; this could lead third-party payers to simply adjust their costs to meet the derived standard (which could result in increased costs) [124]. A more fundamental concern with CER is the issue of differential or heterogeneous patient benefits resulting from broadly applying recommended therapies. For example, a meta-analysis of CER-based studies may establish the best practices therapy for the typical patient yet some will no doubt benefit more or less than others. This concern should be tempered by the relaxation of inclusion criteria for entry into the study, such as greater patient heterogeneity, which may dilute the efficacy while increasing broader effectiveness. The problem manifests when benefits are difficult to quantify exactly; such as reducing pain, anxiety, improving sleep quality, or reducing gastritis. Likewise, differences in drug effects are typically small and as such require large recruitments of patients to study arms to find small effect sizes [125]. Establishing equivalency or superiority of drug therapies is not easy with small effect sizes, often needing a placebo arm, which is a salient impediment to patient consenting when drugs for successful treatment have already been established. However, the CER emphasis goes beyond the traditional RCT efficacy ideal, to investigate the comparison of established therapies to enable enlightening the patient and physician decision-making process as to the most favorable course [126]. Hung et al. provide a recent example comparing metformin, sulfonylurea, and rosiglitazone in a retrospective study of 93 577 patients treated for DM on the composite endpoint of persistent decline in eGFR or ESRD [127]. After four years of treatment, sulfonylurea use compared to metformin revealed a 1.2 greater (HR) (CI: 1.13 to 1.28) risk for the composite endpoint. Critics might question the small effect size (<2.0), yet the 20% increased risk may be quite clinically meaningful given the large sample size involved.

Another related issue is non-ignorable physician skill set variance, which is particularly evident in surgical procedures when comparing low versus high-value organ transplantation programs. The intervention resulting in the greatest patient medical outcome and quality of life benefits may be highly dependent on technical skills and thus create wide variance in measurable outcomes and benefits. Albeit, a stated goal of CER is to advance the public's knowledge of what is effective and what is not, CER intentionality is not to produce a "one size fits all" medical decision-making matrix. There will inevitably be conflicts between the patients-favored outcome, such as quality of life, versus the physicans' biophysical outcomes. Recently Fried and colleagues investigated health outcome priorities in an elderly population and found that staying alive was ranked as top priority by only 11%, with pain management being ranked the top by 13%, and staying independent was number one by 76% of respondents [128]. Obviously when faced with the reality of finitude or chronic pain these priorities may alter substantially, but the study does highlight the need to include quality of life perceptions and needs reported by patients in the medical decision-making process such that a greater individualization of care is the result [129].

Recently a modified RCT design has been proposed as a bridge between RCT and CER to better achieve a balance between efficacy (internal validity) and effectiveness (external validity) called pragmatic randomized trials (PRT) [130]. The design is similar to RCT, with randomization to experimental and control study arms, yet the intervention and the outcome are grounded in meaningful and medical practice-focused research questions. Emphasis is upon external validity, thus a more inclusive patient recruitment strategy is employed in an attempt to generate real-life applied results [15]. PRT design differs in focus from RCT: the aim is investigating patient benefits under usual conditions (versus ideal), maintaining intervention flexibility, focusing on usual care practices, and finding answers to a broad set of outcomes that are of clinical importance to patients [16].

When CER studies are not randomized there is always the potential that observed treatment differences are due not to the intervention (or therapy) but to the preselection conditions that the self-selected patients bring to their respective study groups. Since epidemiologists primarily conduct their research with non-randomized designs, they could be considered a natural fit to assist with CER projects. Epidemiology has a long historical use and sophisticated analysis techniques to manage overt bias, such as matching and stratification analysis. Case-control designs frequently use one of two matching techniques. The first is called individual matching, where cases and control are selected to be similar based on some a priori criteria of deemed importance (e.g. age, gender, blood type, inflammatory markers) [131]. The second technique is termed frequency matching, and involves drawing the control sample in such a manner as to approximate the experiment patient characteristics (e.g. all wait listed patients from transplant center xyz that have similar creatinine levels). Note that in matching designs the test statistic is quite different since we are testing the observed probability of a discordant pair and we use the conditional maximum likelihood estimate of the odds ratio: OR = b/c, tested with McNemar's test, $\chi^2 = (B - C)^2/(B + C)$ [132]. Stratification is another pervasive technique to create balance between the experimental and control groups by segmenting the groups by one or more covariates of interest while making the groups otherwise as similar as possible [133]. Other newly popularized techniques that attempt to manage for selection bias in non-randomized studies include propensity score analysis (based on pretherapy variables, determining the conditional probability of each patient receiving a particular therapy) [134–137] and sensitivity analysis (i.e. determining the effect on an outcome variable by varying input variable or variables, such as changing the discount rate to impact the ICUR).

Summary and future directions

CER began in the USA in earnest with passage of the American Recovery and Reinvestment Act of 2009, which provided funding

and catapulted CER into mainstream research efforts. The principal objective of CER is to reform medical research such that it will enhance the patient-centered reality of clinical practice. Modern medicine has functioned for some time with a certain misalignment with clinical research and its emphasis on "one size fits all" generic focused efficacy versus the day-to-day practice needs of a heterogeneous patient population that presents with their chief complaint and a plethora of comorbidities. EBM was Europe's imperfect attempt at heightening research relevancy and legitimacy in the clinician's utilization of medical research in daily practice. EBM seeks to compliment the pathophysiological knowledge base that clinicians rely on with cutting-edge research evidence. CER seeks to expand the domain of EBM with its emphasis on assessing not only efficacy in the heterogeneous patient context, but quality of patient life vis-à-vis the medical intervention and the efficiency of such interventions. CER emerges partly due to the new found relevancy and successes of personalized medicine in pharmacogenomics, which represents a fundamental shift from generic patient pharmacology to a utilization of drugs in a tailored manner based on the patient's own genetic profile [138]. This genomic relevancy was most dramaticly illustrated by finding that the colon cancer drug cetuximab (Erbitux) was not effective in treatment of metastatic colorectal cancer in patients whose tumor had K-ras mutations on codon 12 or 13 oncogene. Thus, patients screened for K-ras mutation can potentially avoid a futile therapy and clinicians can shift to other therapeutics. Even for effective drugs, genomic information can be used in a dosage-tailored manner; for example a genetic test can reveal patients who are at high complication risk when using normal dosage of mercaptopurine for treatment of leukemia. In solid organ transplantation of liver, heart, and kidneys, it has been shown that patients who are carriers of CYP3A5*1 allele have an adverse impact on the pharmacokinetics of tacrolimus, and need a longer time period to achieve desired blood levels [139]. Recently, Provenzani and colleagues demonstrated that renal transplant patients with the 2677T/A allele needed much higher daily dosing of tacrolimus to achieved targeted therapeutic levels compared to patients who were homozygous for wild-type allele [140]. Finally, Einecke et al. developed a molecular classifier to predict renal graft loss, which demonstrated a RR of 2.70 (CI: 95%, 1.82–4.00) for graft loss with the molecular risk score [141]. The researchers conclude that the gene-based molecular risk score successfully identified active renal injury that can eventuate into graft loss.

A philosophical and economic concern with CER is that results, even from genomic biomarkers, are still frequentist averages, although subgroup based. When CER reveals the individual-level heterogeneity of the treatment effects, how will this affect health consumers and health care providers? Medical information will always have an element of uncertainty in its realized successes; as such patients can over or under estimate the treatment benefits. Simply put, when benefits exceed harms of a new therapy versus control (the mission of CER) and the information is uncertain, patients will jump to get the new therapy manifesting a large moral hazard and a demand upon third-party payers [142]. Some have thus advocated for a greater emphasis on individualization in CER, termed iCER, which will improve patient treatment decision-making [133,143]. The iCER can be brought about by n-of-1 trials, pragmatic randomization trials, use of local instrumental variable analysis to better allow for unobserved confounders, investigation of epigenetic factors, cross-over designs, and by using adaptive randomization techniques, the so called "play the winner" approach to patient allocation to the treatment arms that are demonstrating the largest effect size [144].

There has also been some debate as to whether personalized medicine was in conflict with stated objectives of CER, since the former's emphasis is explicitly patient-centric or individualistic, while the latter professes to investigate effectiveness of competing medical therapies among heterogeneous subgroups of patients. These concerns may be overstated since the very legislation creating the PCORI mandates accommodation between the principles of personalized medicine and CER. Both seek to shift the research emphasis from so called "average group effects" that tends to hide important subgroup effects and move research in the direction of improving patient care in an efficacious and efficient manner. The responsibility of CER researchers is to ensure it is undertaken to capture the relevant issues and nuances germane to the course of the illness being investigated. Patients do not respond to therapies monolithically, there is considerable response variation. It is this fact within medicine that will make it continue as an ongoing mix of clinical judgment (both the art and the practice) and necessitates the continuation of high-quality and relevant research that will inform and develop better clinical predictions [145].

CER in transplantation research will play an important role as it seeks to advance knowledge of effectiveness and patient-centered results. The emphasis needs to shift to complement efficacy with expanded external validity and relevancy for patient care and patient decision-making. Adding the utilities, quality of life measures and quality of adjusted-life assessments, all will help augment the decisional pathway within the complicated maze of transplantation decision-making.

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SECTION 11

Transplant Policy

CHAPTER 136

Transplant Ethics for Clinicians

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Introduction

Transplant professionals are entrusted with a unique position in the practice of medicine, the stewardship of a scarce societal resource: the organ donor. This stewardship entails two major responsibilities for which society holds transplant professionals accountable: the equitable allocation of deceased donor organs to medically suitable recipients; and the evaluation and care for the living organ donor. Both of these issues are addressed in additional detail in Chapters 137 and 138 respectively. This chapter focuses on the amalgamation of these from the perspective of the clinician. The decision to pursue organ transplantation as a therapeutic option entails a complex decision by the physician and patient regarding the risks of the procedure and the expected benefits. Ultimately, the treatment recommended for each patient should be based upon appropriate data and the experience of the transplant physician. Transplant professionals face an avoidable conflict in their stewardship of donor organs when balancing advocacy for an individual patient against the societal responsibility of conserving this precious resource. Data, experience and ethical principles relevant to organ transplantation become invaluable in balancing these competing interests. This chapter "Transplant Ethics for the Clinician" attempts to reconcile the competing interest of the patient and society in organ transplantation. Although the focus will be on kidney transplantation, the principles apply to decisions made for all transplantable organs. This chapter is not intended to convey opinion; on the contrary, it is a review of the application of data and experience that results in a normative course of treatment aligned with principles of bioethics.

The final rule

The Department of Health and Human Services has issued a "Final Rule" [1] as a framework for the structure and operation of the Organ Procurement Transplant Network (OPTN) to oversee the practice of organ donation and transplantation in the US. Section 121.8 of the Final Rule addresses the allocation of organs and states that allocation policies:

- Shall be based upon sound medical judgment;
- Shall seek to achieve the best use of donated organs;
- Shall preserve the ability of a transplant program to decline an offer for a specific potential recipient;
- Shall be designed to avoid wasting organs, avoid futile transplants and promote patient access to transplantation and efficient management of organ placement;
- Shall not be based upon the candidate's place of residence or place of listing.

Thus, the OPTN was directed to set priorities for rankings candidates on the wait-list based upon measurable medical criteria and ordered by medical need. In this setting of the Final Rule, the classic ethical principles of justice, utility, beneficence, and non-maleficence, and autonomy become evident [2]. The Final Rule accounts for the *Justice* principle by requiring allocation policy to be based upon sound medical judgment. Justice calls for the distribution of kidneys to patients with the longest waiting time. Ultimately, all patients with the same medical condition should be treated the same when placed on the wait-list. For extra-renal organs, the Justice principle now mandates medical need or the severity of the patient's illness as the foremost criterion of allocation:

- **Utility** as an ethical principle of the Final Rule, seeks the best use of the donated organ in achieving the best allograft survival; the best outcome.
- **Beneficence** promotes the interest of the patient to undergo transplantation. The Final Rule sustains *non-maleficence*, "to do no harm", by enabling a transplant center to decline a specific organ for a specific patient.
- Finally, **Autonomy** preserves the ultimate decision of the patient to undergo transplantation.

Decision-making in the current system

Beneficence, non-maleficence, and autonomy influence the doctor patient relationship at the time of accepting a kidney for transplantation. The patient trusts the physician's intention to make a recommendation regarding transplantation as a treatment option that is in his/her best interest. This decision is formulated with clinical judgment, knowledge of the data and clinical experience. Recent data suggest that patients and surgeons judge the same factors to

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be important when deciding to accept a kidney for transplantation [3]. These include the quality of the kidney by donor age, risk of transmissible infectious disease or malignancy, the anatomy and function of the kidney, and the degree of HLA match (also accounting for potential recipient sensitization). These data provide essential considerations but the ethical principles elaborated by the Final Rule are important aspects as well.

The application of ethical principles

Addressing the overall quality of the kidney

Justice and beneficence are currently prioritized over utility to enable the allocation of an excellent donor kidney to an elderly patient. Conversely, non-maleficence would reject an older age kidney for a younger recipient. Importantly, the Final Rule enables a transplant center or patient to decline a kidney offer with autonomy as the key ethical component of the decision.

Function of the donor kidney at the time of death

Beneficence compels the acceptance of a kidney with a higher risk of delayed graft function for recipients that are highly sensitized or who reside in regions with longer waiting times and can tolerate the stress of delayed graft function and dialysis. A donor kidney with the same characteristics may be declined for recipients with medical co-morbidities that could not tolerate delayed graft function.

The HLA match of the donor kidney and recipient sensitization

This is a criterion of acceptance or refusal that depends upon the degree of the HLA match, the quality of the donor kidney and sensitization of the recipient. Current practice prioritizes quality over matching especially for the younger recipient. Highly sensitized patients are also afforded a priority for deceased donor kidneys irrespective of donor age and quality.

The risk of transmissible disease

Beneficence supports accepting a kidney at risk for infectious disease transmission for a highly sensitized patient who might otherwise never receive a kidney offer or a patient with failing dialysis access that is in life threatening need of a kidney transplant. However, non-maleficence requires declining such kidneys for recipients with low PRA and those without urgent medical need for transplantation. As the quality of the kidney is improved and recipient factors increase the urgency: such as longer waiting times, higher PRA, and older recipient age, the autonomy of the patient to accept a kidney balances the decision accepting an ill-defined but real risk.

Predicting the survival of the allograft

The expanded criteria donor kidney may be suitable for an elderly patient greater than 65 years of age, because the anticipated mortality on dialysis exceeds the rate of ECD failure. Again, the consideration of non-maleficence would result in the refusal of an ECD kidney for a younger patient. With each occurrence of a kidney offer, the transplant physician must balance beneficence with non-maleficence and determine whether the kidney should be accepted for that specific recipient, based upon sound clinical judgment, current data and guided by ethical principles.

The inadequacy of the current system

The transplantation of a 25 year old deceased donor kidney to a 75-year-old recipient, mandated by current allocation rule exposes the inadequacy of the current system in achieving the best utility of the donated organ. The wait-list has been expanded to accommodate an older age population and patients with more co-morbidities. A system of allocation based mainly upon time waiting overlooks the utility demand of the final rule.

In the past decade, UNOS has attempted to revise kidney allocation policy providing justice to all patients, while exercising utility to maximize the societal benefit of scarce donor organs. However, the potential shift of donor kidneys away from the older age population in the interest of improving the life years gained from a transplanted kidney has not been well received by those who serve the interests of the elderly. To address those concerns, UNOS has proposed a new approach assessing donor kidney and recipient longevity so that the survival of every transplanted organ "can be realized within biological reason and acceptable levels of access for those on the waiting list". The objective is to enhance graft survival for patients with the longest anticipated post-transplantation survival and to minimize the loss of a functioning kidney through an improved matching of the recipient and graft survival.

Living donor organ transplantation

Live donor transplantation has emerged as the predominant practice of organ transplantation throughout the world. This development was anticipated by a pioneer of transplantation, Dr. Francis Moore, who noted 40 years ago, that the live donor kidney would provide "the best tissue in comparison to an organ from a deceased donor. Today, the live donor affords the recipient the best opportunity for successful transplantation and the longest survival. The ethical principle of *beneficence* enables a healthy individual to donate an organ for transplantation. However, the ethical underpinnings of live donor kidney transplantation are not only built upon its success but by a requirement that there be a minimal risk to be a living kidney donor. The ethical principle of *non-maleficence* calls upon clinicians to avoid doing harm by ensuring medical appropriateness of the recipient. Risk to the donor should not be deemed acceptable unless there is a high probability of benefit to the recipient. This ethical axiom is well described by the consensus statement on living donation:

> the person who gives consent to be a live organ donor should be competent, willing to donate, free from coercion, medically and psychosocially suitable, fully informed of the risks and benefits as a donor, and fully informed of the risks, benefits, and alternative treatment available to the recipient. The benefits to both donor and recipient must outweigh the risks associated with the donation and transplantation of the living donor organ [4].

Non-maleficence or medical appropriateness in balancing risks versus benefits is exercised by clear guidelines established for donor protection. These guidelines include donor evaluation and screening and the monitoring of donor outcomes, all codified initially by the Amsterdam and Vancouver Forums. The Amsterdam Forum focused upon consent of the living kidney donor and the Vancouver Forum addressed extra renal living organ donation. These two forums help define core responsibilities of transplant programs (Box 136.1) and the essential elements of informed consent for all

Box 136.1. Responsibilities of the transplant center performing live organ donor transplantation. Data from [9,10]

- Provide information about organ donation and transplantation.
- Educate about the treatment alternatives available to the recipient.
- Provide an independent donor advocate.
- Assess medical, psychological and social suitability for organ donation.
- Ensure the decision to donate is voluntary and free from coercion.
- Implement procedural safe guards to enhance donor safety, understanding and autonomy.
- Provide medical and surgical staff skilled in the evaluation and care of the live organ donor.
- Provide referral and treatment for any medical conditions identified during evaluation.
- Provide psychosocial support throughout the evaluation and donation process.
- Provide medical care until recovery from donation process is complete.
- Facilitate long term follow up of the living organ donor.
- Perform quality assurance and process improvement to decrease risk of organ donation.

Box 136.2. Essential elements of informed consent in live organ donation

- Must be an adult, 18 years of age or greater.
- Must have the cognitive capacity to make the decision to donate.
- Must be a voluntary decision free from coercion.
- Must include assurances to protect the donor's privacy.
- Must include all relevant information inclusive of but not restricted to:
  - risk of death
  - risk of short and long term surgical complications
  - risk of potential long term medical and psychosocial complications
  - risk of being diagnosed with a pre-existing medical condition
  - risk to future insurability; health, disability, life
  - risk that the recipient may not have the hoped for outcome.
- Must include a cooling off period prior to the final decision.
- Must include the right with withdrawn from the process at any point.

living organ donors (Box 136.2). In certain parts of the world, patients with liver intestine or lung failure may not have the option of receiving an organ from a deceased donor so the availability of living organ donors becomes critical. However, the risks to the living donor of liver or lung allografts are significantly greater than that of a living kidney donor. Thus, these donors must especially be able to assimilate accurate information regarding their risks, with information presented at a level of medical sophistication suitable for that individual. With respect to informed consent, the donor should be able to verbalize his/her comprehension of risks and benefits.

Donors are patients

Donors become special "patients" beginning with the testing to determine whether they can donate [5]. In the practice of live donor organ transplantation, the doctor-patient relationship is one of mutual responsibility, which should not be abrogated by the claim of donor autonomy nor the obligation fostered by the recipient's needs. The physician has a responsibility to promote the interest and well-being of the living donor irrespective of the recipient need. The potential living donor has a responsibility to be forthright as to medical history and social encounters that may compromise the opportunity for donation or influence the ultimate well-being of both the donor and the recipient. The donor cannot demand to undergo a nephrectomy or partial hepatectomy without the support of the physician; and thus, the donor is not autonomous in such a decision. Clearly, there are certain instances in which the donor as a parent will present a compelling basis to be a donor despite an isolated medical condition such as hypertension. Nevertheless, if equipoise is not affirmatively achieved in the risk-benefit calculation for the donor and the recipient, then sound medical judgment should override all other concerns and prevent the transplant from going forward. A familial relationship does not impose on the donor (or the recipient) the necessity to take on additional medical risk to accomplish donation but it may influence the ultimate decision to permit donation to go forward (see further on in this chapter).

The psychosocial assessment

Biologically unrelated donors constitute more than 40% of living kidney donors in the US [6]. Most of these donors are spouses or friends with a long-standing emotional relationship to the recipient. Nevertheless, there are an increasing number of living donations that are occurring through relationships established by the Internet or church bulletins that link a recipient to a potential donor. Unrelated anonymous individuals also come forward today with a genuine altruistic intention to provide for another and in the absence of personal appeals. The potential donors that respond to Internet solicitation must receive a thorough psychosocial evaluation to confirm that there is no underlying financial need or expectation. Furthermore, organ donation should not serve as a remedy for a potential donor's psychological malady. Guidelines for the psychosocial evaluation of the unrelated donor were published following a consensus conference on this topic [7]. Risk factors for an anticipated unfavorable psychological outcome following donation were developed to include a history of psychiatric symptoms or disorders or a history of substance abuse or dependence. A motivation reflecting an interest for personal or public recognition or the desire to use the potential donation as a way of fostering a new personal relationship was considered a high risk. Subordinate relationships such as employee/employer in which coercion may be the basis upon which an individual has come forward must be assessed and fully vetted prior to proceeding.

Autonomy versus paternalism

Autonomy is the capacity for self-determination to act intentionally and voluntarily, with comprehension, and without controlling influences coercing the patient. With organ donation, a persons' autonomy is exercised through the process of obtaining informed consent. Respect for autonomy however, does not imply that physicians are obligated to co-operate with someone to enable them to be a donor if medical judgment is not supportive. Clinicians should not be coerced into recommending or participating in a nephrectomy or partial hepatectomy if the medical evaluation renders the potential donor medically or psychosocially unsuitable. It is also important to recall that autonomy is not a principal exercised in a vacuum; it requires a comprehensive assessment of the role of beneficence and non-maleficence in the decision to go forward with organ donation. The responsibility of the physician to uphold non-maleficence is not paternalism but rather the proper exercise of medical judgment. The Amsterdam Forum recommends procedural safeguards such as a cooling off period prior to final approval to proceed with donation in an effort to minimize coercion and yet support autonomous decision-making.

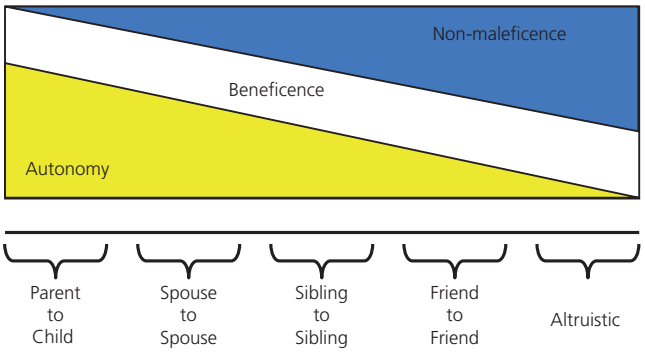

Figure 136.1. Relationship of the donor to the recipient influencing the decision to donate.

The relationship of the donor to the recipient influencing the decision to donate

The relationship between the donor and recipient may be consequential to the ultimate decision for donation. As the relationship becomes more biologically distant, from blood relative — to an acquaintance — to an anonymous stranger, autonomy becomes less influential and non-maleficence becomes the most weighted consideration for the altruistic unrelated or anonymous donor (Figure 136.1). The clinician's concern regarding risk to the donor is not changed by the relationship; however, the exercise of donor autonomy permitting risk is more appropriately sanctioned when there is a strong familial or emotional relationship between the donor and recipient. Isolated medical abnormalities such as hypertension, proteinuria, marginal renal function, or anatomical issues such as renal artery stenosis are not summarily dismissed when the relationship between the donor and recipient is well-established emotionally but rather the good derived from a successful transplant may mitigate such isolated medical conditions. For example, transplant physicians have become more accepting of an older spouse with hypertension to be a donor, because there is a predictable lifespan not adversely affected with successful medical treatment of hypertension and there is a tangible benefit for the donor spouse. Furthermore, the opportunity for donor follow-up simultaneously with care of the recipient helps to mitigate the risk and allows the donation to proceed.

Assessment of outcomes

The transplant community has a responsibility to track and report the outcomes of live organ donors as an integral part of a societal stewardship. Sentinel events such as donor deaths and organ failure should be reported transparently and indemnified from litigation. Similarly, if physician malpractice was not involved, participation in reporting these sentinel events should not place the physician at risk of liability.

The limitations of existing data regarding outcomes of living kidney donors and a defined need for follow-up of donors has been highlighted by a recent consensus conference on these topics [8]. Major reasons to continue the systematic collection and reporting of living donor outcomes were developed:

1 living donors must have accurate outcome information to give informed consent;
2 program specific feedback is necessary for quality assurance and performance improvement;
3 donation is a public trust and the transplantation community has an obligation to continue to collect information on living donor outcomes;
4 ongoing information is necessary to improve the evaluation process and provide a reliable counseling for nontraditional living donor candidates;
5 surveillance may identify individual donor problems at a time when intervention is possible.

The transplant profession must be proactive in ensuring that the donation process is safe both perioperatively as well as long-term. Live organ donation works because the public believes that transplant physicians and surgeons are working for the best interests of donors and recipients.

Summary

The practice of organ transplantation requires the physician to have an understanding of bioethics, and an awareness of current medical information and data that can result in the best outcome for a specific patient. To accomplish those goals, society entrusts the physician to be a steward of the deceased donor organ and to act in the interest of the living donor by providing donor care and assuring safety for the donor's well-being.

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CHAPTER 137

The Ethics of Organ Allocation

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Introduction

This chapter will examine the general ethical principles applied to the development of deceased donor organ allocation policy, as well as other factors that influence patient prioritization; review issues affecting patient access to the waiting list; discuss the relationship between organ allocation policy and organ donation; and review legal and regulatory factors influencing organ allocation policy.

Organ transplantation is unique

Rationing of scarce resources is certainly not uncommon in health care. Due to limited funding some therapies are rationed overtly as a matter of policy to control spending, especially in nations where the government is the primary or exclusive payer. In these cases, expert panels make choices about which available therapies meet an acceptable cost-to-benefit ratio. Insurers may limit access to certain therapies that are considered less "efficient" through higher co-pays, deductibles or even non-coverage or by requiring use of a generic drug or a particular manufacturer's product. In other examples, therapies may be indirectly rationed in a *de facto* manner through unequal access to medical care as determined by such factors as race, educational level, employment or insurance status, or geography.

However, organ transplantation is the only area of medical practice that must be rationed both explicitly and independently of financial concerns. This is due to the severe imbalance between the demand for this therapy and the available supply of organs. Until there are significant research breakthroughs in preventing end-stage organ failure or a dramatic increase in the supply of transplantable organs, this supply-demand imbalance will continue.

The successful development and expansion of hemodialysis in the 1960s provided a life saving therapy to patients with end-stage renal disease (ESRD). However the lack of available dialysis machinery and trained staff forced decisions about who would have access. The so called "death committee" in Seattle created to assess patient "worthiness" for access to hemodialysis was chronicled in *Life* magazine [1]. The public revulsion to this situation led, in part, to the enactment of the US ESRD program, which provided Medicare benefits to all patients in need [2]. This massive infusion of financial resources partially solved the problem of access to kidney transplantation, but could not overcome the inherent shortage of organs. The situation with other transplantable organs that are in short supply (e.g. livers, lungs) is complicated even further by the lack of any widely available, effective alternative therapy other than transplantation. This hard reality forces the medical community, public policy makers, insurers and the public to face difficult ethical and societal choices about who shall live and who shall die.

Ethical principles in the allocation of organs

Given the scarcity of the resource, each national or multinational organ recovery and transplantation system establishes policies for the allocation of organs. In development of these policies, there are certain foundational ethical principles that are commonly considered, although the weight given to each varies according to history, culture and politics. The three primary ethical principles are: utility, equity (or justice) and autonomy (Figure 137.1) [3].

Utility

In the context of organ allocation, the principle of utility requires the maximization of "the expected net overall good, while minimizing harm." [3] Utility can further be subdivided into social and medical aspects. Social utility seeks to rank potential recipients by estimating their potential future contributions to society were they to receive a transplant. In democratic societies, such assessments of "worth" inevitably are controversial and therefore generally not considered in the development of organ allocation systems. As noted by the Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/UNOS) Ethics Committee: "Considering one person more useful to society than another, based on prevailing social values, may be a matter of opinion or good fortune in the random distribution of natural and socially cultivated talents and abilities. We add insult to injury when we withhold the benefits of transplantation from those who may not be as likely to benefit society as those more fortunately endowed." [3]

Medical utility seeks to assess how much medical "good" a transplant will do for one recipient as compared to another. There are at least five factors that commonly are taken into account under the umbrella of medical utility:

- **Patient survival —** Simply stated, this criterion asks: what is the likelihood, relative to other patients awaiting a transplant of the same organ, that the potential recipient will die if a transplant is not received? This criterion assumes that one can fairly accurately predict the likelihood of death without a transplant given certain clinical criteria and within a specific timeframe. It also begs the

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

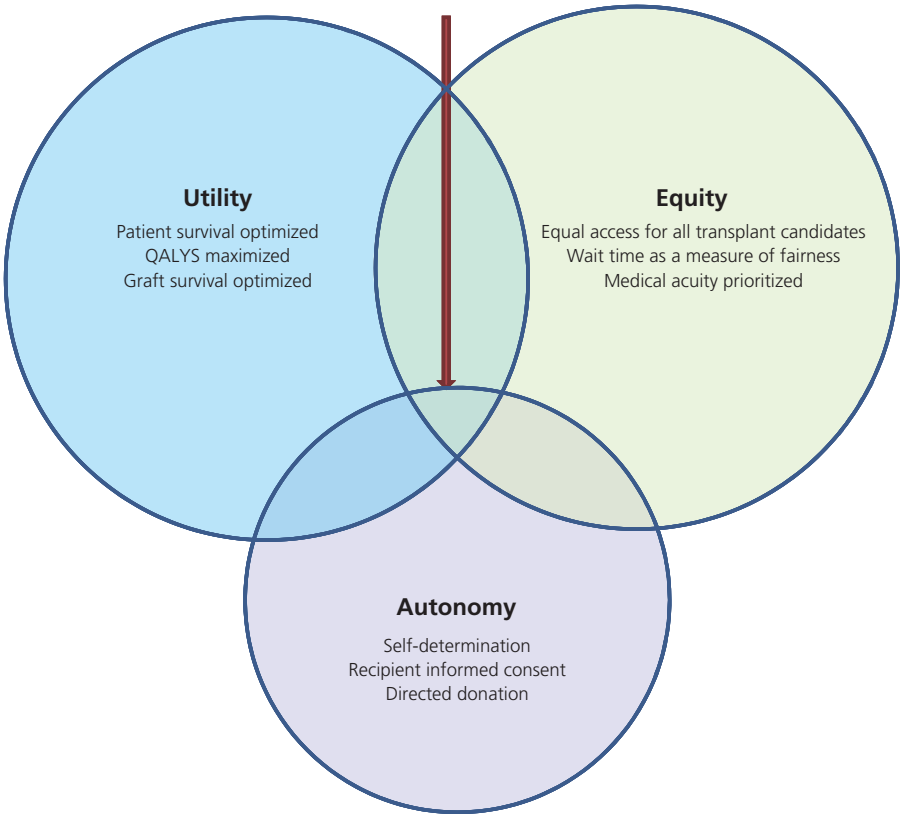

Figure 137.1. Allocation systems are designed to strive for a balance of all three principles.

further question: Is the goal to save a life; or is the goal to maximize the number of years the recipient is expected to live post-transplant? For example, if two potential recipients have the same odds of not surviving without a transplant, but one is expected to live 3–5 years post-transplant and the other 8–10 years, should the second patient receive priority in order to maximize the utility of the transplanted kidney as measured by "life years" added? Specific organ allocation systems often seek to find a balance between these two aspects by providing a "score" that reflects both the likelihood of dying without transplant and the likelihood of surviving for a specified interval post-transplant [4].

- **Graft survival —** Beyond the survival of the recipient, allocation systems also must consider the survival of the graft. An assessment of expected graft survival must take into account both the medical condition of the recipient, and the characteristics of the donor: age, cause of death, process of death (donor after brain death [DBD] or donor after cardiac determination of death [DCDD]) and presence of infectious diseases and/or other medical conditions. Graft survival factors into utility concepts as a measure of how long the transplant is expected to provide medical benefit.
- **Quality of life — I**n recent years, policy makers and clinicians have attempted to assess the usefulness of clinical interventions using the concept of "Quality Adjusted Life Years" (QALY). As its name implies, QALY assesses not only the probability of patient (and graft) survival, but also the quality of life. Although quality of life can be a very subjective assessment, scales have been developed which assume that 0 = death and perfect health = 1, with various states in between along a continuum [5]. Thus comparisons theoretically can be made among potential transplant recipients. For example, a transplant that is expected to keep a patient alive for an additional six years rather than dying within one year, but where quality of life is to varying degrees less than ideal both during the year awaiting transplant and post-transplant, would lead to a calculation as follows:
  - 6 years extra life @ 0.7 quality of life = 4.2
  - less 1 year @ reduced quality of life (1 − 0.6) = 0.4
  - QALYs generated by the intervention = 3.8.

  In the case of kidney transplantation, QALY often is calculated by comparing patients' perception of psychological and physical wellbeing for a year on dialysis, versus a year with a functioning transplant. Various studies have suggested a ratio of 0.8, with transplant the preferred option [6].
- **Availability of alternative treatments —** In establishing priority rankings for transplant candidates a utilitarian approach also considers the availability of alternatives to transplant. The most obvious example is hemodialysis versus kidney transplant although, as noted above, these two therapies generally are not considered equivalent by patients. Similarly, in recent years the increased use of cardiac assist devices (CAD) has complicated the assignment of heart transplant candidate priority. In some cases, CADs have become not just a bridge to transplantation, but a destination therapy.
- **Age —** Age seemingly is the most objective variable as it can be determined precisely without subjective judgments or calculation of statistical probabilities. However, physiologic age is not necessarily equivalent to chronologic age, thus complicating the use of age as a proxy for more subjective variables such as expected

patient survival, expected graft survival and QALY. The application of age as a utility factor in allocation policy may include a comparison of expected graft survival to expected recipient survival. If the graft is expected to survive for more years than the recipient is expected to survive post-transplant, perhaps the graft should be allocated differently to maximize the utility of that graft. For example, a kidney becomes available from an excellent donor and has an expected half-life of 20 years. Should that kidney be prioritized for a younger recipient whose life expectancy with a transplant is at least 20 years? Or should an older transplant candidate with a life expectancy of only ten years have equal priority? In other examples, the choices are clearer. Young children in renal failure will not grow and develop normally on dialysis. Thus most allocation systems provide a priority for children for kidney transplant. For example, in the US, kidneys from standard criteria donors $\leq$35 years old are preferentially allocated to patients listed before their 18th birthday. This policy has effectively reduced the waiting time for a kidney among pediatric patients. However, in a demonstration of the complexity of allocation policy, there has been a concomitant decline in the number of living donor kidneys for the first transplant of a pediatric patient. It is suspected that some parents who are medically suitable donors now choose to save their kidney for the time when their child may need a retransplant [7].

Intuitively, a utilitarian analysis of organ allocation would seek to create a situation where "every allograft is transplanted into a recipient the allograft would outlive, but only by a few days." [8]

On the opposite end of the age spectrum is the Eurotransplant Senior Program (ESP) commonly referred to as the "old-for old" plan. Established in 1999, ESP sought to:

- Find a more effective way to use the increasing number the number of kidneys recovered from donors >65 years old; and
- Reduce the wait time of kidney transplant candidates >65 years old.

A retrospective analysis after ESP's initial five years of implementation demonstrated that these utility goals were achieved with an increase in the total number of kidneys available for transplant for patients of all ages [9].

As we will see further on, the use of age in allocation must be carefully considered in the context of legal prohibitions against age discrimination.

Equity

Counterbalancing the concept of utility is the concept of equity. Sometimes described as fairness or justice, equity in organ allocation acknowledges that there are other criteria, important to most societies, which must be taken into consideration. In general, an allocation system that prioritizes utility above all else probably would not be perceived as the fairest or most equitable by society. If one were to focus solely on utility, then priority might go to: younger patients; patients with stable, intact families able to support them through the surgery and recovery; and patients with insurance (or personal means) to cover the cost of a lifelong regimen of immunosuppressive drugs. Maximizing utility without regard to equity may result in prioritizing shorter waiting times and lower acuity of illness because of anticipated better transplant outcomes (for some diseases). Such an allocation scheme likely would result in de facto discrimination against the poor, the less educated, the underemployed or unemployed, and possibly others with diminished access to health care such as racial minorities.

Because donated organs are considered a public resource to be used for the common good, most societies seek to provide some balance to pure utility factors in order to satisfy a basic sense of fairness. It is relatively easy to reach consensus on some aspects of equity. For example, all other things being equal — expected patient survival, expected graft survival, etc. — generally it is agreed that the patient who has waited the longest should have priority over those who have not waited as long. But waiting time also can be controversial. First, one must agree on a common "time zero" — the moment when a patient is clinically ready for a transplant. Even if that were possible, wait time is heavily dependent on what point in time after time zero a patient is referred to a transplant program, evaluated, accepted for transplant and actually listed. Numerous studies have demonstrated that there is wide variation in patient referral timing, and the timing of transplant center listing [10].

Other factors relevant to assessing patient wait times include:

- Has the patient been offered and previously refused a kidney or liver because they were waiting for one perceived to be from a more ideal donor?
- Has the patient's wait time been extended by periods of wait list inactivity for failure to follow the clinical regimens designed to make them suitable for transplant? (Wait time generally continues to accrue even while the patient is "inactive" on the list.)

Another, more complicated example involves patients awaiting kidney transplantation who are "sensitized" to a large percentage of the population. These transplant candidates may have become sensitized though a blood transfusion, child birth or a previous transplant making it much harder to find an acceptable donor. Thus most allocation systems have provided some priority for kidney transplant patients with increased panel reactive antibodies (PRA). However, even with extra points in an allocation system, high PRA patients still may not have "equal" access to transplant and their graft survival is likely not to equal those not so sensitized [11].

Age can be a factor in equity as well as utility when one considers the concept that everyone should have an opportunity to live a complete life, to marry, to have children and make a contribution to society. This has been termed the "fair innings argument"; that all other factors being equal, younger candidates should be prioritized since they have not had the same number of life opportunities as older candidates [12]. Under this theory, a 20-year-old transplant candidate would be given priority over a 70-year-old transplant candidate. Various proposals have been made to provide some extra access to transplant using a point score that is high for the very young and that diminishes fairly rapidly, but continues to provide some small relative priority to a younger candidate as compared to older candidates at all ages [3].

Autonomy

The principle of autonomy is simply the concept that individuals should be able to exercise self-determination [3]. Although autonomy is the dominant ethical principle in many medical treatment decisions, it plays a less direct role in organ allocation than do the principles of utility and equity. The autonomy principle is most often considered in organ allocation as supporting the potential recipient's right to refuse transplantation or refuse a particular organ being offered for transplant, for example based on medical factors of the donor. This application of autonomy is an essential component of the recipient's informed consent for the transplantation procedure. Note, however, that this only influences allocation indirectly. The initial allocation of a particular organ would be made in accordance with policy and only if the prioritized recipient

declines by exercise of autonomy would the organ then be offered to the next potential recipient in conformity with the allocation scheme.

Another autonomy factor to be considered in organ allocation that is infrequent but has a more direct impact is the ability of a donor to make a directed donation to a specific recipient. In the US, both the state laws governing consent for organ donation (the Uniform Anatomical Gift Act) and the federal law that established the national system (the National Organ Transplant Act) expressly permit organ donors (or their authorized surrogates) to direct a donation to a particular recipient if the organ is compatible and can be transplanted into that individual [13]. If such a directed donation is made, then the organ goes to the intended recipient regardless of the allocation priority such individual would have received. Because a directed donation of organs from a deceased donor will only be facilitated if the intended recipient is in fact listed for transplantation and biologically compatible, it is an infrequent event. Nonetheless, it highlights the importance that the ethical principle of autonomy is accorded given that a direct donation bypasses the allocation scheme entirely.

Legal and regulatory factors influencing organ allocation policy

It is generally agreed that organs are a societal resource, meant to be used judiciously for the "common good." In most nations there are laws and regulations that provide guidance in the development of organ allocation policies.

United States

In 1984 the US adopted P.L.98-507, the National Organ Transplant Act (NOTA), which established the basis for the US Organ Procurement and Transplantation Network (OPTN). NOTA included the creation of a task force to provide recommendations for the development and operation of the OPTN. Included in the charge of the Task Force on Organ Transplantation was a mandate to make:

> "recommendations for assuring equitable access by patients to organ transplantation and for assuring the equitable allocation of donated organs among transplant centers and among patients medically qualified for an organ transplant."

The Task Force report [14] made numerous recommendations concerning the principles that should be applied in developing policy for the allocation of organs. These included:

- . . . donated organs [should] be considered a national resource to be used for the public good; the public must participate in the decisions of how this resource can be used to best serve the public interest [14].
- . . . selection of patients both for waiting lists and for allocation of organs [must] be based on medical criteria . . . [to] be developed by a broadly representative group [and] take into account both need and probability of success. Selection of patients otherwise equally medically qualified should be based on length of time on the waiting list [14].
- . . . the preferential assignment of an organ to a recipient based on objective medical criteria such as HLA matching, a compatible presensitized recipient, or length of time on the waiting list, is well recognized and widely accepted. Other criteria such as urgency of need, age, lifestyle, the presence of social support, or the need for retransplantation must be individualized and carefully applied so that medical judgments are reflected rather than judgments of social worth [14].
- . . . organ-sharing programs . . . [must] be implemented in the interests of the effective and efficient use of organs and justice, and the effect of mandated organ sharing [must] be constantly assessed to identify and rectify imbalances that might reduce access of any group [14].

Although organ allocation policies were developed by the OPTN once it began operating in 1988, the official federal regulatory framework for US policy on organ allocation was not established until 2000 with the adoption of "The Final Rule" [15]. Key sections of the Final Rule call for the establishment of "policies for the equitable allocation of cadaveric organs among potential recipients" to:

- be based on sound medical judgment
- seek to achieve the best use of donated organs
- be designed to avoid wasting organs, to avoid futile transplants, to promote patient access to transplantation , and to promote the efficient management of organ placement
- not be based on the candidate's place of residence or place of listing, except [to] the extent required by [the preceding]
- Distribute organs over as broad a geographic area as feasible [given above] . . . and in order of decreasing medical urgency.

Oversight of the US system is carried out by the OPTN. NOTA requires the US government to contract with a private, not-for-profit organization to carry out the day-to-day management of the OPTN. This includes maintaining a list of all patients waiting for a transplant at all US transplant centers. Since its establishment, the OPTN contract has been held by the United Network for Organ Sharing (UNOS). In addition to prescribing the basis upon which allocation policies are to be developed, the OPTN Final Rule also specifies that the OPTN will seek input from the transplant centers, organ procurement organizations (OPO), histocompatibility laboratories and the public.

Allocation policies are complex and have been subject to change in response to the regulatory requirements and general ethical principles described above, as well as increased understanding of the impact of allocation on various patient groups. Kidney transplant candidates generally are rank-ordered by waiting time, with special consideration for pediatric patients, highly sensitized patients, patients with high degrees of HLA match with the donor and previous living donors. Extra-renal organ transplant candidates are prioritized by acuity of illness, likelihood of dying without a transplant and/or likelihood of surviving with a transplant for at least one year. Allocation policies are designed to attempt a balance between equity and utility and have been slowly evolving to broader areas of sharing.

Although limited by the Final Rule, in practice geography does influence organ allocation. This is a function of historical allocation patterns, the practical limitations on organ transport and preservation given the area of the US and the nature of the US political structure with 50 state governments and a federal government. The US system is three-tiered. There are 58 local OPO service areas, 11 regions and a national list. In general, organs are first offered to medically suitable patients at transplant centers within the service area of the OPO coordinating the recovery. If no suitable recipient is available within the OPO service area, then the available organ is offered to recipients in the OPO's region and then nationally. Thoracic organs not used locally are offered to expanding concentric circles of 500 miles from the hospital where the organ was recovered. Some exceptions to these rules are made to provide improved access to the most acutely ill patients.

Eurotransplant

Eurotransplant (ET) is responsible for the allocation of donor organs in Austria, Belgium, Croatia, Germany, Hungary, Luxembourg, the Netherlands and Slovenia. Its position statement [16] on allocation includes:

- Eurotransplant shall ensure that the allocation of organs under its responsibility be guided by clinical and ethical criteria, and allocation rules that are defined by appropriately constituted committees, based on principles of fairness, equity and transparency, and in accordance with domestic regulations of the member countries.
- Eurotransplant will accept patients on its waiting list for transplantation according to accepted and transparent medical criteria (indication, need, urgency). Allocation of available organs will be based primarily on clinical criteria (need, urgency, and match, other aspects related to both the donor organ and the recipient) and accrued waiting time, taking into account ethical principles, and without regard for personal, social or financial background.
- As Eurotransplant operates on the basis of international cross-border collaboration and exchange of organs, its procedures for allocation across participating countries must take into account the principle of solidarity within each country (to be implemented through a mutually agreed balancing system).

In practice, allocation is based upon two general principles:

1 Expected outcome.
2 Urgency (as determined by experts in an objective and transparent way).

It also takes into account:

1 A reasonable balance among the participating nations and
2 Waiting time of the transplant candidate.

Allocation priorities are developed by panels of professional experts.

Spain

National coordination of the transplantation system in Spain [17] is carried out by the Organización Nacional de Trasplantes (ONT), an arm of the Spanish Ministry of Health and Consumer Affairs. Similar to the US, a three-tiered system of allocation exists. The primary unit of allocation is at the hospital/transplant center level, then to other transplant centers within the region of recovery (there are six) and then nationally. If no recipient is identified, the organ is then offered to other transplant centers within Europe. Unlike the US, each transplant center develops its own medical criteria for prioritizing organ allocation among its waiting patients.

Great Britain

The National Health Service branch for Blood and Transplant (NHSBT) is responsible for matching and allocating donated organs [18]. Rules for allocating organs are determined by the medical profession in consultation with other health professionals, the Department of Health and specialist advisory groups:

"All kidneys from deceased heart beating donors are allocated according to a national system. This is based on five tiers:

1 complete matches for children — difficult to match patients
2 complete matches for children — others
3 complete matches for adults — difficult to match patients
4 complete matches for adults — others and well-matched children
5 All other eligible patients (adults and children).

Within Tiers A and B, children are prioritized according to their waiting time. In the remaining Tiers, patients are prioritised according to a point score, whereby organs are allocated to the patients with the highest number of points. The score for an individual patient is based on a number of factors:

1 Time on the waiting list (favouring patients who have waited longest).
2 Tissue match and age combined (favouring well-matched transplants for younger patients).
3 The age difference between donor and patient (favouring closer age matches).
4 Location of patient relative to the donor (favouring patients who are closer in order to minimise the transportation time of the kidney).
5 Three other factors relating to blood group match and rareness of the patient's tissue type".

Extra-renal organs are allocated by a combination of patient acuity, location of the recovery, waiting time, and a balancing of transplants among the transplant centers.

Access to the waiting list

There exists another key factor that significantly influences how scarce organs will be allocated: access to transplantation. All potential recipients must be carefully evaluated by a transplant center team in order to assess the medical necessity for, and appropriateness of, a transplant. In general, the patient cannot independently make the assessment that the transplant should be considered. Thus patients must rely on their primary care providers to make a referral to a transplant program. Without such a referral and positive assessment, there is no ability to access the deceased donor waiting list. In addition to the medical evaluation of the prospective transplant candidate, transplant centers also assess other factors such as the likelihood that the patient will comply with post-transplant care including taking the necessary immunosuppressive drugs and avoiding other behaviors detrimental to the patient and the transplanted organ. Several of these factors are discussed here.

Organ failure caused by "self-destructive" behaviors

It is well documented that certain behaviors are associated with the likelihood of acquiring certain diseases. For example: smoking is associated with cancer and heart disease; obesity with diabetes; sedentary life style with heart disease; alcohol and drug abuse with liver disease. Health professionals certainly attempt to modify their patients' self-destructive behaviors to minimize the risk of disease. There is rarely a question of whether or not all appropriate treatments should be offered even if the patient's behaviors lead to a diseased state. However, when there is an apparent cause/effect relationship between self-destructive behavior and the need for a transplant, questions arise about the appropriateness of listing these patients.

Because of the organ shortage clinicians and ethicists have debated how best to handle patients in this circumstance. Inevitably part of the debate relates to the "worthiness" of the patient to have access to transplantation as a scarce resource. Liver transplantation for alcoholics is a frequently cited example. Part of the discussion involves whether or not the patient is responsible for their alcoholism, or if it is a disease much like any other disease. In general, the transplant community has focused not on whether the patient is at fault, but rather on determining the likelihood that the transplant will be successful.

Clinicians must take into account the patient's current behavior and the likelihood that the patient will change their lifestyle post-transplant to avoid behaviors that will reduce graft and patient survival. As a result, most transplant programs require the patient to demonstrate their compliance through such things as smoking cessation, weight loss, etc. For patients with a history of alcohol or drug abuse, most transplant centers require a minimum of a six-month abstinence as an indication of the patient's ability to be compliant with post-transplant procedures. Recently, some have suggested that the six-month "rule" should be relaxed in certain circumstances [19]. However, others have noted that "because of the scarcity of organs, we cannot afford to choose candidates in an arbitrary way that cannot be defended in the public eye." [20]

Retransplantation

In the US in 2009, 14% of the kidney transplants, 7.6% of the liver transplants and 4.0% of the heart transplants were into a patient who had received a previous transplant of the same organ [21]. Access to more than one transplant and the priority of access to retransplant has been a source of some contention since the earliest discussions of organ allocation. The 1984 US Task Force on Organ Transplantation noted the debate between those who would limit a patient to a single transplant and those who believed that such a policy would constitute "abandonment" of a patient [14]. The Task Force concluded that medical judgments, not simply the fact of a previous transplant should be the determining factors. Subsequent debates and policy decisions have continued this line of thought.

From a utilitarian perspective, the organ being transplanted saves a life and it is not relevant which life it saves. Except in cases of a recipient deliberately causing the failure of a transplant (e.g. failing to take immunosuppressive drugs) the graft failure is not the patient's "fault." Grafts fail primarily for immunologic reasons or occasionally a surgical technical error, events beyond the patient's control. In certain circumstances, repeat transplant candidates are actually given priority rather than being downgraded. For example, a liver transplant recipient suffering primary graft failure within seven days of transplant is among the highest priority candidates [22].

Others have suggested that the ability to retransplant a patient actually increases organ availability for all. If transplant centers knew that — should it become necessary — they were unable to obtain a second organ for their patient, they would be less likely to use organs from "marginal" or "expanded" donors.

Geography and multiple listing

The location of a patient's residence influences access to being listed at a transplant center and the likelihood that he/she will receive an organ once listed. Factors include:

- the presence of a transplant center close to his place of residence
- the practices of local physicians in referring patients for transplant evaluation
- the medical and social screening criteria used by a transplant center in deciding whether or not to list a patient
- the number of organs recovered by the local organ procurement organization, which is a function of the underlying potential for donation in the region and the efficiency of the OPO [23]
- allocation policy.

In some nations — typically those with a single national payer system (e.g. Spain) — patients are assigned to a particular transplant center, except under special circumstances. However, in the US patients are not only free to list at any center but also are permitted to list simultaneously at multiple centers. Waiting times by organ are publicly available and vary considerably. Patients who are better educated, more resourceful and have better insurance coverage and/or greater financial resources sometimes choose to list at multiple centers, seeking one with a shorter waiting time. In fact, transplant centers in areas with longer waiting times sometimes encourage patients to multiple list if they believe they will not be able to receive an organ in time to meet their needs.

Such practices inevitably raise questions of equity, especially when high profile individuals are transplanted relatively quickly at a transplant center far from home [24]. The ability to multiple list has been challenged as unfair and the US OPTN has proposed banning it. However, significant public opposition to eliminating the practice has maintained the status quo [25].

International patients travelling for transplantation

Travel of patients across borders for the purpose of transplantation may play a role in wait-listing practices and potentially in allocation schemes. The travel of patients for transplant should be considered in the context of the widespread international efforts by transplant professionals, national legislative authorities, and the World Health Organization to counter transplant tourism [26]. Central to this effort has been the widespread endorsement of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [27]. The concept of "transplant tourism" is defined in the Declaration as follows:

> "Travel for transplantation is the movement of organs, donors, recipients or transplant professionals across jurisdictional borders for transplantation purposes. Travel for transplantation becomes *transplant tourism* if it involves organ trafficking and/or transplant commercialism or *if the resources (organs, professionals and transplant centers) devoted to providing transplants to patients from outside a country undermine the country's ability to provide transplant services for its own population.*" (Emphasis added)

The distinction of "transplant tourism" that is of relevance is whether additional non-citizen transplant candidates undermine the ability of the host country to serve the transplantation needs of its own population. National self-sufficiency is a core principle of the Declaration of Istanbul. Specifically, "jurisdictions, countries and regions should strive to achieve self-sufficiency in organ donation and transplantation by providing a sufficient number of organs for residents in need from within the country or through regional co-operation." The call for self-sufficiency includes the concept that "treatment of patients from outside the country or jurisdiction is only acceptable if it does not undermine a country's ability to provide transplant services for its own population" [28].

The issue of transplantation of foreign nationals traveling to the US for transplant was controversial even in 1986 when the shortage of deceased organs in the US was of a different and lesser order of magnitude than it is today. After vigorous debate the Task Force recommended that "non-immigrant aliens should not comprise more than 10% of the total number of kidney transplant recipients at each center" [14]. A substantial minority of the Task Force members entered a statement of exception to the effect that a kidney should not be offered to a non-resident alien unless another suitable US citizen recipient could not be found [14]. The issue of transplantation of "life-saving" organs such as hearts and livers into non-resident aliens was also addressed by the Task Force recommending

that "extra renal organs should not be offered for transplantation to a non-immigrant alien unless it has been determined that no other suitable recipient can be found" [14].

After UNOS was awarded the OPTN contract, it adopted policies taking into consideration the Task Force recommendations and delegated to an Ad Hoc International Committee the task of reviewing the activities of transplant programs whose proportion of non-resident alien recipients for *any* organ exceeded 10%. In 1994, the threshold percentage was changed to 5%. Given that NOTA requires that only medical criteria be used in organ allocation, the "5% rule" did not prohibit a transplant center from waitlisting or transplanting more than 5% non-resident aliens. Rather, the "5% rule" functioned as a threshold that triggered an audit. In June, 2012 the OPTN/UNOS Board voted to eliminate the "5% rule" in favor of a new policy that requires the collection and public reporting of information on all transplant candidates and recipients who are non-residents and non-citizens of the US [29]. The goal of this new policy is to provide the US public, who are the source of all available deceased donor organs in the US, with a measure of transparency regarding how deceased organs are allocated to foreigners.

Despite the clear legal restriction in the US on utilizing citizenship or residency status as an allocation factor, the transplantation of international patients has caused public outcry on a number of occasions, calling into question the fairness of allowing such patients access to the deceased donor list when so many in the country die waiting [30]. Transplant professionals have also noted that patients who travel to the US for transplant may have shorter waiting times although the severity of sickness when such patients arrive may account for the difference [31]. Ultimately, the issue of international patients travelling for transplant may not have a direct role in allocation but rather exemplify why transparency regarding allocation policy and practice is an essential component to an effective organ donation and transplantation system that preserves the public trust.

Relationship between organ allocation policy and the decision to donate

Does local allocation priority encourage donation?

The 1984 Task Force Report described donated organs as a "national resource." The "Final Rule" implemented in 2000 in the US requires that allocation "not be based on the candidate's place of residence or listing, except to the extent required . . .":

- to support sound medical judgment
- to achieve the best use of donated organs
- to avoid the wastage of organs or futile transplants
- to promote patient access to transplantation
- to promote the efficient management of organ placement [32].

However, the current allocation systems still provide a high degree of preference to recipients at transplants centers affiliated with the local organ procurement organization, much as was the case in the initial development of transplantation. Similar arrangements exist in some European nations even though the geographic area is substantially smaller. There are notable exceptions such as broader sharing for the most acutely ill patients and for highly sensitized kidney patients.

The initial justification for local allocation priority was based on several themes:

- keeping organs local reduced cold ischemic time following recovery and improved transplant outcomes
- transplant professionals would be less willing to recover organs if they were less likely to actually have the opportunity to transplant the organs they recovered
- organ donation would be reduced because people would be less willing to donate if organs did not get used in their own community or nearby
- broader sharing would reduce the volume of transplants at smaller centers, ultimately making them non-viable and thus reducing patient access to transplantation

Proposals to dramatically increase the range of sharing have on occasion led to fierce policy debates. One such proposal concerning liver allocation in 1998 following issuance of the Final Rule resulted in US congressional and state legislative action to preserve local priority. The Institute of Medicine (IOM) was commissioned to study the impact. Its report [33]:

- recommended larger local sharing areas
- found insufficient data to draw a conclusion on the impact on transplant outcomes
- found no data to suggest reduced donation rates
- found indications that costs would increase but not so significantly as to outweigh the benefits
- found no conclusive evidence that smaller centers would be closed or, if they were, that there would be a significant negative impact on patient access.

A 1998 Gallup Poll on Organ Donation included this question — Question 4:

> Thinking as if you were going to be an organ donor, if you learned that your organs would go to sick persons within your local region before they were offered to sicker persons elsewhere in the US would you be more likely to want to donate, less likely to want to donate, or would it not matter in your decision?

In response, the report states:

> . . . most adults say it would not affect their decision. However, 32% say if they knew the organ recipient was the sickest person, regardless of location, they would be more likely to donate an organ. In contrast, 10% would be more likely to donate if they knew their organ was going to a sick person in their local region. It may also be noted that those who have signed an organ donor card, are recipients or candidates for an organ, or have donated an organ or bone marrow are most inclined to say the location of a potential organ recipient would not affect their decision to donate [33].

Accordingly, it does not appear that there is empirical support for the concept that local allocation increases donation rates.

Should registered donors receive allocation priority?

Although social worth generally is rejected as a basis for organ allocation priority, there is one notable exception. In the US, a patient who previously has been the living donor of a vital organ or segment of a vital organ is given a higher priority should they themselves subsequently become a candidate for a transplant [34]. Some have suggested that this relative allocation priority concept should be extended to give priority access to organs to those who have previously registered to be deceased organ donors.

In 2006, when the IOM [35] was asked by the US Department of Health and Human Services/Health Resources and Service Administration to examine possible ways of increasing organ donation, one idea considered in depth was priority access to donors. The primary arguments in favor are:

- It would be consistent with the principle of justice (equity). As there is a shortage of organs, it is necessary to ration organs in some way. There is a simple ethical parity in giving some preference to those who have shown a willingness to give as well as receive.
- Presumably it would increase the number of organs available for transplant by increasing the number of donors. By promoting a sense of community and shared responsibility, such a policy could theoretically increase the available supply of organs sufficiently to come closer to meeting the demand.
- Unlike other possible incentive plans aimed at increasing donation (e.g. paying for funerals, a tax credit, etc.) there is a direct relationship between the agreement to be a donor and the "reward." Transplantation is a community endeavor that cannot be accomplished without organ donation.

The primary arguments against such a system are:

- A massive public education program would be required to assure that all were aware of the option to register as a donor and the advantages that would accrue in return. As with all such public education programs there is a fear that those already disadvantaged in access to medical care (the very poor, the uneducated, those whose primary language is different) would be less likely to learn of, and take advantage of, the opportunity to register as a donor.
- Those most likely to potentially need an organ may disproportionately sign up as donors. If this phenomenon of "adverse selection" were to occur, the number of organs available for transplant might not actually increase very much as such individuals are less likely to have transplantable organs to donate. As the committee noted, "any significant degree of adverse selection erodes one of the strong moral arguments for reciprocity-driven approaches: the emphasis on mutuality of interest and the effort to prevent 'free-riding'" [35].
- A reciprocity system raises "slippery-slope" considerations that it would be the start of a system in which moral worth assumed a role in determining access to transplant.
- Although all major religions support organ donation, some individuals have a strong personal, religious belief that donation is inappropriate. Thus a reciprocity system might not withstand legal prohibitions against discrimination on religious and other grounds.

In the end, the IOM Committee recommended against creating a preferential access system, although noting that their recommendation might need to be revisited if the practical problems could be overcome and if better data on the potential for success becomes available [35].

The majority of the arguments against prioritizing donors are operational. Although some are quite significant, others might be less difficult to overcome. For example, one of the practical requirements cited by the Committee has been met now with the development of a system of state-by-state first person consent donor registries. Also, data on the effectiveness of an allocation priority system should become available in the coming years with the adoption of such a system in Israel in 2010. Although still based primarily on medical criteria, the Israeli system gives preference to a registered donor if the medical need is the same. Accompanied by a significant public education campaign, the law already has stimulated a dramatic increase in the number of registered donors in a nation that traditionally has had a very low donation rate [36].

The future/summary

It is likely that the debate over allocation will continue until there are clinical breakthroughs that either:

- dramatically increase the supply of organs from deceased donors; or
- dramatically reduce the demand for organ replacement; and/or
- Provide alternative therapies such as growth of artificial replacement organs.

Some believe that "it is not possible to simultaneously maximize utility, efficiency, equity and predictability, and it is unlikely that any of these can be maximized without the potential cost to autonomy, controversy, trust or live donation" [8]. However, each national or multinational organ allocation system continues to struggle to balance these factors in the context of its political system, its national values and its history.

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CHAPTER 138

The Ethics of Living Donation

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Introduction

The widening gap between the number of individuals in need of organ transplantation and the number of available organs derived from deceased donors has encouraged the growth of living organ donation. The most common living donor organ is the kidney, but liver and lung donors are also increasing in number. Cultural differences and public policies strongly influence donation rates in various countries. In the US, the scarcity of available deceased donor organs, increasing recipient wait times, widespread acceptance of living unrelated donors, and the influence of media and the internet have increased the complexity of ethical considerations. Many policies and practices in living donor transplantation are heavily debated and are still evolving. Here we will review some ethical considerations in living donation. While many specific issues relevant to donation in the US are discussed, the guiding ethical principles are generally applicable. Additional treatment of these principles can also be found in Chapter 136.

Procedural risks and benefits

In the US, healthy living donors accept the risks and discomforts of surgery and loss of an organ to help another individual. It is considered ethically acceptable to allow living kidney donation if the anticipated benefit outweighs the harm. In this case, the living kidney donor may derive psychological and moral benefits that outweigh the short and long-term risks of organ donation. From the inception of living donation, there have been concerns and questions. As Dr. Frances Moore [1], an early pioneer of living donor kidney transplantation, once said:

> [Living donor] tissue transplantation is a unique field of surgery. It flaunts the ancient principles upon which medical and surgical care are based: do no harm and help the patient to help himself. The welfare of a healthy person, heretofore never sacrificed in human medicine, is now jeopardized when tissue is obtained from the healthy donor.

Because of these concerns, and the perception that the risks associated with donation would be more tolerable if the donor and recipient were bonded by family relationships, early living donors were genetically related. Unrelated donors were initially discouraged due to concerns regarding outcomes and fear of commercialization. As outcomes of kidney transplantation improved and criteria for accepting transplant candidates were expanded, the number of patients placed on the waiting list grew and the duration of waiting time lengthened. Numerous studies demonstrated similar recipient outcomes in living related and unrelated kidney transplantation [2]. As a result, living kidney donation and specifically living unrelated kidney donation has grown considerably. In 2009, 38% of kidneys transplanted in the US came from living donors, 23% of whom were unrelated donors [3]. Donor surveys have demonstrated that almost all donors would make the same decision if asked to donate again, and some actually derive the benefit of enhanced self-esteem and satisfaction with the act [4–6].

In living organ donation, one of the guiding ethical principles is non-maleficence. The decision to donate must be voluntary, and donors must have full autonomy, with the freedom to withdraw from the donation process at any time without consequence and with a supportive environment. All reasons for not proceeding with donation, medical or otherwise, must be kept confidential. Donor consent and autonomy are essential to proceed with donation but they are not sufficient. A donor's desire should never trump medical judgment and decision-making.

The major medical risks associated with living organ donation are directly related to the operative procedure. Surveys show a perioperative mortality risk of three deaths per 10 000 living kidney donors [7,8]. However, advances in kidney donor operative techniques, specifically the development of minimally invasive surgery as the preferred method of donor nephrectomy, have reduced hospital length of stay and permitted an earlier return to a normal lifestyle. Several studies have examined the long-term morbidity after kidney donation. Some have found an increased risk of hypertension and non-progressive mild proteinuria [9–11]. However, death rates (for the white donor) are similar to the general population [12]. End stage renal disease (ESRD) rates following kidney donation are slightly increased, ranging from 0.1–1.1% [12–16]. The higher risk is especially notable in the young black donor [17], although a recent analysis revealed that the increased ESRD rates were similar to the increased rates in the general population [18]. Pre-eclampsia may also be marginally greater [19]. Other problems associated with donation include higher premiums or rejection from health insurance companies and possible long-term financial consequences. However, overall, it can be concluded that living donor nephrectomy is, on the whole, safe, with few perioperative deaths, immediate complications, or long-term consequences.

A unique ethical challenge in living donor kidney transplantation was highlighted by the unexpected transmission of HIV and

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

hepatitis C in 2007 by a living donor [20]. Living donors with known behaviors that are associated with an increased risk of HIV or hepatitis C infection raise specific ethical concerns because donors often have a relationship with the intended recipient. In these situations, the donor's right to privacy may be in direct opposition to the recipient's right to full informed consent. In addition, disclosure of risk behaviors may cause psychosocial harm to both the donor and recipient. Consensus guidelines do not currently exist. Some have suggested disclosure to the recipient of these behavioral risk factors with donor consent or, alternatively, determining that the donor is medically unacceptable. Others question the ethics of such actions [21].

As the demographics of the general population have changed and waiting times have lengthened, acceptance criteria for living donors have expanded at many centers. Donor evaluation and selection criteria do vary [22]. Older age, hypertension, obesity, nephrolithiasis, and impaired glucose tolerance no longer preclude kidney donation at many programs. In 2008, 19.5% of living donors were obese, 2.0% had a history of hypertension, and 3.5% had proteinuria, all known risk factors for cardiovascular or renal disease [16]. A recent review of data provided by the Scientific Registry of Transplant Recipients illustrated the changing characteristics of living kidney donors, with steady increases in the number of older, obese, and hypertensive donors between 1999 and 2009 [23]. Other groups that need more study include donors with borderline acceptable glomerular filtration rates and donors who undergo weight loss to meet acceptance criteria. The long-term impact of these changing criteria on donor outcomes is largely unknown. Thus, current informed consent regarding living donation in these subgroups requires discussion about the current limits of our knowledge and understanding regarding long-term outcomes. Better understanding of the impact of these changes will require ongoing comprehensive long-term follow-up of living donors, especially of non-white donors, to determine the safety of these practices and to better inform living donor candidates.

Donor follow-up

Of note, living donors are not required by all centers to have medical insurance, although this relates particularly to countries such as the US that do not provide universal health coverage. In one survey, 15% of US transplant centers reported that they would exclude uninsured donors, and 42% considered lack of insurance a relative contraindication [24]. In 2008, 12.2% of donors were reported to be uninsured, with 14.9% of black and 17.0% of Hispanic donors lacking insurance [16]. Another survey from 2004 to 2006 found higher uninsured rates in younger donors, male donors, and ethnic minorities [11]. Studies have shown that these groups are at highest risk for long-term complications of kidney donation, particularly male black donors under the age of 35 [17], and lack of health insurance could certainly negatively affect their adherence to recommended long-term follow-up. It seems logical to expect that donors without health insurance face significant financial barriers to preventive screening and monitoring of kidney function and blood pressure, potentially further increasing the risk of many donors at highest risk for complications. Short of a change in government policy regarding universal health care, some have suggested "free" lifetime health insurance. This proposal, however, raises the controversial area of compensated donation which is forbidden under federal law.

As the number of non-directed donors, uninsured donors, and donors with medical conditions such as obesity, hypertension, and impaired glucose tolerance has grown, the importance of long-term donor medical follow-up has become well recognized. This led to a recent report from a Living Kidney Donor Follow-Up Conference, which attempted to review current living donor outcomes data, to define what was needed, to identify requirements to fulfill this need, and to propose practical solutions. Reasons for continued systematic collection and reporting of donor outcomes include: accurate outcomes information for appropriate informed consent, especially in regards to donor ethnicity and medical conditions; program-specific feedback for quality assurance and performance improvement; professional obligation to collect detailed donor outcomes; better information to improve the evaluation process and counseling for non-traditional donor candidates; and surveillance to identify donor problems when intervention may be possible [23]. In response to this, the US Organ Procurement Transplant Network/United Network for Organ Sharing (OPTN/UNOS) expanded the amount of living donor data collected and increased the duration of mandatory donor follow-up to two years post-donation. Review of OPTN reports demonstrates that much of the longer-term data is incomplete, and more than 30% of living donors in 2006 were declared, "lost to follow-up."

Further analysis revealed that many characteristics associated with limited access to healthcare and possible greater long-term health risk, such as non-white race, younger age, non-US citizenship, and lack of health insurance, were also associated with lack of follow-up data reporting. Because of this, the OPTN/UNOS living donor data task force concluded that; "As currently collected, the OPTN/UNOS data are incomplete beyond the point when the discharge form is submitted. . .and therefore useless for research or making conclusions about living donor safety." Thus, despite growing concerns about the impact of living donation on donor health, especially in the setting of expanded criteria, and the critical need to understand the medical outcomes after donation to appropriately evaluate living donor kidney candidates and improve the informed consent process, living donor follow-up in the US remains suboptimal. Currently, there are two ongoing NIH-sponsored studies, Assessing Long-Term Outcomes of Living Kidney Donation (ALTOLD) and Renal and Lung Living Donors Evaluation Study (RELIVE), evaluating living donor outcomes. Both have several limitations, including few minority participants but will help to provide more detailed living donor outcomes analysis [23].

A suggested solution has included a proposal for a living donor registry [25], similar to the EULID project developed by the European Commission which created an e-registry database model on organ living donors in order to have a common European database to monitor living donors. The purpose of the EULID project is to establish a European consensus regarding legal, ethical, protection and registration practices in relation to organ living donors, to guarantee their health and safety [26]. Other long-term living donor follow-up efforts from Switzerland, Pakistan, and Brazil have emphasized that adverse renal outcomes are rare and often potentially preventable. The solution to long-term follow-up in the Swiss long-term living donor registry has been the involvement of the donor primary care physician [23]. A significant difference between the European Union nations and the US is the lack of a national health care system in the US. Thus, the biggest question and concern is in regards to the cost and responsibility for longer-term follow-up,

especially for the follow-up beyond two years. Without additional funding, the consensus is that transplant centers could not maintain longer donor follow-up. Some suggest a system of incentives and penalties to more fully motivate transplant centers to complete the current required OPTN/UNOS donor follow-up data and greater incentives and fewer disincentives to living donors to comply with follow-up visits [25]. The importance of donor follow-up visits should be stressed at the time of donor evaluation, prior to the donation procedure.

Altruism

In the US, only altruistic donations are permitted; monetary compensation for organs is prohibited by the National Organ Transplantation Act [27]. Most living kidney donors are biologically or "emotionally" related and include family, spouses, and friends. However, some living kidney donors have no prior relationship with the recipient. Altruism is the guiding motivation for most living donors. Ideally, donors should be autonomous and free of coercion. Altruism is comprised of several types. "No- or low-cost" altruism entails giving one's own resources to benefit others with little or no incurred risk, an example of which is blood transfusion. A second form of altruism is "dutiful" altruism, which involves a sense of duty. Donors may feel a moral if not legal obligation to donate. Familial donors may serve as examples and are generally widely accepted due to well-recognized relationships between donors and recipients. Traditional living related donors are often felt to derive a greater degree of psychological benefit for donating than unrelated donors, but they often face both subtle and overt coercion based on family dynamics. The third type of altruism is "supererogatory" altruism, which involves giving beyond what is expected or asked. Donors with no defined relationship to the recipient fall into this category [28]. This form of altruism is currently well accepted by the transplant community, with a recent survey demonstrating that 94% of transplant physicians surveyed supported non-directed kidney donation from living volunteers [29]. Motivations for supererogatory altruism include a pure sense of altruism, religious beliefs, a wish to reciprocate to society, and a wish to honor a family member or friend.

Religious beliefs are a common component underlying motivations of an individual to donate. It is not uncommon for church members to volunteer to donate to a fellow church member. In these cases, there is generally a pre-existing acquaintanceship with the potential recipient, and most centers accept such donor candidates readily. Other situations, some well publicized, involve religious sects that strongly encourage living organ donation. One such example was a religious community of Jesus Christians whose leader was motivated to donate a kidney based on his interpretation of New Testament scripture calling for "living sacrifices." He then encouraged other members of his religious community to do likewise. Ultimately, these donors were declined due to concerns regarding financial, spiritual, and social dependence of members on the community, which heightened the probability of coercion [28]. Religious beliefs may be a legitimate motivation for donation, but such donors must be carefully evaluated.

Non-directed donations

The appropriate evaluation and selection of non-directed donors requires the support of a multidisciplinary team that is well versed in the implications of living donation. It is incumbent on each transplant program to screen all donors responsibly to assure that the donation procedure is free of financial remuneration, valued consideration and coercive and exploitative elements. Donors must have autonomy to provide full, voluntary, informed consent. All living donor evaluations include a meeting with a living donor advocate. The role of the living donor advocate is to try to ensure that the donor has a full understanding of the donation process and that no coercion exists. It is recommended by some that that donor advocates have "veto power," so that he/she can exclude a potential organ donor if the advocate determines that the donation is not in the donor's best interest. Reasons for donor exclusion include: an unrealistic expectation or demand by the donor that the transplant be free from rejection and failure; the misperception by the donor that if the transplant is not successful, it is due to personal failure of the donor; monetary compensation for donation as prohibited by federal law; a desire for media attention; a response or remedy for a psychological malady, such as depression, or other underlying mental illness; a desired selection of the recipient by gender, race, or ethnicity; and a desired involvement in the recipient's life after donation, possibly unwanted by the recipient [30]. Medical evaluation of non-directed donors is routinely more stringent since the psychological benefit of the donation is perceived to be less, although a recent report demonstrated encouraging psychological outcomes of altruistic living donors who donated to strangers [6].

Recipients of non-directed living organ donation are identified in a number of ways, through unsolicited inquiries to transplant centers, through the Internet, and through third party organizations. The relationship with the recipient is often tenuous or nonexistent. In the past, non-directed donors that were paired with specific recipients through a process that took place outside the transplant center were frequently rejected. Under these circumstances a major underlying concern has been the possible commercialization of organ donation, and examples of this have been well publicized in the media. It has become the responsibility of transplant centers to try to ferret out any coercive relationship, financial or otherwise, between living unrelated donors and their recipient, but the appropriate method of evaluation remains poorly defined. Most programs require a detailed psychosocial evaluation to rule out any underlying psychiatric disorders, to assess the person's competence to make an informed decision, and to explore the reasons for the offer. Psychosocial evaluations are routinely required in all living donor evaluations but are particularly critical in non-directed donor evaluations. In situations where the relationship between the donor and recipient appears ambiguous, centers often require a more in-depth psychological evaluation. Monetary compensation can be particularly difficult to identify as both the donor and recipient are generally coached to deceive the transplant center. Examples of a black market in human organs have come to light in the press and have prompted some members of the transplant community to advocate for federally regulated paid organ donation. Some have suggested that altruistic-directed living unrelated organ donation is a legal fiction concealing commerce or other forms of coercion, at least occasionally [31]. Unfortunately, tests confirming altruism and tests excluding commerce are weak. More rigorous tests would likely diminish the number of acceptable living donor candidates, and many transplant centers continue to debate how best to evaluate the motivations of such donors.

In selection of a recipient for a non-directed donor, most transplant programs have followed the standard algorithm that applies to the allocation of deceased donor kidneys as the most ethical approach [30]. However, some have diverged from standard practice to maximize the chances for success, such as eliminating retransplants and patients with a history of non-compliance. Thus, both equity and utility are often considered in determining the appropriate recipient. In general, centers have not allowed non-directed donors to place restrictions on the characteristics of the recipient to avoid moral complicity in racism or sexism. Some non-directed donors come with recipients identified through third party organizations or internet websites. Commentators have expressed concern that this unregulated situation unfairly favors potential recipients who have greater media savvy and resources. Nonetheless, many such donors are currently evaluated and accepted.

Paired donation

Several strategies have been developed to increase the living donor kidney supply, including non-directed living donors, extended-criteria living donors, and paired kidney exchanges. Rapaport first proposed the idea of paired kidney exchange in 1986 [32]. His proposal involved two donor-recipient pairs in which the two donors were immunologically incompatible with their paired recipient but were immunologically compatible with the other donor's paired recipient. Rapaport suggested that the two donor nephrectomies could be performed simultaneously with an immediate exchange of the two kidneys to the compatible recipient. Kidney exchange procedures have been used to overcome both blood and tissue type incompatibility barriers and are steadily increasing in frequency. Non-directed living donation has allowed the development of a new kidney exchange strategy to increase living donor kidney transplants, namely closed and open kidney exchange chains. In particular, kidney exchange chains bring unique ethical considerations for both donors and recipients because they generally start with a non-directed donor [33]. Historically, non-directed donors have donated to the deceased donor waiting list. Thus, kidney exchange chains may disadvantage the deceased donor waiting list from their only opportunity for a living donor transplant. In closed kidney exchange chains, the last donor donates back to the deceased donor waiting list, but in open kidney exchange chains (or "never-ending" chains), the donor is permanently diverted from the waiting list. Because paired exchanges and chains provide benefits to recipients who provide a living kidney donor in the exchange, these strategies could be considered a violation of NOTA. To avoid this problem, the Charlie W. Norwood Living Organ Donation Act was passed in 2007 [34]. Proponents of non-directed donor participation in kidney exchange chains argue that the increased number of kidney transplants provides adequate justification. They argue that this strategy allows transplantation across a traditional immunologic barrier and therefore ultimately lessens the number of patients needing placement on the waitlist. However, volume considerations and utility have to be balanced with justice and equity to the deceased donor waiting list, particularly for O blood type recipients. One suggestion is to require a minimum number of transplants when a non-directed donor is involved in a chain [33]. Certainly, as kidney exchange programs develop, data must be prospectively collected to better understand and evaluate the implications of these strategies on the waiting list.

Prior to a kidney exchange procedure, the final kidney donor from the kidney exchange procedure agrees not to donate his/her kidney at the same time as their loved one receives a kidney transplant (i.e. within the same kidney exchange procedure), but rather at a later date, whereby he/she donates a kidney to initiate a second kidney exchange procedure. As such, the bridge donor therefore "bridges" two distinct kidney exchange procedures occurring on different dates within a kidney exchange chain. One of the basic problems in kidney exchange chains is "backing out" by bridge donors, which terminates both open and closed chains [33]. "Honor systems" have been developed as a means for minimizing backing out and involve educating the bridge donor about the effects of backing out on a subsequent recipient within the chain. Honor systems are ethically problematic because they are inherently coercive. Suggested solutions include prompt identification and conduct of kidney exchange procedures and institution of predefined limits (time limits or transplant number limits). The primary benefit for living donors is psychological. Even if the transplant fails, donors know that they did everything possible to help their loved ones. Some have suggested that in kidney paired exchange and kidney exchange chains, the donation is indirect, and the psychological benefit may be more diffuse. After donation, some donors experience depression or conflict with family members. Donors may feel angry or guilty if there is an adverse outcome. In the setting of kidney exchange programs, feelings may be exacerbated by the fact that they do not know the result of their own donation. Further study is needed to identify psychosocial concerns unique to participation in exchange programs.

Recommendations for identification of non-directed donors for initiation of a chain have included a requirement that the interest in donating to a chain originate from the donor and solicitation be avoided [33]. However, the multiple examples of mass media stories focused on kidney exchange creates ethical concerns, as some non-directed donors may be enticed by personal exposure in the mass media. This is particularly concerning as transplant centers may drive the media process as kidney exchange chains often attract considerable publicity for the transplant center.

Privacy and confidentiality must also be assured, both for the non-directed donors and bridge donors [33]. Donors need adequate information about the intended recipients to assure that their donated kidney will be used in a medically reasonable manner. Traditionally, pretransplant meetings are not allowed due to the possible negative effects of the meeting on the donor's decision. It has been recommended that non-directed donors and bridge donors should not be told of the number of individuals involved prior to their procedure as this may subject them to an increased degree of perceived coercion. Thus, the problems of overt and perceived coercion, issues of confidentiality, and patient rights need careful evaluation and oversight. In addition, differences in living donor acceptability criteria add increased ethical complexity to kidney paired exchange programs, where donors may be considered candidates in one center but rejected in another. Responsibility for donor evaluation and consent currently lies in the hands of the originating transplant center.

Liver donation

In living liver donation, the basic guiding ethical principles remain the same. Donors must have full autonomy; they must be fully

informed about the process, the surgery, and the short- and long-term implications of donation; they must have the right to withdraw from the process at any time; and donor safety and risk must be justifiable in the setting of chances of a successful outcome for the recipient [35]. The difference that colors the living liver donor evaluation process relates to the higher risk to the donor and the higher acuity of the recipient. Recipient outcomes following living liver donor transplantation have become equal to or, in some studies superior to deceased donor liver transplantation [36]. However, there remains significant recipient morbidity and mortality, particularly in patients transplanted for fulminant hepatic failure, or those with severe portal hypertension. Much of the challenge in assessing donor risk is the lack of an accepted definition of donor exclusion criteria and lack of long-term outcomes data. The first living liver donor was performed in 1989 [37]. After initial excitement and success, several publicized living liver donor deaths [38] led to a precipitous decline in rates after 2002. Since 2000, over 3000 living donor liver transplants have been performed in the US. Most live liver donations do not lead to permanent long-term consequences, though perioperative morbidity rates in the literature range from 9–67% [39]. Currently, the estimated risk of mortality and morbidity associated with live donor right hepatectomy is 0.4% and 35%, respectively [40]. Left and left lateral hepatectomies are associated with lower rates of complication [41]. Nevertheless, the true morbidity remains poorly understood. Formal reproducible grading systems have been developed to allow for more systematic documentation of living liver donor complications and will help better inform potential candidates [39,42,43]. This will be critical in providing a true informed consent to potential living liver donor candidates.

Significant disagreement exists about living donor liver transplantation in high urgency situations. The recipient result is suboptimal, and time constraints place additional pressure on the donor evaluation and consent. However, some argue that the very ill patients are precisely the ones who would most benefit from a living donor liver transplant [44,45]. A real medical dilemma in this situation is the inverse relationship between the duration of donor evaluation and neurological consequences for the recipient [46]. Whether living donor liver transplantation should be considered relates to the efficiency of transplantation through the deceased donor list. In general, it likely should only be considered in experienced centers. In some cases, living liver donor transplantation may be considered the only option. An example would be for patients who would not receive a deceased donor liver transplant due to advanced liver cancer [41]. Patients with early (stage II) liver cancer are afforded an elevated priority on the waiting list for the allocation of deceased donor organs under the assumption that their post-transplant survival is excellent and the risk of cancer recurrence is low. Individuals with more advanced cancers have a worse prognosis and do not receive such priority, even though the likelihood that they will do well after a liver transplant is better than what would be expected without a transplant. The mandate to achieve the most good for the most patients is the guiding principle that excludes patients with advanced cancer from the elevated priority recipient group. Arguably, the organ shortage and the requirement that the transplant community should be a wise steward of organs from deceased donors do not apply when a volunteer live donor has come forward. In such cases living liver donor transplantation may offer an effective oncological option that is ethically justifiable. A potential problem, however, is defining the lower limit for expected recipient survival in a patient with advanced liver cancer that justifies the risks that accrue to the living liver donor.

The vast majority of living liver donors in the US are related, genetically or emotionally, although a few non-directed liver donors have been reported [47]. A significant ethical debate has focused on the ability of parental liver donors to have free choice. The parent-infant relationship is considered by many to be so inherently coercive that there is no other option for parents than to accept the possibility to donate. Parental donors have emphasized their moral responsibility as parents and the impossibility of living with the guilt of refusing to donate. Thus, many have argued that it is irrelevant to discuss living parental liver donation as a choice [48–50]. In particular, some have recommended that parental living donation in the setting of pediatric fulminant hepatic failure should not be proposed as an option [51]. Nonetheless, parental living liver donation is performed and accepted, primarily due to the procedure's life-saving potential far outweighing many of these ethical concerns. As one mother was quoted when asked if she felt pressured to donate to her infant son, "This was a gift. I had the chance to give life to my child twice." Overall, living liver donation accounted for 12% of pediatric liver transplants performed in 2010 [3].

Lung donation

As of 2011, living donor lobar lung transplantation has been performed in approximately 400 patients worldwide. In the US, no donor deaths have been reported, and rates of perioperative complications vary from 7–49% [52]. An unanswered question remains the long-term outcomes and functional effects of lobar donation, though loss of lung function is an expected consequence. A unique aspect of living donor lobar lung transplantation is that two donors are placed at risk for each recipient surgery. This may be the only procedure that carries with it the potential for 300% mortality. Its use in the US has decreased dramatically in recent years due to a change in the OPTN urgency/benefit allocation systems for deceased donor lungs. Currently, most of the literature in living donor lung transplantation comes from Japan.

Commercialization and remuneration

The organ shortage in the US is reflected in varying degrees around the world, but differences in laws pertaining to organ donation have resulted in international problems with commercialization of organ donation. Internationally, the rates of living donation appear closely linked to deceased donor organ availability. In countries with presumed consent, living donor kidney transplantation rates are lower (2.4 vs. 5.9 per million population) [53]. Most countries have signed a World Health Organization statement condemning the practice of buying and selling organs. Various national and international transplant organizations and international consensus panels have rejected monetary compensation as a solution to the organ gap. Despite the illegality of organ markets throughout most of the world, there is a large international black market. In 2007, the World Health Organization estimated that organ trafficking accounts for 5–10% of kidney transplants performed annually throughout the world [54]. In the face of apparently stagnant donation rates and concerns regarding unregulated organ markets, there have been increased calls within the US, including professionals in the transplant community, for ethically controversial proposals to provide compensation for

organ donation [55]. Debate over incentivized donation has polarized many in the transplant community. The main argument in favor of a regulated system of payment to living kidney sellers is that financial incentives will increase donation, so fewer wait-listed transplant candidates will die on the wait-list. The driving force is the benefit to recipients, in terms of mortality, morbidity, success of the transplant, and quality of life. Proponents argue that regulated payment would aid the international campaign against global transplant commercialism by eliminating transplant tourism and thus protecting sellers in unregulated systems. Suggested payments include fixed sums, term life insurance, lifetime health insurance, reimbursement for travel expenses and time off work, or tax deductions. The proposals generally incorporate allocation through a predefined algorithm, oversight, payment by the government or insurance companies, and long-term follow-up. The last is particularly attractive because it would address the ethical dilemma of long-term medical care in uninsured donors. Most proponents favor a single price per organ that does not vary based on age, health, or lifestyle of the donor. Legalization would also eliminate the current ethical and legal problem of directed altruistic living donation. Some have even argued that the donation process is often a financial burden, and that forcing the donor to shoulder the cost is also unethical. They argue that denying legitimate reimbursement is exploitative of the donor's altruistic motivation. Thus, compensation would be for time and incurred expense, rather than for the organ [56].

Arguments against paid donation include the potential exploitation of the poor, the commodification of the body, the use of financial coercion, the lack of sufficient data to guarantee an increase in donation rates, and the potential long-term harm to the donor. Of greatest concern has been the possible exploitative nature of paid donation as it might create an undue inducement for those who are economically disadvantaged. In unregulated or poorly regulated systems, rich buyers could purchase kidneys with little regard for donor safety and follow-up, thus establishing a two-tiered system in which the wealthy benefit and the underprivileged are coerced by monetary incentives. International data has shown that individuals who sold their kidneys and received cash payments were not financially improved five years later. In fact, many described their health, social situation, and overall wellbeing as worse and 80% would not recommend donation to others [57]. One of the primary concerns has been the fine line between compensation and coercive inducements. Intended compensatory measures may result in irresistible financial pressures that encourage impulse donations and violate donor autonomy. A 2006 telephone survey of American households indicated that lower income groups were more accepting of incentives, suggesting a disproportionate effect of monetary payments on the poor [58]. Similar inequities have been demonstrated in surveys of ESRD patients, with more affluent patients willing and able to pay for a kidney than the poor [59]. Additional concerns include the possibility that inducements might tempt the donor to withhold or lie about their medical history, endangering their own and the recipient's health. Of note, physician opinion was an important criterion in deciding willingness to pay for a kidney. In a survey of transplant physicians, 80% were against or strongly against living donor organ markets for kidney transplantation [29].

Additional concerns include the potential for "crowding out" and "bystander effect" [60]. Crowding out is described as the unintended consequence of decreasing the number of donors by the introduction of extrinsic rewards, thus tarnishing the altruistic motivation of many donors and discouraging potential donors. This was corroborated in a study of Swedish blood donors [61]. Whether such a phenomenon would apply to organ donation is unclear. The bystander effect might result in a decrease in the sense of urgency of donors to volunteer in the setting of paid donation programs. It is felt to have resulted in the decrease in live donors to pediatric recipients when a change in policy prioritizing organ allocation to pediatric recipients occurred [60].

Ethical concerns regarding payment for medical risk extend beyond transplantation [29]. In medical research, monetary payments are not proscribed, but Federal Regulations lack specific guidelines regarding compensation. However, the Office of Human Research Protections (OHRP) approved Institutional Review Board Guidebook instructs IRBs to determine whether payments may constitute undue inducement and to avoid it if it could lead individuals to accept risks they would otherwise not take. However, Menikoff, previous head of OHRP, has argued that failure to compensate for degree of risk taken is inconsistent with recognizing an individual's contribution. But Carl Elliot, a bioethicist currently at the University of Minnesota, states that there is a difference between paying for risks when individuals choose to expose themselves to risks for others and paying for risks when such risks involve the participation of a third party. He argues that there is a difference between allowing a person to risk harm and encouraging it. Many argue that paid donation crosses that line between allowing and encouraging risk.

Currently, the focus of debate in paid donation has been kidney transplantation. However, as the safety and outcomes data for living donors for other organs, such as the liver, improves, the debate may expand to include other organs. Per survey reports, 67% of transplant physicians favor non-directed liver donation, significantly fewer than those in support of kidney donation; and 90% were against or strongly against living donor liver markets [29]. Unlike living kidney donation, in living liver donation, donor risk was the major reason cited for opposition.

Summary

Physicians practice under the basic principle of first to do no harm [62], but donor surgeries pose some risk of harm. It remains a paramount responsibility of the transplant community and transplant centers to ensure the safety of living donation and lack of coercion of living donors. However, the shortage of organs continues to grow, and strategies to increase donor availability have included strategies to expand living donor transplantation. Hypertension, obesity, age, kidney stones, and impaired glucose tolerance are no longer considered absolute contraindications. In addition, non-directed donors, paired exchanges, and donor chain strategies are growing in number. Close follow-up of these subgroups is needed to ensure donor safety and provide better education and informed consent. Currently, only altruistic donors are considered in the US, but proposals to incentivize living donation are receiving increasing attention. These proposals encroach on many basic moral and ethical principles and will continue to challenge our current understanding of living donation for many years to come.

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CHAPTER 139

Cultural Variations in Organ Transplantation

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Introduction

Organ transplantation is a life-saving and life-enhancing treatment for patients with end-stage organ failure. In a fair society, it is self-evident that patients who may benefit from organ transplantation should, so far as is possible, have equitable access to transplantation. Variations in access to transplantation should be identified, the underlying reasons explored, and attempts made to minimize differences in access. Unfortunately, when viewed at a global level, it is apparent that very large inequities exist in organ donation and transplantation rates between countries (Figures 139.1 and 139.2). There are also large variations in access to organ transplantation within many countries, even in countries with well-developed transplant programmes [1].

Organ transplantation is one of the more advanced modes of medical intervention and successful national transplant programmes are undertaken in the context of complex legal, ethical, financial and organizational frameworks. They are also based on a widespread understanding of the benefits and ethical acceptability of donation and transplantation in the societies in which they are embedded. Understanding variations in these societal frameworks enables a better understanding of differences in access to transplantation between and within countries.

The success of organ transplantation has led to a global shortage of donor organs, and this increasing disparity between the demand and supply of organs is in part responsible for inequity of access and has also given rise to the concept of transplant commercialism. Wealthy patients with organ failure, when faced with growing waiting lists and perceived inequities in access to organs, may feel that transplant commercialism offers the best chance of receiving a graft. Transplant commercialization serves as a reminder to the international transplant community that reducing waiting lists and improving access to organs are essential.

This chapter aims to examine selected 'cultural' variations that impact on access to transplantation, and highlights selected strategies that have been developed with the aim of minimizing these variations. Transplant commercialism and the related issues of organ trafficking and transplant tourism are explored, with a global overview of the scope of these issues and current attempts to eradicate them. Finally, the arguments for and against the introduction of financial incentives for organ donation are discussed. Throughout the chapter, renal transplantation is examined most closely, as it is by far the most common type of solid organ transplant worldwide, and one in which both living and deceased donors can be utilized. Renal transplantation is also the type of transplant most closely associated with transplant commercialism.

Cultural variations

Access to organ transplantation should be fair, and not influenced by race, ethnicity, religion, or socioeconomic status, which collectively can be termed cultural factors. The definition of culture is broad, but at a societal level can best be defined as the distinctive ideas, social environments, philosophies, customs, attitudes, and behaviours within that society. Individual patients can be considered to have distinctive cultures, as can the organizations and governments that provide the frameworks within which transplantation takes place. Cultural factors are therefore discussed at these two levels.

Organizations and governments

Kidney transplantation is associated with significant long-term cost savings when compared to dialysis [2]. However, the transplantation of organs where long-term organ replacement therapies are not available (e.g. heart, liver, lungs), is expensive and requires major investment in technical expertise. Costly regulatory and administrative infrastructures are also needed for transplantation to be a viable therapeutic option. The high costs involved mean that initiating or expanding transplant programs may not be feasible for many developing countries where financial resources are severely limited. Additional information on governmental transplant management policies are found in Chapter 128.

It is therefore not surprising that there is a clear relationship between the kidney transplant rate within a country and its human development index (a composite statistic incorporating life expectancy, education, and income) (Figure 139.3). There is also a correlation between a country's wealth and its organ donor rate [3]. Interestingly, it should also be noted that there are significant differences in transplant rates even between countries that have a similar human development index, for example Norway and Australia. This implies that factors in addition to affluence are also likely to impact on access to organ transplantation. Data from the WHO Global Observatory on Donation and Transplantation (www.transplant-observatory.org) also show that rates of live and deceased donor transplantation vary with the level of national development. Highly developed countries have higher rates of transplantation from deceased donors than live

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

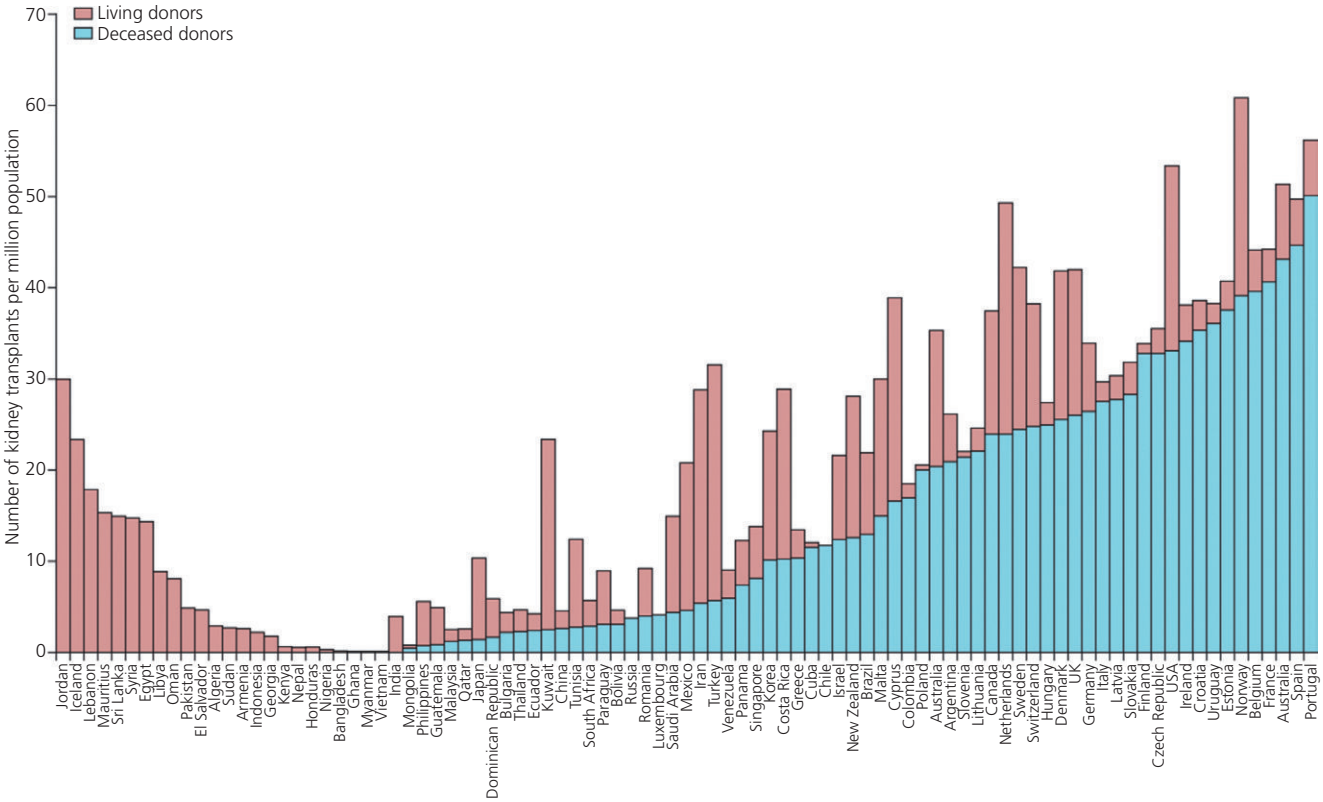

Figure 139.1. International rates of kidney transplantation from living and deceased donor (2009 data). Only countries with data submitted to the Global Observatory on Donation and Transplantation are included. Reproduced from [68] Delmonico et al. A call for government accountability to achieve national self-sufficiency in organ donation and transplantation. The Lancet, **378** (9800):1414–1418, Copyright 2011 with permission from Elsevier. Original figure uses data from the WHO website: http://www.transplant-observatory.org/Pages/Data-Reports.aspx.

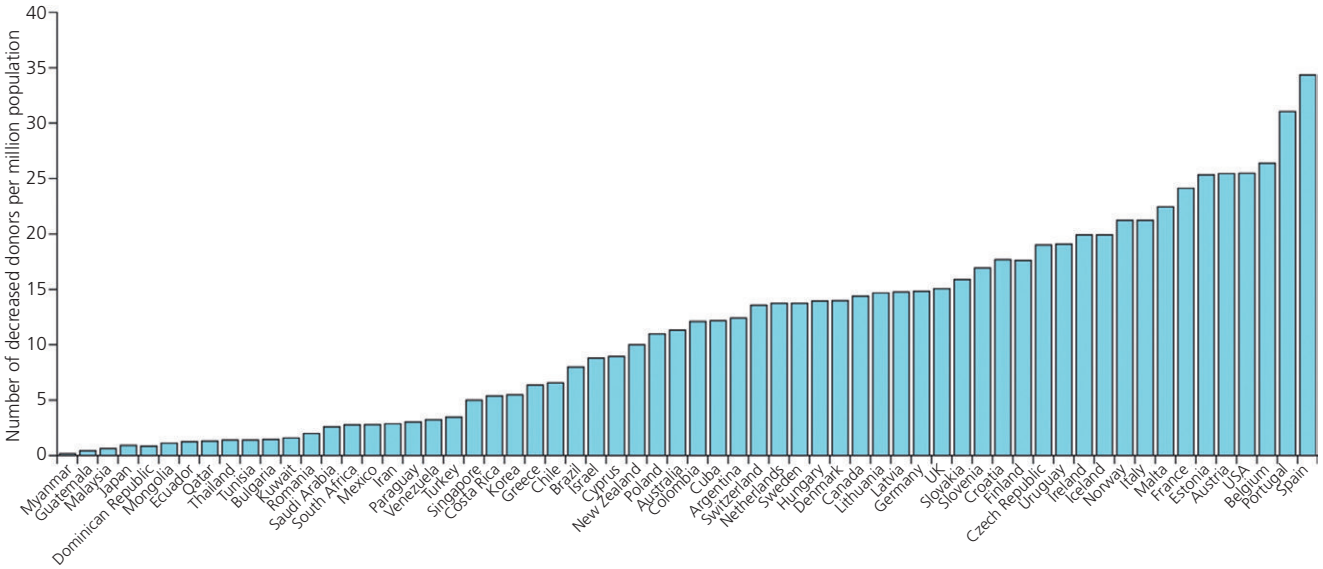

Figure 139.2. International rates of deceased donor organ donation (2009 data). Only countries with data submitted to the Global Observatory on Donation and Transplantation are included. Reproduced from [68] Delmonico et al. A call for government accountability to achieve national self-sufficiency in organ donation and transplantation. The Lancet, **378** (9800):1414–1418. Copyright 2011 with permission from Elsevier. Original figure uses data from the WHO website: http://www.transplant-observatory.org/Pages/Data-Reports.aspx.

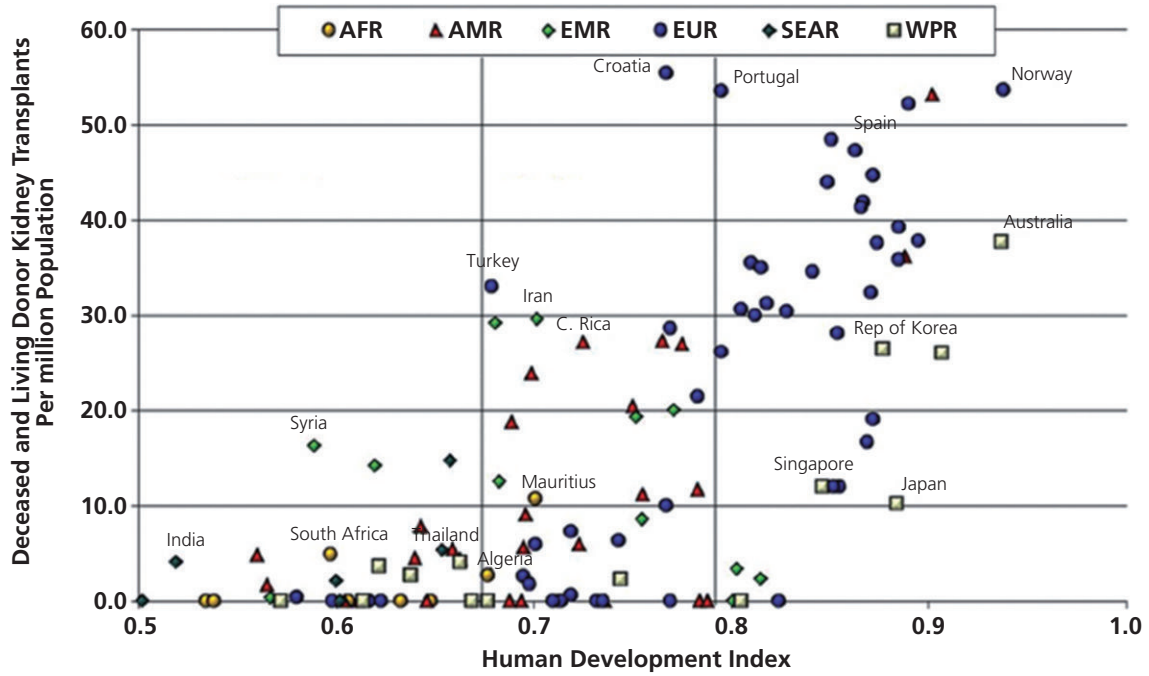

Figure 139.3. Number of deceased and living-donor kidney transplants in WHO member states in 2010, correlated with Human Development Index. Grouped by WHO regions (AFR, Africa; AMR, Americas; EMR, Eastern Mediterranean; EUR, Europe; SEAR, South Eastern Asia; WPR, Western Pacific).
Reproduced from Garcia et al, for the World Kidney Day Steering Committee (2012). The Global Role of Kidney Transplantation. Transplantation, 27; 93(4):337–341, with permission from Wolters Kluwer Health. Original figure uses data from the WHO website: http://www.transplant-observatory.org/Pages/Data-Reports.aspx.

donors, whereas this relationship is reversed in countries with low to medium development [4]. This may reflect a lag between the capabilities to perform live donation, and the development of cultural, legal, and economic systems required to provide a source of deceased donors.

Even if sufficient funds are available within a country to perform costly transplants, effective transplant programmes cannot develop unless there are appropriate legal frameworks in which to operate. Asian countries, in comparison to Western nations, have generally had restrictive laws regarding organ donation after brain death (DBD) [5]. However, this pattern is changing, as India passed laws enabling DBD donation in 1994; DBD donation became legal in Japan in 1997, with Korea following in 2000. Despite these changes, deceased donation rates remain low in most Asian countries.

Even countries with similar legal, historical, cultural and religious backgrounds have markedly different approaches to the concept of donation after circulatory death (DCD; covered in depth in Chapter 22). Dominguez-Gil and colleagues have recently documented the wide variation in legislation and acceptance of DCD donors within Europe [6]. DCD donation is forbidden by law in six member states of the Council of Europe (Finland, Germany, Poland, Portugal, and Luxembourg). In contrast, within the UK a third of all kidney transplants come from DCD donors. Within those countries that permit DCD donation, uncontrolled donors (i.e. patients with a cardiac arrest out of hospital) predominate in France and Spain, whilst in the UK the vast majority of DCD donor organs are from controlled DCD donors (patients awaiting cardiac arrest after withdrawal of treatment, or cardiac arrest in a brain dead patient). Transplantation of organs from DCD donors is far more common in the UK than the US [7,8], perhaps due to the much higher rate of donation of brain dead donors in the US.

Many factors influence deceased organ donation rates and these may include the type of consent required. A recent review determined that the introduction of presumed consent, where all members of a society are assumed to be potential organ donors unless they have registered their objection, increased donation by 25–30% [3]. The degree to which legal changes influence donation rates is controversial, as even those countries with presumed consent laws vary in the extent to which they are enforced. Opt-out systems have been described as either 'hard', that is lack of registration on an opt-out register would result in donation regardless of the preferences of the family of the deceased (e.g. Austria), versus 'soft', where family refusal can over-ride lack of registration (e.g. Spain, Belgium). 'Opt-out' legislation may not cause increased organ donation; rather, it may instead be a marker of a highly developed and effective national organ procurement strategy in a society comfortable with discussing end-of-life issues.

While legal frameworks may have an impact on organ donation, healthcare policies appear to have a major influence on outcomes after transplantation. It is instructive to compare post-transplant outcomes in countries with universal healthcare systems and those with personal insurance-based models. The UK has a health system that is free at the point of care and is funded from general taxation. Risk-adjusted mortality within 90 days after liver transplantation is higher in the UK than the US, perhaps due to poorer investment in high-cost intensive care facilities. However, after one year recipient survival is superior in the UK [9]. Patients receiving a kidney transplant in Canada have similar one-year risk-adjusted mortality

to recipients in the US, but after one year, survival in the Canadian cohort is better [10]. Although retrospective analyses of this type must be interpreted with caution, one interpretation of these findings is that countries with universal health care systems such as the UK and Canada may provide better long-term care than the personal insurance-based US model.

Cultural factors in organ donation

Access to transplantation is directly influenced by organ donation rates, which in turn are determined by cultural factors such as donor ethnicity and belief systems [11].

Donor ethnicity has a significant influence on rates of both deceased and live donation. In a national audit of UK intensive care units, families of white potential DBD donors had a consent rate of 65%, whilst for black and Asian families the consent rate was 23–29% [12]. These findings are particularly concerning given that rates of renal failure are 3–4 times higher in black and Asian populations within the UK, and waiting list patients from these ethnic groups are potentially disadvantaged due to difficulties with HLA matching. Ethnicity is an independent risk factor for unfavourable attitudes towards donation and is not confounded by associations with socioeconomic status or educational attainment [13].

Low organ donation consent rates in black and minority ethnic communities may be due to the lack of awareness of the specific needs for organs within these communities, or problems identifying with the society to which a gift of a donated organ would be made. Concerns about wanting to keep the body intact after death, and removing organs for research without consent are also an issue [13,14]. Interviews with families of potential deceased donors in the US showed that higher consent rates were associated with white ethnicity, younger patients, male patients, and death due to trauma [15]. There was no association with family education or income.

Religious factors and belief systems also influence donation rates. A systematic review of qualitative studies on factors influencing the decision to be an organ donor (both live and deceased) identified a number of major themes associated with reduced donation rates. These included religious beliefs, cultural factors (e.g. superstition, difficulties discussing death, death rituals), family/relational ties, the wish to maintain body integrity, dissatisfaction with the healthcare system, and lack of medical knowledge [16]. The impact of religion on organ donation is variable; Catholicism is associated with increased donation rates, possibly because it recognizes transplantation as a service of life [3], whereas Indo-Asian Muslims in West London had negative feelings towards transplantation and cited beliefs that Islam forbids organ donation by way of explanation [14].

Distinguishing between cultural and religious beliefs can be difficult, particularly in Islam, as a formal religious hierarchy is absent, unlike Christian faiths. Local imams, in contrast to their Christian counterparts, have increased influence, and attitudes towards transplantation are varied within Islam [17]. In Saudi Arabia, both living and deceased donation have been endorsed by senior clerics [18,19], while Egyptian Muslim authorities dispute the religious legitimacy of organ donation [20].

Cultural factors in recipient access to transplantation and outcomes

Recipient ethnicity and socioeconomic status are the two cultural characteristics that appear to have the greatest impact on access to transplantation. Unlike heart, lung, or liver transplantation, the existence of an alternative form of long-term organ replacement therapy makes it particularly instructive to examine the impact of social deprivation on access to renal transplantation, as any bias against socially deprived patients may become more apparent.

Recipient ethnicity is known to have a significant influence on access to the waiting list, waiting time, type of organ transplanted, and subsequent post-transplant outcomes. The reasons for these disparities are both immunological and non-immunological, involving a complex interplay between ethnicity, socioeconomic status, associated co-morbidities, the underlying primary disease process, HLA type, and pharmacogenomics. These inequalities have been observed worldwide, with disparities identified in New Zealand Maori and Pacific peoples [21], Canadian aboriginals [22,23], African Americans [24–26], and UK black and minority ethnic populations [1]. The scale of the problem is significant; one study from Georgia found that African-Americans had a 59% lower rate of renal transplantation than whites, even after adjustment for demographic, clinical and socioeconomic factors [26]. Patzer et al. suggest that this finding may be explained by racial bias, differences in referral for transplantation, or lack of access to healthcare.

Recipient socioeconomic status is also a strong determinant of access to transplantation, although the complex interplay between ethnicity and socioeconomic factors can be difficult to dissect. In the US, prolonged time to placement on the deceased donor renal transplant waiting list has been reported for socially deprived patients [27]. Because of the link between race, socioeconomic status, and co-morbidities, adjustment techniques were utilized. Neighbourhood poverty was synergistic with race; black patients in poor neighbourhoods had increased delays when compared to blacks in wealthier neighbourhoods. Many previous American studies have described similar findings [28–30].

Lack of private health insurance might be a factor that limits access to the transplant waiting list in socially deprived patients in the US. Udayaraj and colleagues investigated the link between social deprivation, ethnicity and access to the deceased donor waiting list in the UK [31]. However, even with a universal healthcare system, as in the UK, the most deprived patients have poorer access to the renal transplant waiting list after adjusting for patient age, sex, cause of renal failure, era, and centre. Interestingly, ethnicity does not appear to influence listing for transplantation in the UK; on the contrary, ethnic minority patients aged greater than 50 years had a higher chance of being listed than their white counterparts. Dudley et al. have previously shown that a wide variety of factors influence the likelihood of being placed on a renal transplant waiting list including age, primary diagnosis, ethnicity, social deprivation, previous transplantation, the size of transplant unit, and the size of the unit's live donor programme [32]. A similar link between socioeconomic status and likelihood of receiving a live donor kidney has also been demonstrated in the UK [33].

The conclusion from the above studies is that multiple cultural factors are important in transplantation access, but the relative impact of these factors varies between countries.

Transplant commercialism

Transplantation has been a victim of its own success, with the availability of organs failing to keep pace with demand. This growing disparity, along with inequalities in access to transplantation, has driven the development of transplant commercialism, organ trafficking, and transplant tourism. These issues are particularly relevant to renal transplantation [34], due to the lesser cost of transplantation compared with long-term dialysis, the relatively

low morbidity and mortality of live donation, the lower level of surgical expertise required, and the increasing burden of chronic kidney disease worldwide.

Transplant commercialism has been defined as a policy or practice in which an organ is treated as a commodity, including by being bought or sold or used for material gain [35]. Where transplant commercialism exists, poor or absent regulation provides the opportunity for organ trafficking to occur. The Declaration of Istanbul (discussed in more detail below) has defined organ trafficking as:

- the recruitment, transport, transfer, harbouring or receipt of living or deceased persons or their organs by means of the threat or use of force or other forms of coercion, of abduction, of fraud, of deception, of the abuse of power or of a position of vulnerability;
- the giving to, or the receiving by, a third party of payments or benefits to achieve the transfer of control over the potential donor, for the purpose of exploitation by the removal of organs for transplantation.

When travel is required for transplant commercialism to occur, it is termed transplant tourism. Specifically, transplant tourism is defined as:

1 the movement of organs, donors, recipients, or transplant professionals across jurisdictional borders for transplantation purposes, and involving organ trafficking and/or transplant commercialism; or
2 devoting resources to transplant patients from outside a country which in turn, undermine that country's ability to provide transplant services for its own population [35].

The true scope of the illegal organ trade is difficult to determine due to its clandestine nature, but it has been estimated to generate illegal profits of between $600 million and $1.2 billion per year, ranking number ten in terms of global illegal activities [36]. A recent review estimated that transplant commercialism conservatively accounted for 5% of all transplants performed worldwide in 2005 [37]. India, Pakistan, Philippines, China, Bolivia, Brazil, Iraq, Israel, Moldova, Peru, and Turkey were all identified as kidney 'exporting' countries, while Australia, Canada, Israel, Japan, Korea, Malaysia, Oman, Saudi Arabia, Taiwan, UK, and USA were thought to be kidney 'importing' countries.

It has been estimated that more than 2000 kidneys have been implanted into transplant tourists in Pakistan alone, and that similar numbers have been transplanted in the Philippines [38]. Despite the Philippine government passing an Organ Donation Act in 1981, there was a rise in unregulated organ sales in the late 1990s; in 2007 there were only 29 deceased donor kidney transplants performed, with more than 1000 live donor transplants in the same year. Regulations for generous compensation for live donors were introduced in 2002, but ongoing organ sales to foreigners continued. Kidney transplants to foreign nationals from unrelated local donors were banned in the Philippines in 2008, but given the high level of poverty and corruption, it seems likely that organ trafficking continues [39].

Commercial live donor kidney transplantation has been reported in Egypt, where donation from deceased donors is illegal, and from destitute tsunami survivors in India [38]. In 2007 it was reported that almost 99% of transplants in China came from executed prisoners [40], and reports of transplant tourists from Taiwan paying for these organs have emerged [41]. This appears to be the only example of deceased donor transplant commercialism. Although Iran allows live kidney donors to be paid, there are strict rules preventing the transplantation of foreign nationals, and so transplant tourism in Iran is not thought to occur. As Iran currently has the world's only regulated system of transplant commercialism, this model is explored in more depth below.

The publication of the Declaration of Istanbul in 2008 has raised the awareness of the international community to the existence of organ trafficking [35], and a number of countries have enacted new laws or strengthened existing legislation outlawing transplant commercialism, organ trafficking and transplant tourism. As a result, Shimazono's 2007 study may already be out of date. It is now illegal for a foreign national to undergo transplantation in China. Israel's 2008 Organ Transplant Act condemns organ trafficking and prohibits authorization of insurance benefits to cover the costs of transplantation in a foreign country that does not conform to the provisions of the Act. Previously, it was legal for Israeli citizens to travel abroad and purchase an organ.

Outcomes after commercial live donation

One of the main concerns regarding transplant commercialism is that it leads insidiously to organ trafficking, and exploitation of the destitute and vulnerable donor. Analyses of outcomes after commercial organ donation are highly relevant given these concerns. Again, as expected for an almost globally illegal activity, relatively limited information is obtainable, and selection and publication bias are likely [42]. However, the available data suggests that commercial donors have poor physical, psychological, and financial outcomes.

A landmark paper surveyed 305 individuals in Chennai, India, who had sold their kidney an average of six years before the survey, which was undertaken in 2001 [43]. More than 90% had sold their kidney to pay debts, and the average amount received was US$1070. Average family income declined by a third after donation, and 86% of donors reported deterioration in health status. The majority of donors (80%) stated that they would not recommend commercial donation to others.

Another large report from Pakistan documented similar findings [44]. Some 239 kidney vendors from the Punjab were surveyed a mean of 4.8 years post-donation. Ninety percent of the vendors were illiterate and almost 70% were bonded labourers. Ninety three percent sold a kidney to repay debt; the average received was US$1377 after paying hospital fees and middlemen. Eighty eight percent reported no economic improvement in their lives, and 98% reported deterioration in health status, most likely because of the labour-intensive nature of the occupations of the vendors. Finally, a survey of 50 Egyptian commercial live donors found that 78% reported a health decline, and 94% regretted their donation [38].

It is clear that commercial live donors have poor outcomes after donation and very few attained the expected improvement in their lifestyle. This contrasts with the overwhelmingly favourable experience of non-commercial live donors found in Western reports, with similar long-term survival to that of matched controls [45,46], and equivalent or better quality of life than the general adult population [47–49].

Recipient outcomes after transplant commercialism

As with commercial live donors, there are similar difficulties in reliably identifying the outcomes of recipients of these organs. As expected, most reports have small numbers of patients, and systematic identification of commercial recipients is difficult due to the covert nature of the transplant.

Overall, recipients of commercial live donor kidney transplants have worse outcomes, with higher rates of infection (including hepatitis B, hepatitis C, and HIV), rejection, and graft loss. In addition, documentation from the implanting centre is often inadequate, which may impair effective care [50–52]. One of the largest studies comes from Pakistan, where recipient outcomes of 126 vended and 180 living-related kidney transplants were compared [53]. Recipients were matched for age, sex, and time from transplant. Acute rejection (33% vs.17%), surgical complications (22% vs. 8%), tuberculosis (11% vs. 6%), and acute hepatitis (16% vs. 2%) were all more common in vended recipients. Five-year graft survival (45% vs. 80%) and overall patient mortality (27% vs. 6%) were also dramatically lower.

A systematic review of recipient outcomes after commercial kidney transplant confirmed that higher rates of graft loss and recipient death appear more common than with non-commercial donation, but that this may be dependent on where the surgery took place [42]. Outcomes of 31 Taiwanese recipients of commercial kidney transplants performed in China from executed prisoners found no difference in ten-year graft or patient survival when compared to 44 non-commercial domestic renal transplant recipients [54]. A larger study of Chinese kidney transplants in 389 Malaysian patients replicated these findings, with no difference in patient and graft survival and rates of bacterial, viral, or fungal infection five-years post-transplant when compared to those from non-commercial live donor recipients [55].

These results suggest that there is a significant variation in recipient outcomes between centres that perform commercial kidney transplantation. As increasing numbers of countries tighten transplant regulations in the aftermath of the Declaration of Istanbul, this may have the unforeseen effect of driving illegal commercial transplant centres further underground, paradoxically worsening live donor and recipient outcomes.

The Iranian model of transplant commercialism

Iran is currently the only country that has a semi-regulated system of transplant commercialism and, as a consequence, is of considerable interest to the international transplant community. Although numerous reports from within Iran have described their model, Iran's internal politics are complex, and the veracity of some reports has been questioned. This, combined with the uneasy relationship between Iran and many Western nations, has made it difficult to reliably verify many of the claims made.

Commercial kidney transplantation in Iran was introduced by the Iranian government in 1988 in response to rising waiting lists; deceased donor transplantation was not legal at that time. Details of the scheme have been provided by Ghods et al. [56]. Patients requiring kidney transplantation without a live donor apply to a non-governmental organization (Dialysis and Transplant Patient's Association – DATPA) run by patients with end-stage renal failure. Potential donors who wish to sell a kidney approach the DATPA, who then perform donor-recipient matching. There is no brokerage fee, and no middlemen. Although the government pays approximately US$1200 to the donor, recipients may give more. The potential donor and recipient meet preoperatively and can arrange additional payments. If the recipient is poor, these payments may be met by a charity. Donor medical preassessment is claimed to be rigorous. Surgery takes place in public hospitals, the transplant team is not aware of the financial arrangements, and donors receive health insurance for a limited time afterwards.

Approximately 80% of kidney transplants in Iran are from commercial donors, although legislation enabling a deceased donor program was put in place in 2002 [57]. Approximately 15% of Iranian organ transplants are from deceased donors [58]. The slow growth of this program has been attributed to cultural reasons and infrastructure issues rather than a negative effect of the commercial donor model [59].

Financial and psychological outcomes of Iranian commercial donors appear to be variable, but are more favourable than reports of commercial donors from India or Pakistan. Malakoutian et al. analysed the socioeconomic status of 478 Iranian commercial kidney donors and found that 62% were living below the poverty line, with financial issues the most frequent motive for donation [60]. After donation, 91% were happy with their decision, and 53% would suggest kidney donation to others. In contrast, a study of 300 Iranian commercial live donors found that donation had negative effects on employment in 65%, and that 71% suffered severe de novo depression postoperatively [61,62]. The goals of vending were not achieved by 75% of commercial kidney donors. Nejatisafa et al. examined predonation quality of life of 424 donors and found that 95% had had at least one stressful event six months predonation; most commonly increased life expenses, low income, or household duties [63]. Postdonation quality of life appeared lower in the donors than the general population, although this was not tested statistically, and the control population was not age or sex matched.

In depth analysis of Iranian recipient outcomes is hampered by the lack of a national registry. However, single- and multi-centre reports generally show that renal allograft survival in Iran is similar to that demonstrated in large Western series, with ten-year graft survivals of 46–68% [64,65]. Grafts from live unrelated (commercial) donors had similar or better survival than live-related kidneys in some reports [56,64], but not others [65].

The Iranian program cannot be called fully regulated as payments to the donor are variable, at least partly dependent on the ability of the recipient to pay, and details of additional payments are not kept by the relevant authorities. Nevertheless, the risks of transplant tourism to Iran have been significantly reduced as foreign nationals are not allowed to be either donors or recipients, unless both are foreign and the donation has been authorized by the Iranian Ministry of Health. Although recipient outcomes are far better than those reported in unregulated commercial programs, concerns still exist about exploitation of poor Iranian commercial donors, and the financial and psychosocial outcomes postdonation. Although the Iranian approach has been credited with elimination of the kidney transplant waiting list in that country [56], the true prevalence of renal disease in Iran is unknown. Also, as a developing country, access to dialysis outside of the major cities is believed to be poor, and it is highly unlikely that many patients who would benefit from receiving a graft die of renal failure having never been dialysed. As a result, the potential Iranian renal transplant waiting list may be artificially low. In addition, some reports have questioned the absence of a waiting list [66].

Strategies to decrease global variation and prevent transplant commercialism

Reducing inequities in access to transplantation

While sociocultural and socioeconomic factors that influence access to transplantation have been relatively well researched, particularly in renal transplantation, interventions to reduce disparities

have been comparatively poorly investigated. The Madrid Resolution [67] and others [68] have called for all countries to address the needs of their citizens for transplantation in a sustainable manner. However, when the world's wealthiest nations are unable to reduce the disparity between organ demand and supply, it is unclear how this aim can be achieved in developing countries with more limited resources.

While practical solutions to this dilemma are not readily forthcoming, opinion leaders in transplantation have described possible strategies to increase equity of access to renal replacement therapy [69]. They have called for improvements at international, national, community, and health system levels. International strategies to improve access to renal transplantation include forming productive relationships between professional organizations, and international collaborations and sharing of data and evidence-based best practice. Of note, The Global Alliance for Transplantation was established in 2006 under the auspices of The Transplantation Society to achieve these aims [70]. At the national level, governments should develop a national transplant policy, a register of waiting list and transplant patients, and maintain oversight and regulation of organ procurement and transplantation procedures. Community interventions should include public education on the risk factors of organ failure, and on the benefits of transplantation. Finally, health systems need to improve the education of health professionals, and to develop locally appropriate deceased donor programmes. Such strategies are already in place in a number of Western countries, but are poorly developed in many underdeveloped nations.

While efforts to reduce the disease burden leading to the need for transplantation are important, increasing organ donation is the fundamental means by which access to transplantation can be increased. Spain has the world's highest deceased donor organ donation rate (Figure 139.2), and consequently the Spanish model of organ donation has been studied intensively over the last decade. However, as noted by the architect of the Spanish system, Rafael Matesanz, an adequate legal, economic, ethical, medical, and political background is needed for donation rates to increase [71]. Spain has adopted a systematic, organizational approach to deceased organ donation, with a nationally-led transplant coordinator network. Donor coordinators in Spain are doctors with a background in critical care, working part-time to allow them to continue their work as intensivists. A rolling audit of brain death is undertaken to ensure that all potential DBD donors are identified. Ongoing training of transplant coordinators is a priority. Hospitals in Spain are reimbursed for organ procurement, and the national transplant organization actively engages with the media to publicize transplantation-related issues. Further improvements in organ donation rates have occurred with the introduction of national protocols for the care of DBD donors [72]. Ultimately, Matesanz also recognizes that large numbers of intensive care beds are essential to high organ donation rates [71].

While the evidence base for interventions to improve donation rates is generally weak, more is known about the factors influencing the decision of relatives to consent to the donation of organs from a deceased relative [73]. Consent rates appear to increase if the relatives think the deceased has received high quality medical care, and if they have an understanding of the concept of brain death. A pause between breaking bad news and the subsequent request for organ donation is also beneficial, as is the presence of an in-house donor coordinator. A recent trial has demonstrated that 'collaborative' requesting, that is a joint approach from an intensive care clinician and a donor coordinator, has no effect on organ donation rates from relatives of brain dead patients compared to requests from the clinical team alone [74]. This is a surprising result, and further research on this issue is needed.

Once on the waiting list, one of the most efficient ways to reduce inequalities in access to donor organs is through evidence-based organ allocation strategies. Organ allocation schemes are most effective in this respect when they utilize scoring algorithms that can be readily refined to adjust for ongoing inequities. Due to disparities in the ethnic origin of deceased donors and those patients on the renal transplant waiting list in the UK, it was recognized that ethnic minorities had longer mean times to transplantation than white patients [1]. The algorithm for allocating kidneys from DBD donors has recently been altered to better reflect the HLA diversity of non-white patients on the waiting list, that is rare recipient HLA antigens are defaulted to a more frequent related specificity. This appears to have at least partially addressed these imbalances [75]. The introduction of the deceased donor liver allocation based on the MELD score, a composite score utilizing objective biochemical markers of disease severity, has removed the association between race and receiving a liver transplant in the US [76]. It seems likely that more countries with ethnically diverse populations will follow these examples in order to increase transparency and reduce inequalities.

Prevention of transplant commercialism

The first step in tackling transplant commercialism is to acknowledge the scale of the problem and for the international transplant community to clearly state its opposition. This has been achieved most clearly through the Declaration of Istanbul [35], the first document drawn up by the international transplant community that defines and condemns transplant commercialism, organ trafficking and transplant tourism. Building on the Universal Declaration of Human Rights, and World Health Organization guiding principles on organ transplantation [77], its primary aim is to inform and promote ethical practices in global organ donation and transplantation.

The Declaration is a document published after a summit in Istanbul was convened by The Transplantation Society and the International Society of Nephrology [78]. Six guiding principles are stated for ethical donation and transplantation:

1. governments should implement programs to screen, prevent, and treat organ failure;
2. in every country where organ donation or transplantation takes place, these activities should be covered by legislation to govern the recovery of organs from live donors and deceased donors, and the practice of transplantation;
3. organs should be allocated equitably without regard to gender, ethnicity, religion, social or financial status;
4. the primary objective of transplant policies and programs should be to optimize short -and long-term care of donors and recipients;
5. countries should strive for self-sufficiency in organ donation;
6. organ trafficking and transplant tourism should be prohibited as they violate the principles of equity, justice, and respect for human dignity, and transplant commercialism leads to inequity and injustice through targeting impoverished donors and should be prohibited.

The impact of the Declaration of Istanbul has been considerable, with more than 100 national transplant organizations endorsing it. Although it is difficult to be certain of causality, after the Declaration's publication, China, Israel, the Philippines, and Pakistan all

passed new legislation or strengthened existing laws that ban organ trafficking and organ sales.

However, the Declaration of Istanbul has been criticized by Ambagtsheer and Weimar for failing to recognize the difficulties inherent in enforcing such legislation, and the lack of criminological and legal expertise in those who drafted it [79]. They have argued that the implications of prohibition of organ trade need to be taken into account. Prohibition of trade in any commodity generates black markets, drives up prices, provides illegal incomes, displaces crime to other regions, and drives trade underground. Investigating alleged organ trafficking is unlikely to be a priority with law enforcement agencies due to a lack of knowledge and training. The authors argue that the World Health Organization and the transplantation societies behind the Declaration of Istanbul should support efforts towards boosting organ donation by initiating regulated trials of incentives for donation, while continuing to combat organ trafficking. In addition, they state that these organizations must assist in training police, prosecutors, and judges, and endeavour to increase cross-border co-operation between medical staff and police. Glazier and Delmonico rebutted these suggestions by stating their continued opposition to both transplant commercialism and organ trafficking [80].

Financial incentives for organ donation

Although no responsible commentators have publicly condoned organ trafficking or transplant tourism, as currently defined, many have suggested that regulated financial incentives for organ donation (transplant commercialism) should be trialled, most commonly for live organ donation. In the UK, France, and Sweden, live kidney donors have their expenses and loss of income reimbursed, with the aim of making the donation process cost-neutral [81]. In the US, live donors are not compensated financially as the National Organ Transplant Act 1984 states 'it shall be unlawful for any person to knowingly acquire, receive or otherwise transfer any human organ for valuable consideration for use in human transplantation'. Proposed financial incentive schemes go beyond reimbursement of costs, and would require changes to existing transplant legislation in countries that currently deem transplant commercialism illegal.

The most pressing argument to support the introduction of financial incentive schemes for live donation is the growing number of patients with end-stage organ failure dying on transplant waiting lists, and, paradoxically, the rise in organ trafficking and transplant tourism [58,82]. It has been noted that all participants in the transplant process benefit materially except for the donor: the recipient is often able to return to employment; the hospital and its staff are paid; and the government benefits from the reduction of numbers needing dialysis (in the case of a kidney transplant). However, the family of the deceased donor, and the living donor, do not benefit financially from the donation process. There are many examples of people who receive extra payments for putting their bodies at risk (e.g. soldiers during active service, healthy volunteers in phase I clinical trials); shouldn't this also extend to live donors? Many different putative schemes are possible, ranging from a true market model, where a live donor would decide who to donate to on the basis of the highest offer, to a system whereby live donors are given a modest lump sum in addition to life and/or medical insurance cover [83].

In the US, Matas and colleagues have been the most prominent proponents of a trial of regulated financial incentives for live donors, and have described the moral and organizational requirements necessary for this to proceed [58,84,85]. These include transparency, national standards for the acceptance or rejection of compensated donor candidates, and rigorous legal and regulatory oversight. They describe a scheme whereby live donors could be offered a package of life and medical insurance, free long-term medical follow-up, and a cash lump-sum (e.g. US$5000) [86]. Donor evaluation and informed consent should be separated from the recipient team. There would be no obligation for a donor candidate to accept the offer, and the organ would be donated to the national deceased donor list by a pre-defined algorithm. Total costs of the package up to the value of US$90 000 would still be cost-effective for society [87].

Other opinion leaders in the US have argued that such schemes would disproportionately attract poor people to donate, and while proponents of financial incentives claim that autonomy is paramount, that there is no autonomy in being poor and using any means to resolve destitution [88]. Such schemes could lead to inherent victimization and exploitation, and risk incomplete disclosure of medical conditions by a potential donor/vendor, as demonstrated by paid blood donors [89]. Concerns have also been raised that organ vending would not co-habit well with non-commercial donation systems currently in place for deceased donor organ donation, for example families of potential deceased donors may resent not receiving the financial incentives that live donors might be given and therefore may not consent to donation [90]. This would have particular implications for organs that are difficult/impossible to be donated by live donors (e.g. heart, lung, liver, and pancreas). On an ethical level, incentivized live donation schemes may compromise the dignity of the human body as it becomes a consumer good [83]. Opponents of financial incentives also claim that alternatives to increase organ donation rates have not been fully explored, including an 'opt out' system of consent, organization of procurement agencies, training of paramedics, intensivists and emergency department staff, and use of extended criteria organs [66].

How might the public react to financial incentive schemes? Perhaps as expected, views vary between surveys, and countries. Public opinions on financial incentives for the families of deceased donors have been investigated by Bryce et al., in a telephone survey of 971 Pennsylvania households [91]. Fifty nine percent of respondents agreed that the state should offer financial incentives or benefits, with much more support for funeral benefits and medical expenses than for direct payment (81%, 84%, and 53%, respectively). Interestingly, almost three-quarters of those surveyed said that financial incentives would not make them change their mind about consenting for donation from a family member. In contrast, in a similar survey, when 720 respondents from across the US were asked:'If a person donates their organs after death, do you believe the donor's family should be compensated in some way for the donation?', just 10% answered in the affirmative [92]. Of particular relevance, Rodrigue et al. surveyed 561 next-of-kin in the US who had recently been approached to consent to deceased organ donation from a relative [93]. Overall, just over 90% of all respondents said that financial incentives would have made no difference to their decision. Twelve percent of those who did not consent stated that they would have consented had financial incentives been offered, but 6% of those who did consent would have refused it. Favourable attitudes towards financial incentives were more common in those who did not consent, the young, those with less than a college education, and less favourable organ donation attitudes.

The public's views on financial incentives towards live donation appear to be more positive, at least in the US. More than 80% of a sample of US adults felt that live donors should have their medical costs reimbursed, and should use sick leave or paid leave to enable donation [92]. Only 28% supported direct payment from the government, however. A Dutch internet survey found that 47% of respondents were against the idea of health insurance companies offering compensation to live donors, either life-long health insurance or a €25 000 lump sum [94].

It is apparent that there are strong arguments for and against financial incentives for organ donation. The only way to adequately determine whether such schemes would increase donation rates is a properly designed and regulated clinical trial. The trial design would need to consider whether to limit payments to live or deceased donation, kidney alone or other organs, the type and size of payment, and the necessary regulatory framework and changes to legislation required. Given the many significant bureaucratic hurdles that would need to be cleared before a trial could be initiated, and the administrative costs likely to be associated with it, it seems unlikely that this will occur, especially in the current global economic climate.

Summary

Inequities in access to organ transplantation probably exist in all countries to a varying extent. Inequity varies markedly according to factors that include national wealth, adequacy of legal, ethical, and organizational infrastructures, efficacy of organ allocation schemes, ethnic and cultural diversity, and belief systems. Even in wealthy countries with well-established transplant programmes, the disparity between the supply and demand of organs continues to grow. The global organ shortage and inequities in access to transplantation are the prime drivers behind transplant commercialism, organ trafficking, and transplant tourism. Global strategies have been developed to address these issues, and recognition of the magnitude of organ trafficking and the international transplant community's opposition are important developments. More controversial solutions include financial incentives for organ donation, although public support appears strongest for incentivised live donation rather than deceased organ donation. The Iranian model provides evidence that financial incentives for live donor kidney donation can satisfy demand, but many concerns remain regarding its applicability to Western transplantation systems. Complex ethical, organisational, political, and legal hurdles remain to be overcome before inequities to transplantation and organ trafficking can be abolished worldwide.

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CHAPTER 140

Federal and Private Insurance Coverage for Organ Transplantation

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Introduction

Performing and providing transplant care is not cost-free. A team of individuals with multiple skills; surgeons, physicians, nurses, social workers, pharmacists, dieticians, therapists, administrative support personnel and others, plus immunosuppressive therapies and facility resources are necessary to respond to peculiar needs of the transplant recipient. Moreover, the processes used to assess and procure donor organs require a distinctly different group of individuals and resources than those used for the transplant procedure and post-transplant care. Irrespective of funding or regulation, every healthcare system that performs complex procedures such as organ transplantation must establish a methodology to support and sustain (1) the individuals providing the care, (2) the resources consumed in providing organs, and (3) subsequent transplantation services. Adequate funding determines whether or not a system will remain in existence.

The simple model in which the individual personally purchases the service is not broadly feasible. Alternatively, a common global model is applied in many parts of the world in which government sponsored healthcare incorporates the transplant and organ costs as a component of a wider societal healthcare imperative. In theory, access to care using this model is universal. However, this model makes organ transplantation a cost center within a healthcare delivery system that has significant impact upon resource availability. Within the cost center model, resources are typically allotted by past demand or desired production. By increasing volumes of procedures, premature depletion of resources may result in restriction of service or diversion of resources from other services. However, the funding system in the US is based on the premise that increasing the numbers of transplants results in increased revenues entering into the system and adds to the prestige of the organization. This paradigm is complicated by the fact that the preponderance of the US funding system is a convoluted mix of governmental and private insurance agencies. The following discussion will concentrate upon requirements necessary to perform organ transplants and how the existing system influences and are influenced by these sometimes competing funding sources. The relationship between legal authority, policy, outcome, performance and payment structure has become inextricably linked. Although other healthcare systems may have a different balance of governmental, private insurance or personal funds that support transplantation, all systems must find ways to compensate for the effort, services and products that are necessary for providing donation and transplant care.

While the goal is not to be "US-centric", analyzing the regulatory and performance components within the US transplant system will allow for a better understanding of the diverse, but necessary elements required for the delivery of transplant care throughout the world. The basic elements of a funding system must address:

1. who can be a provider of transplantation services and what services are essential,
2. how does one obtain organs for transplantation,
3. how do patients find centers and organs for their needs, and
4. how to balance conflicting need-based and financially-based goals?

Additional related discussions on national transplant management and oversight, economic impacts of transplantation, and national healthcare policy as it relates to transplantation can be found in Chapters 128, 142, and 143 respectively.

Requirements to be a transplant program or center

Organ transplantation is highly regulated in the US and in most parts of the world. In part, this regulation is the consequence of the limited availability of organs and the need for public assurance that the system functions within legal and moral structures to benefit those in need of life saving organs. The repugnance of preferential access and/or over abuses of the system is an underlying theme of the social regulation. In order to establish an organ donation and transplantation system, a legal infrastructure is necessary that allows the necessary elements in organ transplantation to function within the legal and ethical mores of society (see Chapters 136, 137, and 138). Recognition of neurologic death, that a person can be dead with a beating heart, is fundamental for multi-organ donation. In the US, this was officially recognized in the 1960s, with the broad acceptance by the states that individuals meeting the Harvard criteria were in fact dead. The passage of the Uniform Anatomic Gift

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Act in 1968 (and multiple modifications since) has given legal structure to the process of organ and tissue donation in the US. Additionally, the donation system has been enhanced by a transportation system to move trauma victims and neurologically devastated individuals to facilities with the expertise and the capacity for specialized care. While these elements are not part of the direct payment system for transplantation, they underscore how intertwined organ donation and transplantation have become with respect to the legal, moral and care delivery fabric of society.

The legal authority for organ transplantation in the US was established in 1984 with the enactment of the National Organ Transplant Act (NOTA). NOTA instituted the national Organ Procurement Organization (OPO) network and the Organ Procurement and Transplant Network (OPTN). The OPO system was created to maximize the yield of organs and tissues from deceased donors. All hospitals within the geographic US were assigned to an OPO for the purpose of identifying and managing organ donors, obtaining authorization for organ donation, coordinating organ retrieval and transporting organs to a designated recipient, hospital and community. Funding for the OPO network was assured through Title XVIII of the Social Security Act (42 U.S.C., 1395 et seq.) which established the process whereby monies and financial accountability for organ retrieval practices are overseen and subsequently identified as through Medicare.

The OPTN was established for the purpose of allocating deceased donor organs to individuals on a national wait-list equitably, adopting standards of quality for acquisition and transportation of donated organs, adopting membership criteria to participate in the national transplant system, and acquiring analyzing and publishing transplant information for the benefit of the Secretary and others needing information about organ transplantation. NOTA specified that only members of the OPTN could register patients on the national wait-list and receive organs retrieved through the OPO system. The OPTN mission is carried out by a private, membership organization which provides the specified services through a contract awarded and overseen by the Health Resources and Services Administration (HRSA), a unit of the Public Health Services. Also included within the authorization of the national transplant network was the creation of the Scientific Registry of Transplant Recipients (SRTR). The intent of this information was to report national and transplant center performance to the OPTN for the purpose of general quality assessment and oversight of specific member's performance. The public release and analysis of this information is meant to aid recipients and their doctors better understand their choices for transplantation care.

OPTN membership is a necessary requirement for a center to list patients and to receive and transplant deceased organs. In 1999, the Final Rule defined how NOTA would be implemented. It specified the areas OPTN policy would have jurisdiction, it required that members comply with OPTN policy and that peer review would be utilized to enforce policy. In 2006, OPTN authority was expanded to include oversight of live organ donation and transplantation. At present, a center is not lawfully authorized to perform deceased or live organ transplants in the US without being an OPTN member. Maintaining the status of "member in good standing" has become a minimum standard for the ability to routinely perform organ transplantation.

OPTN bylaws specify the basic membership requirements for transplant programs, which include having key personnel with minimal levels of experience or training and having defined ancillary professional services (such as anesthesia, infectious diseases, radiology, pathology, blood banking, histocompatibility services and others) and specified hospital/institutional services (including sufficient social work, nutrition, financial, physical therapy and other services). OPTN membership comes with a significant institutional commitment of people and resources, along with the accompanying expense. Membership status is subject to performance outcomes and policy compliance (especially as pertains to following organ allocation algorithms and patient safety). Performing specific numbers of transplants is not necessary for either obtaining or maintaining OPTN membership, but failing to meet expected outcomes triggers a peer review by the OPTN. Furthermore, if a member institution is deficient in key personnel or capacity, fails to comply with OPTN policy or jeopardizes the safety of people on the wait-list or transplant recipients, its membership status can be modified. While the OPTN can discipline programs through a variety of means that include an alteration in membership status, the ability to revoke a program's membership with the OPTN resides solely with the Secretary of HHS. At any one time, there are many transplant programs that are being reviewed by the policy compliance arm of the OPTN. Finally, the costs and requirements of OPTN membership must be met if a program is to participate in the US transplant system. All these elements must be supported for a program to be active.

Government and payment for organ transplantation

The payment structure for transplantation has undergone significant transformations over time. When transplantation first got started in the US, the recipient's institution would bill the recipient for each individual component of care following the procedure. If the patient had insurance, the bill would be passed along but third party payments were variable and individuals and families were put at considerable financial risk. The passage of the End Stage Renal Disease (ESRD) Act in 1972 gave all individuals with renal failure access to dialysis and transplant therapy through Medicare. In 1981, the Medicare Secondary Payer provision clarified the role of government ESRD payments for those patients with private insurance that become Medicare eligible on the basis of having ESRD. It was stipulated that in such individuals with a private employer group health insurance; then even though the individual was Medicare eligible, the first 30 months of ESRD payments are the responsibility of the private plan before transferring payment responsibility to Medicare. However, the lack of predictability of coverage for transplant services and the heterogeneous access to care were significant factors leading to the passage of NOTA in 1984. Under this law, the US government not only validated organ transplantation, but also accelerated the process towards uniform acceptance and payment by the private insurers.

The Federal government compensates participating hospitals and physicians that perform organ transplantation for Medicare beneficiaries. The Medicare ESRD Conditions of Participation were first published in 1976 and stipulated resources, personnel and necessary outcomes. These stipulations were initially quite minimal; however, several amendments expanded the conditions prior to the 2007 Final Rule. Other revisions established survival rates for Medicare reimbursement for hearts (1986), liver (1991), and lung (1995). The Medicare reimbursement strategy to the facility was separated into a payment for the organ-associated costs and those for the transplant procedure. The latter was made to the institution under the Diagnostic-Related Group (DRG). Medicare also developed a

proscribed methodology for the institution to recoup some of the outlier costs in the event that a beneficiary had a complicated and prolonged hospitalization. Physician and surgeon payments were made through a separate payment schedule. This basic model continues today. In 2007, the Medicare Conditions of Participation expanded the resources, processes and outcome elements that were necessary for an institution to provide transplant services for Medicare beneficiaries.

The other major government sponsored healthcare assistance is for those with limited incomes. This is accomplished through a jointly funded federal/state program that is administered by the state's Medicaid program. There are clearly defined financial criteria for individuals to qualify for governmental healthcare benefits which are based on income. Because it is jointly funded, but state administered, Medicaid transplantation benefits vary substantially between states. Considerable latitude is given to the states on how the monies should be allocated for the benefit of its citizens. The criteria to qualify for Medicaid benefits are established by the state and the receipt of care is usually required to stay within the state (assuming that it is available).

The private insurers have had an evolving relationship with transplant programs that has been increasingly influenced by government involvement with transplantation. Initially, transplantation was treated as any other approved medical service provided by an institution and the insurers sought professional and institutional discounts on the "fee for service" provided. However, this model was quickly discarded and the process transformed into "group" buying practices and bundled payments to specific institutions. While insured individuals could access any program for transplant services, the insurance company would only pay full/maximal benefits if the insured patient went to a program that was selected as a preferred program; that is "within network." The qualifications necessary for admittance to the provider "networks" have undergone significant evolution. Like Medicare, there is typically an adjustment formula used to compensate the institution for costs occurred in excess of the initially agreed upon amount. As the numbers of transplant programs increased, the relationships between insurer and healthcare providers evolved and it became apparent that membership in "networks" can be formed or broken rapidly. As noted in the beginning of this chapter, the US system is predicated upon the concept that organ transplantation is a revenue center for a healthcare system, so maintenance of network participation and the accompanying access to patients has become a clinical and financial necessity for most programs. Similarly, it is has become critical to understand the resources expended for transplant care and the associated cost structure. When expenses exceed revenues the system falters.

The cost of obtaining organs for transplantation

The cost of an organ is substantial and warrants individual discussion with each patient. It became a federal felony to buy and sell organs with the passage of NOTA; however, organ donation is an expensive, complex activity that must have a funding source. In response to this need, the OPO and transplant center accounting system was established within Medicare. It defines the allowable costs for organ retrieval. Under this system, the costs of retrieving organs for transplantation are allocated to the recipients of the organs. In the US system, the Standard Organ Acquisition Charge (SAC or OAC) is calculated from the costs accrued from the practice of the organization. The center SAC is derived from "allowable" expenses for the beneficiaries' care. This remains the primary mechanism by which an institution recoups the costs for:

1. procuring deceased organs from the OPO,
2. personnel and tests required to assess an individual's suitability for transplantation,
3. evaluation of live donors, and
4. specific additional allowable services that the transplant center must provide that is not directly billable for patient care.

The SAC varies significantly between centers and is partially explicable by differences in staffing and evaluation protocols. Efficiency in managing the SAC is a central component to the financial stability of US programs. This charge is typically the largest single cost component of a kidney transplant procedure and is a significant portion of the extra renal organ transplant cost.

The mechanism for recouping these costs depends upon the payment source. For governmental payers, the SAC/Organ acquisition charge for Medicare beneficiaries is incorporated into the yearly institutional Medicare cost report. Payment for these products and services will be reimbursed to the institution on an annual basis according to the Medicare cost formula. Under this system the ability to recover Medicare associate costs lags for a year, but the funds are reimbursed to the system.

In the case of privately insured individuals, recouping organ acquisition costs is incorporated into the recipient bill. Private insurers typically do not directly pay for potential or actual donor care, but payment for acquisition of an organ is common. The majority of these donor care costs are then recouped after a transplant is performed, although some insurers will make modest "Phase of care" payments for recipient evaluation and wait-list management. Nonetheless, as recipients can stay on the wait-list for years and not be transplanted, the need for resource stewardship is evident. The uncompensated institutional costs can become significant through a variety of means such as large numbers of inactive people remaining on the wait-list, repetitious histocompatibility testing and follow-up, evaluation of potential live donors that do not proceed to donation and inefficient allocation of costs to the charge center. Under such circumstances, as these costs are allocated into the SAC, the resulting high SAC will either decrease the transplant contribution margin from bundled payment agreements with the payer or will make contracting with the insurer difficult because of the high "fixed" costs. The process of understanding and managing this cost center is critical for the financial wellbeing of a transplant program. Furthermore, because a patient's source of transplant payment may change over the time, the processes to gain compensation for institutional transplant expenses demands an intimate understanding of the financial components of the public and private payers.

Government and private insurance and the individual recipient

The payment system for transplant services has been dynamic and continues to evolve. In past times, charges generated from professional and institutional services for organ transplantation were presented to the patient's insurance company for payment. All or a proportion of the charges were then reimbursed to the professionals and to the facility. In the era when transplantation was an uncommon procedure, the centers could demand lucrative payments for their services. But the numbers of transplant programs has proliferated and cost management must be a significant part of a transplant

program's business plan since transplantation reimbursement has evolved away from the charge-reimbursement model. The private and governmental payment systems evolved along somewhat disparate paths and the institutional strategy to remain financially viable has to adapt to the disparate models.

Governmental providers

In most countries, the government pays for a major portion of the population's healthcare. Care is delivered through government owned hospitals (Ministry of Health hospitals, as in many countries) or through military hospital or Veterans' Administration hospitals, as in the US. However, the most common US government payment for healthcare is as a third party payer, with payments directed to independent providers (hospitals and physicians) that qualify to care for Medicare and Medicaid beneficiaries. The potential government beneficiary must qualify for either the federal Medicare or state Medicaid system. Medicare provides healthcare payments for the largest single group of people in the US (over 100 million people). Even with a more limited reimbursement from governmental payers, there are few healthcare systems that can afford to ignore caring for this expanding population.

Volume and outcome requirements are not new for a program or center to qualify as a Medicare provider of transplant care. However in 2007, the Conditions of Participation (COP) to provide transplant care for Medicare beneficiaries went into effect. These conditions placed a new level of process and personnel requirements upon the institution and transplant center. It became necessary to achieve patient and graft survival outcomes defined by the SRTR in addition to specific volumes of transplants. Failure to meet the expected outcomes jeopardized participation in the Medicare system. While there is an opportunity to argue that mitigating factors explain poor outcomes, failure to convince the Medicare panel places the program at risk of losing Medicare affiliation and payments. In addition to performance volume and outcome requirements, the COP requires enhanced processes for quality assessment and process improvement and live organ donation to name a few, which require additional personnel, such as a live donor advocate. While there is some overlap with OPTN membership requirements and policy, there are sufficiently unique requirements that a transplant program or center must take notice of these differences. Medicare assesses compliance with the COP by reviewing the routine volume and outcome reports provided by the SRTR and by on-site CMS audits/inspection every three years. Losing CMS certification is devastating for a program's reputation and patient referrals so all attempts are made to avoid this possibility. In some instances, transplant programs have shut down because the costs of complying with the COP are too high.

Private insurers

The contemporary trend is for the private insurance companies to place increasing financial liability upon the transplant program/center. The fixed bundled payment provides similar amounts for the transplant procedure irrespective of whether the recipient is the "ideal" patient or the one with multiple co-morbidities. The resources required to provide care for these populations is quite disparate, but the trend is for compensation to remain constant. It has become the program or center's responsibility to effectively manage resources, while maintaining satisfactory (SRTR risk adjusted) outcomes. One of the unspoken options is to restrict transplant care for those individuals that consume/cost too much. It may be that an acceptable outcome can be achieved, but that the cost of providing such care exceeds the reimbursement of the private payer. Managing outcome variability within the center's patient population and the resources consumed by those patients has become essential to the financial viability of the program.

As mentioned previously, most large private insurers have established relationships/networks of transplant centers that provide the majority of care for their insured population. The patients are preferentially directed to receive care at the participating institutions. Because a recipient will have more personal financial liability at an "out of network" center, the financial disincentive drives volume to those centers with optimal payment relationships. Criteria to enter into those relationships are typically dependent upon the stipulations of the private insurer, and include reasonable pricing, adequate transplant volume, satisfactory outcomes and available services. Payment to the transplant center is intended to compensate for pre and post-transplant care, the procedure and hospitalization as well as the cost of the organ. It is imperative that the transplant center effectively manages its costs and negotiate effectively for variable compensation when the patient course generates outlier expenses exceeding base payments. The evolving compensation system demands that the transplant center match its resources and processes with the needs of the population that it serves. If appropriate compensation agreements are not made with the companies providing payments for transplantation, a center will find itself in an untenable financial condition.

A recent transformation has occurred within transplant contracting with the incorporation of the publically available "risk adjusted" program specific outcomes (PSR) provided by the SRTR. These outcomes have become widely adopted as a qualification for network participation, despite the fact that they were not originally designed to be used as such a tool. The metric was intended to be used by the OPTN as a system quality assessment tool and a tool to help patients understand a center's experience and transplant outcomes. However, now programs are removed from a payer network when threshold performance criteria are not met, irrespective of the vagaries of how these criteria were derived. The consequence associated with falling out of networks is a reduction of patient flow, loss of revenue and a damaged reputation.

A significant proportion of smaller and self-insured groups cannot afford the variability of risk associated with the delivery of transplant care. Under these circumstances, transplant coverage can be purchased from a large "reinsurer," such as the United Health Group. In this model, the reinsurer typically will develop even more restrictive networks (Centers of Excellence, COE) that contract with a few high-volume, cost-efficient programs. The insured are encouraged to obtain care at a relatively few centers in the country, while the insurer pays transportation and lodging costs so the insured can be treated at the preferred center. The cost of transplant care in this scenario is reduced by the use of "buying" power and the promise of volume to specified transplant centers. A significant percentage of insured patients receiving organ transplants today have their payments coordinated through such a reinsurer. There is some interest in systems where patients and potential donors are transported to other countries to receive transplant care at a reduced financial liability. These relationships have significant moral, procedural and follow-up drawbacks for systems and patients as greater travel distance imposes personal and family hardships (see Chapter 138).

Balancing patient-need, managing outcomes and finance based expectations

In order for any transplant program to continue to supply care, it is essential that there is money to pay for people, resources, organs and services. It is crucial that one understands how resources are allocated and generated. In systems where transplantation is a cost center, for example a national health system, it is necessary that a program have continuous funding to allow for planned numbers of organ transplants. Knowledge of the resources necessary to provide the ongoing services is critical. If the allocated resources are insufficient to provide for all citizens needing care, then some form of resource allocation/rationing must become operative. In this system, there is commonly budgetary sharing with other services, such that when one program is under budget, either services must be curtailed or monies taken from another, better performing, service. The dialogue about resource allocation/rationing of healthcare monies quickly expands past the strict medical needs of a population and into the realm of economics, politics, ethics, and law. Centers in this type of system are franchised by the state and subject to a different set of dynamics than those in a market driven environment.

In systems where transplantation constitutes a revenue center, such as most US programs, a continual volume of transplants is essential for program viability. Increasing transplant volumes generally leads to more revenue, reputation and opportunity. Reduction in the volume of transplants is followed by programmatic revenue reduction and subsequent need for restructuring of program resources. Understanding patient flow and resource consumption has become a critical business metric. Obtaining program access for a broad population of revenue-generating patients is fundamental to the success within the revenue center model. The first hurdle required to assure patient flow is to have access to those systems providing healthcare coverage for large volumes of patients. In the current US system, this requires participation within Medicare and the large private insurer networks. A common prerequisite for all system participation is to meet OPTN membership criteria and volume and outcome standards. The only independent, comparative program metric remains the publically released SRTR generated Program Specific Report (PSR). Transplant centers must be cognizant of the information about performance that is in the public domain. The variables used for risk adjustment are published and transplant programs must use ongoing assessment of their recipients to assure that risk adjustment is appropriate for their population. Being flagged for outcomes less than expected is damaging to a program. In the event that observed outcomes are less than expected outcomes, it becomes essential to modify practice and selection. Risk adjustment is not perfect, so older patients with multiple co-morbidities may be excluded from transplantation or organ acceptance may become more selective to optimize chances for good outcomes. Careful monitoring of public release of transplant outcomes is not only important for public perception, but also for business. Program discipline to align patient risk, organ quality and organ acceptance is required to improve outcomes after being flagged for poor outcomes. While the public release of risk adjusted outcomes remains an imperfect system, its practice has made program quality assessment and process improvement (QAPI) a necessary component of a transplant business plan.

The other major component for a viable transplant program in the revenue center model is effective management of the cost of transplantation. The cost of providing services cannot routinely exceed the revenue generated by giving the service. While one can attempt to negotiate for a higher reimbursement rate with more financial cushion, as transplant centers have proliferated, the insurers have been able to diminish reimbursement and shift risk to the center. Under such circumstances where the financial margin is shrinking, it is essential that a center understand where inefficiencies occur. Without an ongoing QAPI analysis, the most commonly used technique to improve outcomes is to limit care to patients with diminished risk. This technique is typically effective as most transplant professionals can deal effectively with the straight forward patient. However, such constriction of patient selection limits the access of people who would benefit from transplant care and diminishes volumes to the financial detriment of the program.

Summary

Fiscal balance is a necessary prerequisite for a sustainable transplant program, irrespective of whether the funding source is government or private insurer. Revenues entering into the health system must be balanced with an understanding of how they are spent for people, care delivery, organ acquisition and needs of patient flow. This simple relationship has been confounded by the extreme requirements for regulation, performance metrics, network participation and bundled payments. Knowledge of these integrated systems is useful, but implementation of processes to monitor compliance has become necessary to assure sustainability.

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CHAPTER 141

Drug Development in Solid Organ Transplantation and the Approval Process for Transplant Immunosuppressants

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Introduction

This chapter will describe the general steps from drug discovery to transplant-specific approval, with an emphasis on late stage (Phases 2 and 3) development and advancement through the health authority approval process. It will focus on development of drugs that are explicitly indicated for use in transplantation and is meant to provide the reader with a satisfactory but non-exhaustive awareness of the processes leading to drug availability for general clinical use. The goal for successful introduction of a new immunosuppressant drug product is to offer physicians and their patients an improvement over existing therapy in such a way that it can be specifically recommended for use in the targeted setting. The key steps from discovery to registration are depicted in Figure 141.1.

During the discovery phase, large collections of chemical compounds are screened using various in vitro assays to identify those with the desired biochemical activity. Active compounds that meet the desired criteria are further screened through more focused cell-based assays and in vivo experimental models, preferably in an accepted non-clinical transplantation model. Compounds which demonstrate the intended activity are advanced for further non-clinical evaluation (see Discovery section further on).

The technical research and development of the drug substance (i.e. active chemical moiety), and the final drug dosage form are conducted in parallel with initial screening and non-clinical testing. Initial laboratory scale production is further optimized and scaled for production as the compound advances through late phase clinical development. As development progresses to clinical testing, candidate compounds must be manufactured into the various commercial forms (tablet, capsule, liquid, etc.) which meet regulatory standards for potency, stability, impurities, etc. (see Product characterization, formulation, delivery, packaging development section further on).

Pharmacokinetic studies are used to determine the absorption, distribution, metabolism, and excretion characteristics of the molecule in vivo to select candidates with an optimal profile (see Pharmacokinetics, drug disposition, bioanalytical testing section). In parallel, various toxicological studies are undertaken in vitro and in vivo to exclude candidate molecules with mutagenic/carcinogenic potential or organ-specific toxicities, for example, hepatic, renal, or cardiac toxicity (see Preclinical toxicology testing from discovery to approval section further on). One or more candidate compounds emerge from this extensive selection process for progression to human (clinical) testing (see Clinical trials further on).

The Clinical Trials section in this chapter describes the different clinical trials performed throughout development. Phase 1 studies assess initial human safety, tolerability, and clinical pharmacokinetics. Changes in a biomarker or other pharmacodynamic measure(s) may also be tested to measure activity or toxicity. The first true assessment of clinical activity in man is often called a Proof of Concept (PoC) study. The purpose of the PoC study is to confirm activity of the compound relative to an established standard of care regimen in the intended patient population. If the PoC is confirmed, larger Phase 2b studies are conducted to further profile and refine the safety, efficacy, and optimal dosing regimens for the compound. Phase 3 or 'confirmatory' trials are usually larger, statistically powered studies of 12 months or longer to confirm efficacy, safety and tolerability from the Phase 2b studies. Upon completion of a Phase 3 program demonstrating efficacy, safety, and tolerability, the data generated from the numerous studies conducted during the research, technical, non-clinical, and clinical programs are compiled into an extensive submission dossier or New Drug Application (NDA).

The drug submission review and approval process is complex, including manufacturing, preclinical, clinical efficacy, and safety information. The process is highly regulated and requires detailed documentation to support health authority requirements. Frequent communication between the submitting company (sometimes called the "sponsor") and the health authority, beginning well before initial studies in humans and continuing throughout the entire clinical trial and submission process, permit the relevant stakeholders to negotiate the complete requirements of the NDA or dossier submission. It is important to reach a common understanding with health authorities regarding any potential concerns and risks. As a drug candidate advances through clinical development, health authorities provide valuable guidance regarding assessment of clinical endpoints that will ultimately lead to labeling the product for prescribing information. Today, new drug submissions are often

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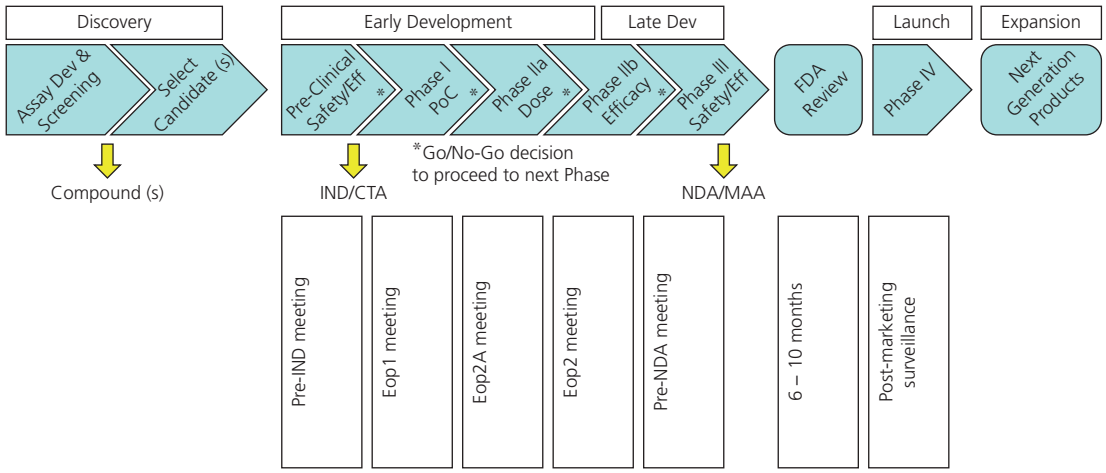

Figure 141.1. Phases of drug development with key regulatory applications and meetings.
Dev, development; Eff, efficacy; PoC, proof of concept; IND, investigational new drug application in the US (Europe: CTA, clinical trial authorization); EoP, end of Phase 1; EoP2A (after IND, but prior to end of phase 2); EoP2, end of Phase 2; NDA, new drug application in the US (Europe: MAA, marketing authorization application).

electronically transmitted to health authorities with a typical submission size ranging between approximately 4–20 gigabytes and organized into various modules (see Road to registration: drug approval process further on). If the application is accepted, it undergoes simultaneous review by different specialty groups within the relevant agency.

This entire process requires at least a five to ten-year commitment and significant resources to adequately characterize the safety and efficacy and to obtain market authorization. Throughout the development, overall quality and validity of results are ensured by the sponsor's compliance with the various guidelines and regulations issued and audited by the global health authorities.

Discovery

Pharmacological targets for drug-based interventions may come from known biochemical pathways or novel targets, such as components of signaling pathways identified through molecular/genomic approaches. In drug discovery, large arrays of compounds are subjected to successive screens seeking a specific activity; for example, inhibitory activity against a target receptor in an in vitro or cell-based system. Compounds with relevant activity may be further modified chemically and then re-screened to identify candidates with improved activity against the specific target. Similarly, rational approaches may derive directly from a known drug-target interactions or optimization of activity based on structural modification(s).

Candidate molecules with activity in vitro and cell-based assays are then tested, for example, in rodent models of solid organ transplantation. Such models provide preclinical proof of principle and a preliminary assessment of safety and activity based on histology and blood chemistry. Since immunosuppressants are used in combination therapy, it is common to assess the potential for additive or synergistic effects. Due to interspecies differences in pharmacokinetics and pharmacodynamics, non-clinical activity may not always reflect human activity. Consequently, positive studies can provide a solid basis for candidate selection, and discussion with health authorities and investigators. However, a restrained and cautious transition into the clinical setting is absolutely essential.

Product characterization, formulation, delivery, packaging development

During discovery and development, a drug product may require scale-up from milligram-sized laboratory batches to multi-ton production scale manufacturing. When producing clinical batches for use in human studies, this process becomes even more complicated and regulated to ensure safety for human use. The drug sponsor must follow stringent regulations called Good Manufacturing Practice (GMP). The details concerning the physicochemical characteristics, analytical methods, as well as the manufacturing of the drug product and formulation(s), must be submitted to the relevant health authorities prior to use in humans. Detailed guidance is provided by the health authorities (www.ema.europa.eu; www.fda.gov). Additionally, updates must be submitted to health authorities whenever significant changes occur at the manufacturing site, and/or in the process or formulation. This information is referred to as Chemistry, Manufacturing and Controls (CMC), and CMC represents a significant portion of a regulatory submission.

Pharmacokinetics, drug disposition, bioanalytical testing

Understanding the disposition and pharmacokinetics of small molecules and biologics is a central component of the drug development process. Pharmacokinetic data generated during the discovery phase in a non-clinical species allows better prediction and understanding of the compound's pharmacology; this is confirmed in later clinical trials.

Bioanalytical testing

The first step in understanding the non-clinical and clinical pharmacokinetics is to develop a reliable bioanalytical assay. Depending upon the properties of the compound, route of administration, and site of activity, assays must reliably measure drug concentrations in biologic fluid and/or tissue samples. The most common research platform for compound measurement relies on the sensitivity and selectivity of high performance liquid chromatography (HPLC) paired with mass spectrometry (MS) which allows for precise, dependable measurement of compound concentrations that can be

transferred across laboratories and developed quickly with minimal time and cost. During later phases of clinical development, solid-phase or immunoassays may be developed to provide a platform for use in clinical practice. In transplantation where therapeutic drug monitoring (TDM) is frequently used, a commercial assay must also be validated in the clinical setting prior to drug approval. The approval process for an assay/device is a separate submission from the clinical dossier or NDA and is usually conducted in parallel.

Pharmacokinetics and drug disposition

The four phases of absorption, distribution, metabolism, and excretion are interrelated; alteration of one component may lead to changes in one or more of the others. Collecting blood or other tissue samples allows for quantification of the various pharmacokinetic parameters that describe the timing and extent of each. Using radiolabeled drug allows the definitive assessment of mass balance in non-clinical and clinical subjects. For orally administered drugs, absorption is the first measureable parameter and is described through serial pharmacokinetic measurements. Concentration data from the entire dosing interval can also be used to calculate the extent of absorption, that is, area under the curve (AUC) or exposure. The distribution of small molecules can be quite complex. A non-physiological parameter associated with this phase is the apparent volume of distribution (Vd/F); this provides insight into the extent of the distribution throughout the body. Computer modeling can predict movement of the drug and metabolites between various tissue compartments. This modeling data can aid understanding the pharmacodynamic effects of a compound in order to optimize dosing and route of administration. For most small molecules, from the moment a compound is administered, various metabolic processes are actively modifying and clearing the compound from the body.

The initial assessment of metabolic clearance pathways can be conducted in silico using the structure of the molecule. These models can be further refined with various in vitro models, such as isolated liver microsomes, human hepatocytes, and metabolic cytochrome binding assays. Assessment of the in vivo metabolic fate of drugs is initially conducted in non-clinical studies using ^{14}C-labeled drug and subsequently confirmed in human subjects. By labeling the compound, both parent (administered drug substance) and metabolite structures and PK can be elucidated and compared across species. This latter point is important when selecting the appropriate species for non-clinical toxicology. Finally, the elimination of small molecules can occur via many routes; the two most common are biliary/feces and urine. Most compounds are eliminated as highly transformed, inactive metabolites.

Toxicokinetics

Measuring drug concentrations and calculating the various PK parameters from samples collected during non-clinical toxicology studies is also key to understanding the concentration-response for various single and repeat-dose toxicology findings. These data also allow prediction of possible future human doses, simulation of human pharmacokinetics, and determination of a predicted safety threshold. Therefore, pharmacokinetic data collected during the non-clinical development program drives many decisions during the drug development process and provides the basis for discussing cross-species differences with regulatory authorities.

Pharmacokinetic clinical trials

Across the phases of development, pharmacokinetic data are collected to first understand how humans process the compound and later, to understand how drug concentrations may be driving efficacy, toxicity, or other relevant biological findings. The impact of disease and other drugs on metabolism and elimination of investigational compounds is the principal focus of pharmacokinetic profiling studies. The potential for drug-drug interactions is more of a concern with small molecules since biologic agents are only rarely affected by other co-administered drugs. The most common type of small molecule interaction is metabolic where a co-administered drug or food, for example, grapefruit juice inhibits or induces the metabolism or efflux of the drug of interest. It is important to profile the potential effects of both the investigational drug and the relevant co-administered drug(s) to provide proper guidance for clinicians in the final use guidance (or labeling). The data defining such interactions are typically generated in healthy volunteers using a crossover study methodology. The extent of drug interaction testing required is driven by the metabolic fate of the investigational drug and the potential to have an effect and/or be chemically altered by metabolism. Global health authorities have released a number of guidance documents to help the pharmaceutical industry standardize and focus the drug interaction profiling process (www.fda.gov; www.ema.europa.eu).

Also, pharmacokinetic profiling studies are conducted in pediatric patients and in various disease states, such as renal and hepatic impairment, to understand the effects of age and disease on the pharmacokinetic behavior of a compound. Other demographic factors that may affect pharmacokinetics can be assessed through dedicated trials, or sometimes through a process called population pharmacokinetics. This utilizes a sparse population pharmacokinetic sampling approach during Phases 2 and 3 clinical trials to gather pharmacokinetic data from a population of interest. The data are then analyzed to assess the effect on large population-related factors, such as age, gender, ethnicity, concomitant medications, or various disease states, on the pharmacokinetics of the compound.

Preclinical toxicology testing from discovery to approval

At first glance, the principles of preclinical toxicology testing for a new candidate in drug development appear standardized and highly regulated. A standardized set of test systems (www.oecd.org) are utilized and must adhere to Good Laboratory Process (GLP) guidelines [1].

Tool box for the toxicologist

Toxicology studies typically involve in vitro and in vivo studies and other test systems to examine the relationship between the dose, exposure of an investigational compound and the resultant toxicity(s). When designing an experiment or evaluating the outcome of a study, the toxicologist must be aware of the potential impact of factors, such as route of administration, dosing frequency, duration of administration, pharmacokinetics, or drug interactions with co-medications that may influence the toxicity potential of a drug. This is particularly important in organ transplantation since the standard regimen often includes a combination of drugs.

Regulatory environment and impact on toxicology testing

Animal research in drug development only serves as a surrogate for efficacy, and similarly safety testing in humans has limitations. The use of animals in research and development is governed by considerations of animal welfare and the ethical requirement to replace,

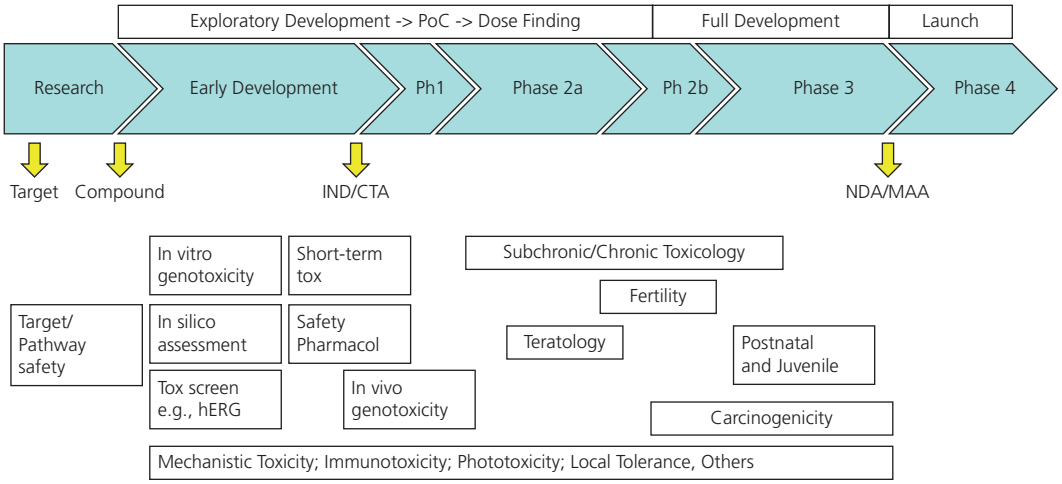

Figure 141.2. Flow chart of toxicity testing in drug development.
Ph, Phase; PoC, proof of concept; IND, investigational new drug application in the US (Europe: CTA, clinical trial authorization); NDA, new drug application in the US (Europe: MAA, marketing authorization application).

reduce and refine (the 3 R's) animal experimentation whenever possible [2]. The most important framework describing guidelines on animal toxicity testing come from the International Conference on Harmonization (ICH) on the technical requirements for registration of human medicinal products (www.ich.org/products/guidelines). Specific guidance is also issued by the US Food and Drug Administration (FDA; www.fda.gov/drugs/), and the European Medicines Agency (EMA; www.ema.europa.eu/).

Based on the experience of limited predictability of drug-induced adverse events in animals with respect to humans [3,4], evaluation is usually required in two or more species (one rodent, one non-rodent). Traditionally, and in line with the ease of animal husbandry and handling, the rat and the dog are the preferred species for toxicity testing. This paradigm was especially useful in developing early transplantation drugs, such as azathioprine and cyclosporine. The introduction of monoclonal antibodies in the late 1990s, however, highlighted the need for an alternative approach; testing was not relevant in a species where the antibody did not cause the pharmacological reaction being studied [5]. Accordingly, the non-human primate was introduced for toxicity testing of therapeutic monoclonal antibodies and other biologics.

In principle, non-clinical safety testing of biologics follows the same considerations as outlined in ICH M3(R2) (www.ich.org/products). Specific aspects and deviations on safety testing for small molecules are described in ICH S6(R1). Additionally, the dog as a non-rodent species for toxicity testing proved extremely difficult for second generation transplant drugs due to the exaggerated toxicity on the gastrointestinal tract [6,7]. Specifically, the (mini) pig has become an alternative non-rodent species which has a high potential for safety assessment [8].

Designing a toxicology program to support drug development

The non-clinical safety assessment strategy in drug development focuses on two major goals; namely, to provide the necessary information for safely testing a drug candidate in man for the first time so that the necessary preclinical safety information is available to extend clinical studies into Phases 2 and 3 and also to support registration. Therefore, deliverables from toxicology are often planned as "study packages", and the execution is triggered by milestones achieved in clinical development. A typical flow chart for a toxicology program is depicted in Figure 141.2.

The IND stage

The safety information needed to support the first dosing of a novel agent in humans is supported exclusively by animal data, placing the burden of proof in decision making, upon the expertise of the pharmacologist, pharmacokineticist, and toxicologist. The majority of adverse drug reactions in man are dose-dependent and predictable from primary or secondary pharmacology [9]. Therefore, a prerequisite for proper safety testing is that the therapeutic agent must trigger a similar pharmacological response in animals being tested and humans. If this is not the case, the differences across species must be elucidated, and these results also become part of the risk assessment strategy and algorithms. Additionally, molecules that exhibit their effects chemically have the potential to cause chemically-mediated toxicity; these toxicities are usually unrelated to the intended pharmacological interactions with enzymes or receptors; they are often called "off-target" effects. An example is the interaction of proteins accumulated in a cardiac potassium channel, which is responsible for repolarization (hERG); blockage can precipitate arrhythmia and even sudden cardiac death. This type of effect has been the reason for a number of drug withdrawals from the market [10] and accordingly, evaluation of this potential effect has become a major focus during development. It is well known that some drugs show fundamental differences in their pharmacokinetic behavior between animal species and humans [11]; similarly, gender differences in absorption, distribution, metabolism, and excretion [12] may influence the safety profile of a compound. Therefore, the preclinical safety strategy must examine and consider species differences in a drug's pharmacokinetic parameters, such as absorption, protein binding or tissue distribution, and elimination before testing in man is undertaken [13]. The basic prerequisite package consists of appropriate pharmacology and pharmacokinetic experiments, plus a number

of toxicology studies covering safety pharmacology, genotoxicity, and acute and repeated-dose toxicity. As needed, exploratory mechanistic toxicity studies are also conducted to provide a good prediction of human safety risk [14] and help to reduce the late-stage attrition rate [15].

The safe starting dose for humans in Phase 1 testing is based on safety margins derived from these extensive toxicity studies. The approach is similar for small molecular weight compounds and biologicals [16], but special considerations are given to immunomodulating compounds [17] or highly pharmacologically active compounds [18,19]. The safety margin (also called therapeutic index) is based on a comparison of systemic exposure from animal efficacy models and results from toxicity studies. Other calculations, such as body surface area, allow a comparison between animals and humans and are sometimes used (FDA guidance on MRSD, www.fda.gov/Drugs/). The key criteria for a Go/No-Go decision for a first-in-man (FIM) study is based on the overall evaluation of the non-clinical safety data relating to the risks identified, as well as a prediction of the likelihood that any observations would occur in man. The benefit-risk assessment is driven by a number of considerations, including the ability to monitor and reverse off-target or adverse events. Following a comprehensive evaluation of these non-clinical safety data, an Investigative New Drug (IND) application is prepared.

Support of clinical development and marketing authorization

If a drug candidate demonstrates promising results in early clinical studies, this triggers initiation of a non-clinical safety program to support continued development in Phases 2 and 3 and eventually, to support registration. Based on international conference guidelines accepted by most health authorities ICH M3(R2), the full development toxicology program centers around covering the duration of expected human exposure. Toxicity studies are conducted in two species (one rodent and one non-rodent) of at least similar duration to that expected in clinical trials. Guidelines dictate a maximum duration of treatment of six months for rodents and up to nine months for non-rodents, even if lifetime treatment is intended in patients. The purpose of these sub-chronic and chronic studies in animals is to refine the dose-response characteristics and to establish the threshold exposure for inducing adverse events upon prolonged drug administration. These studies may additionally identify any new and/or unexpected toxicity upon longer term use. Beyond the standard toxicology studies, two special types of toxicity studies may also be initiated to assess additional hazards or risks; namely, reproductive toxicity and carcinogenicity studies. Since ethical considerations preclude direct investigation, results from such studies serve as a surrogate for the risk assessment in humans. These studies may not be required if treatment in patients is for a short period of time, is life-saving, meets a high unmet medical need (HIV, oncology), or certain groups of patients (women of childbearing potential) will not receive the drug. Results from reproductive toxicity and carcinogenicity studies are presented in the package insert of a compound, together with guidance on the presumed relevance and significance for the intended patients.

For drugs used in pediatric patients, European regulatory requirements on the Pediatric Investigational Plan (www.ema.europa.eu/ema) request toxicity studies in juvenile animals to support clinical trials and registration in some cases. Therefore, careful consideration of juvenile animal studies [20] and an upfront discussion with health authorities would be useful [21].

Reproductive toxicity studies

Reproductive toxicity studies assess the effects of a compound on fertility, embryo-fetal toxicity (e.g. risk of spontaneous abortion or fetal malformations), or pre and postnatal development (e.g. risk of late fetal death, retarded development or functional deficits in early postnatal life). Again, data from animal toxicity studies serve as a surrogate since human data will usually not become available for many years after approval and introduction into general use; low incidence events may take considerable time before relevance is appreciated directly from the human experience. Some drugs have a patient registry program for pregnancy, or preclinical flags have triggered more intense clinical surveillance [22]. In addition, results from preclinical studies help guide the potential nature of effects seen, for example, on fertility. Such results may trigger clinical investigations, which may corroborate preclinical data, as was seen with potential effects on male fertility caused by sirolimus [23].

Carcinogenicity studies

The most time and resource-intensive toxicity studies are those evaluating potential carcinogenicity. These are required for all pharmaceuticals whose intended clinical duration of use exceeds three to six months. This affects all small molecule drugs for transplant patients. Drugs intended for certain oncological indications, biologics, and drugs used for induction therapy (e.g. anti-IL-2 monoclonal antibodies) may not require carcinogenicity studies [24]. Additional studies may be mandated when the chemical is related structurally to other products with known carcinogenic potential, or the biological action, long-term toxicity, or mutagenic study results may indicate a potential for carcinogenicity. In such cases, health authorities may ask for the results before Phase 3 studies may be started. When needed, this type of guidance can significantly extend development program timelines. The association between cancer risk and immunosuppressive therapy was noted well before the cyclosporine era when allograft recipients were treated with azathioprine and prednisone [25]. The increased risk of malignancies associated with immunosuppressants in transplantation is mainly concerned with virus-associated malignancies, such as lymphomas associated with Epstein-Barr virus, human herpes virus 8 (also known as Kaposi's sarcoma-associated herpes virus), and skin and genital cancers associated with papillomaviruses [26]. Aggressive immunosuppression in children with the monoclonal antibody OKT3 or with tacrolimus has been associated with an increased incidence of malignancies, such as lymphoproliferative disorders) [27,28]. Accumulated evidence from clinical experience indicates that the risk of developing certain malignancies in transplantation is associated more with the level of immunosuppression than with a specific drug.

Supplementary studies

Lastly, depending upon the specific intended use of a therapeutic agent, the toxicologist may design and conduct additional studies in vitro or in animals to support the conduct of clinical trials or registration. For drugs being developed in organ transplantation, assays could be required, for example, to elucidate phototoxicity [29], immunotoxicity [30], local tolerability, mechanistic aspects of toxicities seen in animals or man, or combination toxicities of a new drug regimen.

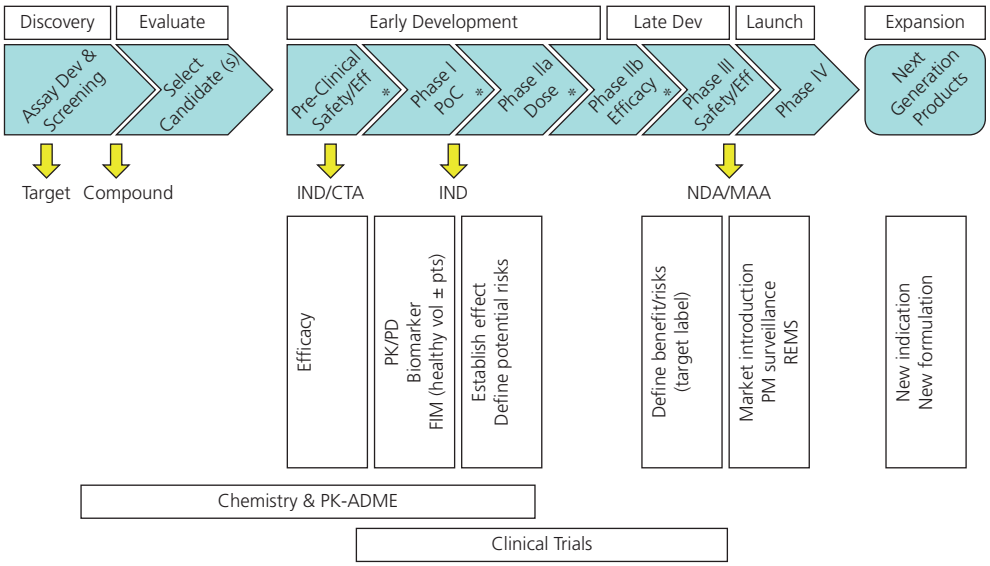

Figure 141.3. Phases of drug development.
Dev, development; Eff, efficacy; PoC, proof of concept; IND, investigational new drug application in the US (Europe: CTA = clinical trial authorization); NDA, new drug application in the US (Europe: MAA = marketing authorization application); FIM, first-in-man; vol, volunteers; PM, post marketing; REMS, risk evaluation and mitigation strategy; ADME, absorption, distribution, metabolism, and excretion.

Clinical trials

To gain health authority approval for novel drug candidates, both safety and efficacy must be demonstrated in the intended patient population. Some approved compounds do not require a full development program as depicted in Figure 141.3; for example, demonstrating bioequivalence is acceptable for generic compounds.

Phase 1 — Clinical development

Phase 1, also referred to as clinical pharmacology, covers the initial clinical profiling of a compound in humans. These studies include the initial assessment of the safety, tolerability, pharmacokinetics and pharmacodynamics of single and multiple doses, and mechanistic profiling of the compound in humans. Depending upon the molecule, five to 40 individual studies may be needed to describe the relevant clinical pharmacology of a given compound. These studies serve as the foundation upon which later phases of development (and eventually labeling) are built, including expected drug-drug interactions and dosing recommendations for special populations.

First in man study

After the biologic activity has been established, biopharmaceutical properties characterized, and potential toxicities identified in non-clinical studies, the compound transitions from research to Phase 1 clinical development for evaluation in human subjects. The human pharmacokinetics, tolerability, and toxicity thresholds (see Pharmacokinetic clinical trials section) are determined from the first-in-man (FIM) study. If the compound is poorly tolerated, exhibits poor biopharmaceutical properties, or subjects demonstrate previously unidentified toxicities, the results may terminate further development.

In general, the FIM study is a randomized (usually 1:1), blinded, placebo-controlled, parallel group, single ascending dose (SAD) design. The initial dose selection and upper boundary of dosing for the FIM study is based on results from non-clinical toxicology studies and the established no observed adverse effect level (NOAEL) using allometric scaling (see The IND stage). Dose escalation is typically time-lagged; that is, one dose-level at a time is assessed prior to proceeding to the next dose. The decision to escalate is based on the overall safety, tolerability profile, and guided by toxicities observed in preclinical studies. Dose escalation typically occurs in half-log or one-log steps, but this may be constrained to a simple doubling of dose depending on the preclinical dose response. This rapid log-based escalation allows profiling of a large dosing range, for example 0.1 to 1000 mg, which minimizes pharmacokinetic overlap between doses. Such a design usually results in a rich dataset, allowing adequate characterization of human pharmacokinetic parameters, as well as dose- or concentration-dependent toxicities.

Depending upon the pharmacodynamic activity and the type of compound (e.g. small molecule or biologic), the FIM study may be conducted in a healthy volunteer or patient population. In the case of first-in-class novel immunosuppressant's, where the extent of pharmacodynamic activity and immunosuppression may be considered too high of a risk for healthy subjects, the FIM study may be conducted in a population where there is a potential benefit. This population might, for example, include stable transplant patients or patients with autoimmune disease.

Proof of concept design

A formal PoC study may be conducted once the tolerability profile has been established and single and multiple dose human pharmacokinetics have been characterized; this signifies the start of Phase 2a. The purpose is to rapidly establish the biological activity of the compound in patients with the disease, or in the case of transplant immunosuppression, as part of an immunosuppressant regimen to determine if the compound warrants further development in the intended indication. Depending upon the primary endpoint being evaluated, there may be two to three dosing cohorts of six to 24 subjects each. An established "gold standard" clinical endpoint is

usually used as the primary PoC endpoint, although a relevant biomarker might be used for decision making in certain settings. Since the primary goal of the study is to assess the potential for clinical activity with a small sample size, innovative statistical approaches may be used to predict success or failure, such as Bayesian methods.

Post PoC activity

When a compound moves into later phases of development, the Phase 1 profiling activities focus on establishing the detailed clinical pharmacokinetics, and pharmacodynamics of the compound. This includes drug-drug and drug-disease interactions, as well as consideration of special populations, such as effects on clearance in patients with renal and hepatic impairment. Additional mechanistic studies to better elucidate the biological activity of the compound in man or the mechanisms behind toxicity may also be conducted at this time. During recent years, depending on preclinical signals (e.g. hERG inhibition; see The IND Stage) a thorough QT study (ICH E14, www.ich.org) has become an industry standard to profile effects on cardiac repolarization, specifically QT interval prolongation.

The scope of the clinical pharmacology package (regulatory studies required to characterize the clinical pharmacology of the molecule) is dependent upon the specific pharmacology. For example, the package for a monoclonal antibody can be quite straight forward since drug interactions and QT prolongation are not expected, and non-specific activity is unlikely. On the other hand, a small molecule, metabolized by the liver which binds to a number of "off-target" receptors, may require 30 or more clinical pharmacology studies to profile the compound under various clinical treatment conditions. The knowledge collected from these studies is essential for both the design of subsequent clinical trials as well as providing labeling regarding the safe use of the investigational drug.

Phase 2 — Dose finding, and the assessment of preliminary efficacy and safety

In solid organ transplantation, the most common study design in Phase 2 and beyond is an open-label, randomized controlled trial against an active comparator. The choice of active comparator is usually the contemporary, approved standard of care in the indication under investigation. If practical, some level of blinding is preferred to minimize potential bias. A Phase 2 development program may include only one study, but the more often utilized 'learn and confirm' construct includes two or more studies prior to Phase 3.

The primary objective of most Phase 2 transplant studies is to demonstrate efficacy of the investigational regimen compared to the standard of care. Study endpoints can be as short as three months post-transplantation, but usually 6 or 12 months are needed to ensure adequate exposure to therapy and to demonstrate that the treatment response is durable. Global health authorities may also require longer-term data for approval; hence, following a primary analysis at 12 months, studies are usually continued for 24 months or longer. A key goal during Phase 2 is to establish the dose (or exposure) that provides optimal efficacy within the context of the planned treatment regimen. Depending upon the size of the study, the scope of pharmacokinetic assessments needed, efficacy, and the task of establishing an exposure-response during the Phase 2 program can be straightforward; at other times, this can be impractical. Results from the single and multiple doses Phase 1 and PoC studies will support the determination of initial dose(s) to be evaluated in Phase 2. It is typical to test two or more doses of the investigational drug against the standard of care regimen to build the exposure-response data set. Sometimes, it is not possible to establish optimal dose and/or exposure data until the end of Phase 3.

Phase 3 — Confirmatory efficacy and safety/registration

Design and endpoints

Phase 3 trials are often referred to as "pivotal" or "confirmatory" because they provide the basis upon which a health authority will make a final determination of the safety, effectiveness, and labeling for the population of interest. With appropriate statistical power, the results need to confirm the correct dose (or concentration range) and demonstrate a clear risk-benefit based on the overall safety and efficacy. The topic of clinical trial design and the specific statistical challenges involved are covered in depth in Chapters 134 and 135 respectively. For transplantation, the duration of a Phase 3 program may span three to five years at a cost of $50–100 million. A common Phase 3 study design tests one or two treatment arms versus control, using a randomized, blinded (when possible) study design with a 12-month study endpoint. The sample size for a Phase 3 program is based upon the effect size and study endpoints (e.g. superiority or non-inferiority) when compared to active, standard of care as control. Acute rejection rates have decreased to the point where it is often difficult to show a significant difference between standard of care treatments and investigational drugs. Consequently, a study demonstrating superior outcome may require thousand(s) of patients per treatment arm, and this is impractical given the limited number of transplants performed each year. Therefore, many sponsors default to a "non-inferiority" approach with the intention of developing new agents that provide a secondary benefit, such as less toxicity or better tolerability.

For drugs intended to prevent post-transplant acute rejection, the traditional FDA endpoint for assessment of primary efficacy failure is a composite of efficacy criteria, including acute rejection, graft loss, and death and "patients lost to follow-up" (see later). Due to the limited potential sample size in Phase 3 transplant development programs, the FDA requires a more rigorous assessment. Therefore, the composite index is comprised of several outcome measures to provide confidence regarding efficacy. Combined (or composite) endpoints may allow better differentiation of the efficacy rate to help delineate non-inferiority between arms. Regardless, the individual efficacy components are also reported. The EMA requires a similar composite efficacy approach, in addition to a functional outcome measure for the transplanted organ. For example, in renal transplantation, this might include a comparison of renal function by estimated glomerular filtration rate (eGFR) as a component of the primary composite or as a co-primary endpoint. The FDA usually requires adequate justification to accept co-primary endpoints.

Since the FDA has tended to also include "patients lost to follow-up" as a component of the composite efficacy endpoint, missing data from discontinued patients represents a potentially significant confounder. This has, in fact, been a major concern for some Phase 3 trials. Understandably, the FDA typically adopts a statistically conservative approach and considers patients who are non-evaluable for the primary endpoint (including those who withdraw consent or are lost to follow-up) as efficacy failures. Therefore, it is also extremely important to ensure study completion for as many enrolled patients as possible. This continued follow-up

within the study, even when patients have discontinued the assigned treatment regimen, allows the efficacy endpoint to be fully evaluated for all patients and also provides valuable follow-up safety information. Differences exist among health authorities; for example, current EMA guidelines do not recommend inclusion of non-evaluable subjects as part of the primary composite endpoint to assess efficacy failure.

Patients and demographics

Health authorities also have an interest in regional demographics, usually requesting that Phase 3 trials include a study population representative of the intended population for use. In Europe, the EMA requests representation from a broad European population. In the US, given the known ethnic differences in response rates to transplant immunosuppression [31], the FDA also recommends that the drug sponsor consider enrollment of a meaningful percentage of African American and Hispanic patients.

Novel endpoints and biomarkers

Biomarkers offer the potential for new insights into transplant biology with potential clinical relevance, but validation using clinical outcome measures is required to gain broad acceptance by the scientific community and regulatory authorities. Standardization of the clinical protocol across regions and centers, as well as strict adherence to prespecified data endpoints and rigorous data collection, are critical components in validating a biomarker for use. Such standardization and clinical correlation provide confidence that results will accurately assess efficacy and safety, thereby helping to inform the overall benefit-risk decision.

Statistical considerations for Phase 3

A comprehensive statistical analysis plan addressing study objectives is prespecified and submitted to health authorities in advance of study completion, preferably during the design phase in order to confirm agreement. Included are measures of study quality (by examination of protocol deviations) and consistency of effect within important subgroups, such as regional and ethnic populations. Statistical concerns are also considered in depth in Chapter 134.

Placebo controls remain the "gold standard" for comparison in clinical research. However, this is not feasible for transplantation since graft survival requires immunosuppression, and it is unethical to withhold immunosuppression. Patients usually receive a combination of drugs to maximize efficacy and safety; the incidence of acute rejection in contemporary kidney transplantation approximates 15% in most recent trials. As described earlier, since increased immunosuppression usually brings improved efficacy (fewer rejections), but more safety issues (e.g. infection), it is difficult to achieve significantly better efficacy versus standard of care regimens without negatively influencing the benefit-risk profile. Therefore, as described earlier, clinical trials in transplantation are usually designed to show non-inferiority (NI) (demonstrating that the test regimen is not worse than standard of care) as the primary efficacy endpoint. With a non-inferiority design, the test agent shows differentiation by secondary measures, such as improved renal function, increased duration of benefit, decreased health care utilization, or perhaps an improved safety profile. Potential areas of differentiation or unmet medical need could also include areas, such as a lower incidence of infection, malignancy or diabetes mellitus or a better side effect profile with respect to gastrointestinal, hematological or neurological systems.

A well-designed NI study, in the setting of combination drug regimens, is based on estimating the effect of the test compound relative to a "putative placebo;" that is, the effect of the test compound must provide an effect over and above that expected from other elements in the combination. This can be challenging to demonstrate if historical data using the drug combination, excluding the test drug, are unavailable or are very sparsely reported in the literature [32]. Sometimes, information can also derive from disease area guidelines, previous trials, discussions with the regulatory agency, and knowledge of historical data on the comparator treatment. Also, the effects seen in the literature should be relevant, for example, in similar populations that are likely to respond similarly to the proposed study population.

According to FDA guidance, a successful NI justification should demonstrate constancy of effect over time. This might be challenging if the characteristics of the "current" transplant population differ significantly from historical data with respect to certain key risk factors. For example, the expanded donor and recipient criteria used in contemporary clinical practice are arguably different from those used previously in the literature. Often the NI margin proposed for Phase 2 transplant trials is more lenient based on operational feasibility, compared to the more stringent NI margin required in Phase 3 pivotal trials for drug approval.

Regardless of the design (non-inferiority or superiority), sample sizes are calculated with the goal to either provide reasonable confidence to estimate a difference in event rates between regimens (Phase 2 trials) or to have higher power to demonstrate the primary objective(s) (non-inferiority or superiority) in Phase 3 trials. Sample size calculation is a function of the expected event rates, estimates of variability, and the level of confidence desired. Typically, for Phase 3 confirmatory NI studies, a power of 90% is desirable, which is a 90% probability of demonstrating that the regimen is non-inferior to control.

Road to registration: drug approval process

Interactions with health authorities

In the US, prior to the first study of a new drug candidate in humans, an investigational new drug (IND) application is submitted to request authorization from the US Food and Drug Administration (FDA) to administer an investigational drug or biological product to humans in a clinical study. In Europe, a Clinical Trial Authorization (CTA) must be submitted and granted from each participating country's health authority and each study center's Ethics Committee. Some FDA divisions, such as the Center for Drug Evaluation and Research (CDER), recommend a pre IND meeting prior to filing an application. This provides early feedback on preclinical information to establish if there is agreement that a particular product has adequate supporting data to meet the requirements for an IND submission and to support early clinical development. It is also an opportunity to assess the overall preliminary clinical proposals and to discuss whether innovative approaches (biomarkers, diagnostics, etc.) will be included.

These early interactions are also important to establish if the product might qualify for special designation or a special review and approval pathway, for example, "orphan drug status" which is intended for rare disease populations (prevalence defined as $<$200 000 cases in the US). Another designation, "Fast Track" can provide a shorter development timeline and review period. This approach can result in conditional/accelerated approval, such as when there is no alternative therapy or there is significant improve-

ment over existing therapy and the investigation compound could treat that specific life-threatening or debilitating condition. All applications to health authorities require adequate documentation to support proposals for clinical study. Also included in a typical application is an Investigator Brochure (IB), which provides a comprehensive understanding of the drug characteristics (chemistry, non-clinical pharmacology and toxicity studies, and any past human experience), a proposed study protocol, and qualification of the investigators being proposed to conduct the planned studies. Full supporting information is also provided for the health authority review, including preclinical reports, manufacturing controls documentation, and any reports of prior experience in humans. Once an IND or CTA is accepted by the responsible health authority, it remains active as long as the drug sponsor intends to maintain an active development program. An annual report is submitted on the progress of the human trial(s), including safety updates, and IB revisions. Depending upon the assigned FDA Division, separate new applications may be required for new formulations or indications. Ongoing opportunities to discuss with the FDA, in person and by telephone, may be requested at each drug development stage and sooner, by specific request (ad-hoc discussions).

Once Phase 2 data are available and a sponsor intends to progress to Phase 3, the sponsor may request an End of Phase 2 (EoP2) meeting with the FDA. At this meeting, the FDA will review non-clinical and toxicology data, as well as clinical data generated during Phases 1 and 2. The purpose of this review is to establish, in consultation with the health authority, that the risk-benefit ratio is justified by the data collected, and that the compound is sufficiently safe for use in larger confirmatory Phase 3 trials. Acceptable clinical endpoints, optimizing the protocol design and appropriate patient types are discussed, as is the justification for the proposed dosing in Phase 3. Any agreements with the responsible health authority at EoP2 are non-binding; they do not guarantee acceptance, for example, if the medical/scientific knowledge and/or standard practice evolve during the Phase 3 development. Although not required, the sponsor may also request a Special Protocol Assessment review meeting with the FDA to discuss and approve the Phase 3 study protocol.

All clinical trial conduct and management must adhere to Good Clinical Practice (GCP) guidelines as a prerequisite to acceptability of trial results for drug registration (approval); this includes local requirements wherever the drug is evaluated, such as the FDA, European Medicines Agency (EMEA) of the European Union or Member states, Japan's Ministry of Health, Labor and Welfare (MHLW), and other local health authorities as appropriate. Some countries have different requirements for conducting clinical research. Guidance for the conduct of most clinical trials are based on: FDA 21 CFR Parts §50, 56, and 312 (http://www.accessdata.fda.gov/) and also the International Conference on Harmonization Harmonized Tripartite Guideline for Good Clinical Practice (http://www.ich.org/) based on the ethical principles described in the Declaration of Helsinki.

Application for market authorization

Before submission of the NDA, the company may request a pre-NDA meeting with the responsible health authority to obtain feedback on the format and content of the proposed review package and to identify potential major interests with the content (efficacy or safety database, chemistry, preclinical, or other administrative requirements). The purpose of this early discussion is to understand any known risks that may cause the health authority to "refuse to file" (FDA) or to validate (EMA) the application (e.g. proceed to substantive). Typically, an original drug application for a new chemical entity contains clinical study reports from a minimum of two adequate and well-controlled Phase 3 trials, a Risk Management evaluation, and submission plans for a pediatric study. All health authorities now require fees for drug applications. For example, in the US, since the adoption of the 1992 Prescription Drug User Fee Act (PDUFA) regulations, the "user fee" funds the NDA review process over a designated target completion timeline: up to six months for a Priority review, and ten months for a Standard review period. Additional review time may be added if necessary and is dependent on the complexities of the documentation.

The FDA review is coordinated by a lead project manager and involves multiple disciplines, including medical, statistical, and/or the clinical pharmacology of the compound, preclinical, and chemistry. The FDA will communicate a filing decision and potential issues concerning the application within a defined time period after submission: 30 days for a Priority NDA review and 60 days for a Standard NDA review. The FDA will also send a formal 74 Day Letter, also known as the Filing Review Notification that communicates preliminary comments on potential issues that could impact approval and also the timeline on providing feedback on labeling, the REMS proposal, post marketing commitments; and an action date that the review will be completed.

In parallel with the NDA review, the Division of Scientific Investigations may conduct a preapproval inspection of the full-scale production facilities to ensure that the infrastructure can produce the quality product. Also, an FDA investigator(s) may conduct a site audit to evaluate the overall integrity of data adherence, compliance, and investigator reporting to confirm the reliability of data submitted by the company. Foreign site audits are becoming more common with the participation of non US investigators to support global clinical trials.

Sponsors of NDAs are required to submit follow-up safety information (typically a 120 day Safety Update) after the initial application; the content and actual timing can be negotiated with the FDA. In the last 45 days of the review cycle, the FDA will also communicate its decision on acceptance of the proposed trade name. It is common that a company will submit alternative names due to the potential for product confusion with respect to sound-alike or look-alike names.

Upon completion of the NDA review, if the benefit does not outweigh the risk, the FDA will issue a "Complete Response" letter listing identified deficiencies. Type 1 letters list minor issues that must be addressed within two months and resubmitted to the FDA for a timely review. Type 2 letters may require additional data, including full study reports or major statistical analysis, within six months. Even if the Action Letter deficiencies are adequately addressed, an approval decision is not guaranteed. The FDA may also require mandatory post marketing requirements or commitments to confirm the clinical benefit and to answer additional safety questions. Termed post approval commitments, these studies are conducted by the sponsor after the FDA has approved the product for commercial marketing. Product approval is posted on the FDA website shortly after the drug sponsor is notified. The FDA may also provide access through the Freedom of Information, which contains summary reviews by the director, including documents and decisions by the assigned review team by discipline, and the REMS document reviews, if applicable. Drug reviews and approval decisions in Europe are also made available to the interested public and are known as European Public Assessment Reports.

FDA advisory committee

As part of the agency's regulatory decision-making process, an Advisory Committee (AC) may be requested approximately two to three months before the NDA action date for drug applications containing a New Molecular Entity. Otherwise, an approval letter may provide a summary of why consultation with the advisory panel was not needed to make a final determination of approval. AC meetings can provide expert advice and are intended to help guide the FDA with discussions and recommendations on any topic related to the drug application itself. The advice of the expert advisory panel is considered useful when data issues are difficult, or of significant public interest, or controversial, or if a special type of expertise is needed, or the division wants confirmation of their own findings. The panel of independent experts is considered knowledgeable in specific areas related to the drug and biologic product and may consist of representatives from academia, the community, patient advocacy, industry, or special governmental agencies.

Benefit/risk analyses

The benefit/risk evaluation is the cornerstone upon which a drug application is approved. In Phases 2 and 3 clinical studies, adverse events and efficacy are analyzed in a fixed population. Following approval, a more diverse and greater number of patients will be exposed to the drug. Health authorities take this increase in exposure, plus additional factors such as the indication, medical need, other available treatment options, prognosis of the untreated disease, mechanism of action (MOA), safety profile, robustness of efficacy data, and likelihood of appropriate use, into consideration as part of the benefit/risk equation. This is considered against alternative treatment options, as well as the prognosis of the disease if left untreated, to arrive at a final assessment. A novel MOA with a better clinical outcome has a clear advantage. However, if less is known about the MOA, the novel setting may become a disadvantage, as there may be unknown long-term risks. Drugs of the same class can be approved providing the drug meets acceptable predefined efficacy endpoints, and safety data are provided to support labeling requirements. A drug with a unique defined characteristic, in addition to the baseline expected demonstration of efficacy and safety, will further improve the benefit/risk profile. The presence of a safety signal, especially if it is not adequately characterized in Phase 3 trials, may require additional post marketing studies to evaluate the safety profile or verify the clinical benefit longer term. A Risk Management Plan (RMP) is being required by an increasing number of countries. In the US, the FDA prefers more succinct documentation known as the Risk Evaluation and Mitigation Strategy (REMS). These documents identify known or potential safety risks, and define specific actions to minimize risks. The REMS may provide additional confidence in labeling the drug to communicate the appropriate use, have a system in place to assure the likelihood of appropriate use, and, under certain circumstances, restrict availability of product use through a controlled distribution system.

Other regulations and compliance

Clinical, non-clinical, laboratory, and manufacturing processes supporting the generation of key data for regulatory submissions and commercialization of investigational drugs must follow specific guidelines often referred to as GxP. Good Laboratory Practice (GLP) describes how non-clinical studies should be planned, performed, monitored, recorded, reported, and archived. Good Manufacturing Practice (GMP) ensures that products are consistently produced and controlled to quality standards (bioavailability, safety, and stability) appropriate to the intended use, as per the product specification. Good Clinical Practice (GCP) is an international ethical and scientific quality standard, dictated by the International Conference on Harmonization (ICH) (www.ich.org/) and FDA E6 Good Clinical Practice Consolidated Guidance (1996) (www.fda.gov/ScienceResearch/SpecialTopics) to protect the rights of human subjects and provide guidance for the design, conduct, recording, and reporting of clinical studies. It also defines the roles and responsibilities of clinical trial sponsors, clinical research investigators, and monitors.

Biologic approval process

The approval process for a biological product in Europe and USA is similar to that for a small molecule. Biologic applications for well-characterized compounds are reviewed by the same CDER divisions as that of drug applications. Applications for novel or more complex biologics are known as a Biologics License Application (BLA) and are reviewed by the FDA's Center for Biologics Evaluation and Research (CBER). In any regulatory environment, there are additional complexities associated with manufacturing and characterization of the biologic; stringent process controls and inspections of manufacturing facilities are pre-approval requirements.

Different health authority approval processes

In general, global health authorities are reasonably aligned with the acceptance of study design, outcome measures, and analyses using data from multi-national studies. The traditional endpoint of composite efficacy failure criteria of acute rejection, graft loss, and death is generally accepted. Based on the number of new drugs under development in transplantation in 2009, EMA introduced comprehensive guidelines and recommendations for the development of transplant drugs in solid organ transplantation (www.ema.europa.eu/docs/). Health authorities in certain areas, such as Asia-Pacific countries, have region-specific interests in evaluating the drug experience from local clinical trial data in order to better assess any potential ethnic sensitivities. This is in addition to reviewing justifications in support of comparable evidence from the global trial database. Each potential new drug candidate, whether a biologic or drug, continues to be developed, with applications reviewed and approved by health authorities on a case-by-case basis, to establish the merits for use in organ transplantation.

Summary

The path from discovering a compound that exhibits biological activity toward a desired pathway/target, to health authority approval of a new drug requires multi-disciplinary teams to translate fundamental science into clinical treatment. Research and development activities are integrated through a complex and lengthy process, to fully characterize and optimize the candidate compound, resulting in a final drug product with demonstrated safety, efficacy, and an adequate benefit-risk profile. After one or more candidates are identified with relevant chemical properties and biological activity, initial safety and toxicology studies are undertaken. When a favorable profile is demonstrated, a clinical PoC study is undertaken to confirm mechanism of action and establish preliminary safety. A positive PoC result leads to full development of the compound, including Phase 2 trials to establish dosing, safety, and efficacy followed by robust, statistically powered Phase 3 confirmatory trials. Health Authorities in countries where

regulatory approval is being sought (e.g. FDA, EMA) interact closely with the Sponsor, using both verbal and written feedback, throughout the process to guide clinical development. Phase 1 establishes the molecule's clinical pharmacology, including the PK, PD, drug-drug and drug-disease interactions; Phase 2 demonstrates the clinical efficacy and preliminary safety of the candidate. After receiving Health Authorities' agreements to proceed, larger Phase 3 trials are conducted to provide enough data to confirm efficacy, safety, and establish the benefit/risk profile needed by them to decide on the adequacy of a product to treat patients. Upon completing Phase 3, the Sponsor submits a detailed dossier to Health Authorities for their review and decision-making. The US FDA process ranges from six months (priority review) to ten months (standard review) and usually includes a public Advisory Committee Meeting in advance of the final FDA decision on a NDA. Even after approval, ongoing pharmacovigilance monitoring programs may be volunteered by the sponsor, or mandated by health authorities, to confirm the ongoing clinical benefit and address drug safety in the intended patient population.

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CHAPTER 142

Organ Specific Benefits of Transplantation: Outcomes and Economics

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Introduction

Organ transplantation is widely cited as one of the key scientific contributions of the twentieth century [1–3]. The Nobel prizes awarded to the landmark contributions of Alexis Carrel, Peter Medawar, and Joseph Murray are a testament to the innovations that have advanced the field from fiction to reality. Beginning with the first successful kidney transplant in 1954, the standard of care for end organ failure changed from symptomatic treatment to functional replacement. Thomas Starzl [4], Norman Shumway and Christian Bernard [5], and Richard Lillehei [6], extended the benefits of organ transplantation from renal failure, to end stage liver, heart, and beta-cell failure, providing millions of years of quality life to recipients around the world. Details of transplantation's rich history are outlined in Chapter 1.

Currently, organ transplantation has been established as the standard of care for advanced organ failure in modern medical systems with sufficient resources to support this complex clinical enterprise. Unfortunately, widespread distribution of transplant services remains constrained by two primary factors; donor organ supply and economic resources. For example, in the US, the waiting list for deceased donor kidneys eclipses the potential supply by more than six fold, resulting in waiting times of greater than 7 years in some locations [7]. As a result, patients experience high rates of waiting list death and prolonged requirements for mechanical organ support with its attendant inferior quality of life and complications. The international community continues to debate key questions regarding the design of the optimal allocation system for the available organs. This debate focuses on the important tradeoffs between equality of access and maximizing the efficacy of the procedure (e.g. benefit of the organ to a specific recipient) [8].

While US domestic variation in access to transplantation is generally a function of organ supply and insurance coverage, variation in care internationally reflects the degree of industrialization and economic infrastructure [9]. Populations with the greatest access to renal transplantation are clustered in Western Europe, North America, and Australia with rates of greater than 30 kidney transplants per million people. By comparison, there is no African nation above ten transplants per million people. Expansion of transplant services to developing nations has been established as the most effective way to address end stage renal disease (ESRD) in countries, as dialysis is not a viable solution given limited health care resources. However, the vast majority of the world's population still lives without access to these life saving procedures [10]. Additional consideration of cultural practice variations, insurance coverage for transplantation and national transplant administrative policy, can be found in Chapters 139, 140 and 143 respectively.

Transplantation is not immune from discussions of about its high cost. Heart transplantation remains the most expensive procedure covered by the Medicare system in the US [11].The substantial resources expended in the care of the relatively few transplant patients prior to and following transplant procedures compared with the total health care enterprise are likely to be increasingly scrutinized in light of an international effort to scale back spending on health care services. Even within the US, transplant care for certain conditions (e.g. liver transplantation for Hepatitis C) was initially removed from the benefits package for Medicaid patients in the state of Arizona [12]. While this decision was eventually overturned, this is likely to be the first of many efforts to restrict access based on cost.

While the challenges of transplantation in the first 50 years were primarily focused on overcoming immunologic and technical barriers, it is likely that the next 50 years will be dominated by addressing the allocation and economic constraints on access. Organ transplantation will increasingly exist within an organized system of care responsible for the allocation of fixed resources across a population in need. By necessity, expansion of transplant care is likely to result in the reduction of resources available for other services. Thus, an accurate understanding of factors affecting the cost and efficacy of specific organ transplants is needed to accurately demonstrate the comparative benefit of these life-extending procedures.

The primary goal of this chapter is to consider the organ specific implications of variation in donor quality, recipient severity of illness, and clinical practice patterns on the cost and benefit of organ transplant. This analysis will focus primarily on kidney, liver, and heart transplant procedures and relevant alternative treatments, as they are the most widely performed and the best studied. The intersection of organ allocation policy and transplant access with cost will also be examined as geographic differences between organ supply and recipient demand substantially influence transplant related resource utilization.

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.

Kidney transplantation

Background

The successful transplant of Ronald Herrick's kidney into his identical twin brother Richard in 1954 marked the beginning of successful clinical kidney transplantation [1]. The procedure extended the life of Richard by 8 years, without the need for immunosuppression. While initially limited to identical twins, the development of increasingly successful immunosuppressive regiments allowed broad application to patients with end stage renal failure, limited only by the ongoing shortage of transplantable kidneys [3].

It is instructive to contrast kidney transplantation with dialysis, which developed concurrently [1,13]. Willem Kolff pioneered mechanical support of patients with dialysis in the 1950s, initially in The Netherlands and subsequently in the US. Access to the Kolff kidney, however, was also very limited as both the machines and the expertise needed were confined to a few centers. Furthermore, there were insufficient resources in the private sector to fund this care. At the University of Washington, an anonymous panel of lawyers, physicians, clergy, and others was appointed to determine who would be placed on dialysis and who would die [14].Soon referred to as the "God Panel," these individuals initiated a system of allocation; limiting care to those over 18 and under age 45 without hypertension, diabetes, vascular disease, and who possessed an appropriate "emotional" character. The panel selected ten of the initial 17 applicants to receive care, leaving the remainder to die. The public outcry over this outcome contributed to the inclusion of support for ESRD care under the Medicare program in 1972. Unlimited by financing or organ supply limitations, dialysis became widely available to all, consuming over $24 billion in Medicare funds alone in 2007 [15].

Clinical and economic benefit of kidney transplantation

The clinical benefits of successful renal transplant have been clearly established. Compared with dialysis, transplantation is associated with prolonged survival and reduced morbidity. In the landmark analysis by Wolfe and colleagues in 1999 comparing waitlisted patients with those successfully transplanted, kidney transplantation was associated with average improvement of 10 years in overall survival [16]. Although the transplant procedure was associated with a brief period of increase risk of mortality, the risk/benefit assessment favored kidney transplantation after 106 days following the transplant. In this analysis, the greatest benefit accrues to the younger patients, who lived on average 17 years longer following transplantation.

Recent analyses have extended Wolfe's original work, through the incorporation of quality of life adjustment and accounting for survival after allograft failure [17]. This work was conducted in support of novel allocation proposals being considered by the United Network for Organ Sharing (UNOS), which are designed to maximize transplant benefit. Benefit was defined as the added survival achieved by patients who underwent transplant (including survival after graft failure) compared with that of patients who remained on dialysis. This measure, termed life years from transplant (LYFT), estimates the contribution of a particular donor and recipient combination to the overall survival impact of kidney transplantation over a ten year time horizon. These estimates were adjusted for quality of life, valuing a dialysis year as 80% of a transplant year. Under the existing kidney allocation system, the average LYFT resulting from kidney transplant varied by age from 9.6 years for candidates less than 17, to 3.5 years for those 65 and older [18]. When aggregated across all patients transplanted under the current allocation system, kidney transplantation adds an estimated 48 850 quality adjusted life years annually. Improved matching of donor and recipient characteristics, for example by placing kidneys from younger donors into younger recipients, has the potential to further improve the benefit of kidney transplant. One proposal examined by the UNOS kidney committee resulted in an annual benefit of kidney transplant that could increase the quality adjusted life years resulting from transplantation by over 3000 annually, although this was achieved at the expense of transplanting fewer older recipients [19].

The increasing benefit of kidney transplantation reflects, in part, the slowly improving rate of long-term allograft survival. Previous analyses have documented that improved immunosuppression has reduced early graft loss from immunologic causes; while, long-term kidney graft survival had remained generally static. However, recent analysis of Scientific Registry of Transplant Recipients (SRTR) data suggests a modest improvement in long-term graft survival for some disease categories [20]. Meier-Kriesche and colleagues reported that that graft half-life for deceased donor transplants increased from 6.6 year in 1989 to 8.8 years in 2005. For ECD transplant recipients, the half live has increased from 3 years in 1989 to 6.4 years in 2005. This improvement demonstrates the benefits of more recent strategies to reduce the impact of chronic calcineurin inhibitor toxicity and corticosteroid side effects, identify and treat both early and late antibody mediated rejection, and, perhaps, better address medical co-morbidities including post-transplant diabetes and cardiovascular disease. Living donor transplant continues to have the best survival, with a half-life that now exceeds 11.5 years.

Similar to the assessment of the clinical benefits of kidney transplantation, the economic outcomes for kidney transplantation must be considered in relationship to the principal alternative treatment, hemodialysis. Dialysis is a highly resource intensive and morbid treatment associated with repeat hospitalizations. Direct costs of dialysis treatment are greater than $73 000 per year per patient under the Medicare system; while private payments can be nearly double [21]. By comparison, kidney transplant is associated with a cost savings of >$200 000 per transplant over the first five years after transplantation [22]. Examination of Medicare spending, suggests that the breakeven point at which spending is equivalent is 2.3 years for patients who undergo living donor transplantation and 3.6 years for recipients of deceased donor transplant. Organ survival beyond this point results in significant cost savings for payers and improved quality of life for recipients. (Figure 142.1). The economic benefits of living donor transplants far eclipse those of deceased donor transplants as a result of the improved long-term survival [23]. Current estimates based on Medicare spending, indicates that each living donor transplant performed for a Medicare recipient saves at least $94 579 dollars. Given the improved quality of life associated with transplant compared with dialysis, the true value has been estimated as $269 319 per living donor transplant.

Impact of donor characteristics on kidney transplant benefit

The clinical success of kidney transplantation has resulted in a dramatic increase in waitlisted patients and has resulted in a significant donor shortage. The desire to treat the greater than 80 000 individuals currently on the kidney waiting list has led to broader use of donor organs with characteristics associated with reduced graft survival and a shorter LYFT benefit. Available allografts were

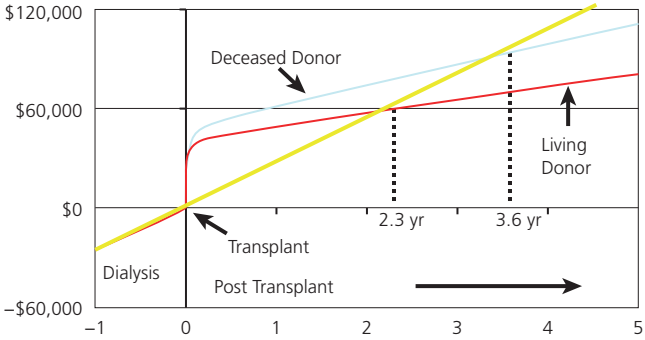

Figure 142.1. Cost effectives of kidney transplant by donor type. Adapted from [100] Smith, et al. Cadaveric versus living donor kidney transplantation: a Medicare payment analysis. *Transplantation*, 2000;69(2):311–314, with permission from Wolters Kluwer Health.

Table 142.1. Donor profile index for kidney transplant. Reproduced from [29] Rao PS, et al. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. Transplantation. 2009;88(2):231–236, with permission from Wolters Kluwer Health

Donor Profile Index
Age
Race/ethnicity
Hypertension
Diabetes
Creatinine
Cerebrovascular cause of death
Height
Weight
Donor after cardiac death
Hepatitis C

initially dichotomized as Standard Criteria Donors (SCD) and Extended Criteria Donors (ECD). The ECD kidney definition was based on donor characteristics that equated to relative risk of graft failure of 1.7 [24]. These higher risk grafts were offered on an expedited basis to candidates who were informed of the added risks and indicated their willing to accept such an offer at the time of listing [25]. Because ECD kidneys are associated with a greater risk of delayed graft function and early allograft loss, the benefit of transplantation with an ECD kidney is confined to waitlisted patients with significantly increased risk of wait list mortality. Through a retrospective analysis of the SRTR database, Merion and colleagues identified a population of patients for whom ECD transplant conveys a significant survival benefit: any adult candidate over 40 years old with diabetes or residence in a donation service area with waiting times greater than 1350 days [26]. Recipients not meeting these criteria did not have a significant benefit from transplantation with an ECD organ. Although this information has been widely disseminated, the proportion of patients who are offered and accept wait listing for ECD kidneys varies widely across US transplant centers [27]. Among the US kidney transplant centers, the percent of candidates meeting Merion's criteria who are listed as willing to accept an ECD kidney ranges from 0–100%. Appropriate candidates willing to accept an ECD kidney appear to have a reduction in waitlist mortality (HR 0.88 [0.85–0.91]) as predicted. However, up to 40% candidates listed for ECD kidneys actually appear to not meet Merion's criteria and may not benefit from the ECD kidney. While patients who did not meet Merion's criteria but were still listed for ECD kidneys were transplanted more quickly, their overall mortality was increased (OR 1.11 [1.05–1.18]) in comparison to similar patients not listed for ECD transplants who remained on dialysis for a longer period.

A continuous measure of kidney graft failure risk offers a more realistic characterization of the continuum of donor risks that can affect graft function and outcome [28]. Unlike the dichotomous ECD versus SCD distinction, the kidney donor profile index (KDPI) compares the relative risk from graft failure of each donor organ to the average kidney risk on a linear scale [29]. The KDPI includes an expanded number of variables, weighted using a regression equation (Table 142.1). There is a significant overlap in KDPI scores across ECD and SCD kidney donors, demonstrating the limitation of a dichotomous classification. The newly approved kidney allocation policy incorporates the KPDI. Using KDPI, the lowest risk kidney donor quintile (the 20% of kidney donors with the lowest KDPI scores) will be preferentially offered to younger, healthier recipients to maximize the number of years with a functioning kidney transplant [30]. Similarly, broader sharing has been proposed for the highest KDPI kidneys. These organs are often harder to place and if offered, are more likely to be used by aggressive centers in regions with longer waiting times. Improved donor risk assessment also offers the opportunity for better informed consent for potential recipients as it may also allow modeling to assess the individual risks and benefit for specific recipients of specific organs. Unfortunately, like other metrics of donor quality, the predictive value of the KDPI remains limited [28,31]. The c-statistic for the full model is 0.62. This value indicates that the KDPI can accurately predict longer survival among any pair of kidneys only 62% of the time, while a model with no information has a c-statistic of 0.5. This lack of predictive ability reflects the fact that significant variation in outcomes occurs because of variation in recipient factors, clinical events, and un-captured variation in donor quality.

An alternative method for effectively utilizing marginal allografts is to explicitly match donor and recipients based on age, as is done in the Eurotransplant Senior program [31]. Under this protocol, kidneys from donors greater than age 65 are offered exclusively to recipients in the same age group. This protocol increases the rate of transplantation in the elderly thereby reducing wait list mortality. Post-transplant patient and allograft survival in older recipients of the older kidneys is similar to that of older patients who received younger donor organs [31]. However, when compared to older recipients receiving younger donor grafts, utilization of the older grafts resulted in increases in the rate of surgical complications (47% vs. 28%, $P = .03$) and reduced rate of primary function (69% vs. 74%, $P = .03$).

Donor characteristics that predict decreased graft survival are also associated with an increased incidence of delayed graft function (DGF), prolonged hospitalization, and increased need for expensive induction treatments with cell depleting antibody [32,33]. A review of 4618 ECD transplants in the UNOS database demonstrated a DGF rate of 36% for ECD kidneys preserved by static cold storage [22,34,35]. This rate can be improved with pulsatile perfusion, which has been shown to reduce the incidence of DGF to 26%. By definition, DGF requires dialysis in the postoperative period resulting in longer hospital stays and higher initial costs. Because DGF is associated with a higher rate of acute rejection, use of induction therapy is believed to be beneficial.

As a result of the increased rate of early complications and decreased allograft survival, ECD kidney transplants are more

expensive to care for in both the perioperative and postoperative period. From a societal viewpoint, ECD kidney transplants remain cost saving, although the time to breakeven in cost is significantly longer than SCD kidneys [22]. Compared with SCD transplants, ECD transplants are associated with a modest increase in the cost per quality adjusted life year ($41 000 vs. $54 000). However, both transplants are markedly less than the cost of maintenance dialysis ($79 000 per QALY). The economic impact of reduced allograft quality reflects both the higher incidence of early graft dysfunction, persistent reduction in the estimated glomerular filtration rate, and early return to dialysis. Compared to kidney recipients with an eGFR >60 mL/min/1.73m^2, the average recipient with an eGFR of <30 mL/min/1.73m^2 incurred over $7000 of increased costs over the first year and more than $10 000 in increased costs between one and 3 years after transplant [36]. Declining renal function increased cost at an accelerating rate based on baseline creatinine and the rate of eGFR change over time.

The economic impact of ECD transplantation on transplant providers is more dramatic. While the outcome and savings benefits of ECD transplant accrue over time, the higher cost associated with the increased complications and early graft dysfunction/primary non-function with ECD grafts is predominantly incurred in the perioperative period. In a recent evaluation of transplant center finances, Englesbe and colleagues demonstrated dramatic erosion in transplant center profitability from 1999–2005 at the University of Michigan [37,38]. While overall reimbursement was static, the cost of transplant rose significantly. ECD donors were associated with a margin reduction of over $8000 per case, resulting a net loss for the institution. Kidney recipients experiencing DGF also resulted in substantial losses for the center ($4947/case) despite an increase in the overall reimbursement from the patients' insurers. One consequence of these financial results is to dis-incentivize the use of higher risk donors, despite their long-term economic benefit. One potential solution is to design risk adjusted payments to transplant centers that reflect the increase initial cost of caring for high-risk patients and transplanting higher risk organs.

Impact of recipient factors on cost and outcome

The clinical success of renal transplant has led to expanded application to older patients with increasing degrees of medical co-morbidities. Segev's data indicates that even patients >65 years of age gain additional life years compared with remaining on dialysis [27,39]. Over the past decade, patients older than 50 are the fasted growing cohort of newly listed recipients, within which those over 65 constitute a proportionally larger share [7]. Additionally, the incidence of diabetes has increased by 17% and hypertension related renal failure by over 30%. These factors have a direct impact on the post-transplant survival. Older patients with diabetes, who do not undergo kidney-pancreas transplant (likely type II), have the lowest expected LYFT post-transplant (approximately 4 years), a 50% decrease compared to young patients without diabetes [17]. Increasing age is also associated with a decrease in expected LYFT, due principally to age related increase risk of death with a functioning graft. Moreover, older patients have a greater incidence of complications, prolonged hospital stays, and re-admissions. However, for all of these higher risk groups, transplantation continues to offer substantial benefits in survival and quality of life compared with remaining on dialysis. Multiple single center studies have documented safe and effective renal transplantation is possible even for octogenarian patients [40].

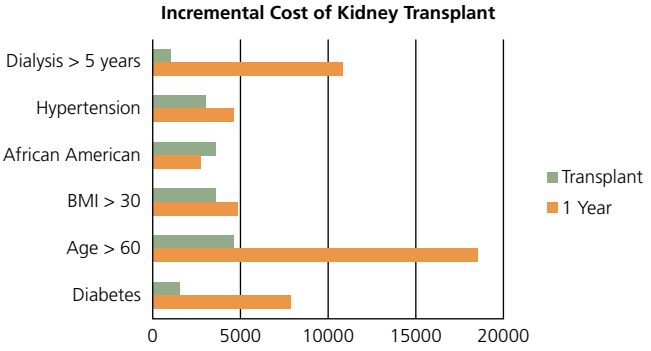

Figure 142.2. Impact of recipient characteristics on kidney transplant cost. Data from [22].

Graft survival outcomes are also closely associated with waiting time, particularly if patients are dialysis dependent [41,42]. Dialysis for greater than 24 months reduced 5 year graft survival to 58% compared with78% for recipients with less than 6 months of dialysis (P <.001) and 10 year graft survival to 29% from 63% (P <.001). Among the factors that predict short waiting times are white race, high socioeconomic status, private insurance, and donation service area of listing [43] (Figure 142.2). Ongoing disparity in organ availability, has led to a marked variability in access to transplant within the US, with median waiting times varying from less than 18 to more than 40 months after listing. Post-transplant patient and graft survival is further impacted by prelisting dialysis time which reduced graft survival markedly. Postlisting time had only a minimal impact on graft survival. Longer prelisting dialysis time reflects late referral and has been associated with lack of private insurance and non-white race. Furthermore, there are marked differences across donation service areas (DSAs) and transplant centers in the amount of prelisting dialysis time reflecting the diversity in local practice patterns and suggesting the need to increase access to early evaluation.

Changing patient demographics have had an equally profound impact on the cost of transplant care both at the initial hospitalization and over the first year after transplant [22] (Figure 142.3). Diabetes increases the cost of the transplant procedure by a mean of $1531 and one-year expenditures by $7884. Similarly, older age, longer time on dialysis, and increased body mass index (BMI) all substantially impact the cost of providing care. High expenditures have also been associated with increased degrees of allosensitization, which requires the use of expensive procedures (plasmaphoresis, intravenous immunoglobulin therapy, and cell depleting antibody therapies) [44]. These techniques, while clinically successful, can add $30 000 or more to the transplant procedure.

Summary

Renal transplantation remains one of the few medical or surgical interventions that has historically proven to be both life and cost saving. This remains true today for young healthy recipients of high quality organs. Reduction in the risk of rejection, improved management of cardiovascular complications, and development of targeted immunosuppression, have all enhanced the benefit of renal transplantation. However, changing demographics of the donor and recipient populations could potentially erode both the clinical and economic benefits of transplantation. Longer waiting times, resulting in a greater burden of co-morbid illness contribute to higher costs and reduced patient survival. Equally significant for

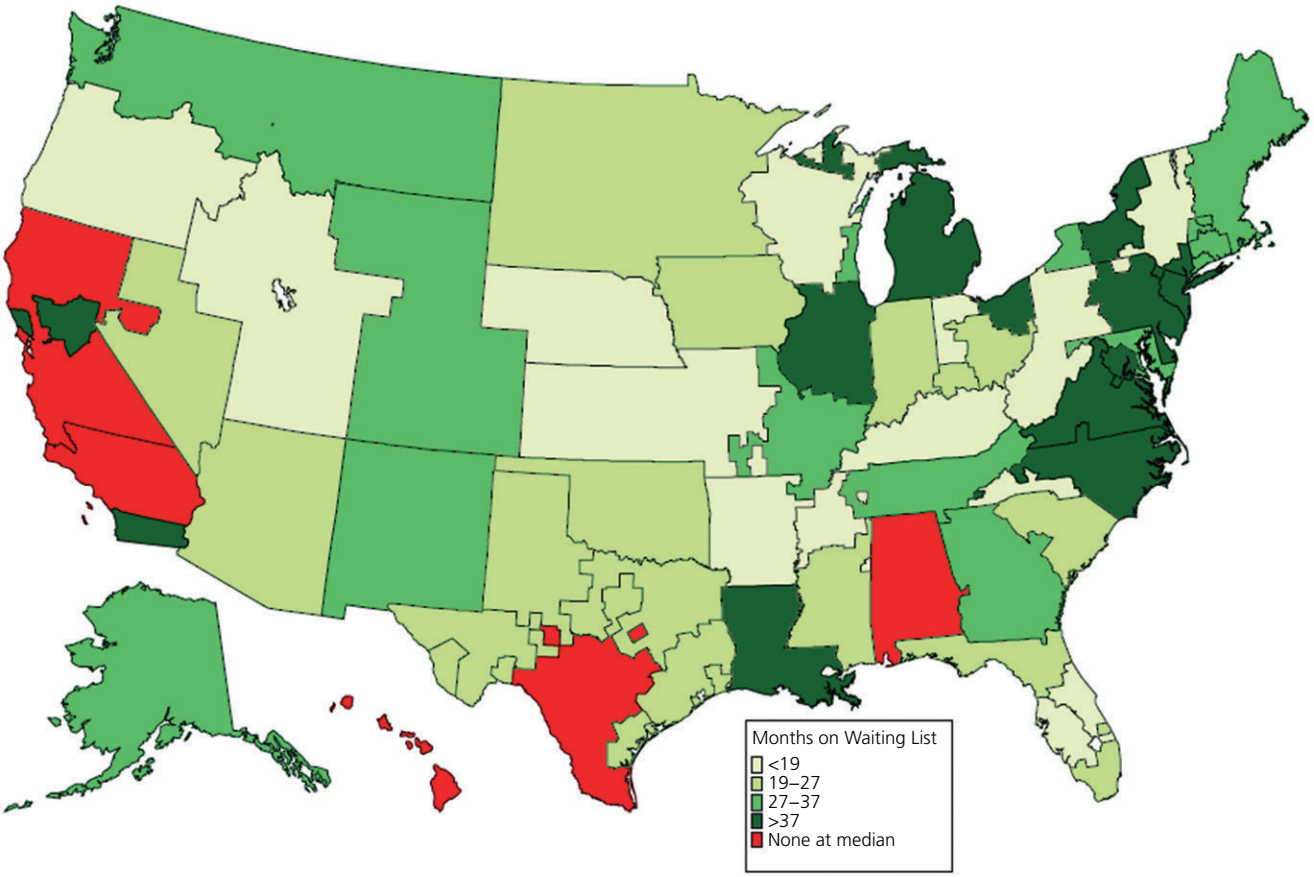

Figure 142.3. Variation in waiting time for kidney transplantation. Reproduced from [7] with permission from Wiley.

transplant centers, these factors interact with donor quality changes to substantially erode transplant center profit margins. Should the proposed kidney allocation system be implemented, there will be an increasing number of more expensive, older, higher risk kidney recipients receiving allografts with a greater risk of delayed graft function. Without adjustment in reimbursement practices to adjust for the risks and these procedures, access to transplantation services for these patients, who would still receive a survival benefit from the procedure, may become more geographically limited as centers with smaller volumes are unable to offset losses with sufficient numbers of higher revenue cases and close.

Liver transplantation

Background

The population of patients with end stage liver disease (ESLD) is rapidly growing throughout the US [45,46]. This growth is fueled by the hepatitis C epidemic, ongoing alcohol abuse, and the consequences of obesity and metabolic syndrome. Although the incidence of new hepatitis C infection appears to have peaked in the mid 1990s, as a result of long standing infection the population of patients with cirrhosis, hepatocellular carcinoma and decompensated liver failure requiring transplantation are expected to grow for at least the next two to three decades. Furthermore, the obesity epidemic has resulted in an unprecedented increase in the incidence of non-alcoholic steatohepatitis (NASH). If untreated, NASH also leads to cirrhosis and ESLD. Current estimates of proportion of the population affected with NASH range from 3–37% [47].

ESLD care is complex and multidisciplinary. Potential liver transplant candidates require a combination of medical interventions to control the manifestations of cirrhosis and portal hypertension [48]. Patients require vaccination, early and repeated radiographic screening for hepatocellular carcinoma to identify lesions that are treatable with liver directed therapy, and treatment of viral hepatitis. While not curative, some patients derive significant health benefits from strategies designed to treat the cause of their cirrhosis including: treatment of hepatitis C infection, abstinence programs to reduce ongoing drug and alcohol abuse, and aggressive weight loss regiments. Progression of ESLD disease is associated with expensive hospitalizations and procedures including therapy for hepatocellular carcinoma or variceal bleeding.

The complexity and severity of clinical care related to progressive chronic liver disease results in significant health care expenditures. Direct health spending for hepatitis C care alone exceeds $5 billion annually and is expected to consume nearly $55 billion over the next decade [49,50]. Similarly, the cost of NASH treatment has risen with the obesity epidemic, adding to the economic burden of ESLD [51]. Severity of liver failure, as measured by the Model for End stage Liver Disease Score (MELD), has been shown to correlate with ESLD mortality as well as spending. Preliminary analysis of charges incurred of pretransplant care of patients transplanted with a MELD score of 25–40 are 14× greater than for patients with a MELD score less than 25 [52] (Table 142.2). Average annual health

Table 142.2. Total charges for liver transplant care as a function of severity of illness in $ thousands. Reproduced from [52] with permission from Wiley

N = 990	Total transplant charges	Pretransplant 1-yr charges	Transplant period charges
Base cost	452.6 (305.2 to 599.8)*	85.2 (36.1 to 134.3)*	274.0 (212.1 to 335.9)*
Female	100.7 (12.3 to 189.1)*	25.8 (−4.6 to 56.1)	44.6 (6.2 to 83.0)*
MELD			
6–14	Reference	Reference	Reference
15–20	72.7 (−33.5 to178.9)	10.7 (−24.9 to 46.3)	30.5 (−15.1 to 76.0)
21–27	177.4 (63.2 to 291.6)*	62.0 (23.4 to 100.6)*	34.5 (−15.1 to 84.7)
28–40	333.3 (205.3 to 461.3)*	145.5 (104.0 to 187.1)*	60.7 (8.1 to 113.3)*
Liver-kidney	212.3 (26.0 to 398.5)*	178.3 (114.8 to 241.7)*	90.9 (7.0 to 174.8)*

* $P < 0.05$.

care charges for a high MELD patient (>25) in the year prior to liver transplant exceed $230 000.

In addition to direct medical expenses, the complications of ESLD, including encephalopathy, impose additional economic burdens on patients and caretakers. Among patients with a history of encephalopathy, 87.5% are unemployed and 85% report that their disease significantly impacts their financial status [53]. Family members also reported high levels of depression, anxiety, and concerns about social support and the burden of caring for their loved ones. These concerns varied by severity of illness, and they accelerate once the patient's MELD score reaches 15 [54].

Survival benefit of liver transplantation

As a result of improved surgical and anesthetic techniques, better immunosuppression, and lower rates of infection, liver transplantation has become a very successful procedure, with 1-year patient survival rates that routinely exceed 80%. This has been accomplished despite a progressive increase in the severity of illness among patients reaching transplantation, as a result of organ allocation changes [55].

Under current allocation systems, US patients waiting for liver transplant are prioritized based on severity of illness as assessed by the MELD score. As a result of increasing waiting lists, the average MELD score at transplant has been increasing across the country albeit at uneven rates in different geographic regions [56–58].

As a result of the complexity of liver transplantation and the potential of operative complications, the benefit of liver transplantation varies significantly by severity of illness. In patients with high MELD score, death without transplantation is nearly universal, justifying the cumulative risks of surgery, immunosuppression, and less than ideal donor organs. However, for patients with well-compensated cirrhosis, the cumulative risks of transplant have been found to exceed the expected benefit. Merion et al. identified a MELD score of 15 points as the point at which the survival benefit of the transplant begins to exceed the risks of transplant and/or continued waiting [59]. As severity of illness (MELD) increases, the hazard ratio (HR) for death with a transplant compared without a transplant diminishes linearly. Comparing transplanted patients with waitlisted patients, the risk of mortality is greater for patients with a MELD score of 6–11 (HR 1.79, $P = .10$) and 11–15 (HR 1.76, $P = .04$) who receive a transplant than it is for patients who remain on the waiting list. In contrast, transplantation reduced the risk of mortality by 94% (HR 0.06, $P < .001$) for patients with a MELD of 30–39.

The benefit of liver transplant has been found to vary by diagnosis [60]. Factors associated with worse survival in multivariate models include age >42 years (relative risk [RR] 3.4), pretransplant hemodialysis (RR 5.1), and diagnosis. In a review of 4000 liver transplants performed at the University of Pittsburgh, diagnoses that predict worse survival include a history of malignancy and fulminant hepatic failure, while biliary atresia, metabolic disease, and autoimmune (PBC, PSC) conditions faired better [61]. The etiology of post-transplant graft failure reflects differences in disease etiology and recipient factors. Older recipients had a high risk of graft failure from PSC and early vascular thrombosis. In younger recipients, graft failure was more likely from recurrent disease including hepatitis C and hepatocellular carcinoma. Chronic rejection was more common among African Americans, patients with PSC, and younger recipients. Finally, graft loss from recipient death due to cardiovascular/cerebrovascular events was most prevalent in older patients and those with a history of diabetes.

Although severity of illness, as measured by MELD at transplant, has been found to correlate with post-transplant survival, it remains a very imprecise measure of post-transplant outcome [62]. Retrospective studies have confirmed that high MELD patients generally have higher rates of post-transplant graft loss than low MELD patients. In a recent analysis of European outcomes, patients with MELD >30 had over 4 times the risk of post-transplant mortality, when compared to low MELD patients. However, the c-statistic for this analysis was only 0.66, suggesting that MELD in isolation did not accurately predict post-transplant survival. Similar estimates have derived from survival models based on US populations [63]. A comprehensive review of studies evaluating MELD based models across the US and Europe, demonstrated that the C-statistic for MELD predicting post-transplant survival was never greater than 0.7. The lack of high levels of correlation between MELD and post-transplant outcome is reassuring in a MELD based organ allocation system. If high MELD patients universally experience decreased survival, allocation based on severity of pretransplant liver failure could result in lower overall utility from transplantation. In fact, increase MELD scores at transplant following the implementation of the MELD system, did not result in reduced post-transplant survival on average [55].

Impact of donor characteristics on liver transplant survival

Given the ongoing shortage of available liver allografts, expansion of the organ supply is vital to decrease waitlist mortality. The impact of donor characteristics on liver transplant outcome has been examined through registry and single center series. Feng et al. utilized the SRTR registry to define a donor risk index that identified six factors that together predicted decreased survival (older age, African American race, reduced donor height, non-traumatic cause of donor death, donation after cardiac death, and partial/split liver) [64]. Using these characteristics and two additional process factors (cold ischemic time, regional or national sharing), these

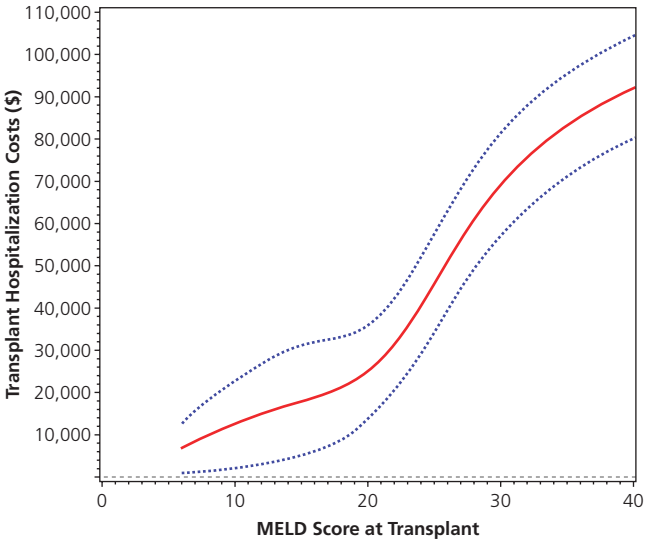

Figure 142.4. Independent associations of MELD score at transplant with transplant hospitalizations costs by multivariate spline regression. Transplant hospitalization costs at each MELD score adjusted for donor and recipient factors including status 1 transplants. Reproduced from [66] with permission from Wiley.

investigators designed a donor risk index (DRI) that can be used to compare the risk of graft failure among deceased donor liver allografts. Additional studies have examined individual factors such as donation after cardiac death to estimate the effects of the duration of warm ischemic time on the risk of non-fatal complications such as biliary strictures. By combining DRI and MELD, Schauble, Merion and colleagues, revised their initial study assessing benefit according to recipient MELD score [65]. They demonstrated that better quality organs (low DRI) conveyed benefit even at MELD scores <15, while riskier organs conferred overall benefit to candidates with higher MELD scores who are exposed to a greater risk of waiting list death.

Liver transplant economics

Liver transplantation remains a resource intensive procedure resulting in inpatient hospital charges that routinely exceed $100 000 per transplant in the US [52,66,67]. The principal cost driver for liver transplantation is patient severity of illness. It follows that the adoption of MELD based organ allocation has resulted in substantial increases in spending for liver transplantation. Retrospective analyses have demonstrated that increasing MELD score is associated with longer length of stay and greater utilization of intensive care units, dialysis, and ventilation [67]. Overall costs increase on average by $4309 per MELD point at the time of transplantation. Similarly, there is a 4% increase in average hospital length of stay per increasing MELD point [68]. The relationship between MELD and cost, however, is not linear. There is a substantial increase in the slope of the cost curve beyond a MELD score of 20 (Figure 142.4) [69].

The increased cost associated with the severity a recipient's liver failure has a direct impact on transplant center profitability. In an early examination of costs and Medicare reimbursement, hospital net income was found to decrease by $1512 per recipient MELD point [67]. These findings were confirmed in Europe, following the adoption of a MELD allocation by Eurotransplant and others [70]. In Switzerland, average transplant costs increased by over 50% following MELD based allocation, driven in part by a marked increase in the use of renal replacement therapy. Once discharged, however, pretransplant MELD is a poor predictor of costs incurred after the initial hospitalization ($P = 0.60$) [52].

The cost of liver transplant care demonstrates marked regional variation in the US, driven principally by differences in organ availability. Currently, average MELD at transplant varies markedly within the US from 21 to 35 across donation service areas [71]. The regions with the highest MELD scores (New York, California, and New England) routinely have higher costs for initial liver transplant care compared with lower MELD regions, largely due to the increased severity illness in the recipients at the time of transplantation. Currently, the UNOS liver-intestine transplant committee is considering proposals to expand organ sharing. One impact of these policies may be a reduction in the rate at which patients with higher MELD scores receive transplants, at least in some regions, as a result of improved access to organs for patients before their liver disease advances to the most severe stages. Without change to the overall organ supply, this proposed broader sharing may result in a corresponding reduction in access to organs for low MELD patients in DSAs that currently have better organ supply. The net economic impact of this shift, would be an increase in the cost of liver transplantation overall because more transplants would be performed at mid to higher range MELD scores; however, given the potential live years saved, the cost of improved access was estimated to be $17 056 per quality adjusted life year saved [66].

Donor selection has also been shown to impact resource utilization for liver transplantation. Multiple studies have demonstrated differential rates of complications and allograft function depending on the type and characteristics of the donor liver transplanted. Use of liver allografts from DCD donors has been extensively studied in single center, registry, and meta-analyses. When compared with DBD donor organs, transplantation of DCD liver grafts increased the need for retransplantation (14.7% vs. 6.8% , P <.001) and reduced 3 year patient survival (71% vs. 77%, P <.001) [72]. The major cause of morbidity in DCD transplants compared with DBD donors is the increased frequency of biliary complications (HR 2.4 [1.8–3.4]), specifically ischemic cholangiopathy (HR 10.8 [4.8–24.2]). These complications increase the need for readmissions, interventional radiology procedures, and retransplantation, raising the overall cost of transplant by as much as 25%, after adjustment for other characteristics [73].

General indexes of organ quality such as the DRI have also been found to correlate with cost, both at the initial admission and over the first year after liver transplant. Compared to low DRI organs, there was a consistent increase in the cost of care associated with the use of higher risk organs [69,74] (Figure 142.5). High DRI organs (>1.8) were associated with $33 627 in additional costs compared to low risk organs. Among the individual components of the DRI, DCD donation ($59 000), Black race ($15 000), Hispanic race ($15 000), nationally shared liver allografts ($30 000), and donor age >40 ($34 000), were associated with an increase in the cost of transplant care.

While the cost of liver transplantation care and quality of life benefits have been relatively well characterized, prior assessment of the overall cost effectiveness of liver transplantation has been limited by the lack of a comprehensive measure of pretransplant costs indexed to severity of illness and by an assessment of

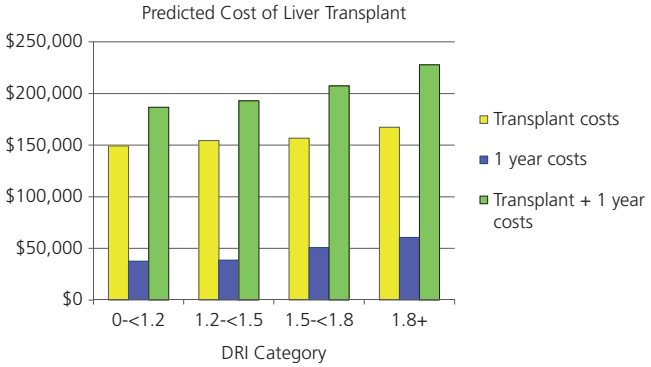

Figure 142.5. Impact of liver donor characteristics on cost of transplant. Data from [52].

the cost of patients who die while waiting of an available liver. Northrup and colleagues recently completed a Markov analysis of liver transplantation cost effectiveness comparing deceased donor liver transplantation, living donor liver transplantation, and medical management [75,76]. In this model, deceased donor LT was associated with an incremental cost of $180 804 compared with medical management. Evaluation for and potential receipt of living donor transplantation added $248 255. Overall, the deceased donor liver transplantation was associated with a cost of $35 976 per quality adjusted life year (QALY), while living donor transplant resulted in costs in excess of $100 000 per QALY. These results are in line with other recent analyses of deceased donor transplants, all of which have been based on Markov models developed from literature review to determine the cost and incidence of complications.

Summary

Continued expansion of the number of patients in need of liver transplantation is likely to result in greater pressure on the organ supply. As a consequence, patients will have greater degrees of decompensating prior to transplant. One likely consequence of increasing disease severity among waiting list patients is an increase in waiting list mortality and perioperative complications. These changes will undoubtedly increase the cost of care for patients with ESLD while waiting and at the time of transplantation. Unfortunately, current efforts to expand the donor supply (e.g. DCD allografts) appear to exacerbate rather than improve these trends. Reallocation of existing livers across a broader area has the potential to decrease wait-list mortality without a dramatic increase in the overall cost of care. As with renal transplant, further use of marginal donors is likely to be limited by financial pressures on transplant centers. Prospective adjustment of payments for complicated patients and donors is needed to ensure that the benefit of all potential organs is realized.

Heart transplant

Background:

While end stage kidney and liver failure represent important sources of morbidity and health care expenditure, their impact is dwarfed by the staggering consequences of chronic heart failure (CHF) worldwide. CHF is the final pathway of a variety of conditions including idiopathic (non-ischemic) cardiomyopathy, ischemic cardiomyopathy resulting from coronary artery disease, chronic hypertension resulting in diastolic dysfunction, acquired or congenital alular disease, and infective myocarditis [77]. Population based estimates predict a 20% lifetime incidence of CHF among individuals greater than 40 years old [76]. Although many patients with CHF can be successfully managed with medical therapy (e.g. ACE inhibitors), others develop refractory arrhythmias requiring implantable cardioverter-defibrillators (ICDs) or progressive ischemic heart disease that benefit from revascularization. Patients failing these treatments are deemed end stage and require consideration for additional interventions including cardiac transplantation.

End stage CHF currently affects between 250 000 and one-half million Americans [78]. End stage CHF is a lethal condition with a very short expected survival. Among patients assigned to the medical treatment arms of recent randomized trials of left ventricular support devices (LVADs), average mortality was 75% at one year and 87% within 2 years [79]. Advanced disease requiring chronic inotrope support, results in an average survival of 3.4 months and a 6–11% 1-year survival rate [80,81]. Currently, between 80 000 and 150 000 US patients would benefit from cardiac replacement therapy with heart transplant if the supply of organs were sufficient [82]. Unfortunately, only 2200 heart transplants are performed annually in the US. While patients who did not undergo transplant were formerly offered palliative care, the development of improved mechanical assist devices provides a growing alternative with the potential for long-term survival, albeit at great cost.

CHF is the most common discharge diagnosis among US Medicare recipients, resulting in over one million hospitalizations annually [78]. Patients are frequently readmitted for ongoing care (25% at 30 days; 50% at 180 days). Among patients with advanced (stage D) heart failure, the overall rate of freedom from hospitalization or death at one year was only 33% [83]. Spending on heart failure appears to be increasing markedly since 2000. Medicare beneficiaries with CHF spend an average of $36 216 during the last 6 months of life in 2007, a 26% increase since 2000 [84]. Despite an increasing use of hospice services, nearly 80% of patients were still hospitalized at some point in this period and 35% died in an acute care setting. Among patients randomized to the medical care arms of the LVAD trials, spending over two years exceeded $200 000, largely driven by inpatient costs [85].

The cost of medical management of CHF is expected to rise further with the deployment of advanced therapies to an aging population. For example, despite population-based analysis that suggest 15–40% of patients with advanced CHF could benefit from ICD therapy, ICD implantation remains limited and demonstrates substantial geographic variation [86]. Comparison of ICD implantation rates, demonstrated a 250% increase in rate between the lowest and high regions, which led to small increases in overall survival (64.8% to 66%). Regions with higher utilization rates had greater numbers of cardiologists and higher per capita income. Expansion of this technology could reduce the incidence of sudden death; however, at a cost of more than $50 000 per device, it is not clear that this approach is cost effective or affordable.

The clinical benefits of heart transplant

Heart transplantation offers patients with end stage heart failure the best opportunity for long-term symptom free survival. The International Society of Heart and Lung Transplantation database estimates that 5000 transplants are performed worldwide [87]. Approximately 50% of the transplants occur in centers that perform

less than 20 transplants per year, and only 5% of centers perform more than 40 transplants.

In the most recent decade, the most common etiology of transplantation for heart failure is non-ischemic cardiomyopathy (53%), followed by ischemic cardiomyopathy (38%), with the remainder composed of varied diagnoses including retransplantation, congenital heart disease, and valvular heart disease. This represents a shift in the dominant etiology leading to transplant. In North America, ischemic cardiomyopathy was the dominant diagnosis from 1997 to 2003.The shift back to non-ischemic cardiomyopathies disease may reflect changes in the management of CHF and the use of mechanical support. Other significant demographic changes comparing patients transplanted in 1992–2001 with patients transplanted 2002–2008 include a greater percentage of heart transplant recipients over the age of 65 (6.6% vs. 10.3%), a higher incidence of diabetes (14.2% vs. 22.4%), and a dramatic rise in the percentage of patients with LVADS (13.4% vs. 20.1%) and RVADS (0.5% to 3.1%).

The median survival of patients following heart transplant has steadily increased from 8.3 years in patients transplanted in the 1980s to 10.4 in the 1990s, and further in the 2000s, though an exact estimate is not possible as greater than 50% of the patients are still alive [87]. Among patients who survive at least 1-year, the median survival has increased from 11.4 to 12.9 years. Multivariate analysis has demonstrated reduced survival among patients who were maintained on preoperative mechanical support devices (RR of mortality: 2.93 for patients with temporary extracorporeal support, 1.33 axial flow LVAD, 1.22 for pulsatile perfusion LVAD). Additional risks for poor outcome include congenital heart disease, use of renal replacement therapy, prior transfusions, and ABO non-identical transplant. Among adult recipients, age has a U-shaped relationship with mortality, with higher mortality from age 18 to 30 and among patients greater than 55. Mortality rates are highest in the 30 days following transplant, largely resulting from graft failure. Long-term mortality is associated with the development of chronic allograft vasculopathy, malignancy, and infection (through the risk declines with time). Acute rejection is now an infrequent cause of graft loss or mortality.

Heart transplant donor selection remains more conservative than that of kidney or liver transplantation [88]. Only 39.2% of Eurotransplant donors were considered for heart donation and only 26% were actually used. However, the pressure to increase access to transplantation has led to limited expansion of "marginal donor" transplants. Registry data comparing transplants performed between 1992–2001 and 2002–2008, demonstrated that mean donor age has increased by 2 years, donor BMI is slightly greater (24.2 to 25.1), and prevalence of hypertension has increased. Interesting, the mean donor age is 6 years greater in Europe than in North America.

The Eurotransplant association has designed a scale to assess donor hearts [88]. Factors associated with an increased risk of graft failure include donor age, cause of death, angiographic results demonstrating CAD, ECHO cardiographic demonstration of left ventricular hypertrophy, high serum sodium, and vasopressor use. This scoring system predicted both donor discard and long-term survival. Additionally, a strong correlation has been noted between donor gender-recipient gender mismatch and outcome. Men receiving female allografts had the greatest risk of long term allograft failure [89].

Economic assessment of cardiac transplant including the impact of donor and recipient characteristics has not been evaluated to the same extent as liver and kidney transplantation. The cost of heart transplant has been compared retrospectively with surgical ventricular restoration (SVR) procedures designed to improve left ventricular function [90]. SVR procedures included coronary artery bypass grafting (CABG), mitral valve repair or replacement, and ventriculotomy with excision of scar and reshaping. A series comparing 69 SVRs with 53 cardiac transplants at Johns Hopkins demonstrated similar survival. Median hospital charges were significantly less for SVR than heart transplant ($45 506 vs. $137 679). However, the recent randomized trial comparing SVR with CABG alone (the Surgical Treatment for Ischemic Heart Failure trial) failed to demonstrate a substantial benefit of SVR over CABG alone which limits the utility of these findings [91]. The single study examining the cost effectiveness of heart transplant was performed in the pediatric population [92]. Published in 2006, the median cost of hospitalization was $221 987 for primary transplant and $285 296 for retransplant. Post-transplant costs were estimated at approximately $18 000 annually. Using survival data drawn from UNOS data, the authors estimated that primary transplant was associated with a cost per QALY of $49 679 ($44 943–$57 628) for primary transplant and $87 883 ($70 834–$103 661) per QALY for retransplantation. These estimates fall marginally under the commonly accepted cost effective threshold of $60 000 per QALY saved for primary pediatric heart transplantation. Corresponding estimates for adults are not available, but may exceed acceptable QALY thresholds given the increased cost and decreased survival potential for older recipients.

Mechanical alternatives to heart transplant:

The development of alternative treatment strategies for end stage heart failure is driven by marked limitation in access to donor allografts. Heart transplantation rates do not exceed 2–3% of the overall need for cardiac replacement therapy. Moreover, expansion of the donor pool to include living donors and donation after cardiac death as has been done for kidney and liver transplantation is not possible for heart transplantation. LVADs are now employed either as a bridge to eventual transplantation (BTT) for waiting list patients with progressive failure or as a permanent treatment for advanced heart failure [82]. Permanent implantation, termed destination therapy (DT), has been prospectively evaluated in several large clinical trials beginning in the late 1990s, leading to FDA and Medicare approval.

The Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial enrolled 129 patients in 20 transplant centers who were randomized to medical management or placement of the HeartMate XV (HM XV) device [79].Entry criteria in the trials were very strict and included only patients with advanced heart failure (New York Heart Association Class IV CHF symptoms, left ventricular ejection fraction $<$25%, and either peak oxygen consumption less than $<$12 mL/kg/min or need for intravenous inotropic infusion). Enrolled patients were deemed not to be transplant candidates. The patients randomized to the LVAD group, had a doubling of their survival (25% to 52% at 1-year, 8% to 23% at 2-years) and improved quality of life. Because the HM XV, is a pulsatile device, patients required long-term anticoagulation which contributed to significant morbidity including bleeding complications, and stroke.

Recent design improvements have reduced the morbidity of LVAD implantation resulting further reduction in CHF related mortality [93]. The HeartMate II (HM II) is an axial, continuous flow pump in which a suspended impeller is used to augment

cardiac output. LVAD placement remains a complicated procedure with a significant risk of operative mortality and morbidity. The majority of the mortality associated with LVAD use occurs during the initial hospitalization for implantation, and has not improved with technology. In the DT trial comparing HM XVE (an improved version of the HM XV) and the HM II, patients had 30 day mortality rates of 28% and 26%. Long-term, however, the HM II design improved the probability of freedom from stroke or device failure resulting in improved 2-year survival compared with the earlier design (58% vs. 24%). In the pivotal trial, only 9% of the HM II recipients required pump replacement compared with 34% of the HM XVE. Based on these results, the HM II was approved by the FDA for BTT in April 2008 and destination therapy in January 2010. Additional devices are currently under development, which utilize magnetic levitation technology to eliminate the need for bearings and improve device life. Also in development are devices without external drivelines and extended battery life.

Despite the complexity of LVAD therapy, many DT patients report a substantial improvement in overall quality of life. The HM II design appears to have limited the duration of rehospitalization and improved patient rated quality of life. Retrospective analysis 30 DT patients treated at Johns Hopkins with either the HM XV or HM II for one year revealed a similar average number of admissions per year (2.9 ± 2.2) but a reduction in average stay (26.3 days for HM XVE to 6.8 days for HM II) [94]. Three patients with HM II were never readmitted after the initial procedure. Patients reported a remarkable reduction of NYHA class (3.9 to 1.4), improved exercise tolerance, and better quality of life scores.

Current indications for DT therapy under Medicare are based on the REMATCH trial criteria. Patients must have advanced heart failure, an appropriate body size to allow implantation, and a contraindication to heart transplant due to age or co-morbidities. However, the choice between DT and BTT may not be clear at the time of original implantation [95]. For instance, in the pivotal trials, approximately 20% of patients who were originally deemed not to be heart transplant candidates due to medical co-morbidities or insufficient period following a malignancy, improved sufficiently to undergo HT. Many of these patients had resolution of severe pulmonary hypertension, improved renal function, or achieved substantial weight loss. Conversely, patients treated with LVAD as a BTT, who are blood group O, highly sensitized, or at the extremes of body size (very small or very large), may never be allocated appropriate allografts.

The overall cost of treatment for the population of patients with advanced heart failure will undoubtedly increase when the constraint of the supply of viable allografts is lifted by further development of mechanical support devices. Like hemodialysis for renal failure, the US is poised to create a large population of patients with end-organ failure who have an indefinite reliance on mechanical support devices. While age was originally viewed as a contraindication to DT, recent studies have demonstrated successful implantation in patients over age 70. A recent retrospective analysis of 2943 patients receiving LVAD within the Medicare program between 2000–2006 assessed the cost and outcome among patients receiving LVAD as a primary procedure compared with those who had undergone a prior cardiac procedure (cardiotomy) in the last 30 days [96]. The mean 1-year payment for inpatient stays alone was $178 714 for patients with primary device implantation and $111 769 in patients who had undergone a previous cardiotomy. Survival in these populations was 73% at one year for the primary implantation and 76% for the postcardiotomy cohort. While the cost of the initial implantation has decreased over the past decade from $210 000 per patient to $126 000 as a result of shorter length of stays and complication rates, reimbursement has continued to climb ($188 000 in 2010 under Medicare). More recently, a Markov model of outcomes was designed using data from recent clinical trials in estimate the cost of complications following LVAD placement compared with medical management [97]. For the continuous flow pumps, they estimated a cost effectiveness ration of $198 184 per QALY. While still expensive, this is a dramatic reduction from the estimates of $802 700 per QALY for pulsatile pumps. Given the clear clinical need and recent Medicare coverage decisions, it is likely that total spending will increase dramatically, if even a fraction of the 80 000–150 000 patients with advanced CHF undergo placement of an LVAD. The return on this health care investment has been questioned given the high cost and relatively short survival of patients following VAD placement. Recent analyses have suggested that while LVAD therapy is not yet cost effective either for DT or BTT given a current standard of $60–100 000 per life year, the cost effectiveness may be improving for the pulsatile perfusion devices [97–99].

Summary

Heart transplantation remains the best alternative for patients with end stage heart failure, but its economic impact is incompletely studied. Confounding aspects of its delivery include an expanding population of elderly potential recipients, and an increasing number of non-transplant alternatives that also have substantial competing expenses. These issues will prompt significant discussions of resource utilization.

Conclusion

Therapy for end stage organ failure has evolved from symptom palliation to functional replacement with the expectation of long-term patient survival. Outcomes following transplant remain far from uniform. Younger, healthier recipients of optimal allografts have achieved levels of success previously unimaginable. This success has led to inclusion of higher risk candidates and expanded waiting lists. As a consequence, transplant professionals are forced to utilize a growing number of marginal organs, and are still unable to meet the demand.

The ability of medical professionals to meet the needs of patients with organ failure is further limited by available resources to invest in high cost services. While challenges of the 20th century were largely proof of scientific principal and technique development, the hurdles of the 21st are questions of scale and sustainability. Expanding indications and further erosion of the quality of the organ supply will increase the cost of care. However, if the constraint of the limited number of organs is removed by the development of mechanical support devices, spending will likely far exceed the capacity of even developed nations. Already, the demand for definitive treatment for end stage organ failure is orders of magnitude greater than we treat today, particularly when considered on a global scale. As we witnessed in the 1970s with dialysis for ESRD, the availability of cardiac mechanical support, unconstrained by the necessity to ration based on a limited organ supply and supported by federal financing through Medicare's approval of LVAD for DT, will far eclipse the cost of organ transplant for CHF. Choosing the appropriate recipient for new technology is crucial to achieving success.

Perhaps the greater challenge both ethically and financially is determining "who is not a candidate" given the limited number of organs and diminishing financial support for health care. Transplantation requires the assignment of a limited resource among a greater number of patients, all of who could potentially benefit to some degree. Absent the barrier of the organ supply, another metric will be needed to restrict access to beneficial treatments based on society's limited resources. In addition, absolute reliance on incremental cost effectiveness as the measure, may limit promising technologies based on today's cost and outcomes. Based on these measures, organ transplantation would never have been approved. However, over decades of perseverance, we have now established a cost effective solution for many patients with end organ failure. Balancing the opportunity to innovate with the necessity to constrain spending may be the greatest challenge facing transplant professionals for the next fifty years.

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CHAPTER 143

National Healthcare Policy, Transplant-specific Statutes, and the Future of Organ Transplantation

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Introduction

During the past century, transplantation and health policy in the US have evolved in ways inconceivable to previous generations. From the first successful kidney transplant performed in Boston, Massachusetts in 1954, to the first successful liver transplant in 1967, to the establishment of the first organ procurement organization (OPO) in 1968, to the 1980 Uniform Determination of Death Act, and to the first successful face transplant in France in 2005, transplantation has changed dramatically. Health policy too has evolved tremendously, from the establishment of Medicare and Medicaid in 1965 and the adoption of employer-based private health insurance, to the current healthcare climate in which healthcare expenditures exceed 17% of the Gross Domestic Product (GDP). Technology has advanced, health insurance coverage has expanded, and delivery systems have improved so that more Americans have access to healthcare than ever before. The Patient Protection and Affordable Care Act passed in 2010 will continue to reshape the healthcare landscape through policies aimed at expanding coverage, implementing market reforms, improving healthcare quality through payment incentives and other demonstrations, and containing costs. These reforms will undoubtedly affect all areas of healthcare, and have important implications for transplantation.

The purpose of most existing transplantation policy is fourfold: to protect the health of recipients and donors, to preserve dignity and protect autonomy throughout the transplantation process, to enhance efficiency in organ allocation, and to ensure equity in availability and allocation of resources. The future of transplantation policy is a broad topic beyond the scope of a single chapter. However, in this chapter, we address a number of key challenges central to the future of transplantation policy, including: implications of health reform for patients and providers, and meeting a growing demand for organs through donation incentives. Given the marked variability of specific laws worldwide, this chapter is presented from a North American perspective, with specific examples from the U.S. legal system. However, the general themes facing transplantation in the U.S. are reflected in similar legal statues in most developed countries. Additional discussions on national oversight in general, and cultural variability as it relates to transplant policy and practice, can be found in Chapters 128 and 139, respectively.

Legal and regulatory structure of transplantation in the U.S.

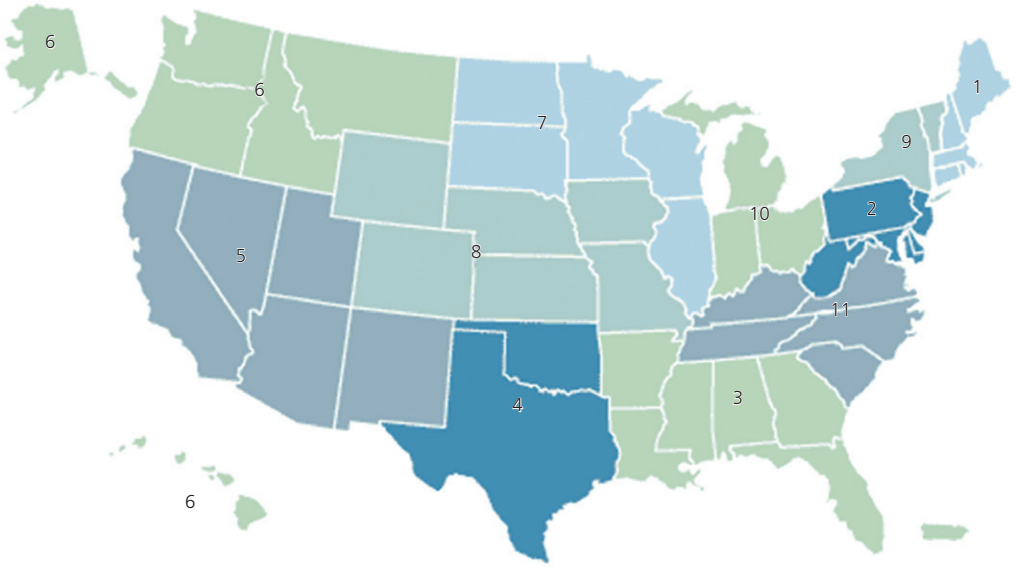
The U.S. transplant system is composed of a network of transplant centers, donor hospitals, OPOs, and ten geographic regions for the purposes of allocation of organs. Each donor hospital is situated within a single geographic region and assigned to a specific OPO (Figures 143.1 and 143.2). Organ donation and transplantation policies are dictated by the Organ Procurement and Transplantation Network (OPTN), which currently contracts with the United Network on Organ Sharing (UNOS) to frame and administer transplantation policies. The Division of Transplantation (DOT) of the Health Services and Resources Administration (HRSA) (a branch of the Department of Health and Human Services (DHHS)), provides federal oversight of the OPTN (Figure 143.3).

Significant changes in transplantation policy have taken place over more than 20 years. For example, the organ allocation algorithms have been devised and revised, criteria for donors and recipients have been expanded, and process, quality and outcomes standards have been established and enforced by the Centers for Medicare and Medicaid Services (CMS). Although some standards have been universally applied, others, such as OPO policy allocation variances, were granted regionally or on an individual basis, creating a patchwork of protocols [1]. Currently, UNOS policy efforts are aimed at reconciling variances and at amending allocation algorithms to better achieve equity and efficiency. Other initiatives involve changes to the kidney allocation rules [2], inclusion of the Kidney Donor Profile Index (KDPI) score in UNet, initiatives to prevent transmission of donor diseases, and for liver transplantation patients, tiered regional sharing to facilitate wider sharing for critically ill patients with MELD/PELD scores above 35 [3].

Despite central oversight for transplantation, laws and rules governing the transplantation process are layered and somewhat fragmented. The legal and regulatory policies that shape transplantation are framed at four levels, in order of most to least granular: UNOS policies, federal regulations, and state and federal laws.

UNOS rules provide detailed instructions and operational protocols for the OPTN, OPOs, and transplant hospitals. UNOS is a private, non-profit organization that is contracted by the OPTN to manage organ transplantation, donation, allocation, and distribution nationally. UNOS rules dictate how transplant data is

Textbook of Organ Transplantation, First Edition. Edited by Allan D. Kirk, Stuart J. Knechtle, Christian P. Larsen, Joren C. Madsen, Thomas C. Pearson, and Steven A. Webber.



The states comprising each region are as follows:

- Region 1: Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, Eastern Vermont
- Region 2: Delaware, District of Columbia, Maryland, New Jersey, Pennsylvania, West Virginia, Northern Virginia
- Region 3: Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, Puerto Rico
- Region 4: Oklahoma, Texas
- Region 5: Arizona, California, Nevada, New Mexico, Utah
- Region 6: Alaska, Hawaii, Idaho, Montana, Oregon, Washington
- Region 7: Illinois, Minnesota, North Dakota, South Dakota, Wisconsin
- Region 8: Colorado, Iowa, Kansas, Missouri, Nebraska, Wyoming
- Region 9: New York, Western Vermont
- Region 10: Indiana, Michigan, Ohio
- Region 11: Kentucky, North Carolina, South Carolina, Tennessee, Virginia

Figure 143.1. Current UNOS Region Map *(Reproduced from http://optn.transplant.hrsa.gov/members/regions.asp)*

transmitted, stored, and disseminated between hospitals, OPOs, and UNOS. UNOS also maintains a national database. UNOS is tasked with managing the national waiting list, running the matching algorithms, and overseeing the public process for continuously monitoring and improving organ allocation. Finally, UNOS is charged with promoting awareness and education related to organ donation and transplantation.

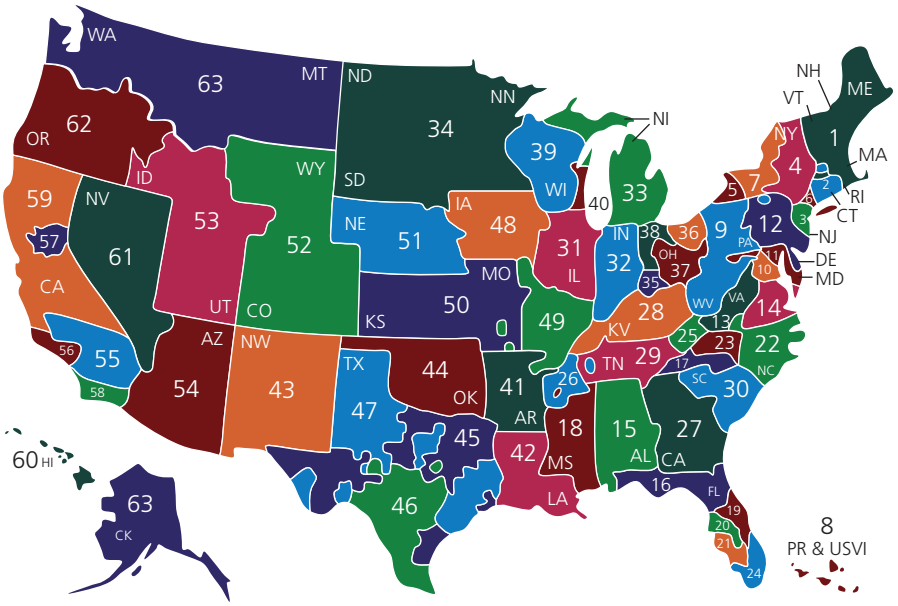
Just as UNOS rules provide practical guidance for transplant providers, federal regulations and CMS requirements also provide concrete direction for the OPTN, OPOs, and transplant programs by clarifying key principles and procedures.

At a higher level, state laws define the donation process. These have been amended to include criteria for declaring death, donor consent requirements, composition of donor registries, the scope of public education programs, and benefits for living donors. In contrast, federal laws govern national processes such as organ procurement, allocation, transplantation, and anatomical gifts. Federal laws outline the principles and constraints at the highest and broadest level; they establish the OPTN and guidelines for OPOs. For example, the federal laws establishing the OPTN stipulate that it is responsible for coordinating, monitoring, and implementing organ transplantation policies and procedures at the national level, while OPOs, which are local or regional organizations are responsible for retrieving organs and notifying potential recipients under the supervision of the OPTN [4]. The sections below discuss in greater detail these types of laws and regulations, as they pertain to organ transplantation.

UNOS

While the federal laws and regulations dictate the framework of the OPTN and its relationship with member OPOs and transplant centers, UNOS is responsible for many of the policies that dictate the action of the OPTN and its members [5]. UNOS has been the organization that administers the OPTN through a contract with DHHS since 1986. While UNOS and the OPTN are legally distinct, they have the same Board of Directors and the same policies.

UNOS is tasked with ensuring that organs are procured and allocated as expeditiously and as fairly as possible through the Organ Center. The Organ Center matches donors with recipients and manages distribution of organs using UNet, a nationwide transplant computer system. UNet maintains information about all wait listed patients. Once the donor organ information is put into UNet, it generates a list of ranked potential recipients for each organ type. Finally, when a match is made and the potential recipient's transplant hospital agrees to receive the organ, the organ is delivered by the corresponding OPO. Importantly, UNOS monitors the matching process and ensures adherence to organ allocation policies. The UNOS Evaluation and Quality Department also



1. New England Organ Bank. Inc.
2. Northeast OPO and Tissue Bank
3. NJ Organ and Tissue Sharing Network
4. Center for Donation and Transplant
5. Upstate New York Transplant Services. Inc.
6. New York Organ Donor Network
7. Univ. of Rochester Organ Procurement Program
8. Lifelink of Puerto Rico
9. Center for Organ Recovery and Education
10. Washington Regional Transplant Consortium
11. Transplant Resource Center of Maryland
12. Delaware Valley Transplant Program
13. Virginia Organ Procurement Agency
14. Life Net
15. Alabama Organ Center
16. The OPO at University of Florida
17. Life Share of the Carolinas
18. Mississippi Organ Recovery Agency. Inc.
19. Translife
20. Lifelink of Florida
21. Lifelink of Southwest Florida
22. Carolina Organ Procurement Agency
23. Carolina Life Care
24. University of Miami OPO
25. Life Resources Donor Center
26. MId-South Transplant Foundation
27. Lifelink of Georgia
28. Kentucky Organ Donor Affiliates
29. Tennessee Donor Services
30. SC Organ Procurement Agency
31. Regional Organ Bank of Illinois
32. Indiana OPO. Inc.
33. Organ Procurement Agency of MI
34. Upper Midwest OPO. Inc.
35. Ohio Valley Life Center
36. Lifebane
37. Lifeline of Ohio
38. Life Connection of Ohio
39. University of Wisconsin OPO
40. Wisconsin Donor Network
41. Arkansas Regional Organ Recovery Agency
42. Louisiana Organ Procurement Agency
43. New Mexico Donor Program
44. Oklahoma Organ Sharing Network. Inc.
45. Southwest Transplant Alliance
46. South Texas Organ Bank
47. Life Gift Organ Donation Center
48. Iowa State Organ Procurement Organization
49. Mid-America Transplant Association
50. Midwest Organ Bank
51. Nebraska Organ Retrieval Systems. Inc.
52. Colorado Organ Recovery Systems. Inc.
53. Intermountain Organ Recovery Systems
54. Donor Network of Arizona
55. Southern California Organ Procurement Center
56. Regional Organ Procurement Agency of Southern CA
57. Golden State Transplant Services
58. Organ and Tissue Acquisition Center of Southern CA
59. California Transplant Donor Network
60. Organ Donor Center of Hawaii
61. Nevada Donor Network
62. Pacific Northwest Transplant Bank
63. LifeCenter Northwest

Figure 143.2. Current OPO region map (Data from Penhoet, E. D. *et al.*, *Organ Procurement and Transplantation*, Washington, D.C., National Academy Press, 1999.)

monitors OPTN members, OPOs and hospitals, and ensures that they are compliant with rules and that they can receive CMS benefits. As efforts to promote paired kidney exchange nationally increase, UNOS may play a role in coordinating a united national system.

In addition, UNOS develops policies for the OPTN. Policy proposals are developed by UNOS committees, distributed as initial briefs, and circulated for public comment.

UNOS then incorporates the public comments and submits the final proposal for approval by the Board of Director to become UNOS/OPTN policy. UNOS has 22 committees: Ad Hoc Disease Transmission Advisory, Ad Hoc International Relations, Ethics, Executive, Finance, Histocompatibility, Kidney Transplantation, Liver and Intestinal Organ Transplantation, Living Donor, Membership and Professional Standards, Minority Affairs, Operations and Safety, OPO, Pancreas Transplantation, Patient Affairs, Pediatric Transplantation, Policy Oversight, Thoracic Organ Transplantation, Transplant Administrators, and Transplant Coordinators. The Board of Directors can also submit approved proposals to the Secretary of HHS, and if approved, the policies are incorporated into

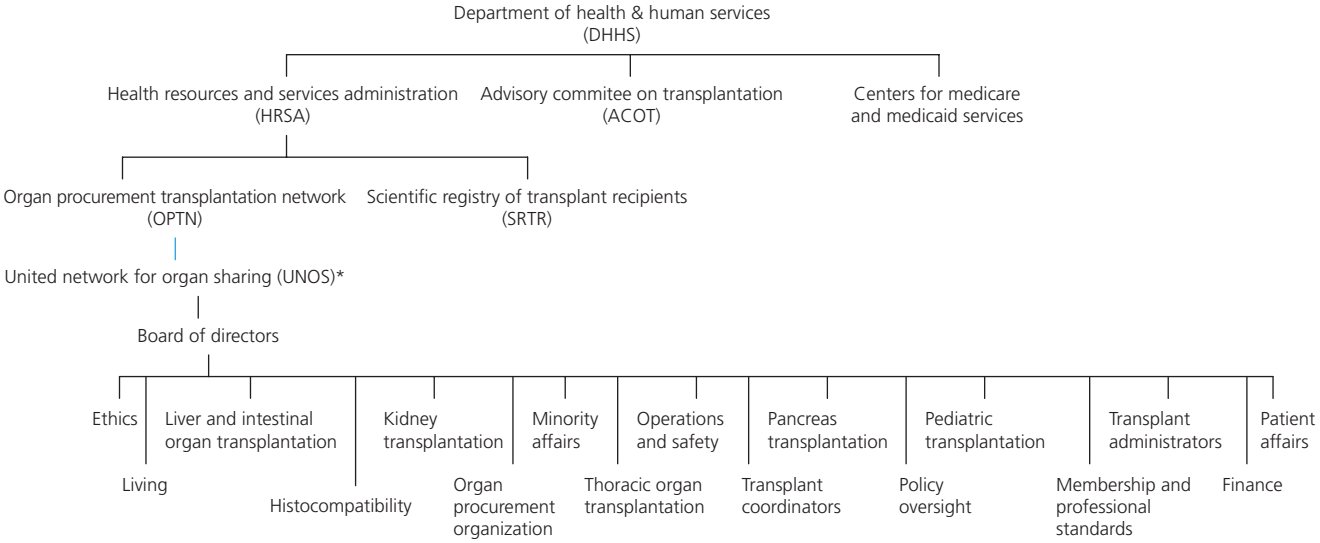


Figure 143.3. Schematic representation of transplantation oversight structure.

official regulations. Most of the committees, including the Board of Directors, are comprised of elected members representing the transplant community. These members include: transplant clinicians and coordinators, histocompatibility experts, OPO representatives, voluntary health organizations, medical or scientific member organizations, and public representatives (including transplant candidates, transplant recipient and families, living donors and families). The Board of Directors is comprised of 41 members and is responsible for overseeing the policy development for UNOS. The diversity of the committees is critical to ensure that all voices and interests present in the transplant community are heard during the policy-making process.

Finally, UNOS has an important role in maintaining public trust and promoting organ donation and transplantation. It provides information and support for patients and their families, as well as for donors. Similar to the Scientific Registry, UNOS collects and distributes information on organ transplantation from UNet. Along with the states, UNOS also promotes the need for organ donation.

Federal regulations and CMS requirements

Federal regulations

These federal laws are supplemented by federal regulations on organ transplantation in Title 42, "Public Health" of the Code of Federal Regulation 42 CFR Part 121 [6]. Effective March 16, 2000, DHHS implemented the "Final Rule", which established the organizational structure and regulatory framework of the OPTN. All OPTN members must comply with the Final Rule, and to future policy amendments added by UNOS, the OPTN, and HHS.

Title 42 Section 121

Describes requirements for transplant programs, guidelines for organ procurement, regulations of the OPTN, and allocation of organs.

Title 42 Section 121.4

Outlines policy guidelines for the OPTN Board of Directors. These include equitable distribution of organs, testing to avoid the spread of infectious diseases, reduction of inequities caused by socioeconomic status, and training of transplant surgeons and physicians.

Title 42 Section 121.5

Establishes listing requirements for an OPTN member. There are three main rules. The member transplant hospital may list a person only for a designated transplant program. The transplant hospital must place individuals on the waiting list as soon as they are deemed eligible to be candidates for transplantation. The OPTN member must pay a registration fee for each transplant candidate placed on the waiting list.

Title 42 Section 121.6

Outlines the determination of suitability of organs donated for transplantation. The OPTN member retrieving the organs must assure that laboratory tests and clinical examinations of potential donors are performed to identify any contraindications for donor acceptance. The OPTN shall have standards to prevent the acquisition of organs from HIV infected individuals. The transplant programs must also establish criteria for organ acceptance and provide them to the OPTN and OPOs.

Title 42 Section 121.7

Explains the identification of an organ recipient, allocation of the organ, and transportation to the recipient. The OPTN member must use the OPTN computer match program, with its organ specific allocation criteria, to identify and rank potential recipients. The OPTN member arranges for the transportation of the organ to the transplant hospital. If the transplant hospital rejects the organ, the OPTN member must offer the organ to the next most eligible recipient.

Title 42 Section 121.8

Establishes guidelines for the allocation of organs and performance goals. The allocation policies must be based on sound medical judgment, preserve the transplant program's right to decline an organ, be organ specific, and avoid wasting organs. Performance goals to ensure equitable allocation include standardizing the criteria for determining the suitable candidates, setting priority rankings

expressed through objective and measurable medical criteria and distributing organs over as large a geographic area as possible.

Title 42 Section 121.11

Requires the OPTN and the Scientific Registry to use a system for managing information about transplant candidates, recipients, and donors. This section establishes rules for rigorous reporting and record keeping through the HHS Secretary, the OPTN, the Scientific Registry, and the OPOs. Information must also be provided to the public on the performance of transplant programs.

Title 42 Section 121.12

Establishes the Advisory Committee on Organ Transplantation (ACOT) for opinions on proposed OPTN policies and any other issue the Secretary deems necessary.

Title 42 Section 482.90

Requires transplant centers to ensure that a prospective living donor receives medical and psychosocial evaluation, to document the living donor's suitability for donation, and to document informed consent.

Title 42 Section 482.92

States that, if a center performs living donor transplants, the transplant surgeon and at least one other licensed healthcare professional at the transplant center must verify that the donor's blood type and other vital information is compatible with transplantation immediately before the removal of the donor organ(s) and prior to the removal of the recipient's organ(s).

Title 42 Section 482.94

Requires transplant centers to write patient management policies including patient care policies for the pretransplant, transplant, and discharge phases of transplantation, and donor management policies for living donor evaluation, donation, and discharge.

Title 42 Section 482.98

Requires transplant centers to identify either an independent living donor advocate or an independent living donor advocate team to ensure protection of the rights of living donors and prospective living donors.

CMS requirements

Requirements fall into three categories: data submission, outcomes measures, and process measures. CMS final regulations (published March 30, 2007) became effective June 28, 2007, establishing conditions of participation (CoPs) for hospital-based transplant programs. These requirements focus on outcomes largely reflecting the clinical experience, quality of care, and resources available at the transplant center. Although CMS did not cede sole jurisdiction over transplant centers to the OPTN, in an effort to fulfill CMS's own role in ensuring patient safety, the additional CoP requirements are meant to complement existing OPTN rules. The data submission requirements largely mirror those of the OPTN (e.g. that 95% of all OPTN-required forms be submitted directly to CMS for all transplants within 90 days of the OPTN's required deadline). Outcome measures are meant to ensure patient and graft survival that is within the appropriate threshold (estimated by Scientific Registry of Transplant Recipient's [SRTR] data). Outcomes falling below the expected threshold will be deemed inadequate and thus may face penalties or sanctions. In addition to these initiatives, CMS instituted process measures, affecting patient and living donor selection and protection of rights, organ recovery and receipt, patient and living donor management requirements, waiting list management, detailed quality assessment and improvement requirements. Important changes include providing potential donors with complete information regarding the short and longer term risks of donation, opportunities for withdrawing from donation, and implications for future insurance coverage [7].

State laws

State laws are fairly uniform and generally govern the donation process, including: education and outreach initiatives, donor consent and donor reimbursement, donor registries, and designation of death. There are numerous state laws that govern donation. The major ones are briefly described below:

Uniform Anatomical Gift Act

The 1968 Act (and its periodic revisions) establishes criteria for donation of organs for the purposes of transplantation and donation of cadavers for medical research and education. The Act describes how the donation process might take place, designating who is eligible to donate the body after death, who has the right to use the body, and the priority list of persons that may donate the body if there is no preference against donation. The Act was revised in 1987, specifying that documentation of a desire to be an organ donor is sufficient for organ procurement (without consent of next of kin), and that witnesses are not required for documentation. The Act also mandated Routine Inquiry (questioning of patients about their organ donation preferences upon admission to a hospital) and Required Request (request from a deceased patient's next of kin in the event that donation preference was not indicated). The Act also prohibits trade of organs, which reiterates the federal ban enacted in 1984 [8]. The National Conference of Commissioners on Uniform State Laws (NCCUSL) has drafted this Act in an attempt to harmonize the law between states.

Recently, all 50 states and the District of Columbia adopted first-person consent laws (also called "First Person Authorization" or "donor designation" to force the honoring of donor intentions. First person consent laws mandate that the indication of an adult's intent to donate on any legally binding document, such as a driver's license, donor card, or online registry be upheld, even against their family's expressed wishes. This legislation frees the OPOs from securing consent from families to donate their loved one's organs, to informing the family of the patient's wishes to become an organ donor and educating the family about the process [9].

Many states have also created computerized donor registries. Revisions of state anatomic death acts allowed people to declare their intention to donate by enrolling in state donor registries, facilitating the identification of potential donors by OPOs. Elements of effective donor registries include: donor designation is considered legally binding consent; consent for tissue donation is included; individuals can enroll through a dedicated website; State Department of Motor Vehicles (DMV) enrolls donors via driver's license and ID card applications and renewals; no follow-up step required for State DMVs or online enrollment; State DMVs export donor records to registry database; organ, eye, and tissue recovery agencies can effectively access donor designations. Other initiatives include the establishment of the National Minority Organ/Tissue Transplant Education Program to increase the rate of donation by minorities, the formation of the Organ Donation Breakthrough Collaborative to raise the rate of recovery of organs in

Table 143.1. State initiatives to promote organ donation

State	Revenue sources		Legal consent				Donation education		Living donor support		
	Voluntary contributions	State-provided funds	UAGA version	Donor designation		ME/C	Schools	Driver education	Tax benefit	Leave of absence	
				Registries	Driver's license					Public	Private
	Total: 36 of 52	Total: 11 of 52	Years	Total: 52 of 52	Total: 51 of 52	Total: 48 of 52	Total: 13 of 52	Total: 15 of 52	Total: 16 of 52	Total: 31 of 52	Total: 9 of 52
AL	√		2006	√	√	√		√			
AK	√		2006	√	√	√				√	
AZ			2006	√	√	√	√			√	
AR	√	√	2006	√	√	√	√	√	√	√	√
CA	√		2006	√	√	√				√	
CO	√		2006	√	√	√				√	
CT	√	√	2006	√	√	√				√	√
DE	√		1968	√	√	√				√	
DC			1968	√	√	√			√	√	√
FL	√		1987	√	√	√					
GA			2006	√	√	√			√	√	
HI	√		2006	√	√	√					
ID			2006	√	√	√			√	√	
IL	√	√	1968	√	√		√			√	√
IN	√	√	2006	√	√	√	√	√		√	
IA	√		2006	√	√	√		√	√	√	
KS			2006	√	√	√				√	
KY	√		2006	√	√	√					
LA	√	√	2006	√	√	√	√	√	√		√
ME			2006	√	√	√		√		√	
MD	√		1968	√	√	√				√	
MA	√	√	1987	√	√	√		√		√	
MI	√		2006	√	√	√		√			
MN			2006	√	√	√		√	√	√	√
MS		√	2006	√	√	√	√	√	√	√	
MO	√		2006	√	√					√	
MT	√		2006	√	√	√					
NE	√		2006	√	√	√					√
NV	√		2006	√	√	√					
NH			2006	√	√	√					
NJ	√		2006	√	√	√	√				
NM	√		2006	√	√	√		√	√	√	
NY	√	√	1987	√	√	√			√	√	
NC		√	2006	√	√	√					
ND			2006	√	√	√			√	√	
OH	√		2006	√	√	√	√	√	√	√	
OK	√		2006	√	√	√	√			√	
OR			2006	√	√	√				√	
PA	√		1987	√	√				√		
RI	√	√	2006	√	√	√		√			
SC	√		2006	√	√	√				√	√
SD			2006	√	√	√					
TN	√		2006	√	√		√				
TX	√		2006	√	√					√	
UT	√		2006	√	√	√			√	√	
VT			2006	√	√	√					
VA	√		2006	√	√	√		√		√	
WA	√		2006	√	√	√		√			
WV			2006	√	√	√				√	
WI	√		2006	√	√	√		√	√	√	
WY	√		2006	√	√	√					
PR		√	2006	√							

participating hospitals, efforts to increase retrieval of organs from cardiac death donors as well as brain death donors, and provision of financial subsidies to donor families, such as contributions to funeral expenses in Pennsylvania [10]. All states recognize the driver's license as a document of gift. Finally, most states subscribe to the same ways of determining death. These states base their definitions on the NCCUSL's Uniform Determination of Death Act.

Although there is consensus among states on areas defined above, state laws diverge in some important ways. Some states have anatomical gift funds meant to increase public awareness and encourage organ donation. These initiatives will be discussed below under "Promoting Organ Donation." Some states have laws aimed at removing disincentives to donation, primarily by addressing the financial burden associated with donation. These initiatives may include: paid leave for state employees, a requirement for private employers to provide unpaid leave of absence for organ donation, and tax credits if the donor received paid leave or tax deductions for unpaid donation expenses. The location of donor laws within state codes also varies (Table 143.1).

Federal laws

Federal laws in the U.S. dictate the legal structure of organ transplantation, and are primarily found in Title 42 of the United States Code, which is called "The Public Health and Welfare" [11]. These laws frame the American system of organ transplantation by establishing the OPTN, the SRTR, defining how organs may be transferred between persons, and assisting federal employees who become organ donors. Further on in this chapter details the three sections and several subsections that together comprise the federal framework for transplantation.

Title 5 Section 6327 of the United States Code

Describes provisions for federal employees donating an organ. The Code states that an employee of any executive agency is entitled to a leave from work without a loss of pay to serve as a bone marrow or an organ donor. This supplements state laws to reduce disincentives and costs associated with organ donation.

Title 42 Section 273

Establishes the roles and structures of transplantation organizations. Section 273 requires that the OPO be a non-profit organization under the jurisdiction of the Secretary for Health and Human Services, and ensures equitable allocation and efficient procurement. OPOs must maintain connections with local hospitals and together identify and consent potential donors. Section 273 also describes federal funding for OPOs, the geographic areas that OPOs must serve, and the policies that they must follow to be reimbursed. Title 42 Section 273a outlines evaluations of the long-term effects associated with living organ donations.

Title 42 Section 274

Establishes the OPTN as the main national entity governing the procurement, allocation, and transplantation of organs. The OPTN must first establish membership criteria and medical criteria for the allocation of organs, then establish a national list of individuals requiring organ transplantation and a national system, including a telephone service, to help match organs and individuals on the list. The OPTN must assist OPOs in the nationwide distribution of organs. And finally, it must carry out studies on how to improve organ procurement and allocation. UNOS has had the contract for the operation of the OPTN since its establishment.

Section 274a

Establishes the Scientific Registry as a national database on organ transplantation that includes national transplant statistics, transplant center-specific reports, and transplant research resources.

Section 274b Establishes general provisions for grants and contracts.

Section 274c and 274d Establishes administration and report.

Section 274e

Prohibits the buying and selling of organs. Organs must be given as gifts without payment. While living donors may receive reimbursement for the costs of giving, only those who provide the necessary services involved in transplantation may receive payment. The Charlie W. Norwood Living Organ Donation Act of 2007 clarified that paired donation is not deemed valuable consideration, ensuring that criminal penalties are not applied to human organ paired donation.

Section 274f

Permits grants for the reimbursement of travel, subsistence, and appropriate incidental non-medical expenses incurred by living donors. These grants can be provided to states, transplant centers, and qualified OPOs. Section 274f-1, 274f-2, 274f-3, 274f-4 contain rules regarding public awareness, studies and demonstrations, grants, and studies and reports relating to organ donation and the recovery, preservation, and transportation of organs.

Multiple levels of oversight are needed to ensure that the transplantation system not only functions effectively and efficiently in allocating scarce resources in acute situations, but also that it upholds the ethical principles of fairness, equality, efficiency, and transparency. To this end, the rules and regulations promote organ donation as a public good while protecting individual autonomy in donation decisions. The laws protect the equal rights of citizens and prevent discrimination in organ allocation. Finally, the laws protect the dignity of human life, and prohibit commodification and trade of organs.

Implications of the Patient Protection and Affordable Care Act (PPACA)

Implications for patient care

The passage of the Patient Protection and Affordable Care Act (hereafter PPACA) (P.L. 111-148) presents the single largest piece of healthcare legislation since the passage of Medicare and Medicaid in 1965. A massive piece of legislation with many goals, PPACA has two main aims: expanding access to health insurance and limiting the growth in healthcare costs and reform the delivery system. One of the primary goals of health insurance is to improve access to healthcare for many Americans, including the 50.7 million uninsured and the 25 million underinsured [12]. To this end, the Act aims to increase insurance coverage to 32 million Americans by 2019 (16 million from Medicaid expansions, and 16 million from insurance exchanges and expansions of private insurance). The major provisions affecting transplant patients in the PPACA seek to expand coverage and reduce exorbitant costs associated with receiving needed healthcare. The Act attempts to do this in a number of ways, most notably, through insurance expansions (both private and public) and through reducing the financial burden of healthcare, especially for those who need it most and cannot afford it. Insurance expansions are a vital part of the PPACA and are achieved using a number of mechanisms. For private insurance, the Act institutes an individual mandate to purchase health insurance, establishes a competitive insurance rate for individuals and the small-group market, and requires employers to "play or pay" with respect to provision of health insurance. More important for vulnerable populations, the Act provides significant funding for Medicaid expansions and subsidies for private insurance for those qualifying. Implementation of the law is staggered, with major milestones taking place between 2012 and 2018 (Figure 143.4). We detail provisions of the PPACA that relate to insurance expansions and regulation of the insurance market further on.

Increased access to care and lower cost liability through insurance expansions and insurance regulation

Private market expansions

PPACA mandates that all citizens and legal residents buy and maintain insurance or face a financial penalty that will be gradually

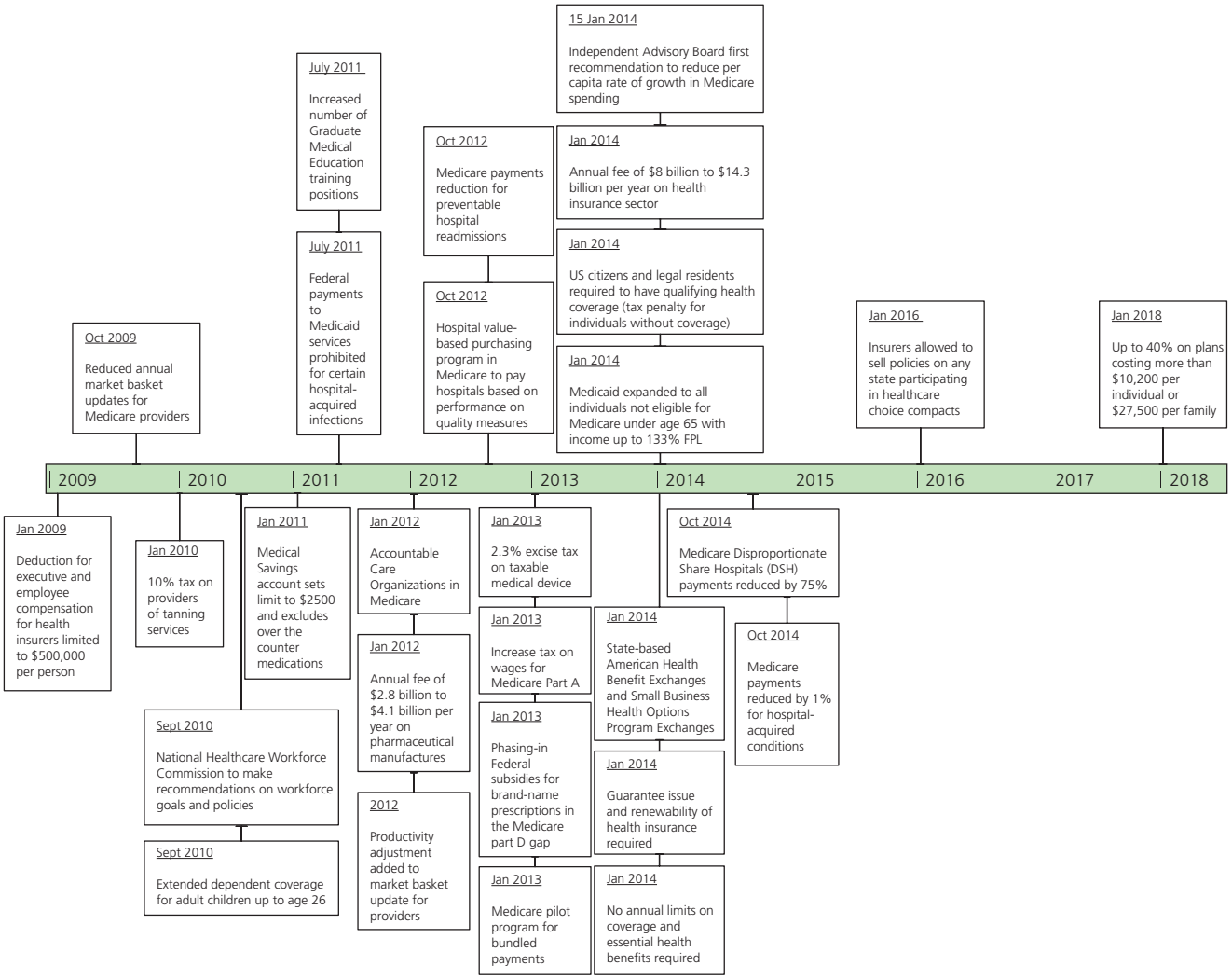


Figure 143.4. Timeline of US health reform implentation.

phased in through 2016. The individual mandate, or "minimum coverage requirement," requires that persons over the age of 18 obtain health insurance, either through their employers or through the state-run health insurance exchanges or public insurance programs if they cannot afford insurance otherwise. Starting in 2016, those who do not have insurance will be assessed at $695 per year, or 2.5% of their income, whichever is higher. Exemptions are available for those who cannot afford insurance.

The rationale for the mandate (and non-discrimination measures) is to reduce moral hazard and adverse selection in the insurance market. Although the goal is to reach universal coverage, persons demonstrating extreme financial hardship, those for whom purchasing insurance would exceed 8% of income, and those with religious objections will be exempt from the mandate. Native Americans would be exempt due to their coverage by the Native American Health Service, and incarcerated persons would be exempted as well. Although PPACA has many implications for healthcare systems and patients beyond the scope of this chapter, we focus our discussion on the potential implications for transplant patients and providers.

Mandating that individuals purchase insurance has been politically and legally controversial. Those opposed to the mandate have stated that it is unconstitutional on the grounds that the federal government oversteps its boundaries by applying undo coercion onto individuals by requiring them to purchase health insurance. In a nearly unprecedented case with three days of Supreme Court arguments, 26 states, the National Federation of Independent Business, and numerous individuals argued that the mandate interferes with personal liberties. The Obama Administration argued that the right to regulate interstate commerce is granted to Congress in the US Constitution, and that this right extends to the case of health insurance. Furthermore, lawyers on behalf of the Administration argued that the individual mandate is a necessary step to achieving universal coverage and preventing discrimination, goals sanctioned by the Constitution and government. In the June 2012, the Supreme Court delivered a final ruling in this case, upholding the individual mandate, leaving much of the law in tact. However, the Court ruled against the right of the federal government to require (or even very strongly incentivize) states to expand Medicaid programs. This may severely impede the likelihood

of achieving universal coverage while keeping the costs of premiums affordable.

On the employer side, consistent with a "pay or play" model, employers with more than 50 employees will be required to provide insurance, and a voucher to employees whose income falls within 400% of the federal poverty level. The exchanges are meant to facilitate affordable health insurance options for those not covered by employers, remedying the problem of unaffordable insurance premiums in the current small-group insurance market. To further ensure affordability for those in need, credits are available based upon income level from 133–400% of the Federal poverty level (FPL). The credits are indexed to keep total premium payment for individuals at a reasonable level (2% of income for <133% of FPL to 9.5% of income for 300–400% of FPL).

Essential benefits package

PPACA establishes an essential health benefits package (termed essential health benefits) that is mandatory for:

1 Health plans offered to individuals and small groups (both in or out of the affordable insurance exchanges);
2 All plans offered in the exchanges; and
3 Medicaid plans.

This benefits package includes ten categories: ambulatory patient services; emergency services; hospitalization; maternity and newborn care; mental health and substance use disorder services, including behavioral health treatment; prescription drugs; rehabilitative services and devices; laboratory services; preventive and wellness services and chronic disease management; and pediatric services, including oral and vision care [13]. This package covers at least 60% of the actuarial value of the benefits, restricts cost sharing to current Health Service Administration limits, and is generally inline with the employer plans. The essential benefits package, which became effective January 1, 2014, also tasks the Secretary of HHS to define and update the benefits package annually through a transparent and public process.

Public insurance expansions

Medicaid expansion is a key feature of PPACA meant to provide coverage for low-income and vulnerable populations and is likely to positively impact transplant and pretransplant patients. The Act extends Medicaid to nearly all individuals under the age of 65 with incomes up to or below 133% of the federal poverty line. For many low-income adults without children, as well as parents at 133% of the poverty line, this expansion marks the first time that insurance coverage is readily available. The federal government will pay 100% of all newly eligible Medicaid patients in financial year (FY) 2014 through FY2016, 95% in FY2017, 94% in 2018, 93% in 2019, and 90% for 2020 and beyond. The federal government also has committed additional transition-making funds to assist states that have expanded Medicaid coverage for adults at least 100% of federal poverty. Although the federal government will pay a very high share of the Medicaid costs in all states, uptake may vary. This is in part due to differences in state policies and their support for the Medicaid program, and partly due to the constrained funding environment and deficits facing many states during difficult economic times. Geographic disparities in Medicaid coverage are likely to augment existing geographic disparities in organ transplantation, and may impede transparency among patients in understanding eligibility and projecting costs associated with care. In particular, lower Medicaid uptake in states with high incidence and prevalence rates of hypertension, diabetes, chronic kidney disease, and substance abuse are likely to exacerbate geographic disparities in organ transplantation.

Relevance to transplantation

Lack of insurance has long been linked to lower rates of transplantation [14–16]. Continued access to care over the life course has the potential to improve health of patients with end-stage renal disease (ESRD) and other chronic conditions that can lead to organ failure by facilitating early diagnosis, and helping patients to better manage their chronic conditions. Furthermore, patients with "pre-existing conditions" can often feel job locked leading to underemployment because they are hesitant to transition to a different job where their insurance coverage may suffer. This is particularly true of patients who have received a transplant and are worried about losing coverage for immunosuppressive drugs. These patients often face a difficult choice between rejoining the workforce and suffering loss of coverage, or applying for disability [17]. For these persons, the promise of insurance is in and of itself, life improving by increasing jobs open to them and reducing anxiety surrounding loss of coverage. Access to insurance and a lower financial burden associated with needed healthcare is likely to solve these problems, resulting in better health and quality of life for transplant (or pretransplant patients).

Expanded Medicaid coverage should increase access to transplantation, offer coverage for uninsured patients in the post-transplant period, and reduce organ loss due to medication non-adherence. Beyond these implications for transplant recipients, expanded coverage will also likely help patients with end-stage organ failure by enhancing their access to transplant services at an earlier stage, leading to better care and more equitable access. This is likely to be particularly important for patients suffering non-kidney organ failure. Unlike patients with ESRD who receive a Medicare entitlement, patients with organ failure, such as those with chronic liver disease, are likely to benefit greatly from this insurance expansion.

Greater access to referrals and chronic care may lead to more patients being listed for transplant. This could lead to proliferation of the waiting list, accompanied by an absolute decline in outcomes for wait-listed patients (e.g. deaths on the waiting list, wait time, etc.). In this sense, increasing access to care will only remedy the situation insofar as there is care to be provided. Without a corresponding increase in the organ supply, longer waiting times and greater use of marginal organs will likely occur and increase the cost of transplant [18]. It is worth noting, however, that while this is a potential outcome, it is unclear whether increased insurance coverage will actually lead to more equitable referral and listing. Some studies suggest that, despite improved coverage, disparities remain [17,19]. Lack of private insurance (in the presence of Medicaid) has been a demonstrated barrier to receipt of quality and timely care for transplant patients [19]. As such, increased access, while alleviating some burdens, is unlikely to solve all of the healthcare difficulties facing transplant patients. Quality and performance measures aimed at providing equitable care are still needed in order to achieve this outcome.

Insurance regulations

Reducing the financial burden and uncertainty associated with paying for care is key to achieving universal coverage. PPACA has been relatively successful at limiting abusive insurance practices, including restricting access, unpredictably increasing costs, and denying claims. Together, these provisions protect the consumer

and aim to reduce the often-ruinous consequences of adverse health shocks.

Private sector reforms such as the elimination of pre-existing condition clauses will likely benefit transplant patients and increase coverage rates among this population. Similar to increasing insurance coverage, defraying the costs of healthcare may result in earlier specialist referral, improved access to transplant evaluation and listing, reduced risk of non-adherence from loss of drug coverage, and improved continuity of care. In particular, elimination of pre-existing condition clauses and annual spending caps may help patients whose immunosuppressive drug Medicare coverage is expiring and those who cannot afford to seek care.

Annual spending caps per enrollee and lifetime limits have also been mechanisms facilitating abusive behavior by insurance companies. These constraints have been particularly injurious for chronically and acutely ill patients, such as those with organ failure, whose annual healthcare costs are substantial. Limits on insurance coverage have transferred the cost burden to the patient, many of whom are forced to liquidate all assets or are driven into bankruptcy in order to pay medical bills [20,21]. For transplant patients in particular, the burden continues even after receiving a transplant. Rodrigue et al. surveyed 333 liver transplant and 318 kidney transplant recipients who were at least one year post-transplant asking whether transplantation caused financial problems, whether income had changed since transplantation, what resources they used to pay for transplant-related expenses, and what their out-of-pocket monthly expenses were. The authors found that 41% of patients reported financial problems after transplantation, and nearly half reported lower monthly income over a year post surgery than in the year preceding transplantation. Average out-of-pocket expenses were estimated to be $47 660 in 2007; this figure has likely increased since. Rodrigue et al. found that patients primarily relied upon savings and credit cards to cover these expenses, however, in the currently constrained lending environment and prolonged recession, patients are likely to face even greater difficulty in paying for care. Provisions to reduce the cost of care, such as elimination of annual spending caps and lifetime limits per enrollee are critical to transplant patients. These patients often experience extended and repeated hospitalizations in addition to requiring expensive medication indefinitely, and as a result, exceed both annual and lifetime limits, resulting in significant financial burden [22].

PPACA also allows for parents to retain their adult children on a family insurance plan up to the age of 26 and limits coverage waiting periods to 90 days. This policy took effect for insurance plan renewals beginning on September 23, 2010. As with the other provisions, this will help transplant patients (and those potentially needing a future transplant) gain and maintain access to care, in particular for younger patients between the ages of 18 and 26 years. Young adults are the age group least likely to have health insurance. This group has benefited tremendously from PPACA, as 18–24 year olds were the only age group to experience a significant increase in the percentage with health insurance, from 70.7% in 2009 to 72.8% in 2010 [23]. Despite the ongoing recession, the two-percentage point increase in coverage for 500,000 young adults aged 18–24 is largely attributable to the PPACA's provision to extend coverage until age 26. Since the fraction of insured adults was stable or decreasing among other age groups, this two-percentage point increase almost certainly reflects the effects of the extension of dependent coverage to age 26.

Effective June 21, 2010, legal immigrants who have resided in the U.S. legally for more than five years or U.S. citizens with pre-existing conditions who have been uninsured for more than six months became eligible to participate in a state managed and underwritten temporary high-risk pool, subsequently replaced by state health insurance exchanges. Premiums will be established based on the standard population and can vary no more than 4 to 1 based on age, geographic area, and family composition. The Act also limits out-of-pocket spending to $5950 for individuals and $11 900 for families, excluding premiums, (on a graduated basis) for families under 400% of the federal poverty level [24]. Up-front deductibles are limited to $2000 for individuals and $4000 per family for plans in the small group and individual market. This will help to assuage the fear of many transplant patients that a 10% or 20% co-payment or high deductibles will exclude them from access to transplantation. Many transplant recipients may be eligible for these plans. However, potentially low reimbursement rates and high costs of organ acquisition may pose a problem for providers, and may pose barriers to access for vulnerable patients [25,26].

PPACA also attempts to reduce administrative costs of healthcare and redirect health insurance costs to pay for healthcare. The Act addresses this partially through instituting a new medical loss ration and premium rate reviews. The Act requires health plans to report the proportion of premium dollars spent on clinical services, quality, and other related healthcare costs, and provide rebates for the amount of premium spent on administrative costs that is over 15% in large group market plans and 20% in individual and small group market plans. Reporting of medical loss ratio was effective in 2010, with the rebate provision effective in January 2011. Additional oversight was included to ensure appropriate increases in premiums. Significant abuses have taken place, with premiums for family coverage having increased by 50% across states, and employee annual share of premiums increased 63% between 2003 and 2010. Were premiums to continue to increase at the rate observed prior to the enactment of PPACA, the average premium for family coverage is estimated to increase by 72% by 2020, to nearly $24 000 [27]. Many insurance regulations in the PPACA are aimed at slowing premium increases, in particular a review process for examining increases in insurance premiums and a requirement for the justification of all increases. Since 2010, all increases in insurance premiums must be approved by the states.

It is likely that these provisions will reduce the cost of care for transplant patients and will increase access, particularly for young patients who may have been uninsured. It will likely also improve quality of life, as young patients may feel more freedom since they will be able to relocate or pursue a different job without fear of losing insurance. However, quality of care and choice of provider may suffer somewhat. Transplant services are often financed as a "carve-out" from medical/surgical premiums because of their high cost and need for highly specialized services. Given heightened scrutiny of the fraction of premium dollar spent on clinical services and restrictions in increasing premiums, it is possible that transplantation coverage may be somewhat limited. Ultimately, transplantation coverage is largely contingent upon the health insurance market (because carve outs are financed by reinsurance) and regulations that more directly relate to transplantation. Depending on the level of reimbursement, plans offered to individual and small group markets and the high-risk pool may restrict choice, reducing care options for transplant patients.

Immunosuppressive drug coverage:

Although kidney transplantation has proven to be superior to dialysis for improving patient survival rates and quality of life and is

cost-effective, its long-term success depends upon ongoing treatment with immunosuppressive drugs. An initial kidney transplant costs Medicare an average of $110 000. Immunosuppressive medications cost $15 000 to $20 000 annually. This is compared to the annual cost of $75 000 for dialysis treatment in the case of premature transplant failure as well as the cost of repeat transplantation. The Medicare extension of funding for immunosuppressive medication for kidney transplantation from one to three years reduced costs and income-related disparities in outcomes [28]. Economic analyses confirm that providing lifetime funding for immunosuppressive medications would lower overall costs by $200 million annually [17]. In addition, long-term survival rates are higher in countries that provide continued immunosuppression. Furthermore, patients experiencing premature transplant failure due to loss of immunosuppression must revert back to dialysis, thereby experiencing poorer outcomes at greater expense. Premature transplant failure is the fifth leading cause of initiation of dialysis in the US [29]. The 2-year mortality rate for patients whose transplants fail is worse than the mortality rate among patients with a functioning transplant and even that among age-matched patients who have never received a transplant.

Transplant failure can result directly from non-adherence to immunosuppressive drugs, which may be due to inability to pay. This link is difficult to confirm using prospective research since transplant recipients are unlikely to admit to poor adherence [30]. However, in a 2010 survey, more than 70% of US kidney transplantation programs reported that their patients had an "extremely serious" or "very serious" problem paying for immunosuppressive medications, and 68% reported deaths and graft losses attributable to cost-related non-adherence [29]. Ensuring lifetime access to these medications with kidney transplants would save lives and reduce the total cost of treating patients with ESRD [29].

Prior to PPACA, in the US, Medicare rules end immunosuppressive drug coverage three years after kidney transplantation for all Medicare patients below 65 years of age or those with work-related disabilities. Upon returning to work and losing disability status, many transplant recipients also lost coverage of their medications. The Comprehensive Immunosuppressive Drug Coverage for Kidney Transplant Patients Act of 2011 is a proposed amendment to the Social Security Act that would grant lifelong coverage of immunosuppressive medications to all kidney-transplant recipients in the US [29]. Currently, lifetime coverage of immunosuppressive drugs is thought to be afforded largely through various provisions in PPACA. Access to immunosuppressive drugs is provided through the high-risk pool (Pre-Existing Condition Insurance Plan), as well as through private insurers who can no longer exclude enrolled patients or institute annual spending caps or lifetime limits. The Act also prohibits discriminatory practices such as exclusion of pre-existing conditions and the lifetime caps on spending. These practices, in particular, have negatively affected transplant patients because they have impeded their ability to receive care, including vital coverage for immunosuppressive drugs beyond the 3-year period covered by Medicare following transplantation.

PPACA also includes Medicare reforms that will result in better drug coverage by closing the "donut hole." Effective starting in 2010, Part D enrollees with any spending in the "donut hole" (coverage gap) will receive a rebate ($250). Subsequently, in 2011 enrollees with spending in the coverage gap will receive a 50% discount on brand-name drugs (provided by the pharmaceutical industry). Medicare coverage is phased in beginning in 2011 to 2013 for generic and brand name drugs, respectively, purchased in the gap. Part D enrollees will ultimately be responsible for 25% of the cost of drugs (brand-name and generic) purchased in the gap by 2020. PPACA also reduces the catastrophic coverage threshold by 2019. Despite these policies, patients may still have outstanding expenses due to the high costs of immunosuppressive drugs, and as such, a special provision addressing the need for full coverage of immunosuppressive drugs may still be needed.

Coverage for living donors

PPACA may also positively impact the health of living donors post donation, as well as potential donors hoping to donate. High rates of no insurance coverage, the transient nature of insurance, and restrictions on pre-existing conditions may have served as deterrents for donation. Lack of follow-up care for living donors may have also been a deterrent. Lack of coverage for long-term donor follow-up may be particularly important due to the relaxing of certain donation criteria (e.g. mild hypertension and higher BMIs) The long-term consequences of donation is unclear for these donors, and coverage for comprehensive follow-up may be both important to their physical and mental health. Insurance expansions may also help living donors, whose long-term follow-up, at least within the 2-year period following transplantation, will be covered by Medicare and is likely to be covered under private insurance as well. A recent study by Kher et al. examining reimbursement rates for donor follow-up at a single center, suggests that private insurance and Medicare reimburse follow-up for donation at rates similar to reimbursements for recipients [31]. This suggests that insurance coverage for donor follow-up should not pose a significant barrier to care.

Importantly, given that people could become donors at any time, increasing coverage, expanding preventative health services and encouraging healthy behaviors is likely to increase the pool of potential donors. This is especially true for previously uninsured populations, many of whom are young and healthy, who may not have considered donation due to lack of insurance. The Act promotes preventative care by eliminating cost sharing on recommended preventive services delivered by Medicare and all new insurance plans; providing an annual free wellness visit under Medicare, contributing more federal Medicaid matching funds to states that offer evidence-based prevention services and requiring coverage of tobacco cessation services for pregnant women in Medicaid; and promoting community preventative services by supporting grants [32].

The transplant community, including professional societies, have embraced living donation and have advocated for supporting living donors. The American Society of Transplantation (AST) has publicly called for policies providing health insurance to donors to address the short- and long-term effects of living donation [33]. Although the recipient's policy typically covers donor-related expenses for a limited time before and after transplantation, the donor is exposed to significant risk by not being guaranteed his or her own insurance. First, the long-term health consequences of donation are not well understood, and as a result, donors may experience a health event related to donation after the recipient's insurance coverage period has expired. Second, if the recipient dies or loses insurance, the donor will also lose coverage. This could result in substantial medical expenses, often totaling thousands of dollars. Prior to PPACA's provision banning exclusions due to pre-existing conditions, donors suffered a further risk that any future medical need might not be covered if it could be tied in any way to donation. With great uncertainty related to future access to medical

care and potentially high out of pocket expenses, living donors might be dissuaded from undergoing surgery. Universal coverage for donors, granted either through the PPACA or through a separate tailored provision, is likely to help alleviate fears surrounding donation and may encourage donors to come forward.

Implications for transplant providers

Reimbursement

Expanded Medicaid coverage through PPACA is likely to increase the number of individuals with access to transplant services (both pre and post-transplant). Furthermore, if this insurance expansion encourages living donation by making living donors more inclined to donate after being insured, then PPACA may indeed increase the number of transplants. As insurance coverage expands though Medicaid and state-run health exchanges, reimbursements for specialists, in this case transplant centers, will likely decrease. This shift in payer mix may lead to revenue deficits, particularly for centers in large, urban regions with long waiting times and those that use a higher proportion of expanded criteria organs [34].

PPACA establishes an Independent Payment Advisory Board (IPAB) to advise the President and CMS using cost-effectiveness analysis. Although this is in line with PPACA's mission to improve efficiency and decrease waste in healthcare, many specialists and surgical organizations have voiced their concern about the potential of President-appointed IPAB to influence Medicare coverage decisions. It has been widely suggested that the IPAB is likely to increase funding for primary care and decrease reimbursement of specialist care [35]. This means transplant and potential transplant patients may have better management of chronic disease and potentially earlier referral and better screening. However, it likely will reduce access to specialized transplant services at the same time. Although the need for Disproportionate Share Hospital (DSH) payments as offsets is debatable depending on the rate of insurance uptake among the uninsured and underinsured and the reimbursement rates offered by safety net payers, Medicare generally provides DSH for large academic medical centers that treat an indigent patient population. Under health reform, hospitals are likely to lose 75% of DSH. Finally, the complexity of transplant patients, infection and readmission may be more common than in other patient populations. This may be construed as "poor quality care," defined by Medicare as high rates of readmission and infection, resulting in lower reimbursement. One way to help both outcomes and reimbursement is through better risk adjustment and enhanced integrated care (perhaps through Accountable Care Organizations).

Restrictions on insurance premiums may lower reimbursement and limit patient choices. These restrictions may increase the market power of large networks, which may limit access to certain transplant centers and may also reduce reimbursement for transplant providers. Thus, restrictions on the overall cost of insurance plans may potentially increase the patient base, but restrictions on high-cost, high-benefit plans will likely decrease reimbursement and choice [18].

Quality improvement

PPACA also addresses stabilization of the sustainable growth rate, creation of medical homes, shift to episode of care reimbursement (already familiar in transplantation), and development of comparative effectiveness research in an attempt to improve the delivery system and enhance healthcare efficiency. The Act also attempts to improve quality by incorporating pay-for-performance incentive mechanisms. Many studies have demonstrated that physicians and hospitals are responsive to such incentives, and this model has been used successfully across a number of clinical domains [36–38]. In PPACA, two provisions aimed at integrating pay-for-performance became effective in 2012: the first provides incentives for the formation of accountable care organizations (ACOs), and the second links payment to quality outcomes, supplying hospitals with financial incentives to improve quality of care.

Accountable care organizations

ACOs promote care coordination and quality improvement by focusing especially on disease prevention, disease management, reducing redundancy, and ultimately reduction of unnecessary hospital admissions. Cost-saving is achieved if ACOs provide high-quality care at lower costs to the healthcare system, in which case the ACOs receive a fraction of the costs saved.

ACOs encourage a disease management approach to healthcare delivery, by linking providers and provider payment (ambulatory, hospitals, nursing and ancillary care facilities) in a defined way to an episode of care. The expectation is that greater communication and ownership over the patient experience will enhance quality care and decrease costs. In many ways, transplant care is a pioneer in this approach, since many of the services surrounding transplantation are integrated and involve a number of different specialties, centers, and routine follow-up care. Transplant centers have acted essentially as medical homes, integrating a number of medical and surgical subspecialties, and even incorporating emergency department visits for patients under their care. Bundled payments, which often accompany discussions of ACOs, will be familiar to transplant centers that have been reimbursed in this way for some time. Provisions aimed at developing the Medical Home Care Models and expanding the Federally Qualified Health Centers may also serve transplant recipients in providing long-term care [34].

The American Society of Transplant Surgeons (ASTS), although supportive of developments proposed in the ACA, suggests that CMS take special care to ensure that Medicare and ACO patients with expensive healthcare needs, such as transplantation, receive proper information about their care options and are not dissuaded from pursuing transplantation [39]. ACOs may have to overcome disincentives to provide such information, particularly if a transplant center is not included in their network.

Pay for performance

The second main quality-related provision incorporates pay-for-performance (P4P), which entails "linking payment to quality outcomes", and offers hospitals financial incentives to improve quality of care. The provision requires public reporting of hospital performance, beginning with measures related to heart attack, heart failure, pneumonia, surgical care, and nosocomial infections. Public reporting requirements will extend to discharges effective October 1, 2012.

P4P has two main components: the first rewards physicians for outcomes meeting a quality threshold, and also often for demonstrating improvement. The second component involves public reporting of outcomes in an effort to increase transparency and information in the market, and facilitate consumer-driven demand. The provision is structured at the hospital or group level, meaning that for physicians individual returns are not directly linked to individual effort. Instead, physician returns depend on the group or hospital quality improvement. Such a structure is risky in that it creates a "free rider" problem, where certain physicians can experience benefits without improving their own quality.

The structure of the incentives is also important. Rewarding absolute quality thresholds can have negative consequences, such as discouraging physicians with complex patients. Without proper risk adjustment, this could exacerbate disparities and lead to fewer physicians accepting patients with severe medical needs. In such a system based on absolute performance, low performers, even those demonstrating significant improvement, would not receive any rewards. In a system rewarding improvement, high performers would not be incentivized to maintain high performance because they would not be rewarded for that. Risk adjustment is crucial, as are both relative and threshold performance incentives. For transplant physicians, this is of particular importance given the range of patients and geographic variation in availability of standard criteria organs. Furthermore, given the importance of equity to transplantation policy, special attention should be paid to incentives and their potential negative consequences.

PPACA is also focused on process-based measures. Process of care measures are intended to improve how care is being delivered, not simply the outcome. Incorporating process measures somewhat mitigates the gaming problems associated with outcome-based quality measures. The timing of incentives (end of the year versus continuous) as well as the relative size of incentives (at least 5% of the capitated salary) will also be important [40,41]. P4P incentives must be carefully structured and implemented. It is currently unclear with these incentives, as they are included in PPACA, will achieve their desired outcome.

Meeting a growing demand for organs through donation incentives

A third area of developing transplantation policy involves stimulating organ donation through incentives and increased public awareness. The current waiting list for kidney transplants has more than 85 000 people, but donated organs allow only about 17 000 transplants each year. Approximately 4500 people die each year while waiting for a kidney transplant, and an additional 33 000 join the list [10]. The growing number of patients on waiting lists is increasingly disproportionate to the supply of donor organs. While the use of organs from donors dying from cardiac death has increased over the past decade, with such donors now exceeding 10% of the total, UNOS policies intended to facilitate the use of expanded criteria donor kidneys (making a second transplant waiting list available for kidneys recovered from older and sicker donors) have met with mixed success [1]. Currently, about 60% of kidney transplants come from deceased donors. Forty percent of kidney transplants are from living donors, with the proportion of living donors increasing in recent years [42].

Legislative attempts to increase organ donation aim to increase the supply of potential deceased donors, to increase the pool of potential living donors, and to improve donor conversion. The transplant community and the US government overwhelmingly support the notion that organ donation (especially living donation) should rely on volunteerism and altruism. This is deeply rooted in legislation in the Uniform Anatomical Gift Act (UAGA) and in Section 301 of the National Organ Transplant Act (NOTA) of 1984. NOTA states that, "It shall be unlawful for any person to knowingly acquire, receive or otherwise transfer any human organ for valuable consideration for use in human transplantation" [43]. In this context, "valuable consideration" has been interpreted as a conditional transfer of money or valuable assets between the recipient, donor, and potential broker [43]. In particular, the law serves to prohibit coercion of inducements of any kind for donation, and explicitly bans the purchase or sale of organs. Policies related to stimulating organ donation can be categorized by the intended benefactor: either living or deceased donors; and, by mechanism: financial incentives, material compensation, non-material compensation. The structure and size of potential financial incentives also vary significantly, with some favoring significant market regulation and non-material compensation, compared to others that promote an open and unregulated market for organs where the price of an organ would be regulated solely by the law of supply and demand.

Conflicting views exist about whether financial compensation for organ donation would alleviate the organ shortage and increase supply. Some have argued that the presence of financial incentives would decrease the pool of potential donors because people would no longer feel ethically compelled to donate, viewing the exchange instead as a transaction. In his "Open Letter" to Senator Arlen Specter of Pennsylvania asserting his concerns related to his proposal for the Organ Donor Clarification and Anti Trafficking Act to amend NOTA, Gabriel Danovitch details numerous concerns related to providing incentives, integrating ethical concerns of commodification (detailed in the Declaration of Istanbul) as well as practical concerns. Of note, Danovitch suggests that in countries where financial incentives or payment for donation are introduced, funded donation crowds out voluntary donation. Danovitch cites examples of Israel, Pakistan, and Iran, among other countries which demonstrate this effect [44]. This line of opposition is well established. Policy makers, including several senators and professional societies such as the National Kidney Foundation, along with religious organizations vehemently and successfully lobbied to exclude pilot programs examining the financial incentives for organ donation in the Organ Donation and Recovery Improvement Act (S 573), introduced by Senators William Frist and Chris Dodd [45]. Opponents of financial incentives caution that financial incentives could reframe the way the public views organ donation from an altruistic behavior to a normal market transaction. This could lead to (and has in some countries) lower familial and altruistic donation rates.

In contrast, proponents of financial incentives suggest that the distinction is not so stark, and that some level of financial incentives may be both ethical and effective in promoting donation. A common ethical argument supporting financial compensation is that all other stakeholders taking part in the transplant process are compensated and paid for their participation, except for the donor. Other ethical considerations include utilitarian arguments that suggest that everyone would gain from a shorter waiting list and that many lives would be improved by increasing the supply of donors. Some ethicists have gone so far as to argue that restricting the ability to sell one's organ unfairly constrains autonomy, and in particular harms those of low socioeconomic status who have most to gain from such a transaction [46]. Matas, amongst others, has called for a regulated system that would compensate living donors. He suggests that the system would have six key elements to be administered by OPOs:

1. ban on donor-recipient contact;
2. donor compensation that includes a 1-year $1 million term life insurance policy and guaranteed lifetime healthcare;
3. reimbursement of travel for donation;
4. fixed compensation for leave from employment;
5. fixed payment or tax break;
6. a payment of $500 for completing follow-up evaluation 1-year post-transplant [47].

He argues that such a system would not undermine altruistic donation, but rather would fairly and ethically supplement this pool with more donors who would be adequately compensated and incentivized, without being coerced.

More recently, many professional transplant societies have come to adopt the view that a middle ground might be most appropriate, both ethically and practically. In 2002, the ASTS sponsored a panel of ethicists, OPO leaders, and transplant physicians and surgeons to consider financial incentives. The panel supported efforts to promote a pilot project to examine the effects of funeral reimbursements or charitable contributions in the name of the donor, as a means of expressing appreciation to the donor and donor family [48]. Roth found sustained support for a certain level of compensation among the ASTS membership, stating that, "in an informal poll following a debate on the subject at a recent meeting of the ASTS, a majority of those polled expressed a willingness to contemplate a trial or demonstration project involving compensation for organ donors" (personal communication, Arthur Matas, January 27, 2007) [49]. Other professional societies, including the AAKP, the PKD, the AST, have also expressed support for various types of supports for living donors, but the enthusiasm for financial incentives per se varies significantly [50–52]. Importantly, the politically powerful American Medical Association (AMA) also joined the chorus of support, urging Congress to amend the NOTA of 1984 to permit demonstrations incorporating financial compensation for families of deceased donors. The UNOS/OPTN have also recently called for study of potential financial incentives for organ donation [53].

The political and policy debates have become increasing complex as the organ shortage has grown and the number of stakeholders and number of proposed incentives has proliferated (Figure 143.5). The policy debate surrounding financial incentives often conflates both living and deceased donation, intensifying concerns about exploitation and extending them even to discussions of deceased donation where they may not be relevant. Five main approaches have been suggested for increasing donation using incentives: payments, tax benefit, funeral reimbursement, and charitable contributions on behalf of donors, and medals honoring donors.

Federal legislation

In 1999, the Organ Donor Leave Act (PL 106-56) was passed, granting federal employees paid leave to serve as organ donors. The law grants federal employees up to 7 days for serving as bone marrow donors, and up to 30 days for serving as organ donors [54]. Following a spirited debate surrounding financial incentives and potential commodification of organs, federal legislation, originally introduced by Senators William Frist (R-Tenn) and Christopher Dodd (D-Conn) was passed. In 2001, two bills were introduced in Congress, one proposed offering a $10 000 tax credit for deceased donation and another a $2500 tax refund for living or deceased donation [55,56]. The Organ Donation and Recovery Improvement Act ((PL 108-216), signed into law in 2004, promotes organ donation by allowing reimbursement of travel and subsistence expenses for living donors, supports long-term follow-up of living donors by facilitating a registry, and provides grants to states and public entities [57]. Importantly, this legislation did not overturn the 1984 NOTA ban on buying and selling of organs. Efforts to expand incentives for donation without providing additional financial incentives led to the enactment of two laws in 2007 and 2008. The Charlie W. Norwood Organ Donation Act (PL 110-144) in 2007 [58] paved the way for paired donation, clarifying that it is not considered valuable consideration for purposes of Section 301 of NOTA. The Act also calls for an annual report detailing advancements in understanding the long-term health impacts of living donation. The Stephanie Tubbs Jones Gift of Life Medal Act [59] (PL 110-413) grants the DHHS authority to award organ donors a National medal in honor of their donation.

State legislation

Financial incentives for living donation

States have enacted a set of financial incentives as well. Despite the prohibition of financial compensation for donors stated in the NOTA of 1984, many states afford living donors some benefits to offset the costs and physical or emotional burden of donation. Beginning in 2000, Wisconsin, followed shortly by Maryland, introduced a law that instituted policies granting state employees 30 days of paid leave following organ donation. In other states, this law often is extended to local government employees as well as public school teachers. Some states have expanded this initiative, adding a requirement for private employers to provide unpaid leave of absence for organ donation. Many states further incentivized living donation by passing a second law that grants donors up to a $10 000 tax deduction to compensate costs associated with donation. The actual tax deduction depends on the donor's marginal tax rate. This varies between states and also depends on income and filing status. The actual monetary value of this deduction depends on the individual's state marginal tax rate, which depends on the state, as well as one's income and filing status. As Wellington and Sayre note, "a single filer in Wisconsin with an adjusted gross income of $25 000 faces a relatively high state marginal tax rate because as one's income increases, the value of the person's state earned income tax credit decreases. However, Minnesota's tax system results in more wealthy individuals facing a higher marginal tax rate [60]". While these examples demonstrate that the value of this benefit varies substantially, in all cases the benefit is relatively small (less than $1500). The transplant community has also called for lifetime insurance benefits for living donors. The ASTS, the AST, and the Declaration of Istanbul have also proposed legislation to provide federally funded lifetime insurance benefits for living donors [48,61]. Although specific guarantees have not been made for living donors, provisions in the PPACA to expand coverage may be enough to encourage donation, likely achieving much the same result as a discrete provision targeting organ donors.

Financial incentives for deceased donation

Efforts to pass legislation related to financial incentives for deceased donation have been less fruitful. According to a 2005 Gallup poll, 95.4% of Americans reported that they "support" or "strongly support" organ donation [62]. Despite this widespread support, less than half of Americans have registered as organ donors [5]. Proposed financial incentives for deceased donation include payments to the donor's family to defray funeral costs or support designated charities as well as tax credits [63]. In 1999, the Pennsylvania Department of Health proposed offering $300 to families of organ donors to defray funeral costs [60]. However, despite multiple revisions and negotiations, the law was never enacted due to concerns about violating NOTA.

The idea of a "futures market" has also been proposed, whereby individuals would receive payment in exchange for the promise of donating their organs after death [64]. As Howard and others have noted, while the concept of a "futures market" is an interesting thought experiment, it would likely require financing out of general

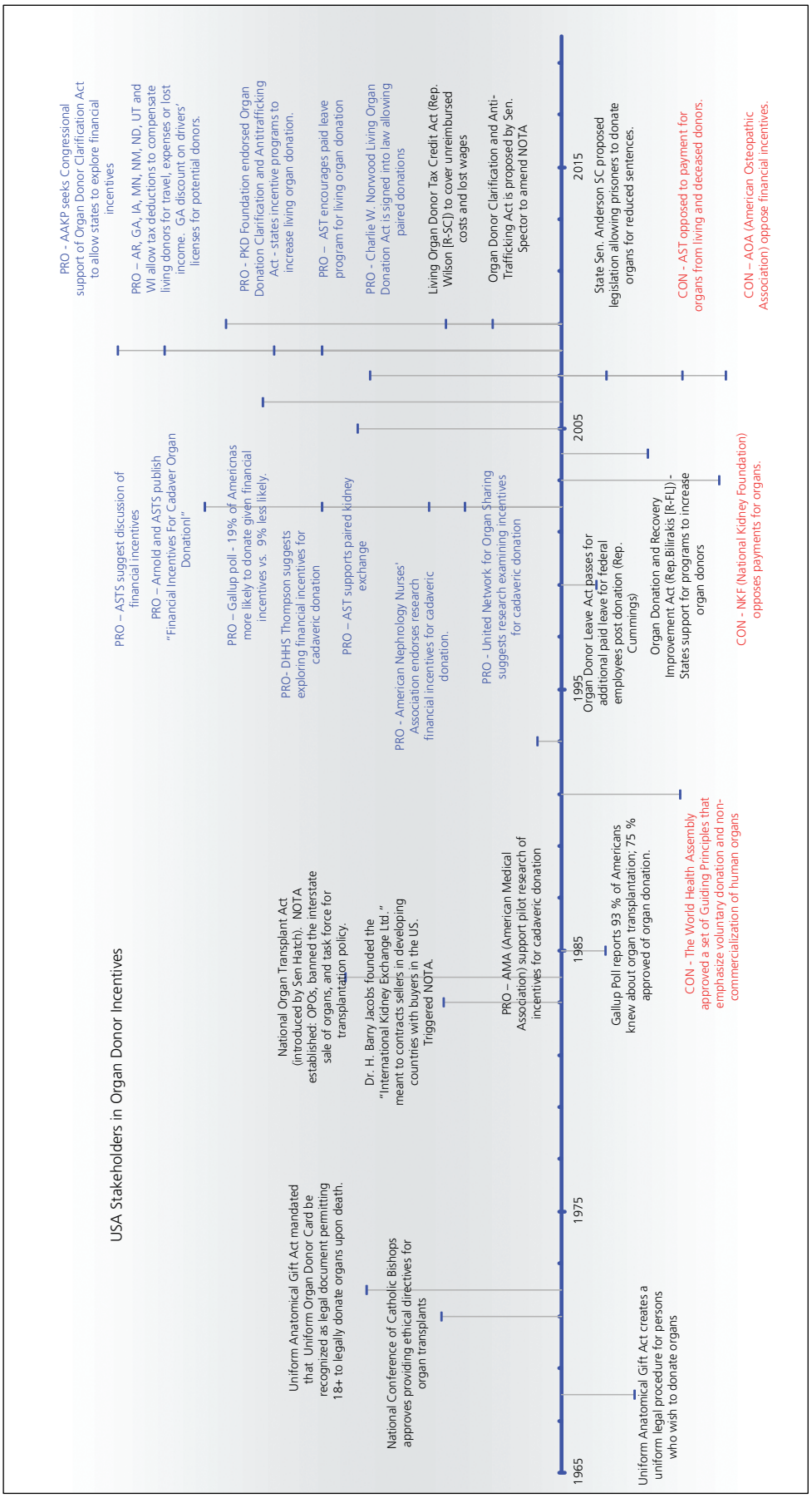


Figure 143.5. Timeline of legislative and policy initiatives related to offering incentives for organ donation in the US.

revenues or tax on hospitals or health insurers [64]. One example of an incentive for a type of futures market can be found in Georgia. Until 2005, Georgia provided a $7 discount on driver's license registration fees to individuals who registered as organ donors. It seems that this small incentive was relatively effective, and in 2005 Georgia boasted one of the highest donor registration rates for organ donation in the US [64]. Although effective, a futures market undermines the validity of the donation designation on the driver's license because the intent of the registrant is unclear with respect to wanting to donate or simply wanting a discount. As a result, Georgia's OPO was reluctant to use the Georgia donor registry data while the driver's license discount was in place. Bryne and Thompson, among others, suggest that such a system may undermine familial consent rates as well [65]. Many in the transplant community suggest that, although financial incentives may increase donation rates, the ethical concerns associated with such measures and potential negative implications public perception, public trust, and living donation render them currently undesirable [66].

Mandated choice

The current practice of requiring family consent at the time of organ procurement is problematic because often families are unsure of the donor's preferences, and even if aware, they may object to donation and refuse efforts by the OPO and clinical team to follow the donor's expressed wishes [67]. Despite the public's strong support for organ donation in public opinion polls [68], suggesting that the stress accompanying the decision-making process may be contributing to the rates of family refusal. Mandated choice would compel all competent adults to decide whether they wish to donate their organs after their deaths. People could document their decision via a number of forums, including: driver's license registration, voter registration, or income tax forms. Donor consent would be indicated on the driver's license or in a state donor register that is accessible at the time of death. For example, in Virginia, three options were available: donor, non-donor, and undecided. In the first six months, 24% were undecided with 31% registering as donors [69]. The main objection to mandated choice is that forcing people to make choices undermines personal autonomy. However, proponents of mandated choice argue that autonomy is actually enhanced because it forces an active decision as opposed to an implied assumption. Mandated choice allows competent adults to make the donation decision for themselves, rather than have it foisted upon them by relatives [70]. Mandated choice may also be beneficial for families too, who often suffer severe stress and internal discord due to donation decisions [71].

Presumed consent

Presumed consent for organ donation attempts to increase organ donation by changing the default option. Changing defaults has been shown to be an extremely effective way to overcome "status quo" bias, where people fail to take action because they are not incentivized to leave the default option. Presumed consent is an opt-out system where, following death, organs can be removed, stored and used in transplant unless a person explicitly refuses to allow such activities. Several European countries such as Spain and Austria have introduced presumed consent. Spain's presumed consent law is considered somewhat soft, because health providers actively check that the next of kin do not object to organ donation, whereas in Austria the organ recovery proceeds unless it is known that the deceased objected before death. Their law actually provides for preservation without consent; the Spanish norm is to use normothermic extracorporeal membrane oxygenation (NECMO) or cardiopulmonary bypass on donors to keep the organs perfused until family located and consent obtained. Still, Spain has relatively high donation rate of 35.5 per million [72]. Abadie and Gay have estimated that donation rates are 25–30% higher in presumed consent countries [73]. Brazil introduced a system of presumed consent in 1998, but returned to a system of informed consent after a year due to systematic failures. In Brazil, the public lacked trust in the organ donation system, stemming largely from misperceptions that organs would be removed before patients were clinically dead. Brazil also failed to institute a system to allow people to object to donation while alive. Evidence from several studies suggests that presumed consent law is associated with increased organ donation rates, but other factors such as availability of potential donors, infrastructure for transplantation, investment in healthcare, and public attitudes may all play a role [74,75].

The Institute of Medicine has recommended that the social climate be changed before any drastic legislative moves aimed at increasing organ donation [76]. The IOM Organ Donation Committee suggested that the long-term goal would be to create a society so committed to organ donation that such presumed-consent or mandated-choice policies would be acceptable. Professional societies seem to be mostly in agreement. A survey of members of the International Society for Heart and Lung Transplantation (ISHLT) in conjunction with the Foundation for the Advancement of Cardiac Therapies (FACT) found that members overwhelmingly favored indirect over direct compensation as a way of increasing organ donation. This is despite the belief among the majority of respondents that presumed consent is the best way to significantly improve organ donation. The majority also favors the wishes of the individual over the family in determining donor status [63].

Promoting organ donation: interventions

Organ donation involves a few components: first, people must volunteer to be organ donors; and second, families must consent to donation once a donation situation arises. This dynamic decision-making process is complex and is influenced by a number of factors and numerous stakeholders. These include: the potential donor, the donor's family, the OPO, the clinical staff taking care of the potential donor, and potentially public figures such as religious or community leaders. This section will review a number policy and clinical efforts aimed at overcoming barriers to organ donation (see Figure 143.5)

Deceased donors: increasing public awareness of organ donation

Organ registries might not be sufficient to expand organ donation rates if awareness among the general public remains low. Public education aims to increase organ donation by improving public awareness of transplantation and the need for organ donation [77]. Approaches include public information campaigns using the media, distribution of donor cards, and providing teaching materials for schools [78]. Increasing the opportunities to register as organ donors, such as during driver's education and licensing, during advance-care planning, and in work, faith, school, and community-based initiatives may help increase the number of potential donors [76].

Changing the social climate would require relieving people of their fears and misconceptions about organ donation and transplantation [79]. Horton and Horton found that lack of religious

support, confusion about the concept of brain death, fragmented healthcare provision by physician teams responsible for the welfare of the donor and recipient, and a mistaken belief that to be valid an organ donor card must be filed with the US Department of Health and Human Services, were associated with organ donor status. The authors found that knowledge of organ donation facts was associated with carrying or requesting an organ donor card, attitudes towards organ donation and willingness to donate their own organs or the organs of a deceased loved one [80].

Willingness to donate also varies by sociodemographic and racial characteristics. Bouleware et al. found that black females were least willing to donate blood (relative to other race and gender groups), whereas black men were least likely to donate organs after death. The authors suggest that mistrust of hospitals and perceived discrimination in hospitals explained much of the racial and gender gap in willingness to donate blood, whereas religious and spiritual beliefs explained the racial gap in willingness to organs after death [81]. Low rates of organ donation among African-Americans has been attributed to: less awareness of transplantation, religious distrust of donation, institutionalized racism and distrust of the medical community, fear of medical abandonment and fear of discrimination [82,83]. Siminoff et al found that, compared to whites, African-Americans reported lower trust in the healthcare system [83] and were more likely to believe that physicians would not try as hard to save lives of donors and that physicians could not be trusted to pronounce death. African-Americans were also more likely to favor compensation for families of donors, such as financial compensation and coverage of funeral expenses. These studies suggest that low levels of trust and lack of awareness and education about the donation process may pose particularly important barriers in minority communities.

Interventions

Despite more research related to identifying barriers to donor registration, few successful interventions have been identified. Given efforts to increase evidence-based policy, HRSA and NIH have committed significant funding to stimulating research promoting organ donation. Future policies may need to incorporate legal and regulatory components of information technology law (specifically internet law), work with various governmental departments, such as the Department of Education and local school committees, and incorporate interdisciplinary teams in order to successfully increase organ donation.

Several studies have highlighted the potential of new media in promoting organ donor registration [84]. Merion et al. studied the effects of an internet-based multimedia intervention (http://www.journey.transweb.org) on donation registration and family notification. The authors found that for the 10 884 participants, knowledge improved after completing the educational module. Furthermore, willingness to donate and join a donor registry increased after the intervention. While increases in knowledge were not associated with changes in attitudes, an increase in pro-donation attitude was a significant predictor of donor registry participation and family notification [85]. Thornton et al. examined the effect of an iPod video intervention on donor registration in DMVs in Ohio. They found that viewing the video was associated with increased rates of donor registration. Importantly, they also found that the video was particularly effective among black participants, although it was also effective for other racial groups. This brief video exposure that took place at the DMV was successful in providing information about organ donation and reducing some of the barriers associated with organ donation, particularly amongst minorities [86]. In a recent unprecedented move, the social networking website, Facebook introduced an "organ donor" option allowing their 161 million members in the US (and over 900 million members worldwide) to advertise their organ donation status. The Facebook option will likely be important for two reasons: first, it will increase awareness and likely lead to high rates (at least initially) of donor registration. Second, declaring one's donation status on Facebook serves as a record documenting consent. Although this may not be legally binding, it will serve as a way for the family to identify the patient's wishes when they are not known. Following this move by Facebook, organ donation registries reported a spike in donation registrations. California alone experienced a 700% increase above the typical number of new registrants [87]. Other states reported a tremendous rise in new registrants following this option, including: Colorado, Connecticut, Maine, Massachusetts, Michigan, Nebraska, Nevada, New Hampshire, Rhode Island, and Wyoming.

School based health education also presents a promising approach for improving organ donation rates, potentially also among ethnically diverse youth. Tailored interventions also hold promise. Piccolil et al. demonstrated that a two hour class and two hour session with patients and experts held in eight schools for 17–18 year old students helped increase interest improve attitudes related to organ donation [88]. Using a randomized-control study design, Cardenas et al. found that in a multicultural high school, following an educational intervention, students in the intervention group demonstrated a significant increase in knowledge scores and improvement in willingness to donate [89]. Positive changes in attitudes and willingness to donate occurred independent of ethnicity and gender, in spite of these both being negative predictors of opinion at baseline. These studies suggest that classroom based interventions may be important in promoting organ donation, particularly among young adults.

Increasing donor conversion

Awareness of a loved ones wishes, and concordance of family members during the decision-making process is also vital to successful donation [90]. Polls have suggested that a key factor in families agreeing to consent to organ donation at the potential donor's death is whether the potential donor had previously discussed organ donation with his or her family [79]. Siminoff et al. found that prior knowledge of the patients' wishes along with discussions of more topics and had more conversations about organ donation were significantly associated with willingness to donate. Families with greater contact with OPO staff and those who experienced an optimal request pattern also were more likely to donate [91]. Rodrigue et al. examined the relative influence of donor and next-of-kin factors, requestor characteristics, communication processes and satisfaction with the healthcare team on the donation decision [92]. Similarly, they found that prior knowledge of preferences, favorable organ donation beliefs were important, satisfaction with the OPO and healthcare team and timing of requests were associated with donation. These studies suggest that there is a need for a sustained commitment to public education interventions aimed at improving beliefs about organ donation, documenting and discussing donation decisions with next-of-kin, and to improving the request process in hospitals.

Even when a patient has a signed organ donation card, the OPO typically seeks family approval to proceed with donation. Although the Uniform Anatomical Gift Act (1968, revised 1987) states that

a signed organ donation card is sufficient to proceed with donation and serves legally as an advanced directive, requesting consent from the next-of-kin remains standard, largely to avoid lawsuits and unfavorable press. This model may be slowly changing, however, with many states passing legislation establishing "first-person consent" laws. These laws establish that the family cannot supersede an individual's expressed wish to be an organ donor. Often times in such states first-person consent registries are maintained, often by the Department of Motor Vehicles (DMV). First-person consent laws support patient autonomy, promoting the ideal that patients should be able to choose what the type of care that they will receive and what should happen to their body after they pass. First-person consent may also alleviate a burden from family members wanting to do the right thing in a stressful and difficult circumstance. In this situation, first-person consent laws relieve the family from making the decision while grieving, and reduces the likelihood of familial conflict related to donation. Given the instability of donation decisions made by families (more than one-third of families who made a decision and declined to donate regretted their decision), avoiding this scenario may provide even greater benefit [90,93].

Laws have been passed that require referral of all potential donors to the OPO or request of the families' permission to obtain organs of potential donors. This requires an effective screening system for identifying potential donors and sharing their information in an expedient way with OPOs. Such systems have required additional hospital development to facilitate donation requests [78]. Demands for organ transplantation may conflict with end-of-life preferences, as organ transplantation may require profusion, receiving or withholding that do not correspond to the patient's end of life treatment plan. Further research is needed to better develop policies that will allow OPOs to intervene efficiently and expeditiously when needed, while maintaining patient autonomy, dignity, and respect during end of life care.

Given that there are such wide range of consent rates from OPOs and transplant centers (from more than 70% to less than 30% of potential donors), the Institute of Medicine has recommended identifying best practices and disseminating them among the institutions. There should also be research identifying new ways to improve the system and increase donation rates. Organ donation could be coordinated with discussions about end-of-life care. Patients and families should be offered the opportunity to donate as standard end-of-life care. There could also be increased efforts to procure organ donations from cardiac-arrest deaths occurring outside of the hospital. According to one estimate, at least 22 000 people each year who die of cardiac arrest outside of a hospital could be potential organ donors.

The refusal of families to grant permission for donation presents a major impediment to organ donation. Three factors that have helped overcome this barrier. First, decoupling the request for donation from the declaration of brain death to allow the family time to cope with the loss of a loved one and fully understand the concept of brain death. Second, a trained OPO representative along with the clinical team should make the request together. Studies suggest that the clinical team should wait for the OPO representative to discuss donation with the family and chart a course of action. Third, the discussion with the family should be discrete, ideally in a quiet and private setting [94]. In 2003, the Secretary of DHHS launched the US Organ Donation Breakthrough Collaborative to formalize national efforts to improve efficiency and outcomes in organ donation. Housed under HRSA's Division of Transplantation, the Collaborative included the national community of OPOs and hospitals. Selecting experts from hospitals and OPOs that had a successful record of achieving and sustaining high organ donation rates to serve as faculty, the Collaborative developed best practices [95]. The intervention yielded positive results, with the number of organ donors in Collaborative hospitals rising 14.1% in the first year compared to merely 8.3% increase experienced by non-Collaborative hospitals. Importantly, sustained increases in organ recovery continued into the post Collaborative periods. Between 2003 and 2006, the number of total US organ donors increased by 22.5%, compared to the 5.5% increase measured over the same duration in the immediate pre Collaborative period. Although perhaps not all of the change is due to the Collaborative, studies suggest that dissemination and implementation of best practices gleaned from the Collaborative were a significant factor in this increase [96].

Living donors

Some have concluded that the waiting list for kidney transplant is too long, growing, and unlikely to be substantially reduced by increases in the recovery of deceased donor kidneys. Of the approximately 2.3 million deaths annually in the US, only between 11 000 and 14 000 produce eligible donors under the standard criteria. Removing barriers for living donors can increase the rate of living donations. These include funding to cover their living expenses, travel, lost wages and the costs of care they provide to other family members, assistance for postoperative care of the living donor, encouraging employers to accommodate employee requests to take time off to donate organs [77]. Minorities are far less likely to be living donors as well. Barriers to minority living donation include: unwillingness to donate, medical co-morbid conditions, trust or fear of medical community, loss to follow-up, poor coping mechanisms, financial concerns, reluctance to ask family members and friends, fear of surgery, and lack of awareness about living donor kidney transplantation [82].

The National Living Donor Assistance Center, administered by the Division of Transplantation of HRSA through a co-operative agreement with the University of Michigan and the American Society of Transplant Surgeons (ASTS), aims to provide greater access to transplantation for persons who want to donate, but cannot otherwise afford the travel and subsistence expenses associated with donation [97]. The program states that, it is "authorized by section 377 of the Public Health Service (PHS) Act, 42 U.S.C. § 274f. The specific authority was authorized by the Organ Donation and Recovery Improvement Act (P.L. 108-216) which provided authority to the Secretary to establish this grant program to assist living donors who need financial assistance to help defray travel and subsistence expenses". In 2006, HRSA awarded the co-operative agreement to the University of Michigan with a subcontract to the American Society of Transplant Surgeons to establish and operate the program, charged with:

1. running a national system to provide reimbursements for "travel, subsistence, and other non-medical expenses that may be authorized by the Secretary, to individuals making living donations of their organs";
2. establishing governing policies together with HRSA and the transplant community;
3. operating the program effectively including efficient payment to living donors;
4. ensuring continued fiscal health; and
5. monitoring and evaluating the quality of the program.

In October 2007, transplant centers were invited to register for the program on the NLDAC website. Between its inception in 2007 and through July 26, 2010, NLDAC received over 1200 applications and funded 86% of them. The expense for travel and lodging has averaged $2900 per application. Payment is granted in the form of an American Express Controlled Value Card (CVC) to purchase airfare, gas, rental cars, hotel rooms, food and other incidental expenses such as parking [98].

In June 2008, the OPTN/UNOS approved a proposal for a national pilot program with interim implementation that began in October 2010 [99]. 77 transplant centers in four regional networks are enrolling patients and potential living donors in the pilot exchange program that includes two- and three-way exchanges.

Current policy and transplant tourism

In 1984, a proposal for kidney sales by a US physician led the National Organ Transplant Act to prohibit monetary compensation for transplantable organs. The prohibition of commodification current policy in the US prevents exploitation. However, data on departures from the US kidney waiting list suggest that from 1987 to 2006, over 335 Americans traveled overseas to obtain kidney transplants. These transplant "tourists" often pay brokers between $15 000 and $150 000 for transplant packages [10]. The current policy thus gives an advantage to those who are wealthy and those with social capital and thus more likely to obtain a live donation. This policy is inefficient due to unrealized willing-but-incompatible donations and losses to potential savings due to these willing-but-incompatible donors. In addition, studies of transplant tourism have found that kidney patients transplanted abroad had a high incidence of serious postoperative infections, although graft survival and function appeared fine [100]. There may also be inadequate communication of information such as immunosuppressive regimens and preoperative information. The extent of commercial transactions and organ theft remains incompletely understood. The WHO estimates that roughly 10% of organ transplants worldwide involve organ trafficking and transplant tourism. Several principles came out of the Declaration of Istanbul World Health Organization Summit on Organ Trafficking and Transplant Tourism. National governments should develop and implement comprehensive programs for the screening, prevention, and treatment of organ failure. Legislation should be implemented to govern the recovery of organs from deceased and living donors, and transplantation, consistent with international standards. Organs for transplantation should be equitably allocated to recipients. The primary objective of transplant policies should be optimal short and long term medical care to promote the health of donors and recipients. Countries and regions should achieve self-sufficiency in organ donation. The World Health Assembly called on countries to prevent the purchase and sale of human organs for transplantation [101].

Summary

Transplantation has evolved from an experimental curiosity to one of the most complex of all human endeavors. This complexity is evident in the exceptional number of statutes and the immense regulatory fabric that covers all aspects of the field, from the formal definition of death, to restrictions on who can give the gift of life. Arguably, no field touches on more of the technical, logistical, social and ethical aspects of human existence, as does transplantation. As such, the study of transplantation will forever uniquely reflect the human condition, and in doing so, make this one of the most satisfying of careers.

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