SECTION 13

The transplant patient

CHAPTER 275

The evolution of kidney transplantation

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Introduction

The history of kidney transplantation begins in 1902 when Emerich Ullman in Vienna, Austria, transplanted a dog's own kidney into the neck using magnesium tubes to connect the vessels. He certainly achieved some success with one kidney putting out urine when he showed the animal at a meeting of the Viennese Medical Society. Alfred von Decastello at the same time carried out dog-to-dog transplants, also in Vienna (Hamilton, 2012).

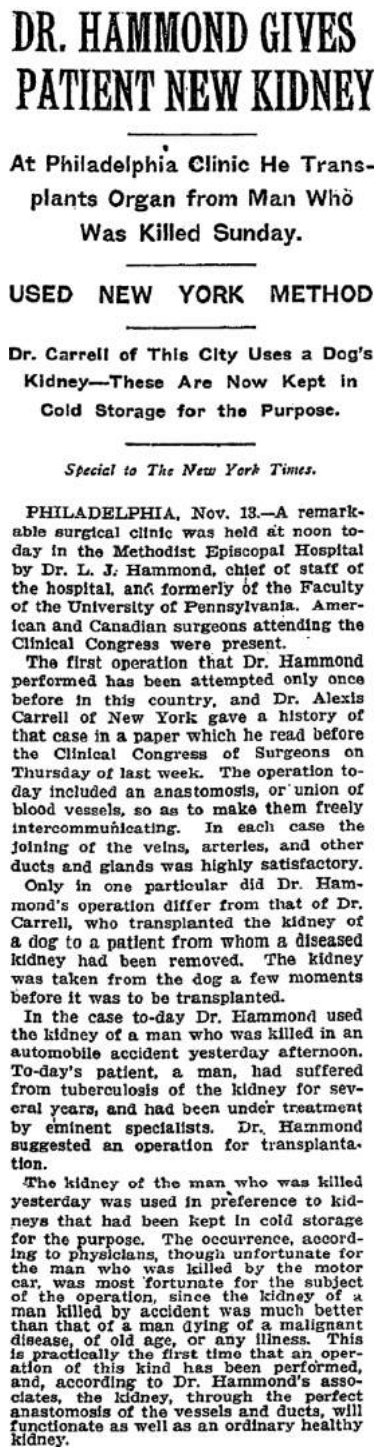
The key surgical advance was made when a young French trainee surgeon, Dr Alexis Carrel, was given a research project by his chief of surgery, Professor Jaboulay in Lyon, to develop a better method of joining two blood vessels surgically. His technique of an end-to-end anastomosis of two vessels was described in Lyon, France, in 1902 and is a seminal publication in the field of surgery (Carrel, 1902). Using that vascular technique, Jaboulay performed the first human kidney transplants in 1906 when he transplanted kidneys from a sheep and a pig into two humans with renal failure, anastomosing the renal vessels to the arm vessels. Carrel later moved to Chicago, Illionois, where, working with Guthrie, he carried out experimental kidney transplants in dogs. After moving to the Rockefeller Institute in New York he delivered an amazing lecture to the International Surgical Society in 1914 in which he stated that the *technical* aspects of transplantation were solved but not the inflammatory response that led to death of the graft. Until then it would have no clinical relevance. However, he went on to say that some of the anticancer cytotoxic drugs being tested experimentally at the Rockefeller Institute might have a place in preventing this inflammatory response. This was a prescient, given that the first immunosuppressive drug, 6-mercaptopurine, was developed as an anticancer drug nearly 50 years later (see Hamilton, 2008).

Early clinical experience

There is a report of a human-to-human kidney transplant as early as 1911, recorded in the pages of *The New York Times* of 14 November 1911 (Fig. 275.1). The surgeon—Dr L. J. Hammond—was reported to have transplanted the kidney from a man dying from a motor vehicle accident into a patient with tuberculosis using Carrel's techniques. The outcome was not revealed. Whether this report is true or not is difficult to establish as there is no report in the medical literature of the time, which is surprising in that the surgeon, Hammond, was writing quite a few case reports at that time! The first acknowledged series of human kidney allotransplants were those performed by the Russian surgeon Yu Yu Voronoy in 1933. The first was in a young woman with renal failure caused by mercury poisoning. The graft was unsuccessful but Voronoy went on to perform five more allografts over the next 6 years. There is little on record, but what is available, is described very precisely by Matevossian and colleagues using notes from archives in the Ukraine, on the occasion of the 75th anniversary of this first human kidney transplant (Matevossian et al., 2009). Voronoy had implanted the kidneys in the thigh joining the renal vessels to the femoral vessels with the ureter being brought out to the skin of the thigh as a ureterostomy (Voronoy, 1936).

In the 1940s and early 1950s, Professor David Hume working in Boston, Massachusetts, performed six cadaver transplants using essentially the same technique as Voronoy. The case histories revealed that, despite the absence of immunosuppression, two of these transplants survived for several weeks and one for 4 months (Hume et al., 1955). Rene Kuss in Paris, France, had also worked on the development of kidney transplantation in the dog and is responsible for developing the technique of implantation of the kidney in the pelvis using the iliac vessels for the anastomosis with the renal vessels and implanting the ureter in the bladder. In his first attempts at human renal transplantation, he used the same technique (Kuss et al., 1951). Kuss performed the first living related kidney transplant in 1952 from a mother to son after an accident on a building site led to nephrectomy for haemorrhage in the son from what proved to be a single kidney. The recipient, Marius Renard, survived for 3 weeks and the graft, a biopsy of which was recently unearthed, was rejected (Kreis et al., 2013). Kuss was disappointed and depressed by both the experimental and clinical results and abandoned work in transplantation, returning to his full-time urology practice. Later, after the successful twin transplant in Boston (see below), he returned to transplantation and was one of the early pioneers of renal transplantation. Indeed, in 1961, he performed the first successful living unrelated renal transplant using 6-mercaptopurine and total body irradiation.

In December 1954, Dr John Merrill, a nephrologist in Boston, was treating a young man dying of chronic renal failure and as the patient had an identical twin brother he thought that there was a real possibility of transplanting a kidney from the identical twin. Dr Joseph Murray, a plastic surgeon, and Dr Hartwell Harrison, chief of urology, at the Peter Brent Brigham Hospital, carried out the transplant. The operation was successful although the procedure was not without its moments of anxiety in that a clamp allegedly



DR. HAMMOND GIVES PATIENT NEW KIDNEY

At Philadelphia Clinic He Transplants Organ from Man Who Was Killed Sunday.

USED NEW YORK METHOD

Dr. Carrell of This City Uses a Dog's Kidney—These Are Now Kept in Cold Storage for the Purpose.

Special to The New York Times.

PHILADELPHIA, Nov. 13.—A remarkable surgical clinic was held at noon today in the Methodist Episcopal Hospital by Dr. L. J. Hammond, chief of staff of the hospital, and formerly of the Faculty of the University of Pennsylvania. American and Canadian surgeons attending the Clinical Congress were present.

The first operation that Dr. Hammond performed has been attempted only once before in this country, and Dr. Alexis Carrell of New York gave a history of that case in a paper which he read before the Clinical Congress of Surgeons on Thursday of last week. The operation today included an anastomosis, or union of blood vessels, so as to make them freely intercommunicating. In each case the joining of the veins, arteries, and other ducts and glands was highly satisfactory.

Only in one particular did Dr. Hammond's operation differ from that of Dr. Carrell, who transplanted the kidney of a dog to a patient from whom a diseased kidney had been removed. The kidney was taken from the dog a few moments before it was to be transplanted.

In the case to-day Dr. Hammond used the kidney of a man who was killed in an automobile accident yesterday afternoon. To-day's patient, a man, had suffered from tuberculosis of the kidney for several years, and had been under treatment by eminent specialists. Dr. Hammond suggested an operation for transplantation.

The kidney of the man who was killed yesterday was used in preference to kidneys that had been kept in cold storage for the purpose. The occurrence, according to physicians, though unfortunate for the man who was killed by the motor car, was most fortunate for the subject of the operation, since the kidney of a man killed by accident was much better than that of a man dying of a malignant disease, of old age, or any illness. This is practically the first time that an operation of this kind has been performed, and, according to Dr. Hammond's associates, the kidney, through the perfect anastomosis of the vessels and ducts, will functionate as well as an ordinary healthy kidney.

Fig. 275.1 Extract from *The New York Times*, 14 November 1911.

slipped off the renal artery of the donor and a considerable amount of blood was lost before control was gained. The recipient also had major problems with uncontrolled hypertension and had to have a bilateral nephrectomy to control the blood pressure. This was a remarkable breakthrough, for although it was realized that there should be no rejection reaction, as the Herrick brothers were truly identical twins, no one was certain whether a denervated transplanted kidney would behave in a normal physiological fashion. Neither physiology nor immunology proved to be an issue and this first successful transplant caused enormous excitement everywhere in the world, demonstrating as it did the proof of the principle that kidney transplantation could be undertaken successfully if the immune response to the graft could be controlled or avoided (Merrill et al., 1956).

Development of transplantation immunology

(See also Chapter 279.)

Cellular immunology

The seminal work of Peter Medawar and colleagues in the late 1940s and early 1950s established that rejection of skin allografts was mediated by leucocytes (Medawar, 1958). Before that observation, it was believed that grafts were probably rejected by antibodies. As a consequence of Medawar's work, antibodies took a lower place in the hierarchy of proposed mechanisms of graft rejection for many years. It was James Gowans, who showed that the constituent of the leucocyte population that caused graft rejection was the circulating lymphocyte also resident in the spleen and lymph nodes. Jacques Miller and others subsequently demonstrated that the lymphocyte population comprised both T lymphocytes arising from the thymus and B lymphocytes arising predominantly from the bone marrow. Two populations of T cells could be identified: the T helper cells and the T cytotoxic cells. Miller had made the discovery that the thymus was the source of lymphocytes, later shown to be T lymphocytes, and that an early thymectomy would render an experimental mouse immunologically deficient. This was a revolutionary discovery in that the thymus had been thought to be a rudimentary or even vestigial organ which disappeared with age in humans (Miller, 1961). Dr Ralph Steinman first showed in 1981 that dendritic cells were essential to presentation of antigen to lymphocytes thus playing a central role in the generation of the immune response that results in graft rejection (Steinmann, 1981).

Histocompatibility and antibodies

In the late 1960s, there were a number of significant developments which improved the outcomes of renal transplantation. Professor Jean Dausset, working in Paris, had already described, in 1954, the development of antibodies against leucocytes after blood transfusion, and he showed that these antibodies were alloantibodies to leucocyte antigens and not autoantibodies. These leucocyte antigens were shown to be histocompatibility antigens and the first such antigen described by Dausset was given the name MAC, later to be renamed as HLA-A2. This led to the application of crude HLA tissue matching to transplantation by Jean Dausset, Bernard Amos, Jon van Rood, Paul Terasaki, and others.

In 1967, Morris and colleagues first described the development of cytotoxic antibodies in man after renal transplantation and showed their association with acute rejection (Morris et al., 1968). This challenged the accepted paradigm of rejection at the time, which was based on the early work of Medawar that held that rejection was caused by leucocytes (and more precisely by lymphocytes). The concept that antibodies were responsible for acute rejection was at the time a rather controversial view. With the exception of hyperacute rejection, it has indeed taken many years for there to

be acceptance that antibodies are responsible for specific forms of both acute and chronic allograft rejection.

This led to the recognition by Paul Terasaki and Fleming Kissmeyer-Nielsen that the clinical disaster of hyperacute rejection of the transplant at the time of surgery was caused by antibodies in the recipients which could be identified *in vitro* through their reaction with donor lymphocytes. Williams and colleagues described the clinical and immunological phenomena of hyperacute rejection in detail in a number of patients in 1968. The introduction of the lymphocyte complement-dependent cytotoxicity crossmatch test improved the results of kidney transplantation, not least by avoiding hyperacute rejection (Williams et al., 1968; Patel and Terasaki, 1969)

Early results of cadaver kidney transplantation did seem to be influenced to some degree by the match of the kidney, crude as the definition of histocompatibility antigens was at that time. The value of matching for human leucocyte antigens (HLAs) in cadaver transplantation was controversial in the early 1970s until the discovery of class 2 antigens and the demonstration by Ting and Morris in 1978 that matching for antigens of the HLA DR series had a very powerful effect on the outcome of cadaveric renal allografts (Ting and Morris, 1978, 1980).

HLA testing relied upon the use of serum from individuals sensitized by pregnancy, transfusion, or transplantation and even from deliberately immunized volunteers, until the advent of genetic methodologies. Restriction fragment length polymorphisms gave way to the more practical and precise techniques built around the polymerase chain reaction in the early 1990s. The precision of individual gene sequencing had to wait another 20 years. The introduction was first in the bone marrow transplant field but has more recently become routine practice in most laboratories. The essential challenge for matching deceased donor transplants is the same as it was in the 1960s—the time available for laboratory testing. Rapid typing and crossmatching must be supplemented by extensive prior analysis of the individuals awaiting transplantation. Accurate and precise HLA typing can be performed when individuals are first listed for a transplant, but the accuracy of the donor typing has to use a rapid and less precise technology.

Screening the recipient for prior sensitization has seen substantial improvements from the initial technique of crossmatching a recipient against a panel of individuals and analysing the positive results to define HLA antigens to which the donor was reactive (Fuggle and Taylor, 2008; Tait et al., 2013). Panel reactive antibody level was the definition of the difficulty that there would be to find a suitable crossmatch negative donor for an individual. Many allocation systems use this convenient number to direct organs to sensitized individuals when a negative crossmatch is identified. Terasaki, having developed the original approaches to screening and crossmatching, was also responsible through his commercial company, One Lambda, for developing the next technology to revolutionize the approach to antibody detection and screening. Coloured beads coated with specific HLA antigens can be used to detect sensitively the antibodies to those antigens using flow cytometry or solid phase immunoassay in the Luminex system (Tait et al., 2010). The application of this technology has yet to be fully understood, but it has exposed the presence of many hitherto undetected antibodies to donor antigens and will probably provide the next improvement in outcomes as it is applied both before and after transplantation.

Immunosuppression

(See also Chapter 281.)

Early development

In the mid 1950s, Dr Gertrude Elion and Dr George Hitchings, working at the Burroughs Wellcome company, developed the anticancer drug 6-mercaptopurine. Schwartz and Damashek, who were two clinical haematologists in Boston, explored the use of this agent in suppressing the immune response and firstly showed that it could suppress the production of antibodies to human globulin and in their seminal paper published in *Nature* in 1958 showed that it could produce tolerance to human globulin when given at the time of the antigen stimulation (Schwarz and Damashek, 1958). They demonstrated that it would significantly delay the rejection of skin allografts in rabbits. Shortly after the publication of this paper, Roy Calne in London, and Charlie Zukoski and David Hume at the Medical College of Virginia, Richmond (to where Hume had moved from Boston), both showed that 6-mercaptopurine could significantly prolong the survival of renal allografts in the dog. Elion and Hitchings went on to develop an analogue of 6-mercaptopurine, azathioprine which then replaced 6-mercaptopurine on the basis that it was less toxic (for further details, see Hamilton, 2008, 2012).

Azathioprine was rapidly introduced into renal transplant units around the world. Despite its use, virtually all patients had acute rejection requiring treatment with high-dose corticosteroid. Thomas Starzl thus introduced the use of corticosteroids as maintenance immunosuppression from the time of transplantation together with azathioprine. From the mid 1960s, azathioprine and high-dose steroids became the standard immunosuppressive therapy for renal transplantation, with rejection being treated with pulses of additional high-dose steroids either intravenously or orally.

By 1964, Michael Woodruff had developed an antilymphocyte globulin in rabbits and showed that it, like thoracic drainage, could be used to deplete rats of lymphocytes, and thereby produce profound immunosuppression (Woodruff and Anderson, 1963; Woodruff and James 1968). This led to the development of anti-human lymphocyte globulins, one of which was first used by Starzl in 1967. They then became the treatment of steroid resistant rejection and a component of induction regimens still in use today.

The introduction of azathioprine and corticosteroids into renal transplantation dramatically improved results. In 1968, the transplant unit in Cambridge, United Kingdom, where Roy Calne had moved as the foundation Professor of Surgery at Cambridge University, and the unit in Melbourne, Australia, led by Vernon Marshall, Priscilla Kincaid Smith, and Peter Morris, both reported 1-year graft survival of 60%. Patient mortality was still significant, most deaths the consequence of infection. The risk of these was substantially increased by the use of long-term high doses of corticosteroids. Low-dose corticosteroid regimens had been used successfully in Belfast, Northern Ireland, in the 1970s (McGeown et al., 1977). In the early 1980s, randomized controlled trials of low-dose steroids versus conventional high-dose steroids were carried out in Oxford, United Kingdom, and elsewhere, confirming that low-dose corticosteroid regimens were just as effective in preventing rejection as those using high doses, but there was a dramatic reduction in the steroid-associated complications (Morris et al 1982). Low-dose steroids therefore became the standard of care.

By 1980, most established transplant units reported cadaveric kidney graft survival of between 60% and 65% at 1 year with the use of azathioprine and steroids and patient mortality had reduced substantially, to around < 10% at 1 year.

Ciclosporin

Ciclosporin was first isolated as part of a drug discovery programme run by the chemists at Sandoz (now Novartis) in Basel, Switzerland, who were looking for an antifungal agent from the fungi that had been collected on a field trip to a plateau above the Hardanger Fjord in Norway. This weakly antifungal molecule was shown to be immunosuppressive by Stahelin and his team at Sandoz and so rejected as an antifungal agent, but recognized by the immunologist in the Stahelin team, Jean Borel, for its potential in transplantation (see Morris, 2013). Borel gave a paper at the British Society of Immunology in 1975 on his experiments with the new agent in rat kidney transplants and within a year ciclosporin was tested in large animal kidney transplants by Roy Calne and David White in Cambridge. Within a very short time by current standards, ciclosporin was first used clinically by Roy Calne in organ transplantation (Calne et al., 1978) and by Ray Powles in London in bone marrow transplantation (Powles et al., 1978). Translating the dose used in experimental studies to 25 mg/kg/day in the clinic rapidly led to the recognition of its nephrotoxic potential and, when used in combination with other immunosuppressive agents, to a 10% incidence of lymphoma (3/33 patients). A dose of 10 mg/kg/day was insufficiently immunosuppressive when used alone and the first phase III trials in Oxford, Australia, and Europe thus used 17.5 mg/kg/day as the sole immunosuppressive agent. By the early 1980s, ciclosporin was licensed as an immunosuppressive drug, first in Europe and then in the United States and globally during 1983 and 1984. The use of ciclosporin led to a dramatic reduction in the incidence of acute irreversible rejection in the early months after transplantation, which in turn resulted in a much improved graft survival (Fig. 275.2). The next 10 years were needed to determine how to exploit this new found control over the immune system, to understand the biochemical pathways of calcineurin inhibition, and to minimize its adverse effects, especially nephrotoxicity.

Combination immunosuppressive therapy

The introduction of ciclosporin changed the practice of clinical transplantation. Physicians could no longer diagnose acute rejection easily, since it was more subtle than had been seen with previous immunosuppressive regimens. The nephrotoxic effect of ciclosporin meant an alternative diagnosis for declining renal function had to be considered. The diagnostic stakes were high, since the two most likely diagnoses—acute rejection and acute nephrotoxicity—required diametrically opposite actions, viz. to increase or decrease drug doses. Transplant biopsies were essential to make this distinction.

The initial idea in the earlier part of the 1980s was simply to replace azathioprine with ciclosporin and argument revolved around whether, and if so at what dose, corticosteroids should be used. A surgeon in Portsmouth, United Kingdom—Maurice Slapak—introduced a new option when he presented the data from a pilot study of low-dose steroids, low-dose azathioprine (1.5 instead of 2.5 mg/kg/day), and low-dose ciclosporin, in a triple combination. His argument was that reduced doses permitted reduced toxicity but the combination of all three agents provided synergic immunosuppression.

The so-called triple therapy regimens were adopted widely during the late 1980s and early 1990s without formal clinical comparison with the previous standard double therapy, but probably accounted for the additional 5–10% increase in 1-year graft survival rates observed from retrospective registry data, over and above the 15–20% improvement brought about by the introduction of ciclosporin (Fig. 275.2). Triple therapy was, and remains, an attractive regimen for transplant clinicians. It is possible to adjust the doses of each drug independently based upon perceptions of the individual patient's toxicity and efficacy needs. Without the formal clinical trial proof that would be expected in later years, physicians and surgeons were given free rein to manage rejection

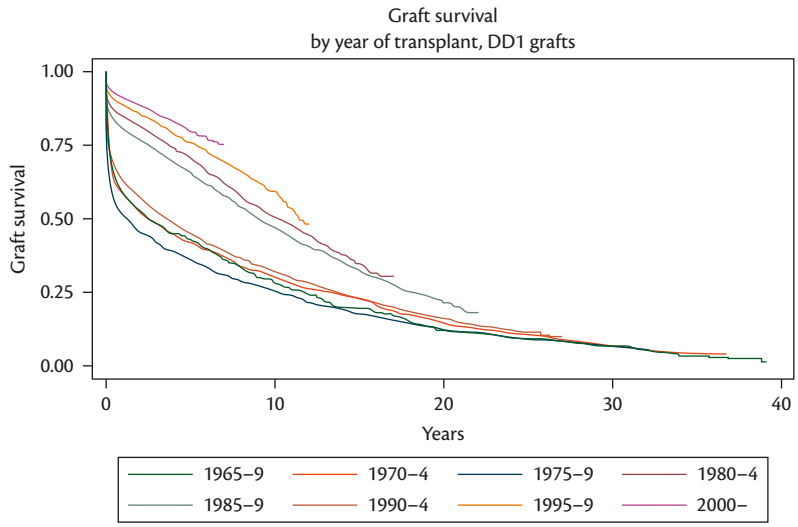


Fig. 275.2 ANZDATA analysis of deceased donor renal transplant long-term graft survival rates in 5-year cohorts demonstrating the enhanced survival with introduction of ciclosporin in 1982.

and adverse events such as infection, through dose adjustment. Some clinicians, for example, Kahan in Houston, Texas, examined the science of drug combination *in vitro*, describing a 'median effect equation', and confirmed as new agents came to be tested, the synergy of some combinations and possible reduced efficacy of others.

The current era of immunosuppression reached its optimum with the addition of two additional approaches: induction agents and infection prophylaxis. Both of these approaches are discussed in more detail below. The concept of an initial 'strong' immunosuppressive agent that is rapidly discontinued had its roots in two practical clinical problems. The first was accelerated rejection and early graft loss from steroid unresponsive rejection. The argument was that it would be better to 'get in first' with use of antilymphocyte globulin and prevent such rejection—especially in sensitized patients—rather than wait for the rejection to develop and allow irreversible graft damage. Hence induction immunosuppression using an antibody preparation could enhance outcomes especially in immunologically high-risk patients. Acute ciclosporin nephrotoxicity, especially in the context of ischaemic acute kidney injury or initial non-function, was the second concept driving adoption of a protocol of antibody induction in the first few days, changing to a consolidation immunosuppressive regimen beyond 10 or 14 days post transplant when the ischaemic injury had resolved.

Infection, especially with cytomegalovirus (CMV) and with what was then called *Pneumocystis carinii* (now renamed *P. jirovecii*) pneumonia, continued to cause early loss of life and much morbidity as well as graft loss, despite or because of the new and more powerful immunosuppressive protocols. New opportunities for regular and prolonged use of prophylaxis were sought and found to complement the power of the chemical immunosuppression achievable at the start of the 1990s. Patients thus started to receive routine prophylaxis against *P. jirovecii* (usually using co-trimoxazole) and against CMV with ganciclovir or valganciclovir. (See Chapter 284.)

Tacrolimus

Fujisawa—a pharmaceutical company in Japan—had discovered an agent with mechanisms of action similar to ciclosporin, which, because of its reputation as the first 'billion dollar molecule', gave the company hope that it had found the next 'blockbuster drug' in the field. Development of the agent, known for years as FK506 before it was named tacrolimus, was not simple and its arrival on the stage of transplantation was protracted. In 1985, a number of programmes had access to the agent for experimental transplantation and their data was presented at a symposium during the congress of The Transplantation Society in Helsinki in 1986. The outcome of that meeting was one of disappointment because of an unexpected dose-limiting adverse event of hepatotoxicity and small vessel inflammation in the dog model. Ochiai from Chiba University, Japan, examined efficacy in the rat, and his presentation convinced Starzl to obtain the molecule and study it in Pittsburgh, Pennsylvania. He developed an exclusive pre-clinical programme to investigate FK506 and quickly expanded it into a clinical research programme. He eventually reported at the 1990 congress of The Transplantation Society in San Francisco, their results of the use of FK506 in multiple clinical settings (Starzl et al 1990). The Federal Food and Drug Administration (FDA) in the United States brought order to what was becoming a rather difficult issue because of the lack of randomized controlled trials. Tacrolimus was clearly an effective immunosuppressant but also a cause of serious neurological and other sequelae, demanding formal randomized double-blinded clinical trials. It was not until 1996 that the FDA accepted tacrolimus for use in the United States. Its use spread slowly around the world but it was not until the middle of the next decade, some 5–8 years later, that the drug would realize its promise and displace ciclosporin as the market leader.

In common with many immunosuppressive drugs, the doses and the blood concentration were high to avoid immunosuppressive failure, but risking adverse events. With time, the blood levels became trusted to reflect immunosuppressive potency and clinical experience with adverse events led to increased confidence in diagnosis of toxicity. The largest clinical trial to date in transplantation was designed and co-ordinated by Henrik Ekberg, a transplant surgeon from Malmo, Sweden. The study nicknamed 'Symphony', compared standard-dose ciclosporin with low-dose ciclosporin, low-dose tacrolimus, and low-dose sirolimus, all in combination with mycophenolate mofetil (MPA) and corticosteroids using basiliximab induction. The results showed that in low-risk patients, triple therapy with tacrolimus, blood levels of 6–8 ng/mL, yielded the best results (Ekberg et al., 2007). The current era of transplant immunosuppression had arrived.

Mycophenolate mofetil

Elion and Hitchings had identified the central importance of purine synthesis in activated lymphocyte function and had explored it successfully not only with the resultant use of azathioprine but also allopurinol for control of urate synthesis in gout. Later Syntex (taken over by Hoffman La Roche) developed mycophenolic acid which also inhibits purine synthesis through reversible inhibition of inosine monophosphate dehydrogenase (IMPDH), specifically in lymphocytes because of a metabolic escape pathway present in all other cells which allows them to avoid MPA-derived IMPDH inhibition. MPA had been trialled briefly in the 1960s in patients with arthritis but proved too toxic to the gastrointestinal tract. Syntex, however, created a pro-drug that was well absorbed, well tolerated, and metabolized to yield MPA acid in the bloodstream. Mycophenolate mofetil (MMF) also had the virtue of being patentable, unlike MPA, and after pioneering clinical trials in the United States, Europe, Canada, and Australia in the mid 1990s, and despite a high price, took over from azathioprine as the antimetabolite in almost all triple therapy regimens globally (Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group, 1996).

Transplantation in the mid 1990s had a problem created by the success of the therapy available at that time. Graft failure and patient death rates had dropped so dramatically in the preceding 10 years that it was very hard to design a phase III study with sufficient power to demonstrate significant improvements in these primary outcomes. The FDA, after some consideration, accepted the concept of a composite endpoint—death, graft failure, acute rejection, and loss to follow up, were combined to provide a primary outcome measure for transplant studies. The three large-phase III studies of MMF recruited around 500–600 patients in each study and used this composite end point to demonstrate superiority over azathioprine. Marketed as CellCept®, it subsequently displaced the much cheaper azathioprine from clinical practice in almost all developed countries by the early 2000s. However a relatively recent systematic review and meta-analysis of all randomized controlled trials

comparing MMF with azathioprine used with the newer calcineurin inhibitors (or their reformulation) showed that the benefits are very modest indeed (Knight et al. 2009).

Novartis—the company created by merging Sandoz and Ciba-Geigy—developed an alternative patentable MPA precursor with different absorption characteristics. Marketed as Myfortic®, it provided competition for CellCept®, until the expiry of the patent and advent of generic competition in most countries by 2012.

Rapamycin

Rapamycin was first identified by Suren Sehgal, an employee of the pharmaceutical company Ayerst, later Wyeth, and tested by Randall Morris in Stanford, California, and by Sir Roy Calne in Cambridge. It was found in soil samples from the beautiful Easter Island—Rapa Nui—the long-eared statues of which provided a symbol for many a lecture on the agent. Now named sirolimus, the active molecule was produced by *Streptomyces hygroscopicus* and was first identified as having not only antifungal properties but also antitumour and immunosuppressive actions (Sehgal, 2003). It combines with the same molecule (FKBP12) as tacrolimus. Sirolimus inhibits the cell cycle kinase, the mammalian target of rapamycin (mTOR), blocking progression of the cell cycle from G1 to S phase.

Wyeth brought the drug to market as Rapamune®, and Novartis followed with a similar drug, everolimus. The initial enthusiasm for these agents as non-nephrotoxic immunosuppressants was dampened by poor patient tolerance and progressive understanding of the impact of mTOR on glomerular podocytes causing proteinuria. However, unlike all other drugs used to prevent rejection, both everolimus and sirolimus proved to have anticancer properties in the phase III studies where lower rates of cancer were observed in post hoc analyses. The two drugs, or their analogues, were developed directly as cancer chemotherapeutic drugs and have indications in renal cell carcinoma and tuberous sclerosis. Unfortunately the synergistic nephrotoxicity, when combined with standard doses of ciclosporin or tacrolimus, probably due to intracellular accumulation of the calcineurin inhibitors when combined with the mTOR inhibitors, has diminished the popularity of the drugs. It may find a role in patients with cancers or a high risk of developing cancer after transplantation (Campistol et al., 2006).

Randall Morris, working in the 1990s on the early experimental development of Sirolimus in Stanford using vascular allograft models, proposed that mTORi may be useful in preventing the overgrowth of smooth muscle occurring in cardiac stents and the so-called drug-eluting stent became a legacy from transplantation inherited by cardiology.

Therapeutic antibodies

The first clinical use of immunosuppressive antibodies in transplantation was in the 1960s as the role of the lymphocyte, in particular T lymphocytes, was understood. Injection of the target cells into a horse, goat, or rabbit led to production of anti-human lymphocyte serum which could then be injected into a patient depleting the target cells. Antilymphocyte globulin (ALG) and antithymocyte globulin (ATG) thus joined the immunosuppressive armamentarium of the 1960s and 1970s, before being developed into a standardized pharmaceutical product. The initial ALS and ATG products were produced in a number of university facilities. They were subject to research-level scrutiny and standardization of complement mediated *in vitro* lymphocyte killing, cross-reactivity testing, and sterility assurance, but were still very variable in efficacy and adverse event profile from batch to batch. The first problem was not the level of *in vitro* cytotoxicity, since that could be managed by adjusting doses according to *in vivo* effect, but the cross-reactivity than came from impure inoculating preparations and from the multiple shared antigens between lymphocytes and other cells. Anaemia, thrombocytopaenia, and broad leucocyte depletion were just some of the problems encountered. Despite these problems it was often the dramatic efficacy in cases of severe rejection that ensured their continued use. Trends in the United States and European transplant programmes took different directions during the 1980s. American programmes usually used ALG from Minnesota (MALG) as an induction agent for the first 5, 7, or 10 days of transplantation, both to avoid nephrotoxicity of ciclosporin in the early vulnerable days of a transplant and to reduce the incidence of acute rejection in the first 2 weeks. By contrast, European centres concentrated on the use of ALG and the more reliable ATG preparations as treatment for steroid resistant rejection.

During the 1980s, an Australian scientist, Gideon Goldstein, working for the pharmaceutical company 'Ortho', later part of the Johnson and Johnson conglomerate, used the then newly invented technique for clonal production of antibodies to produce the first 'designer' monoclonal antibody to reach clinical practice. 'Orthoclone OKT3', directed at the CD3 molecule on T lymphocytes, was introduced into clinical practice in trials in the early 1980s and the results published in *The New England Journal of Medicine* in 1985 (Multicenter Transplant Study Group, 1985). The first recorded cases of death from a 'cytokine storm', provoked by rapid lysis of T lymphocytes, were seen in this study and conditions on use of OKT3 included ensuring patients were not fluid overloaded prior to treatment, for that reason. OKT3 was used as an alternative to ATG for steroid-resistant rejection. Through the next 20 years it gradually fell from favour because of the severe adverse event profile and production was discontinued and supplies were exhausted by 2010.

Another early monoclonal agent was produced in Cambridge in the 1980s by Herman Waldmann, Geoff Hale, and colleagues—'Campath', re-engineered to be humanized as Campath-1H and renamed alemtuzumab as the convention for naming of monoclonal antibody pharmaceuticals was introduced (Hale et al., 1986). Alemtuzumab targets CD52, producing a profound lymphocytopenia and as a result is used in the treatment of chronic lymphocytic leukaemia. But it has also been used as an induction agent for prophylaxis of renal allograft rejection (Morgan et al., 2012). Produced by Genzyme which has recently been taken over by Sanofi-Aventis, alemtuzumab has now found a new indication in treatment of multiple sclerosis and the company has lost enthusiasm for its use in renal transplantation.

Leaning on the haematology sector, renal transplant programmes have explored several other agents with haematological indications. Of these, rituximab has seen the most use because of its B-cell specificity through targeting CD20. Several indications have been explored especially around the prevention or treatment of antibody-mediated rejection and in desensitizing patients with anti-HLA or anti-ABO blood group antibodies. The proteasome inhibitor bortezomib, and anti-terminal component complement inhibitor eculizumab, have also found favour amongst transplant programmes exploring methods of preventing graft loss from antibody-mediated rejection.

The only enduring monoclonal agent in clinical renal transplant practice today is the Novartis drug basiliximab (Simulect®), which is directed at the interleukin 2 receptor. Initially in competition with daclizumab made by Hoffman La Roche, basiliximab is widely used for induction. Used on days 0 and 4 after renal transplantation it reduces acute rejection rates and in meta-analyses improves graft survival rates. It is one of the few agents which have yet to have any adverse events described either in clinical trials or in extensive clinical practice (Webster et al., 2010).

Failed drugs and the future transplant pipelines

A large number of drugs have been tested for their role in transplantation. The pharmaceutical industry noted the transformation of Sandoz from a small to a large company based on the success of ciclosporin and sought to imitate their success. The early phase of this research in the 1980s and 1990s was the search for a non-nephrotoxic calcineurin inhibitor, a quest that remains active. A Canadian biotech company is today developing what they hope will replace tacrolimus. Greater understanding of the scientific basis of allograft rejection led to discovery pipelines of drugs with different mechanisms of action. The failures in those pipelines were many, dispiriting, and expensive, though not all were consigned to the filing cabinet and waste bin. Some, like the Novartis drug FTY720, were repackaged for treatment of autoimmune diseases such as multiple sclerosis. In the last decade, janus kinase inhibitors (JAK) were trialled and as a result, work was focused onto JAK 3 inhibition. JAK 3 inhibitors have been developed by Novartis, Astellas, and Pfizer but did not demonstrate sufficient efficacy to succeed in transplantation, though at least two of them are likely to become treatments for psoriasis. Sotrastaurin, which is a pan-protein kinase C inhibitor, was trialled by Novartis in phase II but not phase III transplantation studies. A monoclonal antibody that had promise to protect kidneys suffering from ATN and reduce fibrosis, was the first in a line of drugs targeting the problem of fibrosis in allografts, but is not being further developed.

The most recent drug to be brought to market with a transplantation indication is illustrative of the problem now faced by pharmaceutical companies. Belatacept is one of the drugs that emanated from knowledge of immunological mechanisms rather than a discovery programme designed to identify naturally occurring immunosuppressants (Vincenti et al., 2005). The drug is a designer molecule that targets co-stimulation receptors to block lymphocyte activation. Belatacept had an expensive development phase which lasted for at least 9 years before approvals were achieved. Marketed by Bristol Myer Squibb it has faced the reality that the price that could be sustained in the United States dropped markedly with the advent of generic standard of care drugs, despite the several advantages that a once-a-month injectable agent could bring, not least that it allowed minimization of calcineurin inhibitors and so better renal function.

The sad reality is that today none of the major pharmaceutical companies have research and development groups orientated towards transplantation and there are essentially no active pipelines for investigation of new drugs in transplantation. It is not clear where advances will be made over the next 10 years. Perhaps it will be in repackaging the more effective immunosuppressants registered for treatment of autoimmunity.

Organ preservation

Development of sophisticated histocompatibility testing in the 1960s created a major problem for its implementation for cadaveric organ transplantation, in that a suitable method for organ preservation was required to provide the time for typing, recipient selection, and transport of the preserved organ to distant locations. Folkert Belzer had developed an effective preservation system for kidneys using a bulky perfusion machine in which the kidney was kept cool and constantly perfused with cryoprecipitated plasma (Belzer et al., 1968). This method was effective but too cumbersome to be helpful in achieving the above objective. To answer this challenge, the Waters company produced a portable version of the Belzer machine that could be used to transport kidneys, but it required an operator to accompany the machine, limiting its applicability. Geoff Collins was asked by Paul Terasaki to develop a simple cold storage technique for kidney preservation. The thoughts at the time were that the damage induced by cold storage using typical electrolyte solutions such as Ringer's lactate would include loss of intracellular constituents, osmotic swelling, and ultimate rupture of cell membranes. So after establishing the lack of efficacy of extracellular compositions, Collins tried an intracellular solution. Working with a medical student, he formulated an intracellular solution, and added glucose as an additional osmotic agent to control cell swelling. He first tested this solution for 24-hour ice storage using dog kidneys and was amazed by the results. Collins solution did indeed allow cadaveric kidney to remain viable so that they functioned immediately after even 24–30 hours of storage (Collins et al., 1969). It turned out that there was an error in the magnesium concentration as a result of confusion between mM and mEq, and it was originally set at double the actual intracellular content accounting for reports of magnesium phosphate crystals in kidneys flushed with Collins solution. In response to this, European transplant centres removed the magnesium and substituted mannitol for glucose earning the solution the name 'EuroCollins'. This appeared to be as effective as the original solution, and these solutions remained the standard preservation technique for organs until development of the University of Wisconsin (UW) solution by Belzer and Southard. The key advance in UW solution was the recognition that prevention of cell swelling in the preserved organ was critical and this required much larger molecules than had been selected for Collins solution, namely lactobionate as the principal anion and raffinose instead of glucose or mannitol (Belzer et al 1990). UW appears to be superior to EuroCollins but equivalent to Celsior (O'Callaghan et al 2012). Hypertonic citrate (Marshall's solution) is widely used in the United Kingdom and United Kingdom registry data suggests it to be equivalent to UW but there are no randomized controlled trials (J. O'Callaghan, 2013, unpublished data)

Static cold preservation has been the standard of care but may not remain so for long as the concept of organ resuscitation becomes a reality. Improving an organ damaged by ischaemia and the cytokine storm unleashed by brain death may soon open dramatic new opportunities for organ donation. Whether hypothermic machine preservation is superior to static cold storage is still under evaluation (O'Callaghan et al., 2013) as is its role in resuscitation of potentially damaged kidneys from extended criteria donors or donation after circulatory death donors.

Kidney donation

The original studies of kidney transplants explored all three potential sources of viable kidneys—the living donor, the deceased donor, and an animal donor. These three sources remain viable options while a fourth—construction of a kidney through directed differentiation of stem cells—remains a source of grant funding but is not yet on the immediate horizon. Xenotransplantation, experimented on in man from the early 1900s to today, remains mired in two problems: full understanding and suppression of xenograft rejection, especially related to the presence of natural cytotoxic antibodies in man against all species except the higher order primates; and prevention of cross-infection from the selected animal species to man (McKenzie et al., 1968; Heneine and Switzer, 1996; Lin et al., 1998).

The two sources of kidneys for clinical transplantation thus remain the deceased and living donor (see Chapter 277). There have been significant advances in the ability of communities to provide for the care of people with end-stage kidney failure such that the rate of transplantation has been unable to meet the needs of the population in almost all countries in the world. The mismatch between donor rate and recipients on waiting lists is often the introduction to many a lecture and grant proposal. Spain has led the way to maximizing the potential for donation from deceased donors, followed now by countries as diverse as Croatia, Portugal, Italy, Austria, United States, United Kingdom, and Australia where the lessons of Spain have been applied (Matesanz et al., 1994).

Donation from living donors has followed two trends—one legal and one illegal. The technical advances that have opened up the possibilities for related donors are several. Firstly, the exclusion of a relative from donation by virtue of blood group or HLA sensitization barriers have been reduced markedly as discussed below. Secondly, the procedure has advanced through development of the better accepted laparoscopic technique for donor nephrectomy (Ratner et al., 1995). Thirdly, the development of paired exchange programmes in Korea and then in Europe and United States provided mismatched donor recipient pairs to avoid HLA sensitization or high-titre blood group antibodies by donating between two or more pairs of individuals (Park et al., 1999). The capacity of paired exchange programmes to avoid expensive and hazardous desensitization protocols has proved attractive to patients and transplant programmes and is now widely applied.

ABO incompatible transplantation

In their seminal 1955 paper, Hume et al. noted that ABO blood group compatibility was probably required for successful renal transplantation (Hume et al., 1955). The presence of blood group substance on the endothelium of the kidney reinforced the view that ABO compatibility was an essential condition for renal transplantation. Allocation systems implemented ABO compatibility, but also may only allocate to ABO identical recipients, since biologically acceptable incompatibility unfairly discriminates against blood group O recipients, as their only donors are O, but O donors are compatible with any recipient.

In Belgium, Alexandre questioned the fundamental tenets of ABO incompatibility and undertook ABO incompatible transplants through the late 1970s and 1980s with some success. He eventually settled on a protocol involving splenectomy and plasmapheresis, and noted that if the donor was blood group A2 the density of blood group antigen on the kidney was reduced, permitting more frequent successes (Alexandre et al., 1985).

In both Sweden and Japan the protocols for successful ABO incompatible transplantation were explored extensively. A significant advance on plasmapheresis for removal of antibody was developed in Lund in Sweden, where specific immunoadsorption columns were placed into a dialysis circuit and depending upon the glycoprotein in the column, blood group antibodies were removed with greater efficiency than could be achieved by plasmapheresis. The other advantage of the columns was the lack of removal of other blood proteins such as clotting factors, making them safer to use in the context of the transplant operation. A protocol employing the blood group adsorption columns and rituximab in place of splenectomy and conventional triple therapy, used in Sweden, was so successful that there was not only no graft loss from ABO antibody but also essentially no acute allograft rejection either. The Japanese experience, spearheaded by Tanabe in Tokyo, was successful in large numbers of patients (Tanabe et al., 1998). ABO incompatible transplantation spread across the United States, Europe, and Australia during the period from 2000 to 2010. The critical elements of success were accurate measurement of the titre of anti-blood group antibody in the recipient and ensuring a low titre at the time of transplantation. It appears not to matter if the titre rises after the transplant has been *in situ* for a few weeks, implying some form of accommodation to the presence of antibody.

Desensitization

HLA sensitization by pregnancy or transfusion and especially by a failed transplant has consigned many individuals to long-term dialysis without a practical alternative strategy to waiting for the miracle of a matched donor offer. Large donor-sharing programmes in Europe, the United States, and elsewhere have allowed some to be provided kidneys from distant donors when a fortunate gap in the individual's sensitization pattern meant that they do not have antibodies to the particular donor HLA antigens (De Meester et al., 2002). The alternative explored in the United Kingdom in the 1980s and then implemented in the United States as more powerful immunosuppression became available, was to remove antibody by plasmapheresis and prevent recurrence through immunosuppression (Taube et al., 1984; Montgomery et al., 2011). The role of intravenous immunoglobulin in management of HLA antibody has yet to be fully elucidated but has been widely used in post-transplant therapy.

Anti-infective prophylaxis

Many clinicians ascribe some of the improvements in outcome typified by the improved graft survival seen in Figs. 275.2–275.5, not to the improved immunosuppressants but to the ability to control and prevent the worst infective complications that result from patients' altered immunity. The first universal prophylactic drug to be used from the late 1980s was also derived from the work of Hitchings and Elion—co-trimoxazole or combined sulphamethoxazole and trimethoprim. This reduced to zero the incidence of pneumonia due to *Pneumocystis jirovecii*. There was the side benefit of a 50% reduction in urinary tract infections.

The other scourge of transplant programmes in the early 1980s was CMV which regularly caused severe illness at about 6 weeks in

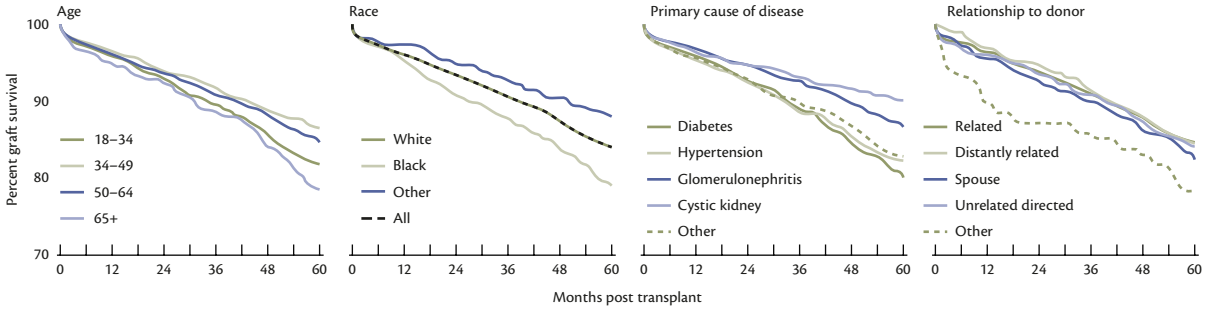


Fig. 275.3 Scientific Registry of Transplant Recipients (SRTR) analysis of graft survival in adult kidney transplant recipients transplanted in 2006 from living donors.

CMV-naïve recipients with primary infection contracted from the donor. Reactivations and *de novo* infections in previously infected recipients, typically manifested at about 3 months after transplantation and caused less severe but still troubling disease. An intravenous drug treatment became available in the mid 1980s, ganciclovir, and a well absorbed oral version of aciclovir became a practical but still not very effective prophylactic alternative. There was a period in the 1990s when alternative strategies were examined to prevent CMV infection: pre-emptive therapy in which blood was sampled regularly for CMV and when positive results were detected intravenous (IV) ganciclovir was administered; oral valacyclovir (an orally bioavailable aciclovir preparation) used in CMV-naïve recipients of CMV-positive donors; or short-term IV ganciclovir prophylaxis in such high-risk individuals. All such strategies were resolved with the introduction of valganciclovir which provided the efficacy of ganciclovir and the bioavailability of the intravenous preparation (Kotton et al., 2013). (See also Chapter 284.)

Tolerance: from laboratory to clinic

More than 60 years ago, tolerance to skin allografts in mice was achieved by Medawar's group in London (Billingham et al., 1953) and then an experiment in dogs first realized the 'holy grail' of drug-free renal allograft tolerance by the induction of bone marrow-derived mixed allogeneic chimerism (Mannick et al., 1959). The experiment has been repeated many times since then in different large animal models, including the pig, dog, and monkey, and has achieved the same result. In the clinic (in San Francisco, Chicago, and Boston) three groups have studied this approach. The original intent of mixed chimerism was to separate graft-versus-host disease from graft versus leukaemia after bone marrow transplantation. Since those early experiments, the protocols have been refined to deplete host T cells and then infuse haemopoietic stem cells of the donor including, in the latest iteration, specially prepared facilitator cells (Leventhal et al., 2012).

The most recent available data from the Boston group show that out of 10 patients, six have lost their grafts or restarted immunosuppression. The renal experience is thus different to those of drug withdrawal after liver transplantation where up to 50% of patients with grafts lasting > 10 years and who have stopped immunosuppressive drugs have not restarted them. Defining the biological profile of patients who can achieve withdrawal safely has proved difficult, despite ongoing endeavours in both Europe and the United States. They do appear to have a different B-lymphocyte profile to those who fail withdrawal.

Tolerance has thus not yet reached the clinic in anything other than carefully monitored clinical trials in the best-resourced centres in the United States. If the tolerance studies had been clinical drug trials the strategy would have been discarded as a failure, but because it has worked in large animals and has the tantalizing promise of stable, immunosuppression-free therapy the trials continue (Chapman and Alexander, 2012).

Chronic rejection

Progressive improvement in short-term results, achieved through the 1980s and 1990s, exposed the problem of long-term attrition through both premature death of recipients and chronic graft loss after progressive and seemingly unalterable decline in renal function. The term used to describe this clinical course of progressive

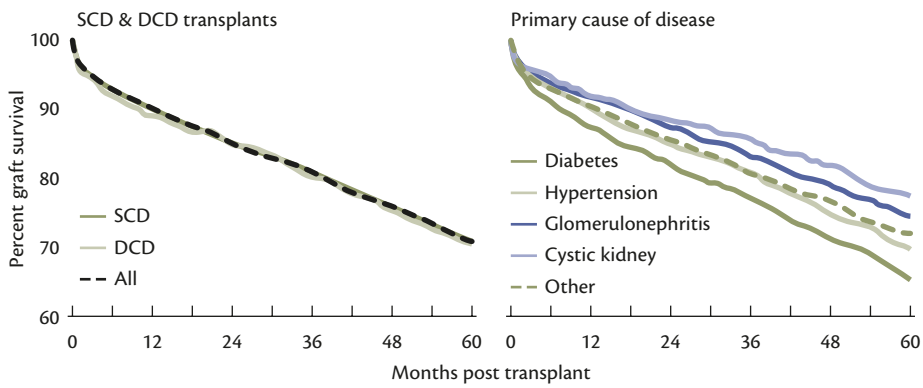


Fig. 275.4 Scientific Registry of Transplant Recipients (SRTR) analysis of graft survival in adult kidney transplant recipients transplanted in 2006 from deceased donors.

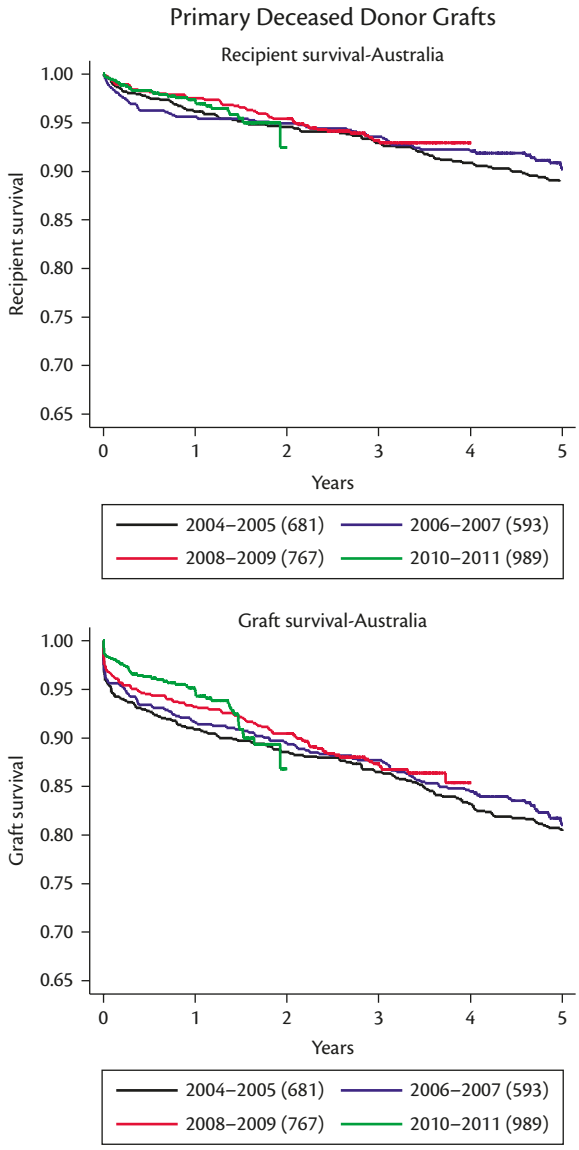


Fig. 275.5 ANZDATA analysis of deceased donor renal transplant patient and combined graft and patient survival in Australia 2012.

graft loss has changed like fashion. 'Chronic rejection' gave way to 'chronic allograft nephropathy' as the pathologists gained supremacy over immunologists. The physiologists in turn displaced the pathologists and 'chronic allograft dysfunction' became common in discussion and publication until the return of the immunologists and their approach to definition of 'chronic rejection' once more. A more detailed discussion of the understanding of the mechanisms of chronic graft destruction is provided in Chapter 284. The progress in understanding has relied upon the application of new technologies. The data underpinning the evolving view of the importance of chronic graft loss came from registries, which collected data on large numbers of patients across many countries. Pathology of kidney transplantation required classification and codification to move from opinion-based observation to science. This was driven by Solez and his colleagues who developed the Banff classification system (Solez et al., 1993). Pathologists were only as good as the material provided to them and so understanding of natural history of factors influencing graft pathology relied on biopsies taken by protocol at fixed times after transplantation. Pioneered in the 1980s by Morris and others (Morris et al., 1987), developed by Rush and colleagues (1994, 1998) in the 1990s and applied to chronic graft processes by the Westmead group in the early 2000s (Nankivell et al., 2003), protocol histology has become the gold standard against which the newer technologies can be assessed. The next steps in understanding have been delivered by genetic techniques examining urine, blood, and the graft for RNA signalling cellular processes operating in grafts undergoing rejection or other processes leading to fibrosis (Muthukumar et al., 2005). It is likely that biomarkers of underlying disease mechanisms will become available in clinical practice to provide the opportunity for intervention before irreversible kidney damage has occurred.

Outcomes of kidney transplantation

Data accessibility

What results can patients expect of renal transplantation today and how will their decisions be informed? The medical answers are written in the reports of the national and international registries of transplant outcomes and in the clinical trials and meta-analyses to be found in the scientific literature. The statistical techniques of analysis are such that only a minority of the clinicians working in the field actually understand them. Furthermore the availability of level 1 and 2 evidence, namely systematic reviews, meta-analyses, and randomized controlled trials, is sparse. How then can patients be expected to understand data and make the complex decisions?

An example of the questions that a patient on the waiting list may need to answer is: 'Should I accept the offer of the kidney transplant presented to me or would I be better waiting for another offer?' The information base for decision may be scanty because of confidentiality provisions in the law—'The donor died of a cause of death that cannot be divulged and had a number of medical illnesses before death that remain a secret. The donor is classified as an "extended criteria donor", which is a phrase used instead of "marginal donor", since that might put you off accepting this kidney and we want someone to accept it.'

The data are accessible through a number of routes—firstly, the Transplantation Library contains a bibliography of randomized clinical trials and meta-analyses in Transplantation and may be accessed through the Internet (<http://www.transplantevidence.com/library.php>). Secondly, the Cochrane Review Library contains a large number of continuously updated meta-analyses of transplantation questions, amongst other areas of work (<http://www.thecochranelibrary.com/view/0/index.html>).

The reports of the major registries are also available online, for example:

1. The Scientific Registry of Transplant Recipients (United States) <http://www.srtr.org>
2. Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) <http://www.anzdata.org.au>
3. The Collaborative Transplant Study (International) <http://www.ctstransplant.org>
4. The World Health Organization (WHO) Global Observatory on Donation and Transplantation <http://www.transplant-observatory.org/Pages/home.aspx>

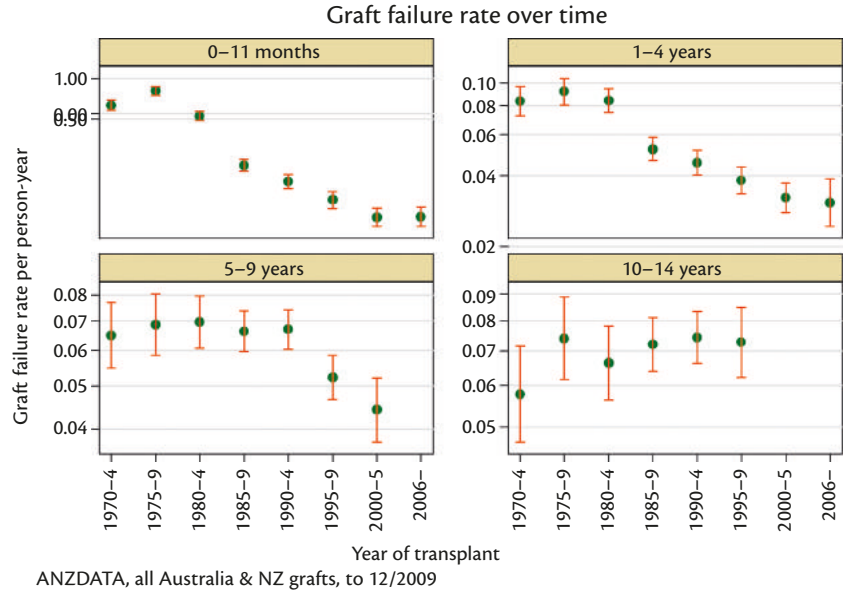


Fig. 275.6 ANZDATA analysis of renal transplant graft failure rates in the first year, 1–4 years, 5–9 years, and 10–14 years by 5-year cohorts of year of patient transplantation, showing the reductions in graft failure rates achieved over time, but absence of improvement in long-term outcomes > 10 years.

5. United Kingdom Renal Registry: <http://www.renalreg.com>
6. ERA-EDTA Registry: <http://www.era-edta-reg.org>.

Results of transplantation

The average graft and patient survival after renal transplantation should be known for most countries and the relative success of individual transplant programmes should also be known, a benefit of assiduous data collection and analysis that typifies renal transplantation.

Analyses of graft survival are presented in several formats, the commonest being a Kaplan–Meier actuarial survival plot which shows the proportion of grafts still functioning by time after transplantation. In this representation, due weight is given to grafts that have been transplanted for short periods of time and have not failed, as well as grafts that are lost to follow-up, that is, the outcome is only known to a certain point. A difference in the presentation is seen with the way in which death with a functioning graft is handled. In a 'death censored' analysis, death is not treated as a graft

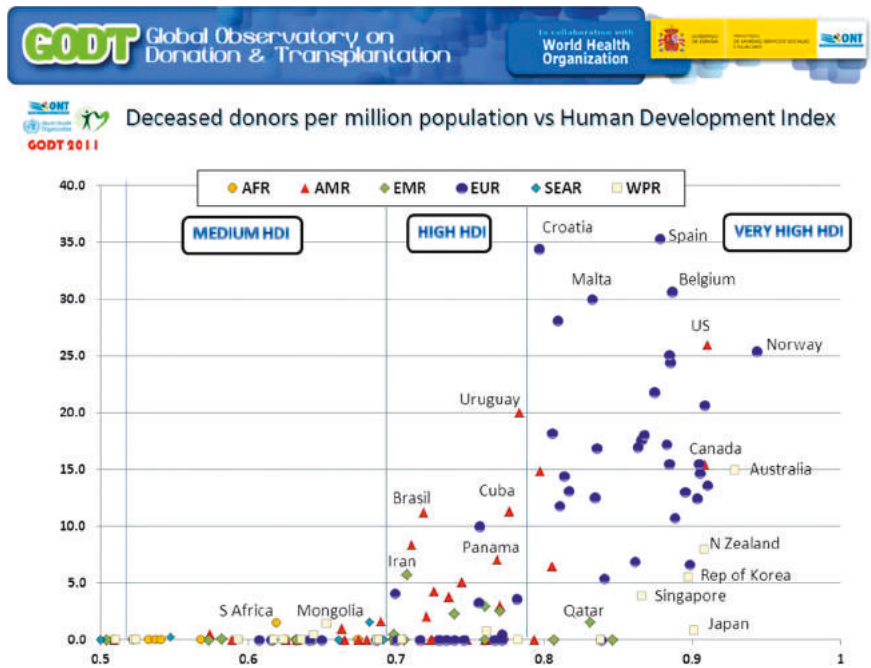


Fig. 275.7 WHO Global Observatory analysis of the number of kidney transplants performed in 2011 in different countries and shown in relation to the Human Development Index.

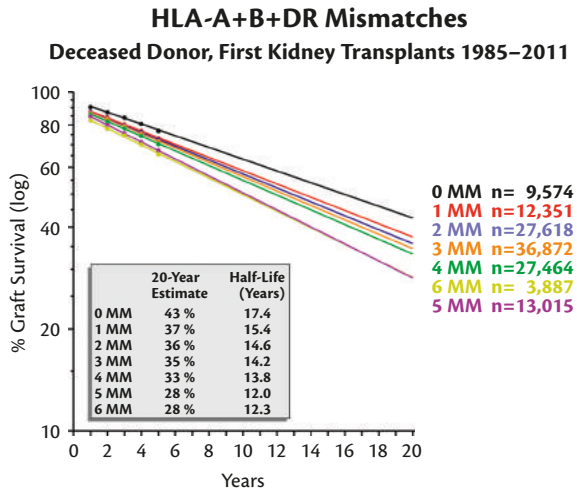


Fig. 275.8 Collaborative Transplant Study (CTA) analysis of the outcome of deceased donor kidney transplants based on degree of HLA mismatch.

failure but as lost to follow-up at the point of death, while combined 'graft and patient survival' treats death as graft failure. Both have validity but it is important not to compare the results of one type of analysis against the other. For example, if 10% of patients die with a functioning graft by a certain time point, then death censored graft survival will be 10% better than 'graft and patient survival'.

The other common formats of presentation of results include projections of outcomes based on short-term data to derive half-lives, or analyses that compare the outcomes of survivors at a certain point such as the 10-year results of 1-year survivors. Unlike the problem of actuarial graft survival, both of these types of analysis are usually presented clearly in graphical format so that there is no disguising the type of analysis. Examples of some of these analyses are shown in Figs 275.3–275.8 in which representative data are shown from a variety of sources around the world.

The current number of kidney transplants performed worldwide is between 75,000 and 80,000 with > 90% of these grafts functioning at 1 year after the transplant (Fig. 275.7). Transplantation has evolved from a single successful transplant between identical twins 60 years ago, through a rare experimental procedure 50 years ago, to being the optimal therapy for end-stage kidney disease today.

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CHAPTER 276

Pre-transplant assessment of the recipient

Christophe Legendre

Indications for kidney transplantation

Kidney transplantation must be considered for any patient with a stage 5 chronic kidney disease, either already on dialysis or just before (pre-emptive transplantation), provided they wish to be considered, the risks do not exceed the expected benefits, and there is no absolute contraindication. Ideally, this information should be delivered during the decline through stage 4 of chronic kidney disease as the glomerular filtration rate reaches 20 mL/min depending upon the rate of decline (Abboud and Henrich, 2010).

Age alone is no longer a contraindication and some patients > 80 years old have been transplanted successfully. The only absolute contraindication is the presence of a cancer with metastases. Some contraindications are only temporary such as infectious diseases while they are unresolved, or a past history of cancer. HIV infection is no longer a contraindication to kidney transplantation provided there is no HIV-1 viral replication while being treated with a stable antiretroviral regimen, the CD4+ cells count is > 200/mm^3, and there is no past history of severe opportunistic infection (Stock et al., 2010).

However, there are some relative contraindications which together amount to an absolute or relative contraindication, for example, severe congestive heart failure, unstable coronary heart disease, or unstable psychiatric disorder. In such cases the decision must be a shared multidisciplinary one.

Finally, severe concerns about future adherence to the treatment may be a reason to delay listing.

Preparation of the potential and future recipient

Education

The first step is to deliver balanced information to the future recipient. This will be delivered by the transplant physician (nephrologist, surgeon, or both) and the transplant nurse coordinator in a face-to-face meeting and supplemented with a written document. The patient needs information about:

- the various categories of donors (Rao and Ojo, 2009): living donor (related (human leucocyte antigen (HLA)-identical, haplo-identical, or mismatched)), deceased donor (standard criteria donor (SCD) or expanded criteria donor (ECD): donor age > 60 years or donor aged 50–59 years but with any two of the following criteria: cause of death is a cerebrovascular accident, past history of hypertension, serum creatinine > 130 μmol/L), heart-beating donor (DBD: donor after brain death) or non-heart-beating donor (DCD: donor after cardiac death be it controlled or not), and finally dual transplantation (Snanoudj et al., 2009) (Fig. 276.1)
- the consequences of choosing a given donor category with regard to anticipated life expectancy of the transplanted kidney, short- and long-term risks for the living donor, expected duration of hospital stay, immunosuppressive regimen, and surgical strategy
- the listing process and the expected waiting time according mainly to ABO blood group (which varies a little by country but usually with a decreasing length: B > 0 > A > AB), and the degree of anti-HLA sensitization (as well as the various options to desensitise)
- the allocation process for SCD and ECD kidneys (Pascual et al., 2008) which are different from one country to another
- the call at time of transplantation with a focus on minimizing cold ischaemia time
- the surgical procedure (see Chapter 278)
- the initial hospitalization with a focus on the various catheters, drainage systems, and the management of pain
- organization of the follow-up (shared or not with the local nephrologist and how)
- the extreme importance of adherence to the immunosuppressive treatment (Denhaerynck et al., 2007; Fine et al., 2009)
- the risks of under-immunosuppression (rejection) and over-immunosuppression (increased risk of infection (Fishman, 2007) and cancer especially of the skin) as well as the main side effects of the various immunosuppressive drugs
- the expected benefits and risks when comparing with dialysis (either haemodialysis or peritoneal dialysis)
- the risk of disease recurrence in case of focal segmental glomerulosclerosis (FSGS), atypical haemolytic uraemic syndrome (aHUS), membranoproliferative glomerulonephritis
- the risks of obesity and persisting tobacco use increasing morbidity
- the possibility of falling pregnant (Armenti, 2011; Deshpande et al., 2011)

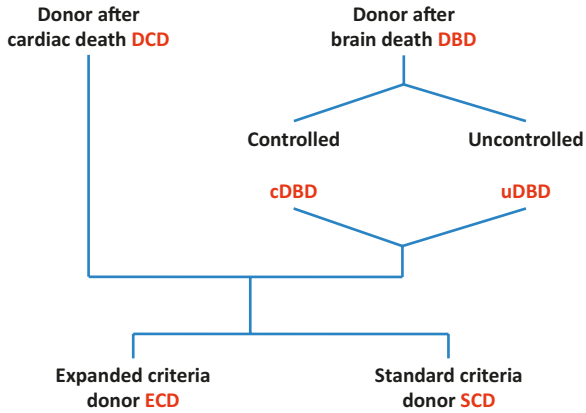

Fig. 276.1 Various categories of living and deceased donors.

- the concept and need for clinical trials and research and if relevant, the concept of randomization.

Medical history and examination

A thorough medical history and examination is essential with recording of past illnesses, including:

- cause of chronic kidney disease and duration, treatments received, dialysis mode (haemo- or peritoneal dialysis), time spent on dialysis, residual urine volume, and size of the kidneys in case of adult polycystic kidney disease
- previous transplantation(s): number, side, vascular and urinary anastomosis, duration, and main cause of graft loss
- gastrointestinal tract: gastric or duodenal ulcers, bleeding, biliary stones, acute pancreatitis, diverticulosis, and colonic polyps
- cardiovascular: coronary artery disease, myocardial infarction, cardiac arrhythmias, thrombophlebitis, pulmonary embolism, cerebrovascular accident, and lower limb arterial disease
- allergy to various drugs or latex with the clinical symptoms encountered
- psychiatric: psychosis, bipolar disorder, anxiety and phobias
- addictions to tobacco (Hurst et al., 2011), drugs, alcohol, and the current state of weaning
- cancer: with details on the localization, the precise category and the various treatments received, as well as the overall prognosis
- infections: tuberculosis, urinary tract infections, pneumonia, septicaemia, herpes zoster, dental sepsis, surgical procedures with a focus on urological (urinary tract, nephrectomy, implantation of one or more previous grafts), gynaecological, abdominal ones as well as subtotal parathyroidectomy
- sensitizing events: blood transfusion, pregnancy, miscarriage and abortion, and previous transplantation
- a thorough physical examination including height, weight and body mass index.

Investigations

A number of tests have to be performed (Table 276.1):

- *ABO blood group* with complete phenotype and agglutinins.

Table 276.1 A non-exhaustive list of tests to be performed prior to listing for a kidney transplantation

Immunological workup	ABO blood group (complete phenotype and agglutinins)
	HLA typing: A-B-DR-DQ (C and DP optional)
	Anti-HLA antibodies: microlymphocytotoxicity, ELISA, Luminex® (screening or single antigen)
	Crossmatch (if living donor): microlymphocytotoxicity, flow cytometry
Infection risk workup	Viral serologies: HIV1, HTLV1 and 2, CMV, EBV, HCV, HBV, HHV8
	Toxoplasmosis, syphilis
Cardiovascular health workup	ECG, echocardiography
	Myocardial scintigraphy, stress echocardiography, MRI, CT scan
	Coronary angiography
	Pelvic CT scan without injection
	Carotid Doppler ultrasound, aorto-femoral Doppler ultrasound
Cancer workup	PSA in male > 50 years
	Gastroscopy and colonoscopy (same as in general population)
	Mammography (same as in general population)
Urological workup	Cystourethrography, cystoscopy, urodynamic analysis, bladder ultrasound only if indicated
Miscellaneous	Ca, Phos, parathyroid hormone, vitamin D
	Coagulation tests (PT, aPTT, fibrinogen)
	Thrombophilic factors (factor V Leiden, anticardiolipin antibodies, anti-ß2gp1, antiphospholipid antibodies, etc.)
	Complement workup (CH50, C3, C4 and genotype)

- *HLA testing*:
  - HLA typing: A,B, DR, DQ in most cases, DP and C if possible especially in case of a living donor together with anti-MICA antibodies.
  - Anti-HLA antibodies using a screening test then a single antigen flow-bead assay (Luminex®) (Gebel et al., 2009).
  - Cross-match in case of a living donor: CDC-AHG test and flow cytometry crossmatch.
- *Infectious disease screen*:
  - Viral status about: HIV, HTLV-1, cytomegalovirus (CMV), Epstein–Barr virus (EBV), HHV-8, hepatitis B (HBs Ag, anti-Hbs abs, anti-Hbc abs, HBV-DNA), hepatitis C (anti-HCV antibodies, HCV-PCR, HCV genotype), E (if endemic area).
  - Screening for toxoplasmosis, syphilis, tuberculosis (Currie et al., 2010), bilharzia (if patient is coming from endemic area).
  - Vaccinations (Avery and Michaels, 2008; Kotton, 2011): listing of local and regional requirements is probably the ideal situation to check the various vaccinations performed and to update them.

- Oral and dental status (but teeth need not be removed systematically).

- *Coagulation testing*:
  - Routine coagulation tests (prothrombin time (PTE), activated partial thromboplastin time (aPPT), fibrogen).
  - Screening for thrombophilia routinely or in patients with a past history of thrombosis (Friedman et al., 2001; Ghisdal, 2010): Leiden factor V, antiphospholipid antibodies (Canaud et al., 2010), anti-ß2gp1 antibodies, anticardiolipin antibodies, homocysteine, etc.).
- *Cardiovascular assessment*: for all patients: electrocardiogram (ECG), chest X-ray and echocardiography.
- *Coronary artery assessment*: there is no specific guideline for preoperative cardiovascular evaluation for renal transplant candidates (Lentine et al., 2010; Kahn et al., 2011; Kittleson, 2011)). The potential benefits of screening are a prolonged survival and a reduced risk of postoperative death while the drawbacks are cost, delayed listing, risk of the revascularization procedure, and risk of precipitating dialysis. There is no prospective trial testing the usefulness of screening versus non screening. Thus in practice, in subjects > 45–50 years of age, coronary angiography will be needed where there are cardiovascular risk factors other than dialysis, and whenever there is ischaemia on a non-invasive stress test (stress echocardiography, myocardial scintigraphy, magnetic resonance imaging (MRI), computed tomography (CT) scan).
- *Once coronary artery disease has been diagnosed*:
  - In candidates at high risk (patients with unstable cardiac symptoms and patients for whom coronary intervention offers a long-term survival benefit), intervention improves the survival of the patient irrespective of the decision to wait-list.
  - In asymptomatic candidates with an intermediate risk, there is a choice between coronary revascularization (coronary artery bypass graft or percutaneous coronary intervention) and medical treatment. Which option is better remains unknown because no prospective randomized trial has been performed. There is, however, a relatively recently published prospective study (Kumar et al., 2011) in which coronary angiography was performed in all patients over the age of 50 years, patients with diabetes mellitus, patients with any cardiac symptom or disease, and patients with an ECG showing changes suggestive of ischaemia or previous myocardial infarction. Cardiac event-free survival for revascularized patients was similar and good at 1 and 3 years whether or not they had been transplanted. Those who refused the procedure had a worse outcome. Coronary calcium score using CT scanning is being assessed for its role in pre-transplant evaluation.
- *Vascular assessment:*
  - Pelvic CT scan without contrast medium injection in order to assess the number and localization of vascular calcification of the aorta and iliac arteries.
  - Doppler ultrasound of the aorto-femoral circulation and carotids in order to diagnose an asymptomatic stenosis (Ploussard et al., 2010).
  - In case of past history of venous thrombosis, venous iliac Doppler ultrasound or MRI may be useful.
- *Urological workup*:
  - Prostate-specific antigen level in males over the age of 50 years.
  - Bladder ultrasound, cysto-urethrography, urodynamic analysis, cystoscopy are performed only in patients with an anatomical or functional abnormality.
- *Cancer screening*: a high number of cancers are more frequent in dialysis patients, especially those of the kidney and urinary tract (Lemy et al., 2008).
- *Miscellaneous*:
  - If relevant to a patient with possible disease recurrence: complement levels, ANCA antibodies, antiglomerular basement membrane antibodies, anti-phospholipase-A2-receptor (PLA2R) antibodies.
  - In order to predict the risk of post-transplant diabetes mellitus, several tests may be performed such as fasting glucose, glycosylated haemoglobin, glucose tolerance test, etc.
  - It may be useful to genotype CYP3A5 in order to optimize the initial dose of tacrolimus (Thervet et al., 2010).
- *Anaesthetic evaluation:* all patients require referral to the anaesthetist in order to evaluate the risk of anaesthesia and to anticipate any specific difficulty in intubation.

Pre-transplant procedures

All of the patient's information must be collected to define the strategy and the prognosis before wait-listing and transplantation, and must be available at the time of the call for transplantation.

In young patients (<45–50 years) without increased cardiovascular or anaesthetic risk, without risk of disease recurrence, and without anti-HLA sensitization, the listing process is straightforward and nothing is needed before transplantation except for regular updating of the medical chart.

If information about transplantation has been delivered early enough during stage 4 of chronic kidney disease, it is possible to plan pre-emptive transplantation before dialysis is needed. Pre-emptive transplantation is usually possible when a living donor is available, especially in children. This avoids potential morbidity, unnecessary vascular access, cost of dialysis, and provides longer allograft survival.

Sometimes there is a need for a surgical procedure before listing:

- Unilateral nephrectomy may be needed to provide the physical space for the transplanted kidney in case of large polycystic kidneys (Jacquet et al., 2011) which may be undertaken laparoscopically or by embolization (Cornelis et al., 2010) (Fig. 276.2).
- Rarely bilateral nephrectomy is needed because of recurrent and severe UTIs or urosepsis in the presence of stones.
- Bilateral nephro-ureterectomy in case of aristolochic acid nephropathy (Lamy et al., 2008) to obviate future screening for urothelial cancer.
- If the patient has a non-functional bladder and post-transplant self-catheterization is not feasible, a urinary diversion must be created before transplantation. A small bladder as a result of long-standing anuria will usually regain a normal size quickly after transplantation and does not need surgical augmentation or diversion procedure.

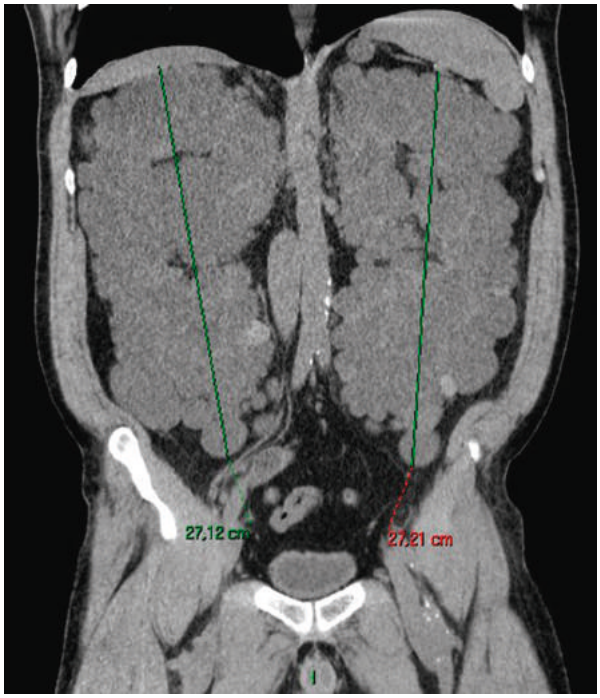

Fig. 276.2 On this tomodensitometric image, the size and volume of both polycystic kidneys are largely increased (> 22 cm) leading to an indication of either unilateral nephrectomy or embolization at time of listing or at time of transplantation.

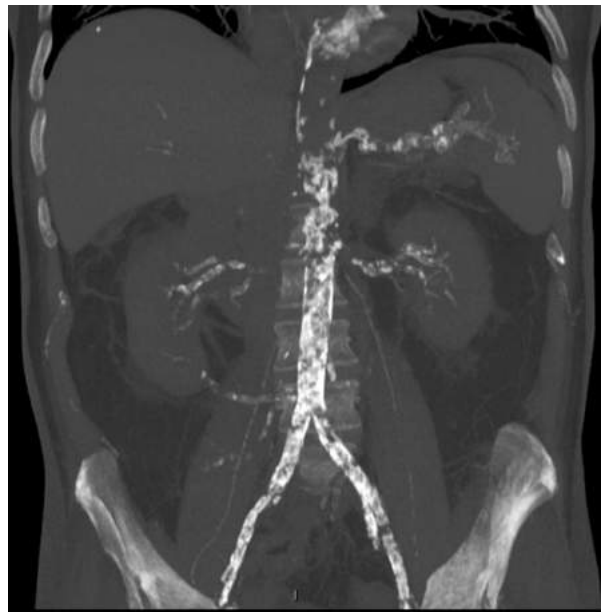

Fig. 276.3 This tomodensitometric image performed prior to listing reveals diffuse vascular calcifications on the aorta and the iliac vessels leading to almost impossible implantation of a kidney transplant. In this case, surgical replacement of native vessels prior to listing remains the best option.

- It is often preferable to wait for the post-transplant time period to treat pre-existing prostatic hypertrophy.
- Very occasionally, in an otherwise suitable patient, calcification of the aorta and both iliac arteries is so diffuse (Fig. 276.3) that an aorto-bifemoral bypass must be performed on whichever side the transplant artery will be anastomosed.

The sensitized recipient is more complex. In the Necker Hospital, Paris, 46% of currently listed patients are defined as sensitized. There are several options to transplant these sensitized patients (Vo et al., 2008; Lefaucheur et al., 2011; Montgomery et al., 2011).

HLA matching

Waiting for a well-matched kidney will mean a longer waiting time for a suitable kidney. It is also well recognized that the waiting time on the list is an adverse prognostic factor for transplantation outcome and survival (Meier-Kriesche and Kaplan, 2002).

Acceptable mismatch: this strategy consists of defining, very carefully, the specificities of anti-HLA antibodies to avoid any donor with unacceptable specificities (virtual crossmatch) and to define which specificities are acceptable. This policy has been used and evaluated in Eurotransplant with excellent results allowing an increase in the number of transplants as well as improving long-term results which are no different from those of non-sensitized patients (Frei et al., 2008; Claas et al., 2009).

Desensitization: it is possible to consider decreasing the level of antibodies in order to perform the transplantation without hyperacute or accelerated rejection (Marfo et al., 2011). Desensitization may be performed before transplantation, usually when a living donor is available, using a combination of intravenous immunoglobulin (IVIG), anti-CD20 monoclonal antibody, and plasma-exchange or immunoadsorption. Transplantation will then be performed if the cross-match becomes negative or if the level of antibodies is deemed to be at a safe level. It is also possible to perform post-transplant desensitization with a combination of the approaches listed above, as long as the cross-match with the current serum is negative. In these cases, information regarding an increased risk of antibody-mediated rejection must be given to the patient and the donor. Very recently, the use of eculizumab, an anti-C5 monoclonal antibody, has been demonstrated to decrease the risk of rejection significantly (Stegall et al., 2011).

Paired kidney donation

The paired kidney donation strategy involves finding a pair of donors and recipients, each of which is unacceptably mismatched for blood groups or HLA antibodies, but are mutually compatible when each donor provides a kidney to the other recipient (Gentry et al., 2011).

Cancer

In the past, guidelines recommended a 2- to 5-year waiting time for candidates successfully treated for their cancer to be transplanted (Wong and Chapman, 2008) (see also Chapter 287). These recommendations were based on retrospective data with an overall cancer recurrence of 21% (54% within 2 years post-transplantation, 33% between 2 to 5 years, and 13% after 5 years). The highest risks were observed among symptomatic renal cell cancers, sarcomas, melanomas, bladder cancers, and multiple myeloma. A 5-year wait was therefore recommended in these cases and at least 2 years in other categories of cancer. However, more recent, interesting and concordant data from both Australia-New Zealand and US registries (Kauffman et al 2005) found a much lower incidence of cancer recurrence of around 5% and 2.1% respectively. It is therefore very difficult to define recommendations by simple cancer category. For example, in patients with renal cell carcinomas, the risk is

considered to be nil in incidental tumours. The best recommendation to be given is therefore to undergo thorough pre-transplant evaluation for any signs of recurrence of their malignancy in patients with a previous history of cancer before listing and until transplanted. The specific advice of their oncologist with respect to recurrence risk is of utmost importance. On the other hand, it has been shown that patients with a past history of cancer are at higher risk of developing a *de novo* cancer post-transplantation suggesting that these patients must be screened very closely after transplantation.

Evidence shows that patients with a past history of post-transplant lymphoproliferative disease can be listed and that the waiting time for listing is short as long as EBV replication is negative and that the workup is negative (Karras et al 2004). Finally, patients with skin cancers which had developed during a previous transplant have a decreased risk after dialysis, but it will increase after a further transplant. There may be a role for mammalian target of rapamycin (mTOR) inhibitors in the prevention of cancer occurrence and recurrence in such high-risk recipients.

In countries or areas with a high prevalence of Kaposi sarcoma (Francès et al., 2009), the issue of re-transplantation may need to be considered. There are currently insufficient data to predict the risk of recurrence which, in our own unpublished experience, is as high a 50%. An mTOR inhibitor is indicated in this situation.

Graft loss due to disease recurrence

Graft loss due to disease recurrence is estimated to be around 15%, mostly due to the recurrence of primary glomerulonephritis (FSGS, membranous nephropathy, and membranoproliferative GN) (Chailimpamontree et al., 2009; Lorenz et al., 2010; Ponticelli and Glassock, 2010; Ponticelli et al., 2011), aHUS, and primary hyperoxaluria. (See Chapter 289.)

In the patients with primary FSGS, there is so far no reliable test to predict the recurrence in a specific patient although this is a matter of intense research. This risk is estimated to be around 40% and recurrence usually occurs early after transplantation. The most predictable factors are aggressive primary disease and recurrence in a previous graft. In spite of this high recurrence risk, there are now efficient treatments such as the one proposed by our group (Canaud et al., 2010) (Fig. 276.4).

In case of the other glomerulonephritides, there is not yet a specific marker of recurrence although the presence of anti-PLA2R antibodies in peripheral blood at time of transplantation might to be a marker of an increased risk of membranous nephropathy (Debiec et al., 2011).

The rate of recurrence of aHUS is very high and leads to graft loss in > 50% of cases. Approximately 60–70% of patients with aHUS have mutations in regulatory factors of the complement system (CFH, CFI, C3, CFB, and thrombomodulin) or circulating anti-factor H antibodies. The risk is low (15%) for mutations in membrane cofactor protein (MCP) and high (80%) for mutations in circulating proteins especially factor H. It is therefore important to explore the complement system in order to detect the alternate pathway activation, the presence or absence of factor H and I, the various mutations and polymorphisms, and the presence of anti-factor H antibodies (Zuber et al., 2011). However, the prognosis of recurrence has been dramatically improved mostly due to the use of eculizumab, an anti-C5 monoclonal antibody. It is still advised to avoid a living donor in such cases except if a complement mutation has been determined in the recipient and is absent in the donor. There remains, however, a small risk of HUS in the donor at time of the surgical procedure.

In type 1 or 2 primary hyperoxaluria, when it has been diagnosed before transplantation, there is a consensus that combined

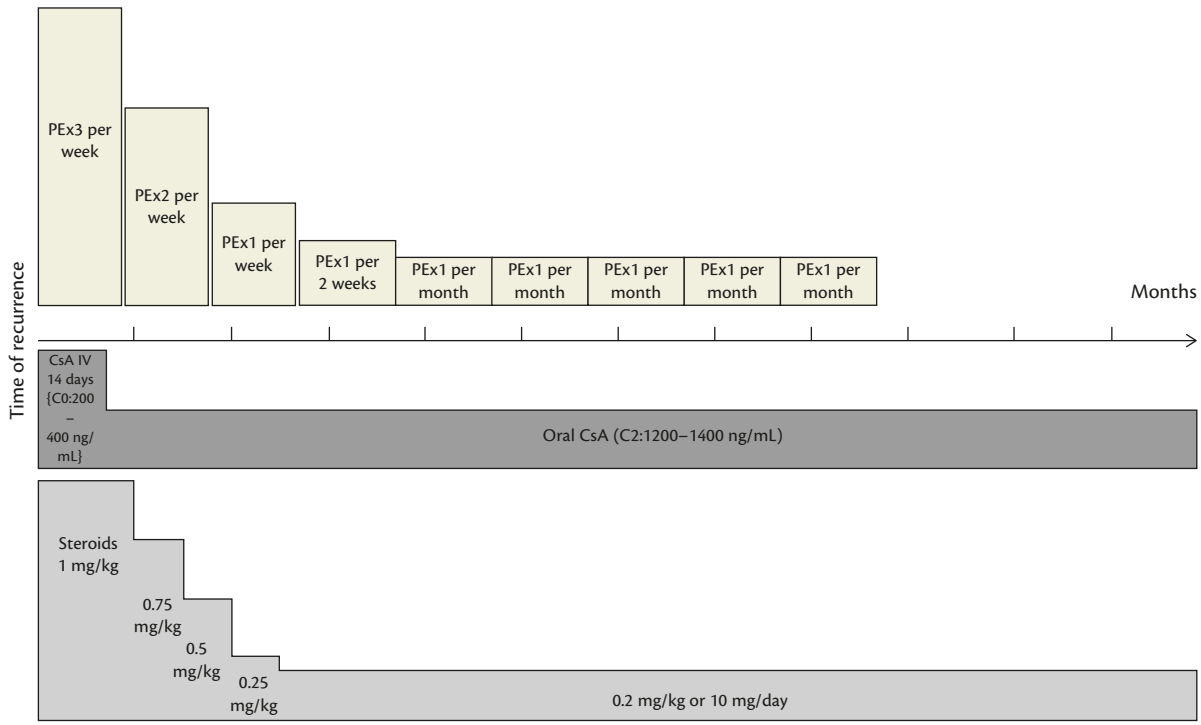

Fig. 276.4 A protocol for treatment of recurrence of FSGS after transplantation.

liver–kidney transplantation is indicated since it cures the deficit in the liver enzymes responsible for the accumulation of oxalate (Bergstralh et al., 2010). There is still a need in the post-transplant period to perform daily dialysis in order to eliminate the burden of oxalate and to avoid its deposition on the grafted kidney. Unfortunately, sometimes the diagnosis has not been made pre transplant and immediate recurrence leads to renal failure very quickly.

The recipient who has had previous transplant(s)

In these patients, there are three specific issues to be discussed before listing: anti-HLA sensitization, since > 95% of these patients are sensitized; strategy with regard to transplant nephrectomy (Loupy et al., 2007); and risk of BK virus (BKV) nephropathy recurrence. Transplant nephrectomy may be necessary because of space constraints if multiple previous transplants remain in place. There remains little consensus on whether a failed graft should be removed routinely or only if it is causing local or systemic symptoms and the useful literature is sparse. Patients who have lost a previous graft due to BKV nephropathy, can receive a repeat transplant with at least as good medium-term results (Dharnidharka et al., 2010). Two issues are still not solved: first, is it necessary to remove the native kidneys, the ureters and the previous transplant; and second, is it mandatory to wait for a negative BKV PCR blood and urine test? This may be a wise recommendation but success is possible despite continued BKV replication.

The patient with diabetes mellitus

In patients with type 1 diabetes mellitus, the main question is to decide which option is better: simultaneous kidney–pancreas transplantation (SPK) (Richter et al., 2011), deceased donor kidney transplantation, living donor kidney transplantation with pancreas after kidney transplantation (PAK), pancreas transplant alone, or islets alone. Most centres regard the best option as SPK in patients aged < 50 years without anti-HLA immunization. There is still controversy whether SPK is better than a living donor kidney transplant.

The patient with viral hepatitis

In an HCV-infected potential candidate, since there is no effective antiviral treatment which can be used during the course of transplantation, every effort must be made to clear the virus while on dialysis because viral infection will not recur following transplantation. After 6 months with a negative marker for viral replication, the patient can be regarded as cured. However, data from the literature are quite clear about the increased risk of death due to liver failure or infection in cases of prolonged viral replication after transplantation (Vallet-Pichard et al., 2011). It is also important to determine if there is cirrhosis or not, as this is an indicator for combined liver–kidney transplantation. In HCV-infected patients, it is also possible to use HCV+ donors with safe long-term results (Morales et al., 2010) even though it would be more logical to use these kidneys mainly in patients with active viral replication.

In an *HBV-infected potential candidate*, the presence of cirrhosis is an indication of combined liver–kidney transplantation although the availability of several efficient antiviral drugs may change this advice because there is some evidence that the liver may heal after a period of prolonged negative viral replication (Vallet-Pichard et al., 2011). There are data supporting the safe use of HbcAb+ donors, provided the future recipient is aware of the risk of conversion, has been vaccinated with an adequate response and surveillance serology are performed (Ouseph et al., 2010).

Combined organ transplantation

For pancreas and kidney, see above.

Combined liver and kidney transplantation is indicated (Papafragkakis et al., 2010) in cases of HCV-cirrhosis, HBV-cirrhosis, liver involvement in autosomal polycystic dominant disease, primary hyperoxaluria, and aHUS due to factor H deficiency. Mortality in these instances is mainly that observed in liver transplantation but the overall kidney prognosis is considered to be better in cases of combined liver–kidney especially in HLA-sensitized recipients. The main difficulty is to decide the best timing since transplantation must be performed earlier than in an isolated liver transplantation in order to avoid ascites infection and malnutrition.

Heart and kidney

In patients waiting for heart transplantation with apparent chronic kidney disease, it is important to determine the degree of reversibility of renal failure in order to carefully select the best candidates (Labban et al., 2009).

Kidney transplantation after a non-renal organ transplantation

The risk of multifactorial (diabetes, ischaemia, calcineurin inhibitor nephrotoxicity) chronic kidney disease is estimated to be around 5–10% at 10 years in heart transplant recipients and a little less in liver transplant recipients. Such patients may therefore need to be listed for a kidney transplant. The listing process is not different, but the risk of infection must be carefully assessed since the risk of over-immunosuppression is higher.

It is of utmost importance to stress that in all difficult cases, the decision must be shared with all physicians who will be involved in the care of the patient, always bearing in mind that transplantation is almost always preferable to dialysis.

Prognosis

At the time the patient is listed, it is important to define, as accurately as possible, the overall prognosis of the proposed transplant according to the various risks (sensitization, cardiovascular, post-transplant diabetes, disease recurrence) and both published and transplant centre-specific results. Continual surveillance while active on the waiting list is important and consideration is needed as to which tests have to be repeated and at what intervals, to avoid specific difficulties at time of transplantation such as the management of oral anticoagulation, a surgical procedure anticipated to be difficult, or an additional planned surgical procedure to be performed at the time of transplantation must be borne in mind.

The individual prognosis is of course difficult to predict because it depends on the analysis and comparison of results of kidney transplantation reported by single centres (homogeneous, small-sized populations); registries with data originating from regional, national, or international allocation agencies (e.g. Eurotransplant); and, finally from registries gathering data from several countries all over the world often on a voluntary basis (e.g. Collaborative Transplant Study in Heidelberg. This study is heterogeneous but includes huge numbers of patients). Results are expressed as patient survival, graft survival censored or not for death, half-life of a transplant, and also various scoring systems (Foucher et al., 2010).

Overall, 1-year graft survival has improved steadily over the past 20 years (Lodhi and Meier-Kriesche, 2011), with percentage of graft loss decreasing from 20% to about 8%. This has been achieved in most countries all over the world. The results have also improved in high-risk patients, in diabetic patients, and in second transplant recipients. This is due to the decreased incidence of acute rejection, to the control of CMV disease and *Pneumocystis jirovecii* infection through prophylaxis.

Long-term results have improved to a lesser extent. The main explanations are the discrepancies between projected and observed half-lives as well as uncertainties about the main causes of chronic allograft dysfunction, especially the role of chronic antibody-mediated rejection which is now considered as the leading cause of graft loss (Sellares et al., 2011) and for which there is no effective treatment.

The main considerations are the following:

- Living donor kidney transplant recipients have better results than deceased donor recipients (especially in case of HLA identity). ECD kidney transplant recipients have inferior results compared to SCD kidneys.
- Pre- and/or post-transplant sensitization (HLA and non-HLA), delayed graft function, increasing age of recipients and donors, and diabetes mellitus are strong adverse prognostic factors.
- Ethnic background seems to have a limited influence on transplant results in Europe (Pallet et al., 2005), but is significant globally.
- Results of second and third transplants are almost equivalent to first transplants as long as patients are not sensitized (Loupy et al., 2007).
- Finally, the effect of specific immunosuppression regimens is probably minor (Opelz et al., 2009).

Admission for a transplant

When the patient receives the call to be transplanted, it is important to minimize the duration of cold ischaemia in order to reduce the incidence of delayed graft function (Kayler et al., 2011; Siedlecki et al., 2011). The patient may be asked to participate in a clinical trial for which informed consent is needed. Even though he may have been informed of this possibility earlier, giving an informed consent shortly before transplantation is an inevitable but obviously non-ideal situation. The patient needs to be physically examined, his current treatment noted, a dialysis performed if necessary, and the immunosuppressive therapy and the antibiotic prophylaxis begun.

A thorough pre-transplant assessment of transplant candidates is an essential precursor to the admission and a vital step in the overall process of kidney transplantation. It is the only way to define the pre- and post-transplant strategies as well as estimate the individual prognosis. It must never be forgotten that kidney transplantation is by definition a highly multifactorial process.

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CHAPTER 277

Organ donation

Thomas Mone

Current state of organ donation

A country's organ donation performance correlates roughly with its general development status and medical infrastructure. The United States, the countries of Western Europe, Australia, and Canada consistently report higher deceased donation rates than the developing world. Annual donation rates in these countries range from 8 to 32 donors per million population (DPM), with Spain and the United States reporting the highest rates (32 and 26 DPM, respectively) (Organ Procurement and Transplantation Network (OPTN), 2010; US Census, 2010; IRODAT: European Transplant Coordinators Organisation, 2011). Regions where general access to healthcare and funds to support transplant programmes are limited (e.g. Africa and the Indian subcontinent) report donation rates of 0–3 DPM. Between these two poles, are countries reporting donation rates ranging from 0 to 15 DPM where adequate to sophisticated medical systems have been established but traditional cultural, legal, or structural barriers to donation exist (e.g. Japan, Russia, and much of Latin America).

Although DPM is commonly used for comparative measurement, it is a relatively crude measure of donation practices across regions, because donation is influenced by multiple factors, including death rate, in the United States, for instance, state death rates average 8.2 per million but range from a high of 14 per million in West Virginia to a low of 5 per million in Utah (US Census CDC, 2010). In addition cause of death, age, population, and availability of intensive care unit (ICU) beds can vary dramatically (Sheehy et al., 2012). Therefore, DPM should be supplemented with additional measurements, such as transplants per million population (TPM), in which world leaders include the United States (92.8), Croatia (88.9), Norway (88.3), Portugal (83.5), and, Spain (75.3) (OPTN, 2010; Nanni et al., 2011a).

Ideally, donation performance should be measured by the conversion rate now used by the US OPTN, which assesses the percentage of declared brain-dead donors without contraindicating diagnoses (e.g. cancers, viral meningitis) who actually donate upon death. In the United States, where this statistic has been the standard measure of donation effectiveness since 2004, the conversion rate averages 76% (OPTN, 2011). In other countries, however, it is not routinely and consistently measured, so DPM is the only common measure. To make this statistic more reliably comparable, normalizing DPM to a mean death rate (normalized DPM) enables a more accurate assessment of donation performance by region (see Table 277.1).

Ultimately, the success of organ donation in a region is judged by the status of the transplant waiting lists. Clearly, even in the most successful regions (e.g. the United States and Austria), donation is not keeping up with the need for organs. This shortfall is attributable partly to an ageing and affluent society experiencing organ failure secondary to hypertension, diabetes, and partly to advances in medical technologies such as dialysis and left ventricular assist devices, which enable patients to wait years for an organ. In fact, even if 100% of potential deceased organ donors donated, waiting lists would not disappear, not only because of the current high demand but also because over the past 20 years, any increases in the availability of organs have routinely been matched or exceeded by increases in the number of potential recipients.

Consequently, organ donation programmes (ODPs) have ventured into the arena of living donation. Focusing almost exclusively on kidneys, ODPs now incorporate living donation into public education and promotion. In addition, they have begun to assist transplant programmes in recovering and transporting living donor organs between recovery and transplant facilities and, as seen in California (Carlson 2010), they are establishing living donor registries through which altruistic living donors can be connected with transplant programmes. Nonetheless, even in areas with high transplant rates, living donation accounts for < 20% of all transplants (OPTN, 2010; Nanni et al., 2011b).

With the increasing demand for transplants, the limited supply of possible donors, and the rising waiting lists, it has been difficult to find a simple intervention that will bring about the 'cure' (i.e. increased donation rates). In fact, no single intervention—presumed consent, payment for organs, use of prisoner organs, or donor cards—has been shown to shorten waiting lists (Mone, 2010). What does make a demonstrable, measurable difference in best-practice countries is a multifaceted, coordinated approach that engages social, political, religious, cultural, and medical communities to create well-managed donation organizations; to modify laws, regulations, and care practices; and to increase societal understanding, acceptance, and active support of donation.

Countries that have invested substantially in well-established nationwide programmes of this type (e.g. Spain, the United States, Austria, and France) have been among the world leaders in donation for > 20 years. Over the past 5–10 years, these and other countries have increased their efforts and achieved statistically significant results through programmes such as the US Organ Donation Collaborative and the Barcelona-based Transplant Procurement Management international donation training programme (Shafer, 2008; Manyalich et al., 2011). Such investments have proved to be economically viable and, in fact, are a relative bargain, compared with the cost of treating chronic organ failure (Mendeloff et al., 2004; Monaco and Morris, 2004). More recently, donation improvement grants from The Transplantation Society

Table 277.1 Organ donors, donation rate (normalized by death rate), and transplantation volumes in 2010 (among countries with verifiable routine reporting of donation and transplantation data)

Country	2010 deceased donors	Population (millions)	2010 donors/million pop. (DPM)	Death rate	Death rate normalized nDPM	Total trans-plants (Tx)	2010 Tx/million Pop. (TPM)	Deceased donors (DD) transplants	DD Tx/million Pop. (DDTxPM)	Living donors (LD) transplants
Spain	1502	47.1	**31.9**	8.8	**33.3**	3781	80.3	3521	74.8	260
United States	7943	309.6	25.7	8.4	**28.1**	28663	**92.6**	22102	71.4	6561
Portugal	323	10.7	**30.2**	10.8	**25.7**	893	83.5	842	**78.7**	51
France	1538	63	24.4	8.8	25.5	4747	75.3	4447	70.6	300
Croatia	135	4.4	**30.7**	11.9	23.7	391	**88.9**	369	**83.9**	22
Austria	196	8.2	23.9	10.1	21.7	762	**92.9**	701	**85.5**	61
Italy	1298	60.5	21.5	9.2	21.4	3146	52.0	2952	48.8	194
Norway	102	4.9	20.8	9.2	20.8	432	88.2	349	71.2	83
Australia	302	21.8	13.9	6.9	18.5	1279	58.7	727	33.3	552
Belgium	221	10.4	21.3	10.6	18.4	900	86.5	818	**78.7**	82
Argentina	583	40.5	14.4	7.4	17.9	1586	**39.2**	1317	32.5	269
Canada	495	34.1	14.5	8	16.7	2114	62.0	1565	45.9	549
Czech	206	10.5	19.6	10.9	16.5	573	54.6	556	53.0	17
UK	1015	62.2	16.3	9.3	16.1	3946	63.4	2896	46.6	1050
Finland	92	5.4	17.0	10.2	15.4	265	49.1	254	47.0	11
Brazil	1934	193.3	**10.0**	6.4	14.4	6422	**33.2**	4599	**23.8**	1823
Netherlands	227	16.6	13.7	8.9	14.1	1136	68.4	659	39.7	477
Germany	1296	81.6	15.9	10.9	13.4	5194	63.7	4439	54.4	755
Israel	60	7.6	**7.9**	5.5	**13.2**	235	**30.9**	150	**19.7**	85
Denmark	73	5.5	13.3	10.2	**12.0**	332	60.4	230	41.8	102
Sweden	118	9.4	**12.6**	10.2	**11.3**	641	68.2	465	49.5	176
Mean	908	48.0	18.4	9.2	18.4	3183	66.3			
California, United States[a]	785	37.3	21.0	6.2	**31.2**	3222	86.4	2454	65.8	768

Donor data:

European Transplant Coordinators Organisation (2011). *Organs, Tissues, & Cells*, 14(3).

UNCS/OPTN: California, United States: <http://optn.tranplant.hrsa.gov/latestData/viewDataReport.asp>

Death Rate Data: *CIA. World Fact Book* <https://www.cia.gov/library/publications/the-world-factbook/index.html>

2020 population data: <http://www.ration.online.org/onworld/>

[a]California induced to demonstrate impact of low death return on donor availability and DPM.

and the Transplant Donation Global Leadership Symposium have enabled sharing of best practices across countries by teaching the management and leadership skills needed to increase donation and end deaths on the waiting lists.

There are critical processes, organizational functions, and fundamental principles that have been shown to yield the highest sustained donation rates, with the aim of establishing well-tested foundational building blocks of donation in areas with fledgling programmes and to identifying donation-limiting practices and preconceptions in areas that already have relatively successful programmes.

Critical processes for maximizing transplantation

In the earliest days of transplantation, it was the responsibility of the treating doctor or transplant surgeon to ask potential donors' families to consent to organ donation. At that time, donation was seen as a discrete event, a component of end-of-life care for a select few patients suffering cardiac death in hospital, and a part of the bad news the family had to digest. Ideally, family members' innate altruism would be triggered by the physician's personal explanation of the need for and value of their gift.

With the establishment of neurologic criteria for death (brain death) and the increased frequency of donation opportunities, the intervals from the terminal prognosis to the declaration of death to the decision to discontinue mechanical ventilation increased. This change, coupled with ever-growing demands for physicians' time, necessitated the use of 'physician extenders', usually critical care nurses, as 'approachers' who would explain donation to families and seek their consent. Moreover, reliance on brain death declaration expanded donation from a single event to a systematic process that begins with the identification of potential donors and involves numerous independent but coordinated skill sets and steps.

Most ODPs now employ all of these steps; larger ODPs have dedicated specialists for each step, whereas smaller ODPs typically expect generalist nurses, technicians, or doctors to be competent (if not expert) at each one. In addition, as donation has grown, the sharing of organs beyond the recovering transplant centre's organ allocation systems has made the process substantially more complex (see Fig. 277.1).

In addition, several national donation programmes and coordinators groups have published donation process and donor management guidelines. Among the more concise and complete is the Australian Transplant Coordinators Association *National Guidelines for Organ and Tissue Donation* (Cunningham, 2008).

Identification and referral

Because of the stringent requirements of severe neurologic injury, mechanical ventilation, and declaration of brain death, the likelihood that any given dying individual will be a deceased organ donor is minute (about 0.5% of all annual deaths) (Sheehy et al., 2003). Because fewer than half of all deaths occur in hospitals and fewer still are ventilated patients in ICUs, the chance that an average hospital will have an organ donor is small, and this scarcity of potential donors makes it extremely difficult to train hospital staff in donor identification. Consequently, successful ODPs typically rely on referral criteria such as a Glasgow Coma Scale (GCS) value of 3–5 (Earle 2006), though successful ODPs opt for the much broader criterion of 'all ventilated patients who have suffered a neurologic injury'.

Upon identification of a patient that meets the referral criteria, the hospital notifies the ODP with the aim of clarifying the patient's condition. To help the hospital comply with the referral criteria, the ODP reviews the medical records of all ICU deaths of ventilated patients (excluding specific diagnoses such as active cancers) to identify any 'missed potential donors'; it then shares this information with the hospital as part of regular donation education. In the United States and Spain, this procedure is supported by national regulations requiring hospitals to refer potential donors in a timely manner that allows for donor assessment and family support and approach.

Assessment

When a potential referral is not ruled out by comorbid diagnoses, the ODP dispatches clinically trained 'procurement coordinators' to perform a more comprehensive review of the medical record and, equally important, to re-emphasize to the treating staff the potential for donation if brain death ensues. This initial assessment can help remind the treating physicians that even if the patient's terminal condition appears not to benefit from intervention, continuation of basic measures to maintain fluids and pressures is essential for preserving the opportunity of donation and integrating donation into end-of-life care (National Health Service, 2011b). These visits, which should occur at least daily for each referral, enable the ODP coordinator to assemble caregivers, develop a plan for supporting and informing the family, and, ultimately, facilitate the approach for donation authorization.

The training of the ODP's referral responders varies significantly. Whereas some programmes rely on the donor hospital's in-house physicians or nurses, many high-performing ODPs rely on their own coordinators, who travel to or may be based at the potential donor hospital.

Declaration of death

Brain death declaration is driven by the laws of the region. Because the donation process ultimately relies on that declaration, most ODPs make a point of training their coordinators in the fundamentals of clinical examination, apnoea testing, and, possibly, cerebral blood flow assessment and ensuring that they can distinguish spinal reflexes so that they can verify that the declaration was accurate.

Declaration of brain death routinely occurs in the ICU, before the family is approached for authorization. In the vast majority of cases, national laws require whole-brain death, including brainstem death. The practice of brain death declaration is routinely re-evaluated on the basis of advances in neurology and ethics; the most comprehensive and current review is *Controversies in the Determination of Death*, which 'reaffirm(s) and support(s) the well-established dictates of both law and practice in this area' (Pelligrino, 2008). Countries with cultural and legal practices that do not recognize brain death face a fundamental hurdle in deceased donation and have extremely low deceased donation rates, as evidenced by China (Siu et al., 2011), and Japan which historically recognized it only for purposes of organ donation (Aita, 2011).

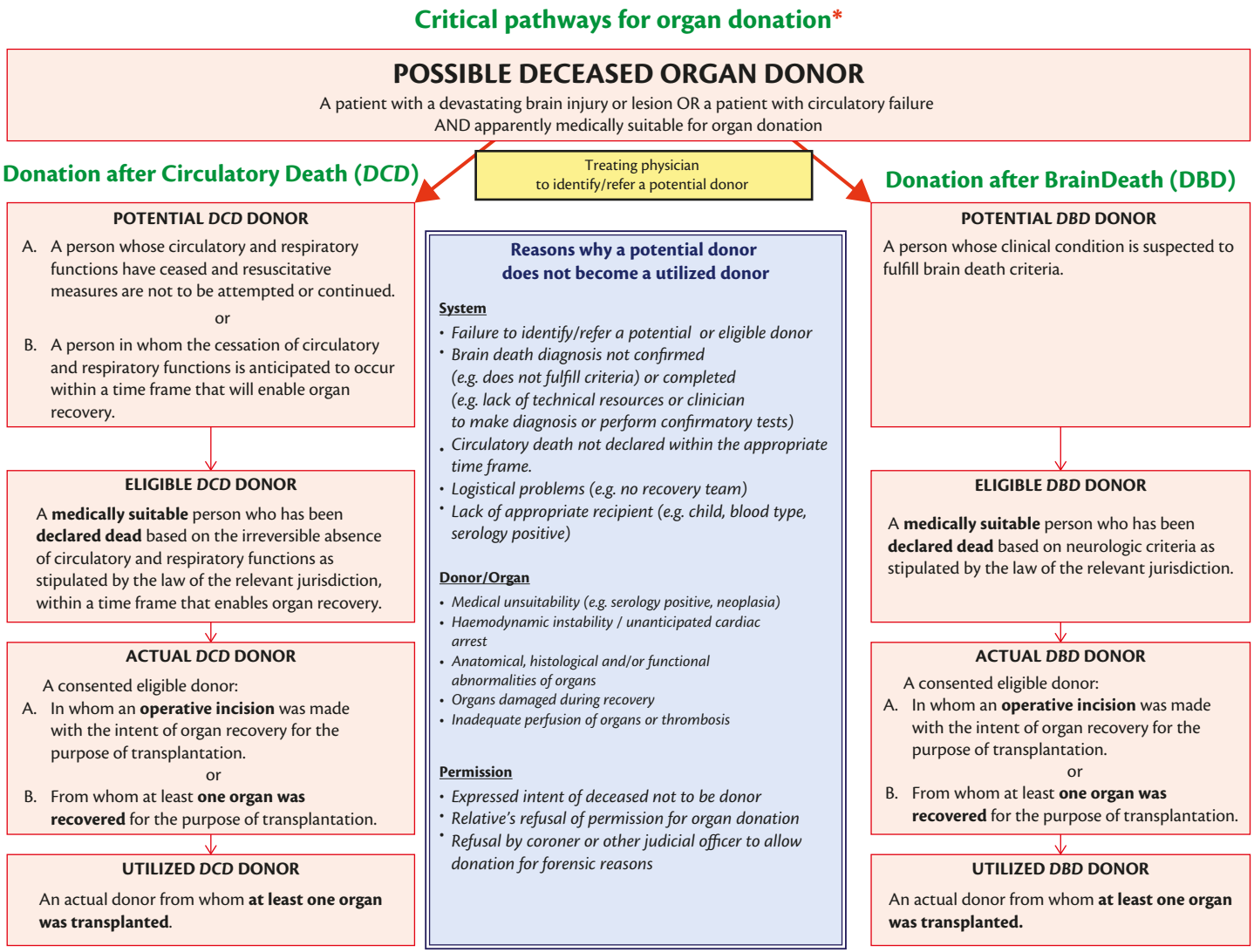

Fig. 277.1 The World Health Organization schema for the critical pathways for organ donation.

Finally, although <5% of deceased organ donors have their organs removed after declaration of circulatory death (DACDD), it is important to emphasize that this option is available. In most cases, the DACDD procedure is performed by the surgeon in an operating room (OR) so that organ recovery can occur as soon after cardiac arrest as possible (National Health Service, 2010). DACDD has the potential to increase organ donation significantly. In some ODPs, DACDD accounts for 20–30% of total donors (OPTN, 2011). However, reduced organ function and ongoing controversies over the timing of the determination of death have limited DACDD donation worldwide. The United States and United Kingdom primarily recover organs from Maastricht III 'controlled' donors, and Spain recovers from 'uncontrolled' donors; Germany largely prohibits DACDD donation, with the exception of Maastricht V Uncontrolled Cardiac Arrest In-Hospital (Stadtler 2007; Vogel et al., 2011), and each of these counties is in the midst of efforts to enable both controlled and uncontrolled DACDD.

Family support and approach

Few doctors, nurses, hospital staff, and (especially) families are psychologically prepared to manage the transition from treatment of a patient to organ donation. Donation family support and approach practice has been designed to address this transition through consciously applied strategies and practices.

From the earliest days, successful donation practice has included a period known as decoupling, which is designed to separate the communication of the patient's death from the approach to the family for potential donation (de Groote et al., 2011). Today, standard practice is for the care team, including donation coordinators, to 'huddle' and plan end-of-life guidance for the family. When informing the family members of the death, the treating physician refers them to 'a member of the team who will help them deal with end-of-life issues' (Ehrle 2006).

This approach enables the coordinator to support family members in their immediate grief, answer their questions (e.g. why the monitor still shows a heartbeat when the 'patient' is brain dead), and, ultimately, offer them the opportunity to fulfil the life that was cut short by authorizing the donation of organs. (This process has traditionally been referred to as obtaining 'consent' for donation, which implies a similarity to informed consent for a medical procedure. However, all principles of informed consent are invalid in the setting of organ donation, because a deceased donor's life cannot be further endangered. Consequently, both terminology and practice are shifting to 'authorization'.)

Obtaining authorization for donation may take only minutes, but it is more likely to take hours or even days if families are in denial of the death. In fact, the dedication of time is highly correlated with increased family authorization (Shafer, 2008). Family support and approach was traditionally the responsibility of treating doctors (MDs) or registered nurse (RN) donation coordinators; who have always been able to dedicate the time required. Therefore, successful ODPs routinely provide healthcare personnel who are dedicated to this specific task. Frequently these individuals may not have clinical licensure, but come with exceptional interpersonal skills and specific training in death and dying and grief counselling.

In the United States, authorization for donation has been assisted by legally binding organ donor registration programmes (so-called first-person authorization), recorded in registries associated with driver's licence applications; which made up 33% of the US annual 8000 deceased donors in 2010 (Donate Life America, 2011). In these cases, family members are informed of the donor's decision to donate rather than asked to authorize donation. However, efforts are made to provide the family grief support, help them understand the donation process, and they are encouraged to provide a medical and social history. When a donor's first-person authorization is identified, US ODPs report a near 100% rate of support for this authorization from the donor's family and very few cases in which families seek to prevent donation.

While donor registries have been a valuable tool in the United States, in Europe, they play little or no role in donation authorization; which has continued to rely on family approach and authorization; even in countries that have 'Presumed Consent' laws (Rithalia et al., 2009; Boyarsky et al., 2011; Rosenblum et al., 2012). The research shows that while an available recorded choice to 'opt-out' from Presumed Consent will prevent donation, the family's support is routinely required to proceed whenever an individual has not opted-out. Interestingly, little to no data exists on the frequency of opt-out decisions preventing donation, because few if any formal and easily accessible systems exist specifically to capture this decision by residents of Presumed Consent countries. This is likely to be a recognition that public and individual objections to donation are usually found to be based on myths and misinformation which, when corrected through family conversation at the time of death can be overcome and donation proceeds., Thus, while some transplant professionals and waiting patients promote Presumed Consent as the panacea for ending waiting lists, there is no evidence that it has ever accomplished this.

Donor management and organ assessment

Once authorization has been obtained, donor and organ assessment begin in earnest. This process takes various forms in different ODPs and countries. In the US model, the treating physician signs off, and an ODP coordinator (either an MD or an RN with organ procurement training) takes on the responsibility for medical management of the donor. In Spain, a hospital-based physician usually manages the donor and in Australia, United Kingdom, and Canada, both systems are used.

The coordinator's task begins with testing of physiology, organ function, and potential for infectious disease transmission. Evaluation may include creatinine, alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatine phosphokinase (CPK), computed tomography (CT), and testing for infectious diseases. Worldwide biovigilance efforts have dramatically heightened the attention paid to infectious disease transmission, as illustrated by the NOTIFY project of the World Health Organization and initiatives from the Council of Europe (Kirste, 2011), the Centers for Disease Control and Prevention (CDC), and the United Network for Organ Sharing (UNOS). As a result, numerous ODPs have implemented real-time nucleic acid testing in addition to serologic testing to reduce window periods of viral infections (Aswad et al., 2005) and have added testing to address regional vectors (Nowicki et al., 2006).

Concurrently with testing, coordinators must address any after-effects of the initial injuries and their treatments, such as acid–base imbalance, dehydration, blood pressure abnormalities, infection, and possible sepsis (Menza and Geraghty, 2006).

Management is further complicated by the necessity of addressing the physiologic and metabolic effects of brain death, the resulting hormonal surge that inhibits organ function (especially of the heart and lung), and the ensuing blood pressure drops that can injure the organs. Accordingly, the coordinator must be thoroughly familiar with the specific abnormalities of deceased donor physiology and metabolism and must have a specialist's knowledge of the ventilator settings, fluids, and medications designed to offset them, as well as of antimicrobial regimens to address chronic and acute bacterial and fungal infections that may be well outside the ranges of live patient treatment.

All coordinators require specialized training to develop the diagnostic and interventional skills required for addressing the unique and sometimes esoteric medical requirements of deceased donors. To this end, some ODPs have established integrated 'intensivist consultation' programmes that use hospital-based intensive care physicians with subspecialty training and experience in donor management to supplement coordinators (especially non-physician coordinators). Systematic approaches to medical management of donors, including paediatric donors, have been described in several sources (Wood et al., 2004; Powner and O'Conner, 2006; Nakagawa, 2008).

While laboratory testing and donor management efforts to improve metabolism and physiologic function are under way, the coordinator supplements the laboratory data by ordering diagnostic imaging (e.g. echocardiography, bronchoscopy, CT, magnetic resonance imaging (MRI), or ultrasonography) for further assessment of the function of specific organs, usually at the request of the transplant physicians. On occasion, this can be done in as little as 3–6 hours, but in multiorgan cases with donors who have post–brain death complications, 24–36 hours may be required in addition to the time already needed to obtain authorization for donation.

Organ allocation

In the process of determining which waitlisted patient should receive a donated organ, blood typing and crossmatching (ABO and human leucocyte antigen) is a relatively simple task; the greater challenge lies in determining which patient has the greatest need. Historically, the local surgeon made this decision on the basis of the patients in the local transplant centre. However, there have always been recovered organs for which there are no suitable recipients on the primary centre's waitlist; consequently, sharing between centres became essential.

As a fundamental principle, ODPs that maximize organ donation are guided by a national set of allocation rules for determining the neediest patient (as determined by clinical urgency and time on the waitlist) until a match is found. In the United States, UNOS generates the priority list for each organ from each donor, and organs are allocated to local patients with the highest need and then geographically further away until a match is found. In United Kingdom, a similar system exists, though the geographic factors are less challenging (National Health Service, 2011a). In northern Europe, EuroTransplant facilitates inter-country allocations, and most other countries manage their own allocation (European Transplant Coordinators Organisation, 2011), with informal cross-border sharing between ODPs and accepting transplant centres. In most areas with developing donation programmes, organs are shared between centres via ad hoc communications as they become available.

The historical priority of local centres and regions has given rise to substantial variations in patient need at transplant. For example, in the Model for End-Stage Liver Disease (MELD) system for liver allocation in the United States, some regions have an average MELD of 22 at transplant, whereas others have a MELD of 32 or higher; this implies the existence of a 45% need differential that the current system fails to recognize (Yeh et al., 2011). Additionally, efforts in some countries to achieve fairness by relying on waiting time for kidneys often lead to transplantation of younger donor organs into older recipients, with the result that graft survival is lowered and older donor kidneys are discarded as older recipients elect to wait longer for younger donor kidneys to become available.

Such challenges are difficult to address: any change in allocation will extend the wait time for some while reducing it for others and can shift transplantation volume from one centre or surgeon to another. However, the value of allocation system refinement is well demonstrated by the 'old for old' allocation system employed in Germany which resulted in transplantation of more kidneys from older donors. Other countries could increase transplants by adopting this system (Moers et al., 2009). Thus, allocation remains in a state of constant re-evaluation, modelling, political discussion, and manoeuvring, as with any item in short supply and in this case with life and death consequences.

Organ recovery, packaging, and transportation

Surgical recovery of organs is fairly straightforward, though complicated somewhat by the number of surgical teams needed for a multiorgan donor. Generally, the transplanting team sends its own surgeons to assess the organs for size and condition, though in the United States, it is routine to have dedicated kidney recovery surgeons in each ODP to minimize travel costs. The donor hospital routinely provides anaesthesia services to help maintain intraoperative pressures. Given the rarity of organ recovery in many hospitals, the role of the ODP coordinator—sometimes filled by the donor management coordinator and sometimes by a dedicated organ surgical recovery technician—is critical.

This coordinator's task is to ensure that appropriate instrumentation, flushing and preservation solutions, and packaging materials are used for each organ. Packaging procedures follow national regulations or transplant centre policy but routinely require double, triple, or quadruple containers, adequate slush to maintain a temperature of 4°C for up to 8–12 hours, and clear labelling to confirm donor identification and ABO. In addition, the donor chart is routinely included with each organ (Peterson et al., 2006).

Family aftercare

By the time the donor's organs have been recovered, packaged, shipped, and transplanted, the family members are usually home and dealing with their loss. Providing aftercare for these families greatly enhances their perception of the value of donation and reduces any ambivalence that might harm this perception and, ultimately, the public view of donation. ODP aftercare programmes range from follow-up letters of thanks to offers of ongoing access to or referral to grief recovery services, donor medal ceremonies,

and opportunities for continued volunteer involvement with the ODP. An especially valuable practice is to include donor families in donor hospital and public education efforts, where they can share their first-hand experience with donation and the benefits it provided them as a counterweight to the common concerns of inexperienced hospital staff that an approach for donation may be seen as an imposition (Post, 2011).

Essential functions of donation organization

The critical processes discussed above are now employed for every organ recovery, whether in a small hospital-based donation agency or in a large multisite organization. Worldwide, best practices are found in countries that have created national standards for donation law and practice, with local ODPs that are independent of transplant centres and assume full responsibility for donation, from promotion to education to recovery. In larger donation agencies, these elements, like the aforementioned critical processes, are functions of individual departments; in smaller organizations, they may be functions of a general management, clinical, or education department or may be outsourced to independent providers.

In some countries (e.g. the United States), the components are itemized in formal regulations and practice standards used by accreditation and licensing bodies (Association of Organ Procurement Organizations, 2012; Centers for Medicare & Medicaid Services, 2006) to ensure that they are present and active in all of the local programmes. In Spain, the national transplant agency authorizes and oversees the practice (Matesanz et al., 2011). The goal of these top-down approaches is to establish some standardization of practice and, ideally, encourage widespread adoption of best practices.

Hospital donation development

Because of the relative rarity of organ donation in even the busiest of donor hospitals (Sheehy et al., 2003), hospital staff and systems typically are not organized to integrate donation into routine practice and thereby maximize it. Accordingly, ODPs developed the function of hospital development to ensure that hospitals were cognisant of circumstances necessary for donation, systems for identifying and referring potential donors, and policies and practices for integrating donation into end-of-life care.

International practice in hospital development differs widely, but best practices established in Europe with Donor Action (Pugliese et al., 2003), enhanced and promulgated by TPM (Manyalich et al., 2011) and resulting in a 30% increase in the Collaboration for Organ Donation in the United States (Shafer, 2008), have yielded referral rates higher than 99% for brain-dead potential donors in countries following these practices. In best-practice models, hospital development staff members ensure that hospitals formally adopt policies to ensure early referral, timely brain death assessment, frequent end-of-life planning meetings with ODP and hospital staff, and full training of ICU and OR staff in donation medicine and surgery.

Without continuous hospital development, the exigent needs of new patients, the turnover of staff, and the rarity of donation opportunities lead to a decline in donation. Active, supportive, and inspirational hospital development creates environments where donation is an integral part of end-of-life care and is celebrated for the benefits it bestows on donor families and recipients.

Public education and promotion

Although the hospital is the site of donation, it should not be the place where a suddenly grieving family first learns about it. Unfortunately, this is routine in many areas. Negative reactions to donation are partly a function of people's general distaste for issues dealing with mortality and partly a function of the myths and mysticism besetting the topic of death. Therefore, to encourage donation, organ donation professionals must endeavour to overcome these fears and myths.

The need to overcome perceptual barriers to organ donation has given rise to social change programmes aimed at presenting donation as a social good that provides tremendous value to the recipient. Public surveys on the value of transplantation routinely find wide support for donation; those specifically addressing the personal decision to donate one's own organs find measurably lower, though still substantial, support (Donate Life America, 2010). More advanced public education programmes, whether they are supported by governments, ODPs, or individuals, directly confront misconceptions about donation (e.g. 'They will let me die to get my organs'), incorporate personal testimonials from donor families on the value of donation in grief recovery, demonstrate successful transplantation in healthy recipients living active lives, and public calls to action (Slim, 2012).

Supplementing these initiatives are efforts to position organ donation as an integral component of end-of-life care that provides comfort to families. ODPs help donor families to share their donation experiences with the public and healthcare professionals, with the goal of demonstrating the benefit to families who authorize donation. One of the best-known examples is that of Reg Green, whose sharing of the loss of a son in a shooting in Italy led to a threefold increase in organ donation in that country (Green, 1999). For the past 10 years, the public awareness event with the largest international impact has been the annual Donate Life float in the Pasadena Tournament of Roses Parade, which generates more than 1000 news stories internationally and is watched by an estimated 50 million viewers. By focusing on recipients' lives and organ donors' gifts in the celebratory atmosphere of a parade, it is possible to position organ donation as a positive part of the fabric of modern life (Stadtler, 2005).

In addition to these broad-based promotional efforts, ODPs in many regions must address the unique needs of diverse communities that often include recent immigrants form cultures with little exposure to donation. Finding cultural antecedents and role models is critical for sharing a culture of donation with these new audiences (Garcia et al., 2006).

Medical direction and quality oversight

Besides the need for advanced medical skills in donation (see above), there is also a need for multidisciplinary oversight and direction to ensure that problems are identified and opportunities for performance improvement are maximized. Although an intensivist can help correct metabolic problems and ensure donation potential, only transplant physicians can determine acceptable levels of organ function and infection risk for their patients, which can

vary dramatically, depending on patients' needs and the capacity of the transplant centre.

The necessary oversight is often provided via full- or part-time medical director positions, quality management committees, and organ-specific councils that bring together multiple transplant physicians for the oversight and establishment of protocols. Hospital-based ODPs are routinely integrated into medical staff oversight, whereas independent ODPs are routinely required to include physician oversight (Centers for Medicare & Medicaid Services, 2006; UNOS, 2011). Integration of quality management into organ donation has become a requirement of US programmes (Centers for Medicare & Medicaid Services, 2006) and a specialized training area in European programmes (Manyalich, 2010).

Organ donation programme performance monitoring

Like any service, organ donation requires that performance be monitored over time and against benchmarks and peers. In too many places, this measurement has relied on DPM, which is limited by highly variable death rates and access to ICU services between regions and countries. Alternatively, donation success may be measured via TPM or as a percentage of the waiting list, but such measurement is also influenced by varying death rates and listing practices. The most reliable measures consider donation as a percentage of potential ('eligible') deaths in hospital ICUs with brain death declaration or signs and symptoms of brain death. This measure, in international best practice, appears to yield a donation rate in the range of 75–90%, but it will require broader adoption to establish its reliability.

Donation professional education

Because organ donor management often involves practices that would not be acceptable or viable in a living patient, it is not a part of routine medical or nursing education. Fortunately, organizations such as the European Transplant Coordinators Organization, the North American Transplant Coordinators Organization (NATCO, 2009), Transplant Procurement Management (Barcelona, Spain), the University of Toledo (Ohio, United States), and OneLegacy (Los Angeles, California, United States) offer specialized materials and facilities for this unique area of medicine.

Donation science research

Research into alternative practices of donor identification, authorization, and medical management is sparse. In large part, this is because the field does not lend itself to double-blind trials: cases are rare, variables cannot be controlled for, and each case carries life-saving demands. Nonetheless, efforts to achieve statistically significant improvements in transmissible disease prevention, inflammatory reaction reduction, organ function enhancement, and graft survival extension are increasing.

An area that has become a model for donor research is pulsatile perfusion of kidneys (Moers et al., 2009). Efforts to build on this approach are being institutionalized with the creation of organizations like the Organ Donation Research Consortium in the United States in 2011 and the initiation of grant-funded research aimed at evaluating donor management protocols for the use of medications and treatments to combat the effects of brain death on organs.

Information technology

Too often, donor information continues to be communicated via a series of discrete telephone calls and faxes. In recent years, however, Web-based systems have been employed, and in some cases required (Tuttle-Newhall et al., 2009), to allow simultaneous transmission of full donor information to multiple transplant centres. This approach improves safety and speed and increases the number of offers that can be made, but the data must be properly formatted to keep from overwhelming transplant teams with heaps of data that bury critical information. Some early studies found automation to be associated with increased organ transplant rates (PalmSource, Inc., 2004), but subsequent research has yielded mixed results (Gerber et al., 2010), probably because of expansion of the donor pool to include extended criteria or marginal donors. Nonetheless, research generally supports the increased safety provided by the more substantive electronic offer versus the telephonic summary.

Management, financial systems, and governance

Regardless of whether organ donation is managed by non-profit organizations, hospitals, or governments, it must operate efficiently and be able to document the need for the resources it uses. That is, ODPs must be run like businesses, with internal controls, management, budgets, regular reporting, and staff supervision and oversight. This may be easier with a large independent ODP that relies on reimbursement for funding, because by its very nature it is already acting like a business. Smaller hospital-based or governmental ODPs can usually access the services they need, but at times they are such a small component of a larger organization that they must clarify their unique needs to their leadership to ensure adequate budgeting and investment (Mone, 2002).

Foundational principles of best practices in organ donation

All ODPs include most or all of the critical processes previously discussed, and most incorporate the essential functions. Nevertheless, donation performance still varies dramatically, even in countries with well-established transplantation programmes. It is therefore essential to try to identify the factors distinguishing the better-performing countries and regions from those with lower donation rates.

Certainly, these factors include widely shared values of altruism and community, which may help explain why the European countries with the highest donation rates have populations that are 80–95% Catholic (Mone, 2010). Even against a background of shared values, specific regional and national action is required to create a donation system in which the critical processes and essential functions can be promoted, acted on, and enhanced. In best-practice countries, the following foundational social, legal, and organizational elements have been identified, without which recovery organizations struggle and donation and transplant founder.

Ethical underpinnings

While deceased organ donation has occupied the minds of ethicists from the beginning, the focus has narrowed over the years as practice has become codified, transparent, and audited. Early discussion

focused intently on the determination and verification of death and most importantly that deaths were never hastened in order to facilitate donation (Veatch, 2000). While brain death has been adopted as a clinical and legal determinant of death in nearly all countries practising donation, a minority in the ethics and religious communities question its validity, but most often based on evidence that speaks to mis-diagnosis of incomplete brain stem death. To ensure that this concern does not undermine organ donation, the donation community has adopted a practice of verifying brain death declarations (as discussed earlier in this chapter). An area where this concern remains a subject of ethical discussion and concern is Asia. After some 30 years of debate the Japanese Diet (parliament) in 2010 finally implemented a brain death and donation law that begins to mirror Western practice by recognizing brain death for organ donation upon individual prior designation or next of kin authorization for donation.

Ethical concern with the assurance of the 'dead donor rule' has more recently focused on DACDD of patients declared dead in the OR based on cardiac cessation. While there exists no clinical measure or standard period of time of heart cessation that is required in the declaration of death outside of organ donation, in the United States the generally accepted practice is to wait 2–5 minutes post arrest to ensure that the heart does not auto-resuscitate. Recent research has supported these time periods and have helped address ethical concerns that a donor may not be truly deceased (Hornby et al., 2010, Sheth et al., 2012).

The second area of ethical concern that has existed in donation is the debate of salvage versus donation of organs. Salvage assumes that the deceased's organs would routinely be recovered, without concern for an advanced directive or family determination of the disposition of the remains. Ethicists and religious leaders have uniformly supported donation rather than salvage as it supports the rights of the individual as higher that the rights of an unknown recipient or society (Veatch, 2000). This relative unanimity of opinion is likely highly associated with the practice of Presumed Consent countries to disregard their 'salvage' laws in favour of family choice.

The third area of significant ethical debate, allocation of organs, remains a largely unresolved issue. Fundamentally, the scarcity of available organs confronts a growing need for transplants; thus a rationing system must be developed. To avoid obvious personal, racial, and socioeconomic biases, countries have opted for allocation systems that aim to balance degree of illness, likelihood of post-transplant survival, waiting time, and organ viability as measured by time from recovery to transplant. Of course, these measurable factors are confronted by a doctors's obligation to care for an individual patient rather than someone else's listed patient and by a transplant team's need to maintain a minimum volume to ensure clinical performance. Thus, efforts to find an ethical solution to organ allocation is routinely complicated by new research, data, and anecdotal experience that is presented to meet individual perceptions of fairness; a work that shall remain 'in progress'.

More recently, donation ethics has shifted its focus to the widely reported and formally acknowledged practices of: (1) the use of the organs of executed prisoners in China and (2) paying the poor for living 'donation' of a kidney in Egypt, Pakistan, India, the Philippines and potentially other countries. The use of prisoner organs has brought into question whether the 'donors' are in fact deceased at the time of organ recovery and whether any 'authorization' for donation was by default coercion. Equally concerning has been question of the cause for execution being tied to political opposition and the speculation that some prisoners may be executed for their organs. Paying the poor for kidneys has been shown in numerous studies to result in a financial and not an altruistic decision and the 'donors' routinely have clinical challenges given their limited access to medical services. Each practice has been publically condemned with the World Health Organization Declaration of Istanbul providing a unified voice of international condemnation of these practices on ethical grounds (Participants in the International Summit, 2008).

Transparency in donation practice

The societies, medical professionals, and governments that manage organ donation and transplantation rely on individual and public trust, and they can only earn and maintain that trust when the system is transparent with respect to policies, practices, and access to services. The persistence of myths about donation and the unavoidable association of the process with death give rise to misconceptions that undermine donation (Morgan et al., 2008). Modern media can exert dramatic effects, both positive and negative, on public perceptions of donation (Morgan et al., 2009), and the donation field has used the media to counter misconceptions through organizations such as Donate Life Hollywood (2012), which strive to ensure that donation and transplantation are accurately portrayed. Public access to and participation in the establishment of standards, routine communication of outcomes, and public testimony from donor families can help ensure that trust is maintained.

Individual and societal altruism

Countries and cultures with pre-existing social and religious traditions that value charity and public good, have demonstrably higher donation rates than those with traditions that emphasize family, clan, or individual good. Thus, the relationship between high donation rates and largely Catholic populations may explain variations in European performance, but it does not explain the high US donation rates, which may derive more from an underlying communitarianism in the populace (Etzioni, 2003). However, if the source of these values does not fully embrace donation (e.g. Catholic authorities before the papal encyclical supporting organ donation, as well as certain current orthodox and fundamentalist religious leaders), the followers cannot be expected to embrace donation.

Fortunately, influential religious leaders have been providing guidance that can be built on among lower-donation-rate religious groups such as Orthodox Jews, Muslims, and Buddhists (Islamic Religious Council of Singapore, 2007; Tzu Chi, 2011; Halachic Organ Donor Society, 2012). Meanwhile, some maintain that altruism will never be enough and argue that organ donors should be paid to increase the supply of organs (Matas, 2006), while others point to abuses and exploitation where payment for organs has been practised (Harmon and Delmonico, 2006). However, with organ donation rates of 75% and higher in the United States for instance and as high or higher in Spain and Austria, it could be argued that there has never been any voluntary and intentional human action that has been as widely adopted, even when coerced by law or incentivized by payment.

Legislation to support critical processes

In support of critical donation processes, laws are required to establish (1) brain death declaration, (2) potential donor referral by hospitals, (3) authorization by coroners or medical examiners for recovery, (4) authorization by families for donation, (5) ODP donor management authority, (6) surgical recovery of organs, (7) allocation of organs, (8) designation and licensing of ODPs, (9) funding and reimbursement systems for ODPs, and (10) performance standards. British (Price, 2012), US federal (USDHHS, 2012), Australian (Neuberger and Thomas, 2011), US state (National Conference of Commissioners on Uniform State Laws, 2006), and local (California Health and Safety Code, 2012) laws provide examples of the types of legislation needed to remove legal impediments to donation.

Allocation systems based on equity

A population can be expected to donate organs only if there is reason to be confident that organ allocation is fair. Historically, allocation systems relied on waiting time as the standard on which this confidence was based. As medical assessment of the urgency of transplant and the ability to maintain the graft have improved, systems for the liver (MELD) and the lung (ALS) have been developed to optimize transplant outcomes and maximize the fairness of organ allocation (Graham et al., 2006).

Equally important is to ensure that organs are allocated as over as broad a geographic areas as possible so as to maintain equivalent waiting time within a country or a region. This remains a challenge in large countries such as the United States, where, despite efforts to improve allocation formulas, waiting times in some areas can be two to three times longer than average because of lower donation potential and larger waiting lists (UNOS, 2014).

Specialization of donation professional

The development of organ donation specialties (e.g. procurement coordinators, hospital development specialists, and family care specialists) has advanced donation practice by ensuring that donation professionals can pursue their goals without conflict and can dedicate the time to ensure that donation happens. Associated with specialization is functional or organizational separation of donation from transplantation. Just as the skills of donation are distinct from those of hospital patient care, they are also distinct from those of transplant care. And just as the demands of living patients limit the availability of hospital staff to donor families, the demands of waiting and transplanted recipients limit the time available for recipient transplant coordinators to apply the specialty skills of donation. In small hospital-based donation programmes, it may be difficult to achieve the necessary separation and to offer professionals the specialty skills and professional training needed to maximize donation (Mone, 2002).

Collaboration among 'three estates'

Because organ donation occurs in various hospitals, relies on donation professionals from ODPs, and serves transplant surgeons from multiple transplant centres, it requires agreements, shared knowledge and practices, and common goals to be successful. By the very nature of institutions, however, the missions, purposes, and orientations of staff are not necessarily fully aligned, and in some cases they are actively competitive. There may be conflict with neighbouring donor hospitals or academic medical centres, or there may be difficulty in balancing the immediate need for ICU beds for stricken patients against the needs of organ donors. Through examination of these conflicting goals, healthcare professionals can be prompted to focus on their shared basic purpose—saving lives—and to place the same value on transplanting distant patients as on caring for critically ill local patients.

To address these challenges, the Organ Donation Breakthrough Collaborative, using collaborative research initiated in the United States in 2006 by the Institute for Healthcare Improvement and expanded to Canada and Australia (Chapman, 2008), began its work by identifying the 'three estates' involved in donation (donor hospitals, transplant centres, and ODPs) and explicating their distinct purposes and motivations. This process enabled the three estates to find common ground, with the result that US deceased organ donation rates increased by 33% over the 4 years of the Collaborative (Shafer, 2008). Although these results were specific to the United States, their thrust is universal: organ donation requires a common set of values, shared responsibilities, and distinct roles. It is significant that the Collaborative's success was not immediately transferable to other countries where a uniform national donation system had not been established and where roles and responsibilities varied.

It is reasonable, therefore, to conclude that the basic work of establishing a uniform set of laws and practices around donation is essential for maximizing the benefits of collaboration. This lesson is reinforced by the Spanish model, in which standards have been established by Madrid (Report of the Madrid Consultation, 2011) and integrated into a national organ donation system.

Future of organ donation

Some 17 years ago, Sir Roy Calne made the now famous comment, 'Xenotransplantation … it's just around the corner but it may be a very long corner'. This sentiment remains true today and probably can also be applied to organ cloning and to the replacement of human organs with mechanical devices that make organ donation obsolete.

Doctors, researchers, and those suffering from organ failure look to these potential advances with hope, but mimicking the complexity of organ function remains a formidable challenge. Dialysis was the first and most successful mechanical replacement, but the mobility restrictions and debilitating side effects have actually created longer waiting lists for transplant because patients with kidney failure now live longer. Advances in cardiac assist devices have enabled some patients to live outside the hospital while awaiting heart transplants, have helped end deaths on the waitlist in some regions, and in specific cases have reduced the need for transplantation, but they have not decreased the overall need for heart transplants.

Further progress in nonhuman transplantation will probably take place in the cardiac arena; functional replication of cardiac tissue primarily focuses on nerve and muscle replication, whereas functional replication of liver and kidney tissue requires the creation of complex filtration and chemical production systems. For each of these organ systems, research seems to be coalescing around the use of stem cells for auto-generation of organ function. However, despite some dramatic advances in stem cell research, most scientists view

this work as being decades away from human application and, like xenotransplantation, requiring experimental and clinical validation before it can actually be used to treat organ failure.

Of great interest to the organ donation community is genetic research into the identification, matching, and (ideally) replacement of a donor's genetic fingerprint with the intended recipient's immunologic code (Sarwal et al., 2011). Progress in this area would yield dramatic benefits for allograft organ function and graft survival; antibody-mediated rejection remains the primary cause of long-term organ failure, and immunosuppression side effects (e.g. cancers) significantly shorten the lives of transplant recipients. If this research becomes clinically viable, the organ donation process will probably have to be modified to allow time for the genetic recoding. This change is likely to raise expectations regarding the levels of graft performance and survival that would have to be demonstrated by any xenogeneic, mechanical, or cloned alternatives to human allograft transplant.

Therefore, it is reasonable to predict that whereas human organ donation ultimately will be supplanted by new biotechnological processes and devices, it is likely to remain an essential part of the treatment of organ failure for decades to come. Accordingly, if the goal is to reduce or even end deaths on waiting lists, it is necessary to redouble worldwide efforts to share and implement the critical processes, essential functions, and foundational principles of best-practice ODPs while supporting broader efforts to increase the pool of potential donors and inspire all such donors to choose donation.

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CHAPTER 278

Donor and recipient kidney transplantation surgery

Richard D. M. Allen and Henry C. C. Pleass

Introduction

The observation of a transplanted kidney changing from a flaccid and pale appearance to one that is firm and pink, and within seconds of removing vascular clamps, is an impressive sight for the first timer in the transplant operating theatre. It is an unforgettable experience. But even better is the sight of urine, minutes later, dribbling from the end of the divided donor ureter. It puts a smile on the face of everyone in the operating room, no matter how many times they have seen it before. More importantly, the sight of transplant urine is the ultimate test of the quality of the organ donor procedure, organ preservation, the vascular anastomoses, and, the quality of the recipient anaesthesia. The appreciative transplant surgeon always turns to the head of the operating table and thanks the anaesthetist.

For the electively transplanted living donor kidney, this should be the norm. For heart-beating deceased donor kidneys, primary transplant function occurs for about 75%, and for extended criteria donor kidneys, the figure is closer to 25%. This is a reflection of the less than optimal passage to transplantation for the computer-allocated deceased donor kidneys that arrives on the doorstep of a transplant centre, many hours after the multiorgan donor procedure, for a procedure performed in emergency operating time in a dialysis-dependent waiting-list recipient. If the kidney is not passing urine by the end of the transplant procedure, the expectation is that it will at some time in the coming days to weeks. At the operating table, the experienced surgeon relies on observational assessment of the perfusion of the transplanted kidney for reassurance that all is well. Thereafter, colour Doppler ultrasound (CDUS) and percutaneous core biopsy are used to monitor the progress of an initially anuric transplanted kidney.

The transplant surgery team

Over the last four decades, the role of transplant surgeons has gained in significance and to the point where they are now the most important variable in kidney graft loss within 6 months of transplantation (Fig. 278.1). Progressive improvement in kidney graft survival, resulting from more sophisticated tissue typing techniques and better immunosuppression, has focused greater emphasis on surgeon-related causes of kidney graft loss. Surgical misadventure is now three times more likely than rejection to result in graft loss at 6 months. Good transplant centres select their new surgeons carefully!

Surgeons involved in kidney transplantation come from varied backgrounds. In Europe and the United States, they are more likely specialist trained and credentialed in multiorgan transplant centres. Because of its unpredictable workload and onerous hours, it is not always seen as an attractive option for surgical trainees. For many however, particularly those who enjoy working in a multidisciplinary team environment and at the interface between the research laboratory and clinical practice, the rewards are great. The involvement of vascular surgeons and urologists in the transplant surgical team is important for care of the live kidney donor and management of technical complications in the transplant recipient. They are also an important part of the large team of transplant clinicians necessary to facilitate separation of decision-making and care of the live donor from that of the recipient.

An historical perspective

The world's first successful kidney transplant surgery was performed in Boston, United States, in 1954 (see also Chapter 275). All previous attempts at kidney transplantation from deceased donors had had been failures because of rejection. Success was made possible only because no immunosuppression was required. Twin brothers, aged 23 years, were verified as identical by comparison of their fingerprint patterns at their local police station and subsequent skin grafting from donor to the recipient that did not reject. Hartwell Harrison, a urologist at the Peter Bent Brigham Hospital, removed the left kidney of Ronald Herrick using an open technique that was common for removal of a diseased native kidney. It involved a loin incision through the bed of the left 12th rib and came close to disaster when the vascular clamp slipped off the renal artery pedicle causing dramatic haemorrhage. Fortunately for the Herrick twins and for transplant history, catastrophe was averted and both the live donor and his twin brother the recipient, made a full recovery. The recipient went on to marry one of his caring nurses with whom he had two sons. Receiving no immunosuppression, he survived another 8 years before succumbing to recurrent glomerulonephritis in the transplanted kidney. The donor lived for more than another 50 years and without evidence of renal impairment (Tilney, 2006).

Appreciating that the role of kidney transplantation could only be widened if kidney failure patients could be transplanted with organs from genetically non-identical individuals, the Boston team explored the use of total-body irradiation and bone marrow replacement for suppressing the immune system. In 1959, a 24-year-old man was transplanted with a kidney from his non-identical twin

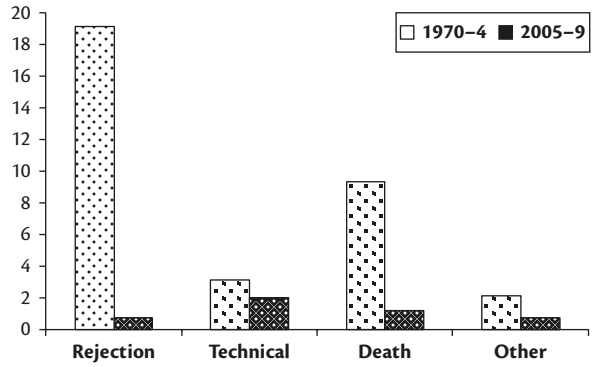

Fig. 278.1 Causes of graft loss in first the 6 months after kidney transplantation reported to ANZDATA in Australia for recipients of living and deceased donors, comparing 5-year time periods 1970 through 1974 (N = 1118 transplants) and 2005 through 2009 (N = 4014 transplants).

brother using this technique and survived 29 more years (Merrill et al., 1960). The immunosuppressive drug azathioprine was also used for the first time, also by the Boston team, to provide a safer and more effective means of overcoming immune rejection. In 1962, they transplanted a 23-tear-old man with a deceased donor kidney (Merrill et al., 1963). It is indeed a remarkable testament to pioneering transplant clinicians from Boston and elsewhere, that despite almost universal recipient mortality, a few never gave up hope of achieving the dream of transplant success between genetically unrelated individuals. Joseph Murray, the surgeon leading the Boston team, was awarded the 1990 Nobel Prize in Physiology or Medicine for his contribution that began with an interest in skin grafting burns victims (Tilney, 2006).

As knowledge and understanding of transplant immunology has moved forward, so has the practice of transplant surgery. To minimize the trauma of major surgery, the open nephrectomy has been replaced by a variety of minimally invasive surgery techniques. However, the risks of major surgical morbidity of donor nephrectomy remain significant and were recently reported as being 3%, with a worldwide risk of mortality ranging from 1 in 1600, to 1 in 3300 across large series (Segev et al., 2010). Despite these risks, patients remain remarkably selfless and often doggedly determined to donate a kidney to a family member or friend. Perhaps because of these statistics, living donation of kidneys has not found universal ethical acceptance. Nevertheless, it has become the predominant source of kidney donors in many countries without deceased organ donor programmes. The benefits to the donor are purely psychological and the risks of haemorrhage, pulmonary embolus, pneumothorax, wound infection, and hernia are very physical. By necessity, the techniques of live donor nephrectomy have had to evolve (Buell et al., 2001).

Living donor patient assessment

A multidisciplinary team, independent of that involved with care of the potential recipient, undertakes assessment of the living donor. The donor surgeon separately ascertains that the proposed donor is related, spouse, partner, or friend, and is making a free and informed decision in full knowledge of the facts and without any form of coercion. This is not an easy task, particularly in clinical environments where living kidney transplantation is the only transplant option. Donor-recipient pairs do not always tell the truth. It is tempting for some donor surgeons to abrogate this responsibility to others in the assessment process, and to act only as a surgical technician or 'hired gun'. Equally, the surgeon should be assured that the donor has lifetime access to medical care which is at least comparable, if not better, to that of the recipient.

Collectively, live kidney donors are in many ways fitter than the general population and live longer, at least in developed countries (Fehrman-Ekholm et al., 2001; Ibrahim et al., 2009). Transplant surgeons are prone to literally 'sizing-up' potential kidney donor patients when they walk into the consultation room. It is with good reason for their nephrology colleagues sometimes have unrealistic expectations of what can be achieved. Morbid obesity is a general risk factor for surgery and development of type 2 diabetes mellitus in the long term. However, data to support use of an absolute 'cut-off' body mass index (BMI) is not strong because of variations in muscle mass, body shape, and distribution of adipose tissue. Absolute criteria are hard to work with. Hence, for the obese potential living kidney donor, the authors' suggestion is to provide an individual donor target BMI based on the potential donor's exercise and dietary patterns, and a clinical examination with the donor in a supine position. For example, the target might be a BMI of < 30 kg/m^2 in an apple-shaped middle-aged man and < 35 kg/m^2 in a multiparous pear-shaped female. For the committed donor with scope to decrease carbohydrate intake and increase daily exercise, weight loss before the elective donor surgery procedure can be easily achieved. Indeed, it is a good test of a prospective donor's preparedness to be a donor. If a target is set, regular personal review of the donor by surgeon is beneficial. Once achieved, the authors' experience is that donors invariably appreciate and maintain their changed lifestyle and benefit in the long term.

In addition to evidence of excellent kidney function, normal glucose homeostasis and satisfactory cardiorespiratory testing, the surgical team requires donor anatomical information to assist decision-making for side of kidney to be donated—left or right. The overriding principles are that the best kidney remains with the donor, and after consultation with the recipient surgeon, the donor surgeon has the final say. Information is provided by abdominal CDUS examination, nuclear glomerular filtration rate (GFR) assessment and computed tomography (CT) angiography with vascular reconstructions to demonstrate kidney arterial venous anatomy (Fig. 278.2). The correlation between CT angiography findings and surgical findings is about 97% (Holden et al., 2005). It is exceedingly unusual to turn down a prospective donor on the basis of anatomical issues (Crane et al., 2010). Most large series report that 80–95% of living donor kidneys are left sided, implying a surgical preference based on anatomical grounds. The right kidney is the 'fallback' second choice in the presence of multiple renal vessels or bifid ureters in the left kidney, or pathology, such as calculi and benign tumours, in the right kidney. The argument to support use of one kidney over the other based on unacceptable differential kidney function (< 45% of nuclear GFR) is problematic because of the accuracy of the test (Fig. 278.3). Measurement of differential kidney volume using computer software is likely to be more accurate (Fig. 278.4) (Miyazaki et al., 2010). Individual surgeon bias is also likely to have a role with some recipient surgeons preferring to anastomose two left-sided renal arteries than use a right kidney with a short vein.

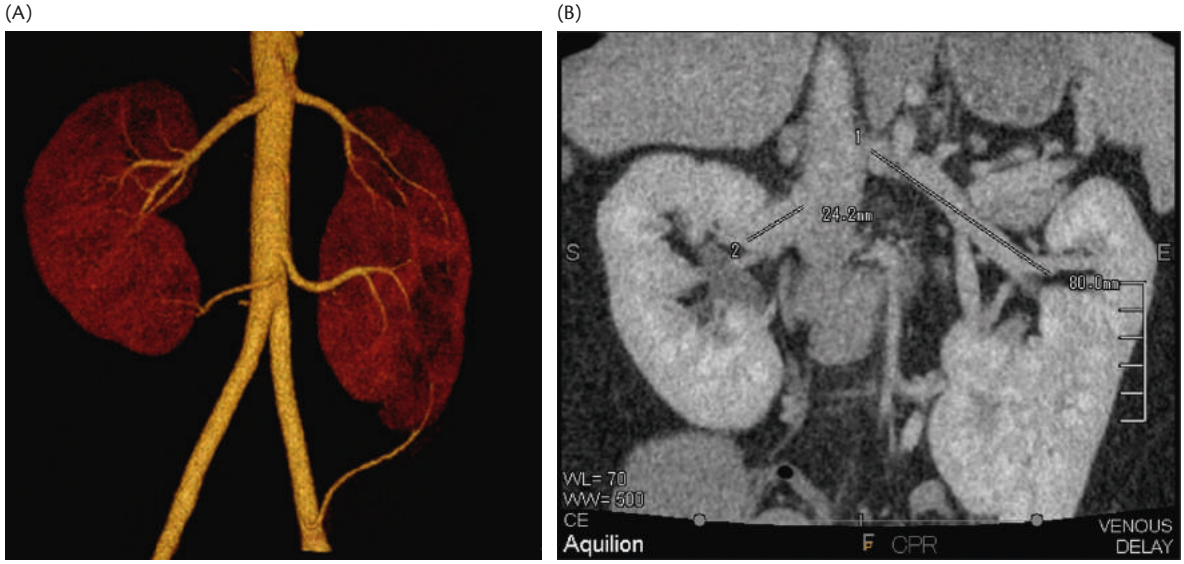

Fig. 278.2 CT angiography of a prospective 28-year-old male living kidney donor. (A) Arterial reconstructions to demonstrate donor kidney arterial anatomy. (B) Late venous picture to evaluate venous anatomy.

Living donor surgical techniques

Between 1954 and 1995, all live donor nephrectomy procedures were carried out by some form of open incision, usually in the loin and extending from the bed of the 12th rib towards the umbilicus, as far as the lateral border of the rectus abdominis muscle (Fig. 278.5). The long muscle-cutting incision was associated with basal atelectasis, hernia formation. and chronic pain. Not surprisingly, 15% of patients undergoing open donor nephrectomy were of the view that they would not consent to the procedure if they had their time over again. Hence, with the introduction of laparoscopic living donor nephrectomy by Ratner and colleagues in 1995 (Ratner et al., 1995), the open procedure was replaced rapidly by the cosmetically more acceptable, and less painful minimally invasive technique (Fig. 278.6). Inpatient stays became shorter, as did time to return to work. Like the introduction of its cousin, the laparoscopic cholecystectomy, patients and referring physicians voted with their feet, with all live donor nephrectomy programmes experiencing an increase in patient numbers over the last decade. Because of these market forces, and despite claims of higher rates of vascular and ureteric complications with donor kidneys with multiple arteries (Kuo et al., 1998), few stalwarts of the open nephrectomy procedure remain. They tend to be in economically deprived regions, particularly in the setting of transplant tourism. Furthermore, the authors have shown that with careful technique and experience, there are few anatomical barriers to laparoscopic donor nephrectomy (Crane et al., 2010). However, they also accept that initial kidney function of donor kidneys retrieved by laparoscopic means is not as impressive as it is for the open procedure,

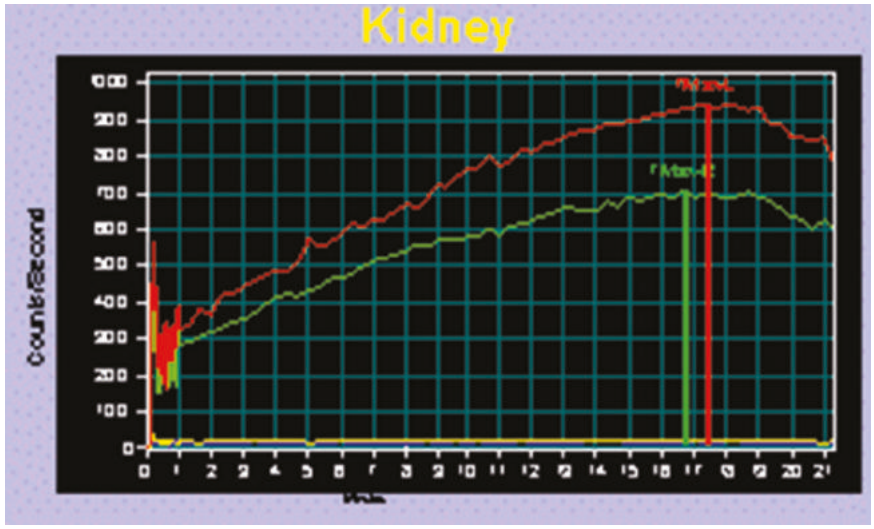

Fig. 278.3 ^{99m}Tc-DTPA dynamic nuclear scan to assess split renal function with 54.8% of kidney function attributed to the left kidney.

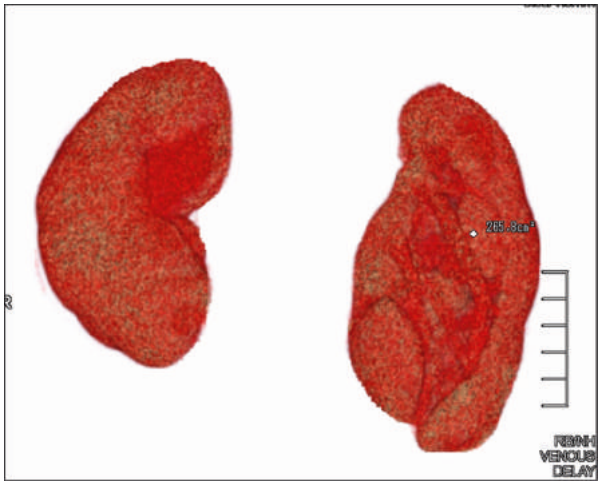

Fig. 278.4 Three-dimensional reconstruction of kidneys from original data sets from original coronal and axial images of Fig. 278.2 are evaluated with three-dimensional (3D) tools to isolate renal parenchyma (*outlined areas*) from adjacent structures, including the renal pelvis. The generated model can be manipulated in space using 3D software to ensure accurate generation and delineation of parenchymal borders. The abnormally left kidney is estimated to measure 265.8 cc. The right kidney measured 239.6 cc.

likely because of longer donor operating time, greater manipulation of the donor kidney, and the need for a pneumoperitoneum.

There are several variations in the technique of laparoscopic donor nephrectomy, likely a reflection of surgical training influences. Choices are either a pure laparoscopic approach or one that is 'hand assisted'. Surgeons with a limited laparoscopic surgery training background likely opt for latter because of the perceived

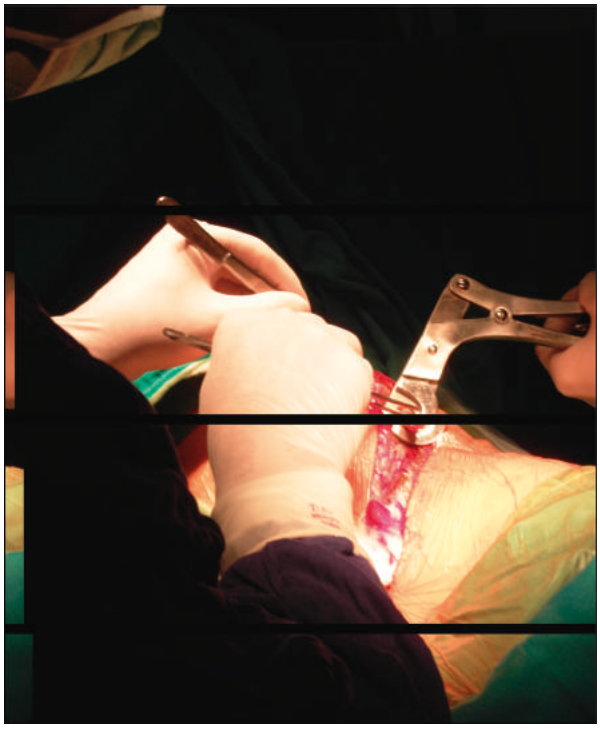

Fig. 278.5 Urology surgeons removing the left 12th rib during a living donor nephrectomy by open technique in Vietnam in 2009.

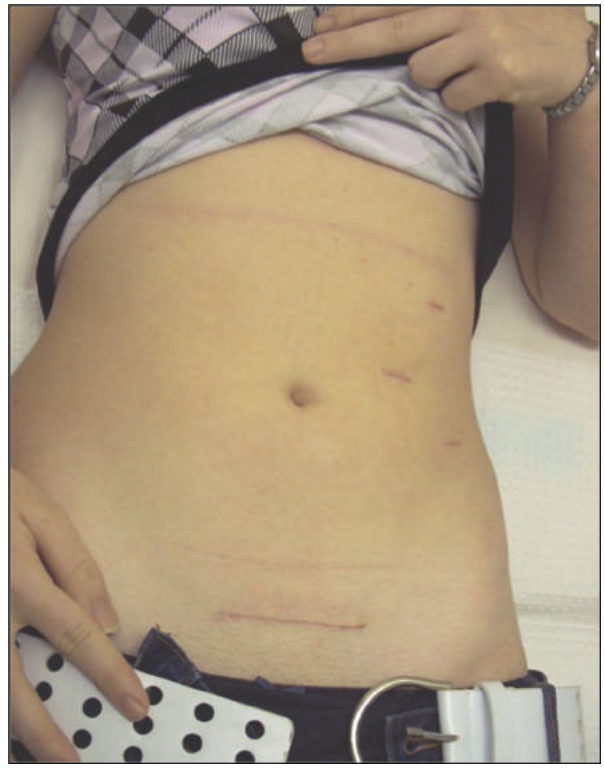

Fig. 278.6 Wounds associated with left laparoscopic donor nephrectomy 6 weeks after surgery.

ease of being able to control bleeding. For either, the procedure can be intra- or extraperitoneal with the latter thought to reduce potential for intraperitoneal misadventure such as small bowel perforation and adhesion formation (Greco et al., 2010). Careful attention is given to preservation of the kidney vasculature and blood supply to the ureter. The recipient surgeon should never be too far away and is always present when the kidney is removed to facilitate cool preservation of the donor kidney and preparation of kidney vasculature for subsequent transplantation.

The authors' preference is to use 3 × 5 mm ports, an intraperitoneal technique, and a 1 × 10 mm port for clip and stapler deployment. A 30°, 5 or 10 mm camera is used as standard. For a right donor nephrectomy, an additional 5 mm port is required to retract the right lobe of the liver. Dissection is usually performed with a combination of diathermy scissors and harmonic scalpel. A 5 mm diameter blunt metal rod is used to retract the kidney on its vascular pedicle. Hence, there is no need for the surgeon's hand to enter the abdomen during the dissection phase of the procedure. A segment of the gonadal vein is normally taken with the left renal vein and ureter. The gonadal vein on the right side is usually left *in situ*. The donor ureter is divided at the level of the pelvic brim.

After fully mobilizing the donor kidney, an initially peritoneum-preserving, 7 cm suprapubic transverse and non-muscle cutting incision is made to facilitate subsequent removal of the donor kidney. Heparinization of the donor is not routine and is reserved for instances of multiple renal arteries. The donor renal vessels are controlled with a combination of a plastic clip device and metal clips for the renal artery, and a 30 mm vascular endovascular stapler for the renal vein. Depending on surgeon preferences, the donor kidney is then removed from the peritoneal

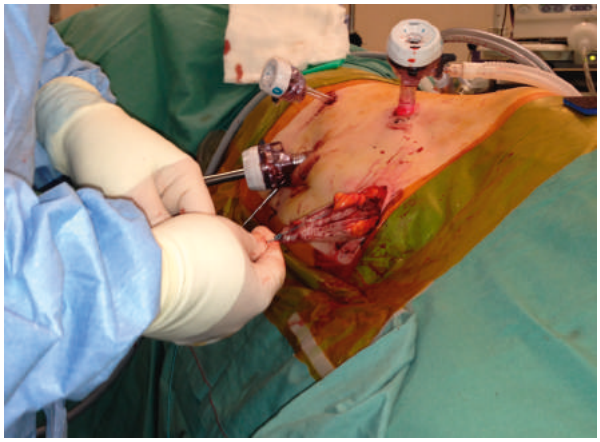

Fig. 278.7 Removal of a left-sided living donor kidney through a suprapubic midline incision and using an EndoCatch™ bag, during a purely laparoscopic donor nephrectomy procedure.

cavity using either an EndoCatch™ bag (Fig. 278.7) or by hand extraction using a hand port (Fig. 278.8). Either can be inserted through the peritoneum exposed by the suprapubic incision. The time between dividing the donor renal artery and initial cooling of the donor kidney with organ preservation solution by the recipient surgeon is usually between 3 and 5 minutes.

Live donor nephrectomy is clearly a challenging surgical technique to learn, but one with obvious benefits to the patient. It is not an operation for the beginner. Credentialing guidelines in Australia include the need for training in other laparoscopic surgery followed by formal training and mentoring by an established nephrectomy surgeon. The incentives, of small incisions, discharge from hospital on postoperative day 3, and return to work within 2 weeks, are both persuasive and real to patients (Simforoosh et al., 2005). However, it is a very different operation to an ablative nephrectomy and prospective donor patients should be encouraged to seek information about an individual surgeon's specific training and experience.

Horgan et al. from Illinois, Unite States, reported the first successful series of robotic-assisted laparoscopic living donor nephrectomy, using the da Vinci™ surgical system in 2002 with what appears to be equivalent morbidity and mortality rates (Horgan et al., 2002). Initial purchase price and consumable costs related to this technique have, so far, prohibited widespread use of this technology. More recently, extraction of a live donor kidney through the vagina has also been reported, avoiding the need for the abdominal extraction site scar, and is another potential evolution of the technique (Pietrabissa et al., 2010).

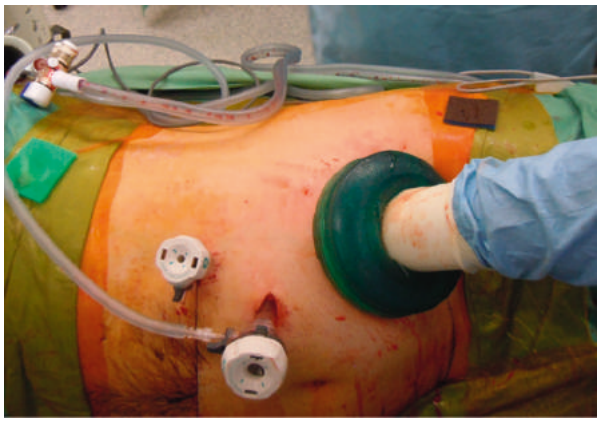

Fig. 278.8 Use of a hand port to facilitate dissection of the donor renal vessels and subsequent removal of the left donor kidney through a muscle cutting incision in the left flank.

Deceased kidney donors and retrieval surgery

It can be argued that living donor surgery is a procedure of necessity only because there are insufficient deceased donors to meet the demand for kidney transplantation. Organ donation from deceased donors requires community acceptance, legislation to protect intensivists to make a diagnosis of brain death, and financial resources to support intensive care units and donor retrieval teams. Community acceptance can be both adversely affected by prevailing cultural norms and positively influenced by building community trust with transparent and clinician-led organ allocation protocols that are not dependent on money changing hands and political influence. Deceased donor organs are considered a precious community resource that save lives, and not a commodity that can be bought and sold.

Deceased organ donation is both complex and expensive, and in economically deprived communities, is unlikely to compete with limited health dollar expenditure that might save a greater number of lives (White et al., 2008). It is dependent on sophisticated cardiorespiratory care and equipment, regionalized organ retrieval teams, and access to emergency virology and tissue typing services. Furthermore, and because donor kidneys are allocated according to computer-driven algorithms based on negative donor-recipient lymphocytoxicity crossmatching, human leucocyte antigen matching, and waiting time, infrastructure is required to collect, store, and distribute sera for recipient waiting list patients. It is estimated that a single after-hours multiorgan donor will have more 150 healthcare professionals out of bed all night! Only a small part of that number will be the on-call donor retrieval surgery team that includes an experienced transplant surgeon able to evaluate the macroscopic appearance of the donor organs, two surgical assistants, and an organ perfusionist who often doubles up as the driver of the transport vehicle. The nocturnal nature of deceased organ donation is well illustrated by the 15-year record of timing of commencement of donor organ cool perfusion by the NSW Deceased Donor Organ Procurement Service—82% of aortic cross-clamp times were outside normal office hours (Fig. 278.9). The unsociable working hours are likely the result of time required for diagnosis of brain death, initial laboratory investigations to exclude donor infection transmission, and coordination of donor and recipient surgeries to minimize total organ ischaemia time.

The existence of national legislation to allow organ donation after the declaration of brainstem death facilitates organ donation surgery to take place in an organized and optimized manner and maximizes the number of usable donor organs. Clinical testing of brainstem death is dependent on demonstration on two occasions, by two senior clinicians independent of the transplant team, of absence of all responses to stimulation of the brainstem respiratory and reticular activating centres. Termed 'Donation after Brain Death' (DBD), it allows use of cardiorespiratory support in

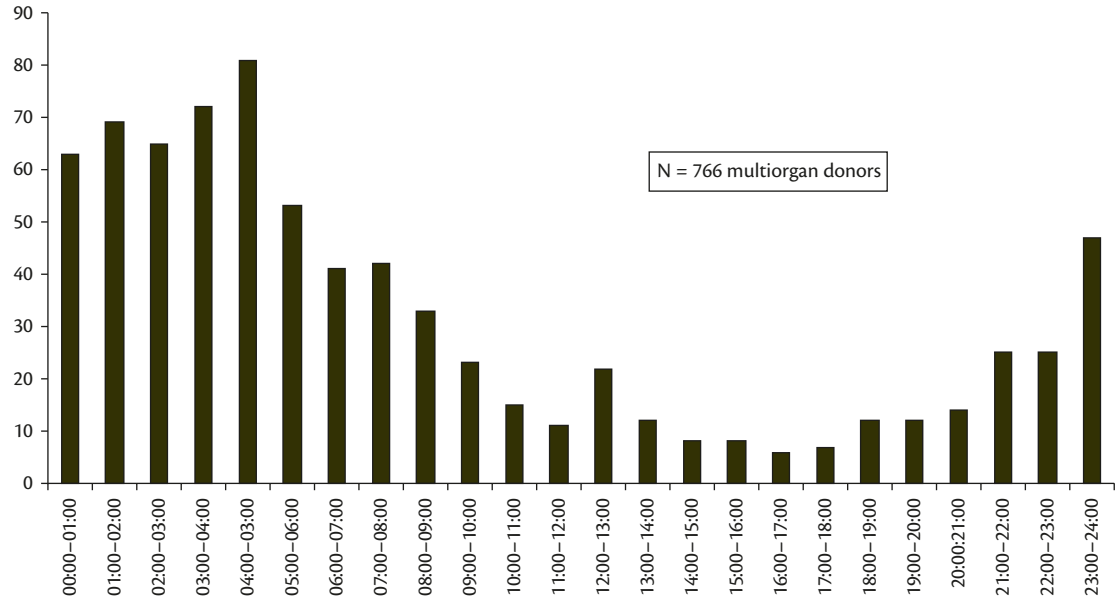

Fig. 278.9 The 15-year record (1996–2010) of timing of commencement of donor organ cool perfusion by the New South Wales Deceased Donor Organ Procurement Service.

the operating theatre until separate cardiothoracic and abdominal surgical teams complete mobilization of respective organs in readiness for cool perfusion of the donor organs with preservation solutions (Fig. 278.10). This includes identification and partial mobilization of the two ureters. About 85% of DBD donor surgery involves retrieval of organs in addition to the kidneys and, depending on whether right and left lobes of the liver are split *in situ* and the pancreas is retrieved, can take 3–4 hours of operating time. For kidneys-only donation, the retrieval procedure is simplified with the aorta clamped above the superior mesenteric artery (SMA) after its ligation and division.

Next, and virtually in a sign of reverence towards the deceased organ donor, a quiet hush descends upon the operating theatre, as cardiac monitoring is ceased and ventilatory support withdrawn from the donor followed by departure of the anaesthetic team. Hardly a word is then spoken as the donor surgeons go expeditiously about their work, simultaneously cross-clamping the aorta and commencing cool perfusion of the abdominal organs through an isolated segment of aorta from the descending aorta above to the aortic bifurcation below. The liver, kidneys, and pancreas are cooled rapidly, usually with 2 L of isotonic crystalloid solution at 4°C and followed by 2 L of University of Wisconsin (UW) solution, a purpose-made solution that reduces cell swelling in the absence of function of the cell membrane sodium-potassium pump (Anaya-Prado and Delgado-Vazquez, 2008). The latter is expensive and comparatively viscous because of its high potassium content. Hence, the initial use of the crystalloid solution such as Ringer's lactate. Indirectly, the liver is also cooled via the SMA and venous return through the portal vein. Venous effluent from the inferior vena cava (IVC) is drained either by suction from the chest or by gravity through a separate distal caval drain to a container on the floor. After placement of iced saline slush in the abdominal cavity, the abdominal surgeons stand back as cardiothoracic surgeons remove the heart and lungs.

The liver and pancreas together with the segment of aorta containing the coeliac trunk and SMA are then removed *en bloc*. The small and large bowel are then retracted into the chest cavity to expose the kidneys in the retroperitoneal plane. They are separated *in situ* by dividing the left renal vein flush with the IVC to expose the aorta behind which in turn is divided anteroposteriorly down to the level

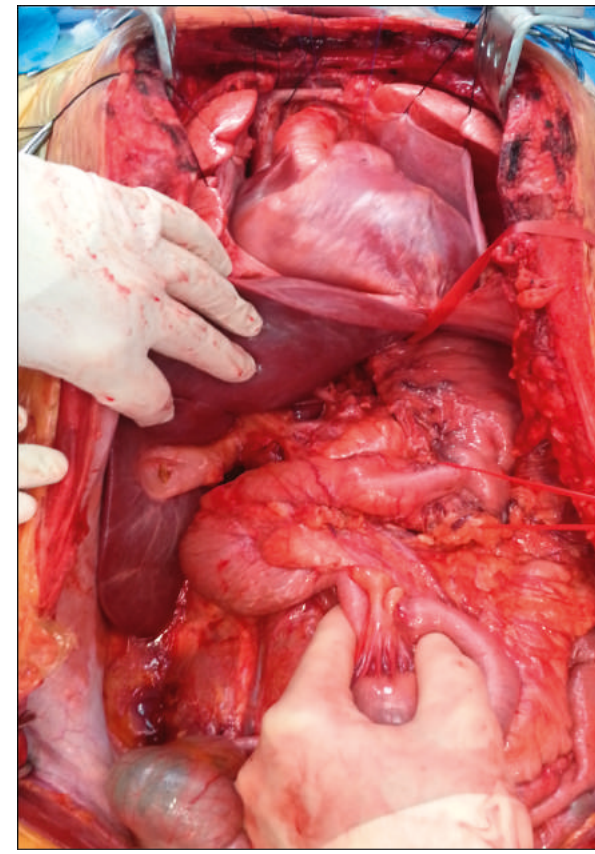

Fig. 278.10 Thoracic and abdominal cavities open during a multiorgan donor retrieval procedure to display heart above the diaphragm, and the liver, pancreas, and intestine below.

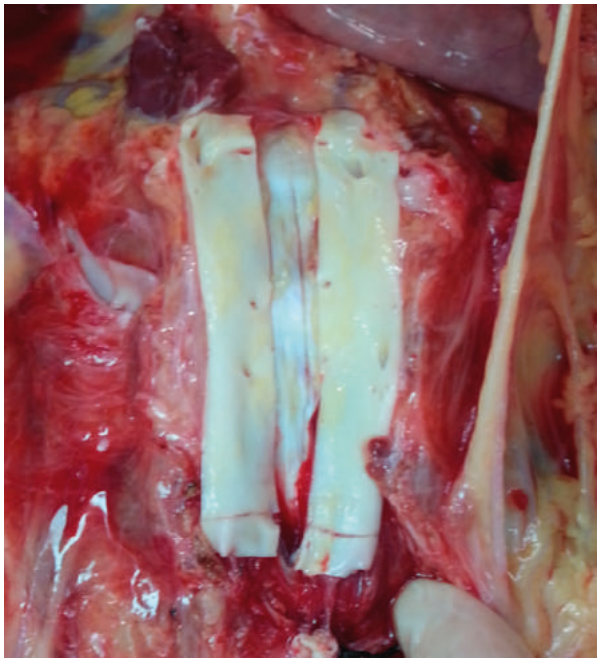

Fig. 278.11 The kidneys are separated *in situ* by dividing the left renal vein flush with the IVC to expose the aorta behind which in turn is divided anteroposteriorly down to the level aortic bifurcation.

aortic bifurcation so as to ensure retrieval of any accessory renal arteries (Fig. 278.11). On a separate back table, the liver is flushed with about 1 L of UW or equivalent solution through the portal vein and bagged for transportation in the UW effluent. The kidneys are separately perfused again with UW or equivalent solution, checked for vascular anomalies, incidental pathology, and completeness of perfusion, before bagging and labelling, left or right, for transportation in ice boxes to the transplanting hospital (Fig. 278.12).

Donation after circulatory death

In instances where intensivists are unable to diagnose brainstem death in a ventilator-dependent patient with severe and irreversible brain injury of known cause, but are nevertheless confident that cardiac standstill would occur within 60 minutes of withdrawal of ventilatory support, they may seek permission from the patient's family to proceed with 'Donation after Circulatory Death' (DCD). In other words, organ retrieval surgery occurs in a controlled setting and only after the cardiac standstill occurs. Because of the effect of hypoxia on the organs to be retrieved, the nature of that controlled setting is important and guidelines vary from country to country. At one end of the spectrum, in Australia, for example, intensivists, ethicists, and the community have decreed that ventilatory support is withdrawn in the intensive care unit in the presence of the donor's family. Lines that might facilitate rapid cooling of organs are unable to be inserted beforehand and no heparin can be given intravenously. Five minutes after cardiac standstill occurs, death is declared and the donor is taken to the operating suite where the donor surgeons have been scrubbed and ready for action. In donors under the age of 45 years, and when the time between ventilation withdrawal and cardiac standstill is < 30 minutes, liver, kidneys, and pancreas can be retrieved. Kidneys only are retrieved if the time is < 60 minutes and in donors up to the age of 65 years. At the other end of the spectrum, as is the case in China without brain death laws where ventilatory support is withdrawn in the operating suite and extracorporeal circulatory support commenced in heparinized donors when cardiac standstill occurs. The resultant shorter warm ischaemia time is likely to maximize the potential number of viable organs for subsequent transplantation.

By necessity, the donor surgery technique is very different for DCD donors. Rapid dissection is possible in the non-bleeding donor. The initial step is to clamp and cannulate the distal aorta to infuse tissue plasminogen activator at room temperature in the expectation that it will promote lysis of thrombus that is likely to have occurred after cardiac standstill. Cool perfusion is then commenced with Ringer's lactate followed by UW or equivalent solutions and up to 10 L can be infused. The abdominal cavity is filled with iced saline slush as the donor is progressively exsanguinated (Fig. 278.13). The cooled and preserved organs are then mobilized

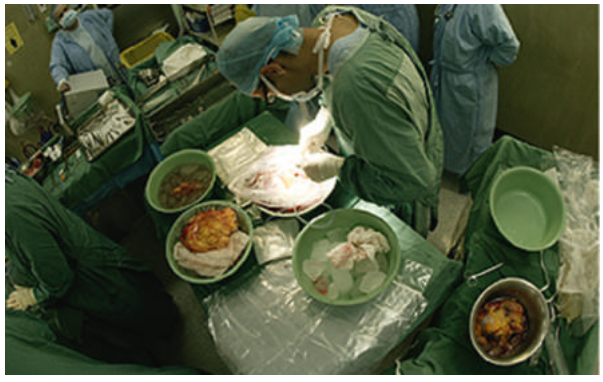

Fig. 278.12 The senior donor surgeon examines the donor organs for quality of cool perfusion with preservation solution and checks for vascular anomalies, incidental, before bagging and labelling.

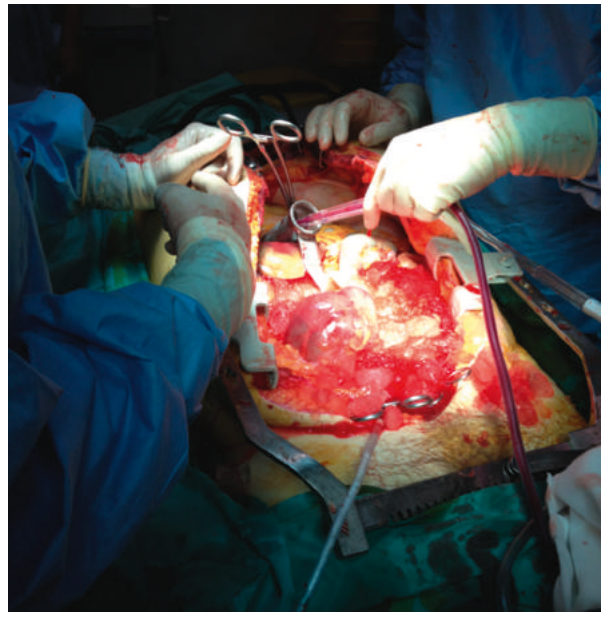

Fig. 278.13 The abdominal cavity is filled with iced saline slush as the distal donor aorta and abdominal organs are perfused from below with organ preservation solution. The venous effluent is removed from the divided thoracic IVC by suction.

in a manner similar to that for DBD and examined on the back table for completeness of perfusion. Some donor retrieval teams routinely place DCD donor kidneys on machine perfusion in the belief that better organ preservation can be achieved (Moers et al., 2009).

The results of kidney transplantation using DCD kidneys are comparable with those of DBD kidneys except for the recipient over the age of 60 years undergoing re-transplantation (Summers et al., 2010). However, because of the hypoxia associated with the necessary wait for cardiac standstill to occur and the subsequent warm ischaemia time, DCD kidneys are associated with poorer initial graft function. In Australia, the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) reported that dialysis was required in the first 72 hours after transplantation in 52.5% and 21.5% of transplanted DCD and DBD kidneys respectively ($P < 0.001$). For this reason, it also makes sense to minimize cold ischaemia time by transplanting DCD kidneys locally rather than shipping long distances to other transplant centres.

Organ donor numbers have increased substantially in recent years in both the United Kingdom and Australia. Much of that increase can be attributed to the introduction and acceptance of DCD (Fig. 278.14). This has primarily benefited the kidney transplant community with liver transplant waiting list patients missing out because the restrictive criteria for use of DCD donor livers. In the last 5 years in Australia, the average number of transplanted organs from each donor has dropped from more than four to less than three in 2012. As a result, some argue that many DCD donors could be DBD donors but for the comparative ease of the intensive care units to proceed to DCD compared DBD. Australian data suggests otherwise, demonstrating that the cause of the brain injury in DCD donors, when compared to DBD donors is more likely to be the result of a hypoxic/anoxic event or head trauma, and, less likely the result of a stroke ($P < 0.001$). This implies that DCD donors are likely to be an additional source of organ donors rather than a replacement of DBD donors.

Kidney transplantation techniques

The principles of transplantation of a living donor kidney and deceased donor kidney are similar although with some important differences related to the quality of the donor kidneys, timing of the procedure, and vessels available to facilitate anastomoses. Both involve placement of the donor kidney into a heterotopic position in one or other iliac fossa, necessary because of the limited length of the donor ureter. Vascular anastomoses are to the iliac vessels (Fig. 278.15). In comparison, cardiothoracic and liver transplant surgeons have an easier technical task, placing size-matched donor organs into an orthotopic position after removal of the failed recipient organ.

For deceased donor kidney transplantation, the surgeon must cope with a computer-allocated pairing of the donor kidney and recipient, both sight unseen. Donor kidneys, particularly from an extended criteria deceased donor, are not new engine parts that can be taken off a spare parts shelf. They are pre-owned and have no regenerative capacity. The clinical status of waiting list recipients has not been optimized and the transplant procedure is always performed in emergency operating time and often with junior anaesthetic staff and a non-specialist surgical scrub team. In contrast, living donor kidneys are in great shape, the recipient is either pre-emptive or buffed up to best clinical status, and the procedure is performed in elective operating time with a specialist team in daylight hours. However, the expectations of the donor, recipient, and surgeon are much greater for living donor kidneys, perhaps making the procedure tougher for all involved.

Either way, the good kidney transplant surgeon is one who recognizes the small margin for surgical error and avoids difficult

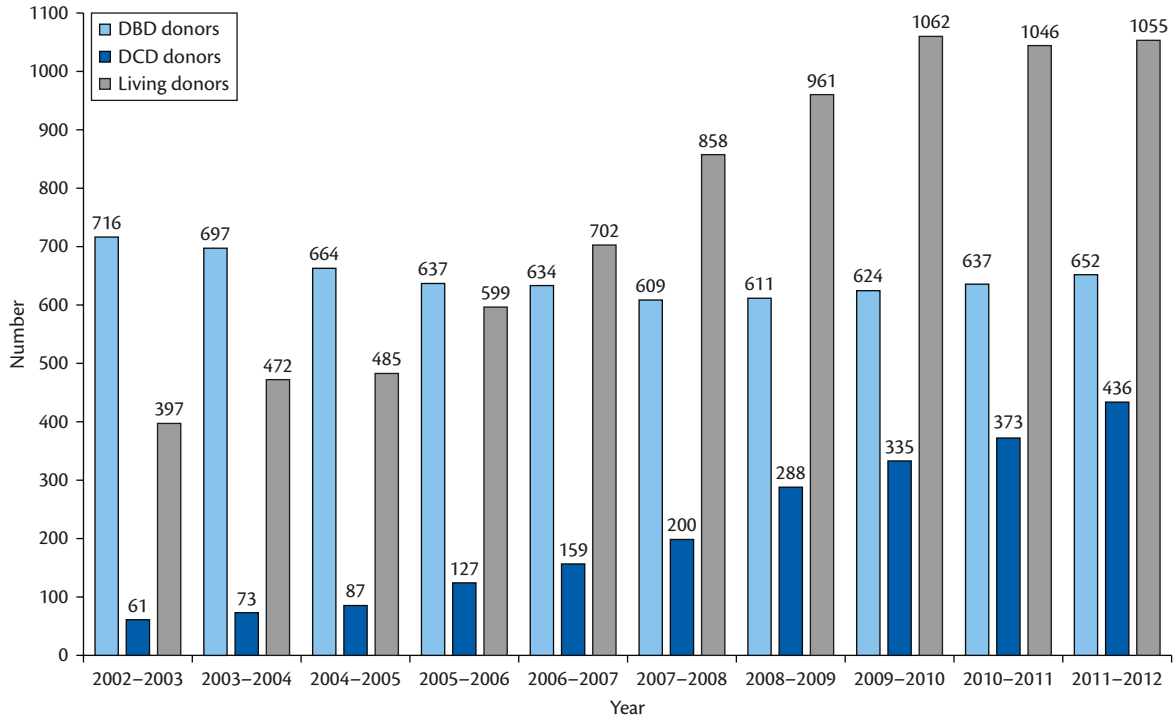

Fig. 278.14 Composite graph demonstrating progressive annual increases in both live kidney donor numbers and DCD donors in the United Kingdom between April 2002 and March 2012. Comparatively, there has little change in the number of DBD donors.

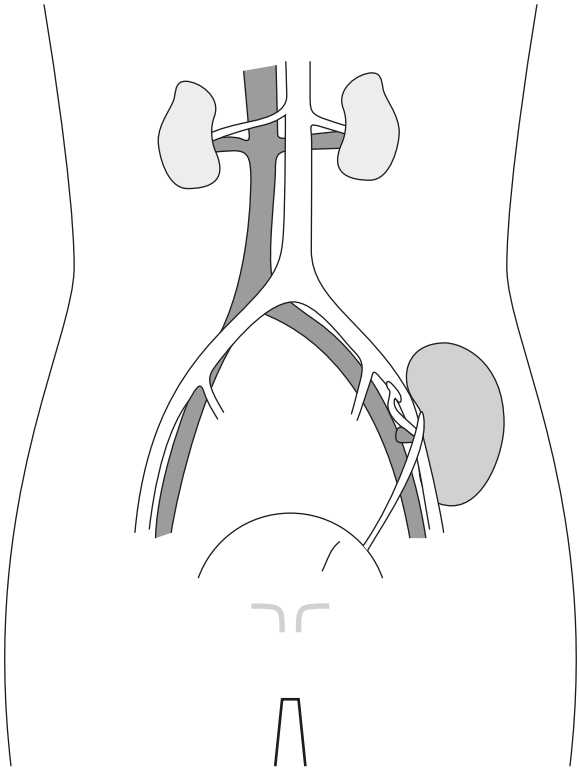

Fig. 278.15 Schematic representation of the heterotopic transplantation of a right-sided deceased donor kidney into the left iliac fossa with vascular anastomoses to iliac vessels.

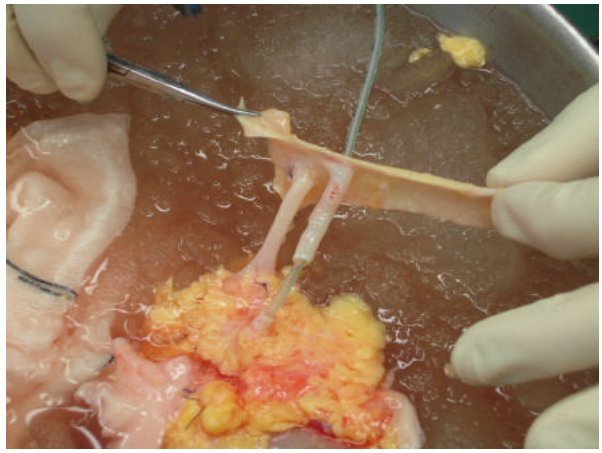

Fig. 278.16 Divided lower pole artery identified at the time of back-table preparation of the deceased donor kidney. Subsequently, the artery was repaired by end-to-end anastomosis using interrupted Prolene® sutures, and an aortic patch created to allow anastomosis of both arteries together.

situations by careful preparation and anticipation of the potential pitfalls. The transplant nephrologists have it easy, and the good ones are not quick to find fault! Equally, a description of the possible complications of kidney transplantation surgery to a patient when obtaining informed consent before surgery can cause alarm (see Chapter 276). They are best put into the context of the individual transplant centre's published results of patient and graft survival at 1 year. Ideally, the individual transplant surgeon's own results will be peer reviewed on a regular basis, within and outside their own transplant centre.

All donor kidneys require back-table preparation and failure of the surgeon to examine the deceased donor kidney before starting the recipient procedure can create problems if the kidney is not 'as advertised' by the donor surgeon. Accessory arteries may have been missed or divided (Fig. 278.16). Atheromatous plaque, clot, or an intimal flap may be impinging on the lumen of the renal artery. If problems are identified and corrected before surgery, operating and anastomosis times are kept to a minimum and surgical options are retained. Donor artery and vein are mobilized as necessary, with perirenal adipose tissue trimmed, gonadal vein removed, and, in the case of a deceased donor kidney, adrenal gland removed. Haemostasis after revascularization of the transplanted kidney is easier if venous tributaries and small hilar vessels associated with trimmed tissue are ligated.

Living donor kidneys, particularly in the era of laparoscopic donor nephrectomy, are more likely to have more than one artery to anastomose, leaving the option for the surgeon to undertake two arterial anastomoses or join the two together to fashion a single orifice for anastomosis. The short renal vein of right-sided living donor kidney is more of a challenge but anastomosis can be facilitated by either lengthening the donor renal vein using recipient long saphenous vein or recently banked deceased donor iliac vein. Easier however, is mobilization of the recipient external iliac vein by dividing the internal iliac vein tributaries. The decision is best left until the recipient iliac vein is exposed at time of recipient surgery.

Deceased donor kidneys, in comparison, present more options with use of the donor aortic patch. The artery of a right-sided kidney may be too long and result in kinking if not shortened. The vein of a right-sided kidney can be easily elongated if necessary using adjacent donor IVC (Fig. 278.17). Nevertheless, objective evidence to support the greater ease of transplantation of the left kidney is found in Australian registry data that compared outcomes of left and right deceased donor kidney pairs (Vacher-Coponat et al., 2013). Recipients of right-sided kidneys were at significantly greater risk of developing delayed graft function and had inferior graft function because of greater risk of graft loss in the 3 months after transplantation, and principally, because of surgical misadventure. The authors recommended that the more experienced surgeons within a transplant centre be allocated the right donor kidneys to implant.

The next decision is the side of recipient surgery. By convention, and the authors' preference, is to place the left-sided donor kidney into the recipient's right side and vice versa. The rationale is that transplant ureter is more likely to require surgery to correct a complication than the transplant vasculature and is more easily performed if the collecting system is medial to the vessels. Also the alignment of the transplant renal artery and vein always seems to sit better this way around. However, most surgeons are right-hand predominant and find it easier to operate when standing on the right side of the operating table. Hence, always check the original operation report when re-operating on a kidney transplant

Limiting the extent of the dissected iliac artery limits disruption of adjacent lymphatic channels returning about 300 mL of lower limb lymph each day to the central venous system. Meticulous ligation of even the smallest lymphatic trunk with non-absorbable or

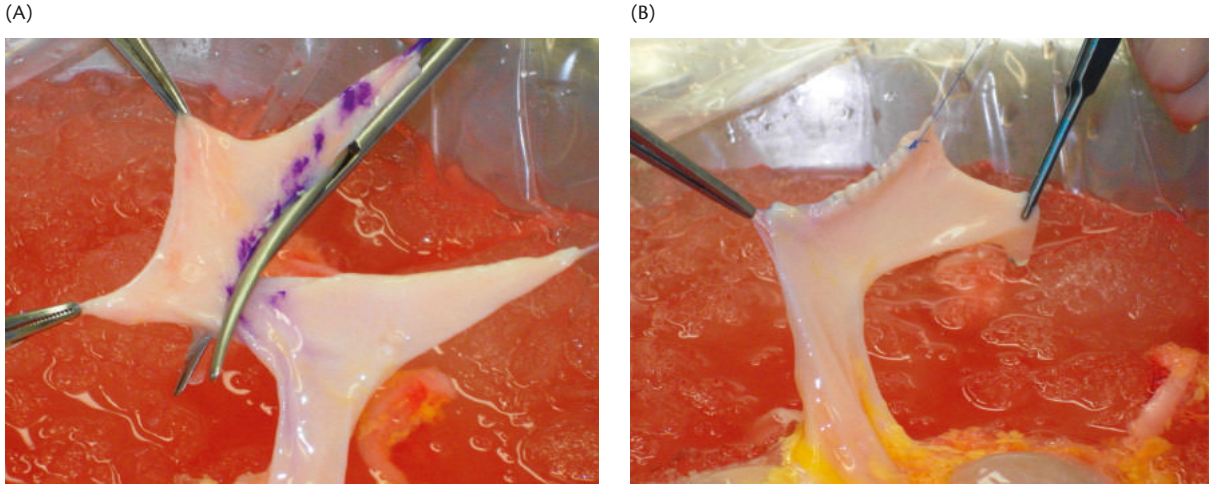

Fig. 278.17 Anastomosis of the deceased donor right renal vein can be facilitated by elongation of the vein using the adjacent inferior vena cava (IVC). For this reason, the IVC is always left attached to the right kidney at time of donor kidney retrieval surgery. (A) The IVC is trimmed and (B), fashioned into tube.

slowly absorbed ligature material during mobilization of the iliac vessels is crucial to the prevention of lymphocoeles. If the internal iliac artery is to be used, the authors' preferred option for living donor kidneys (Fig. 278.18), the surgeon inspects the bifurcation of the common iliac artery and carefully examines the origin for atheromatous plaque. Use of the internal iliac artery is avoided if the opposite-side artery has been involved in a previous transplant. The bifurcation of the internal iliac artery should be preserved to reduce the risk of buttock claudication. If both internal iliac arteries have been used for transplantation, claudication is inevitable, as is impotence.

The renal vein is anastomosed first and followed by the artery. The time taken for anastomoses is deemed warm ischaemia time. An achievable target for the time between removal of the donor kidney from ice saline slush to completion of revascularization in the recipient is 30 minutes. Well-preserved kidneys, in particular those from a live donor source, can better tolerate longer ischaemia times. This is fortunate because living donor kidneys are more likely to have multiple arteries to anastomose (Fig. 278.19). Reperfusion is the high point of the transplant procedure—there is no turning back. Before completing the arterial anastomosis, air is excluded from the clamped vessels by injecting heparinized saline. Fixed

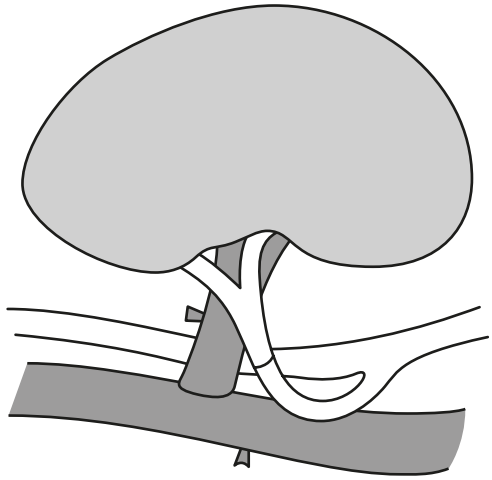

Fig. 278.18 Schematic diagram to demonstrate the authors' preference for the anastomosis of a left-sided living donor kidney with a single artery to the divided right internal iliac artery.

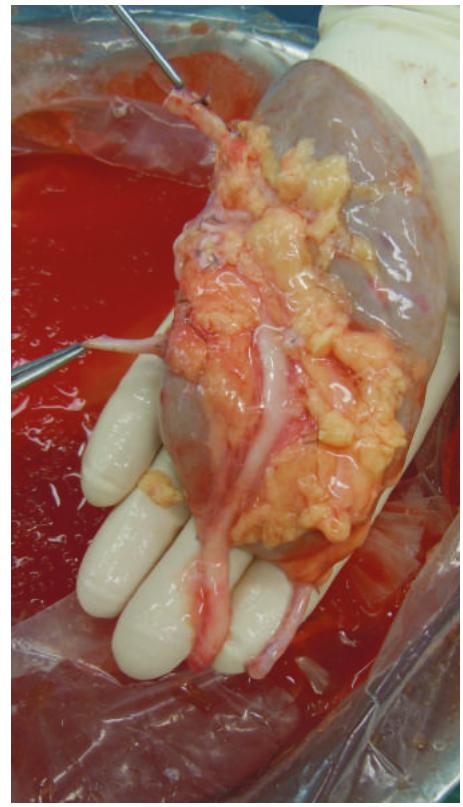

Fig. 278.19 The left-sided living donor kidney previously demonstrated in Figs 278.2 and 278.4. Note the three renal arteries and the single donor ureter. The two medial arteries were anastomosed separately to the recipient iliac artery, end-to-side, before revascularization after 45 minutes. The third artery at the lower pole was then anastomosed to the end of the donor inferior epigastric artery.

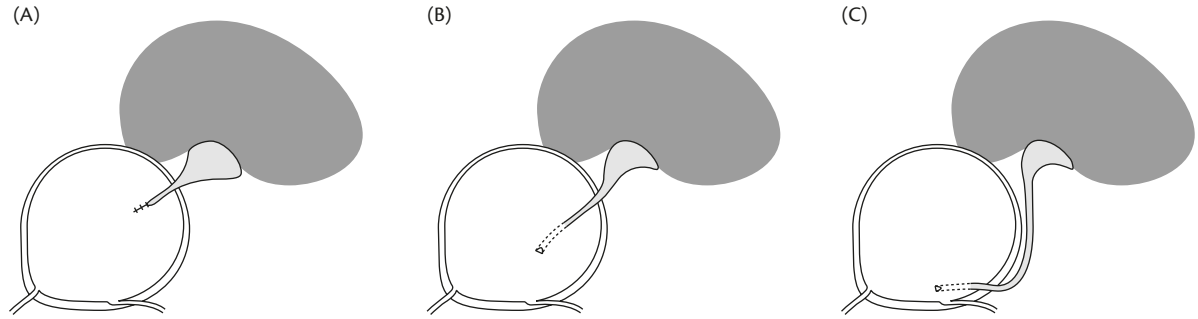

Fig. 278.20 Three commonly used ureteric anastomosis techniques are schematically demonstrated. (A) Submucosal tunnel for the donor ureter is fashioned to minimize vesicoureteric reflux, with placement of the end of the ureter near the bladder trigone. (B) A shorter length of ureter is tunnelled on the lateral wall of the bladder. (C) The extravesical technique involves anastomosis of the dome of the bladder.

retractors that might compress proximal iliac vessels are reviewed and individual anastomoses are tested before revascularization of the transplanted kidney. Imperfect anastomoses are managed more easily beforehand. The proximal arterial clamp and venous clamps are released first. The last clamp removed is the distal iliac artery clamp after systemic blood pressure has stabilized following reperfusion of the kidney. Observation of urine within a couple of minutes is a reassuring sight—a pink, firm, and well-perfused kidney is the next best thing. If neither is observed, the surgeon actively looks for mechanical problems. Kidneys from marginal donors or with long renal ischaemia times may have a 'blotchy' or mottled appearance with dark, less well-perfused areas. An encouraging sign is the gradual reduction in extent of the dark areas until the kidney is uniformly pink. In the era of sophisticated tissue typing and lymphocytotoxicity, and crossmatching, poor perfusion as a result of hyperacute rejection must now be close to being a non-entity.

The ureteroneocystostomy (UNC), the anastomosis of the ureter to the bladder, should be the relaxing part of the kidney transplant operation. The kidney is positioned to avoid compression of the vascular pedicle and all being well, urine is being produced. The best technique for UNC, either extravesical or intravesical, remains unresolved with only a single published randomized study and no systematic reviews to support one technique over the other (Pleass et al., 1995). Hence, without clear direction, an individual surgeon's preferred UNC technique is likely to be based on their training background in urology, vascular surgery, or in a multiorgan transplant programme.

Urologists are likely to favour their more accustomed intravesical UNC technique, a posterior approach used when re-implanting a native refluxing ureter. A submucosal tunnel for the donor ureter is fashioned to minimize vesicoureteric reflux, with placement of the end of the ureter near the bladder trigone (Fig. 278.20A). Proponents of intravesical UNC argue that the technique is more likely, at least on a theoretical basis, to offer fewer problems associated with ureteric reflux. Its major advantage, however, is the ease of cystoscopic access to investigate and manage ureteric complications. The disadvantage is the need for a longer length of donor ureter that places it at greater risk of ischaemia and greater likelihood of requiring complex reconstructive ureter surgery to resolve (Raman et al., 2013). The authors favour a compromise UNC technique, involving placement of a shorter length of ureter on the lateral aspect of the bladder (Fig. 278.20B). It minimizes the risk of ureteric stenosis and is still easily accessible for retrograde assessment of the transplant collecting system (Fig. 278.21) (Raman et al., 2013).

The extravesical UNC technique is less challenging and involves a simple mucosa-to-mucosa anastomosis using a comparatively short length of donor ureter to the recipient bladder mucosa at the dome of the bladder (Fig. 278.20C). It tends to be the procedure favoured by surgeons without a formal urology training background. Advocates claim fewer problems with haematuria in the early postoperative period and its role is likely to be more important in the anticoagulated recipient and those dependent on antiplatelet agents and recipients with small bladder capacity (Veale et al., 2007). Irrespective of UNC technique used, it behoves the transplanting surgeon to carefully assess the quality of the blood supply of the ureter following revascularization, particularly if dependent on a small lower pole accessory renal artery. Distal ureteric stricture prevention is based to limiting the length of transplanted ureter.

For the great majority of kidney transplant surgeons, the use of a transplant ureteric stent is not controversial because of its role in reducing urine leaks and kinking of the transplanted ureter (Nicol et al., 1993; Pleass et al., 1995; Wilson et al., 2005). They are ordinarily removed by cystoscopy under local anaesthesia as an ambulatory procedure 4–6 weeks after transplantation. Alternatively, some

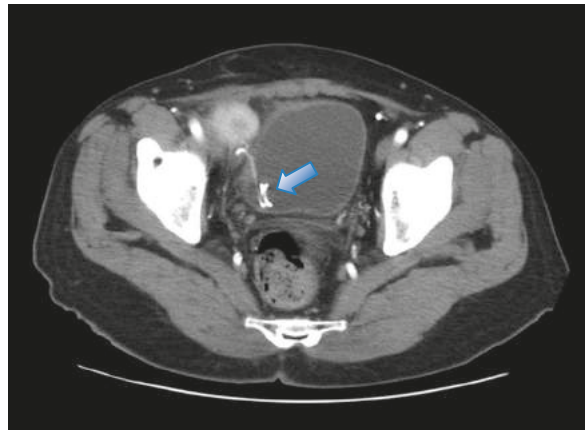

Fig. 278.21 A 4.7 G, 8–22cm double pig-tailed ureteric stent is demonstrated passing from the transplant ureter into the bladder through a submucosal tunnel on the lateral wall of the bladder.

surgeons suture the stent to the tip of the urinary catheter and it is removed along with the catheter some time after transplantation. Detractors exist however, and likely urologists, claiming that the use of the stent covers up poor surgery and exposes recipients to discomfort from the stent, urinary tract infections, need for a second procedure, and added cost (Damji et al., 2013). The authors' greatest concern is the forgotten stent that almost always has an adverse outcome. Every kidney transplant unit must have a stent register.

Finally, it is time for wound closure. A suction drain is inserted with the transplanted kidney on view and a last check is made of the alignment of the transplant vasculature. Hopefully, the rectus abdominis muscle has not been divided on the way in. The muscle layer can be sutured with a continuous absorbable suture followed by subcutaneous fascia and a subcuticular absorbable stitch for the skin, the only part of the operation that the recipient gets to see. Routine use of subcuticular skin closure in Sydney, Australia, has almost eliminated wound infections in the kidney transplant recipient population. A bladder washout is performed on the operating table. If there is no urine, be worried and organize an ultrasound examination in the recovery ward. An attempt should be made to remove the suction drain the next morning—prolonged use encourages lymph drainage and lymphocoele formation. The indwelling urinary catheter is removed in the morning of the fifth day after surgery.

Conclusion

No two kidney transplant procedures are the same. They warrant the input of thinking surgeons who enjoy both the surgical challenges and being part of a multidisciplinary team. Improved short-term kidney graft survival has placed greater importance on their role within that team.

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CHAPTER 279

Immunology, sensitization, and histocompatibility

Thangamani Muthukumar, Darshana Dadhania, Choli Hartono, and Manikkam Suthanthiran

Introduction

The term *immunity* is derived from the Latin word *immunitas* that means exemption or protection. In this formulation, allograft rejection is an immune 'protection' from the histoincompatible allograft and involves a highly orchestrated action of multiple cell types and mediators (Suthanthiran and Strom, 1994). Among the host's cellular elements, lymphocytes are the principal immune cells responsible for the identification of the foreignness of the allograft and mediate rejection by cell-to-cell interactions and via their secretory products including antibodies that bind to antigens displayed by the allograft (Fig. 279.1 and Table 279.1). Alloreactivity—the immune response of the recipient's immune cells directed against the donor—is primarily but not exclusively directed at the proteins encoded by genes located in the donor's major histocompatibility complex (MHC) region that are inherited from both parents in a Mendelian fashion and expressed co-dominantly (Felix and Allen, 2007).

T-cell biology

Transmembrane signalling of T cells: the immune synapse

The immune synapse or supramolecular activation cluster is a structure that forms at the point of physical contact between the T cells and the antigen-presenting cells (APCs). At the synapse, multiple T-cell surface proteins form clusters thereby creating a platform for antigen recognition and generation of T-cell activation-related signals (Davis and Dustin, 2004; Dustin and Depoil, 2011). Depending on the type of APCs, the synapse can appear like a bull's eye or can be multifocal (Dustin and Depoil, 2011). The immune synapse serves several functions:

1. Establishing checkpoints for lymphocyte activation
2. Enhancing signalling
3. Terminating signalling
4. Balancing signalling
5. Directing secretion (Davis and Dustin, 2004).

Besides T cells, interactions involving B cells, natural killer (NK) cells, and phagocytes also form immune synapses.

The T-cell synapse can be considered to have three functional layers: (1) the receptor interaction layer that includes the T-cell receptor (TCR), adhesion molecules, co-stimulatory and co-inhibitory molecules and co-receptors (TCR–CD3 complex, CD4 or CD8, CD2, CD28, and LFA-1); (2) the signalling layer that includes a tyrosine kinase cascade, a nuclear factor kappa B (NF-κB)-activating oligomeric complex, and ubiquitin-dependent signal termination (LCK, ZAP-70, ITK, PLCγ, and PKCθ); and (3) the cytoskeletal layer containing the three filament-forming proteins actin, myosin II, and tubulin (F-actin, talin, paxillin, vinculin, FAK1, and PYK2) (Dustin and Depoil, 2011). The signalling molecules found in synapses are localized in the lipid rafts of the plasma membrane and the signalling initiated in these rafts cause cytoskeletal rearrangements that allow the rafts to coalesce and form the immune synapse. Cell-to-cell communication at the immune synapse is usually bidirectional and the immune synapse is considered to be the focal point for exocytosis and endocytosis (Griffiths et al., 2010; Mittelbrunn et al., 2011).

The first step in the formation of an immune synapse is the slowing down of the T-cell mobility in the circulation. The synapse begins to form when antigen-induced upregulation of T-cell surface protein LFA-1 binds to ICAM-1 on the APCs (Table 279.2). *In vivo* imaging shows that the time from first antigen recognition to arrest is related to the quantity of antigen present with higher concentration of antigens resulting in a faster initiation of stable contacts (Henrickson et al., 2008). These adhesions create intimate contacts between T cells and APCs and thereby provide an opportunity for T cells to recognize antigen. In addition to adhesion, repolarization of T cells is also a critical event. Chemokine signalling is the first type of polarization that prepares the cells for extravasation from the circulation. Subsequent polarization of the migrating lymphocyte results in a protrusive leading forward edge, the lamellipodium, which is highly sensitive to the detection of antigen (Sanchez-Madrid and Serrador, 2009).

The clontypically determined α and β chains on the T cell that recognize the peptide–MHC complex on the surface of APCs comprise the TCR. Following the physical contact, the TCR complex (TCR, CD3, and ζ chains), CD4 or CD8 co-receptors, co-stimulators such as CD28, and enzymes such as PKC-θ are rapidly mobilized to the centre of the synapse. Antigen recognition is called 'direct' when the recipient's T cells bind to donor MHC molecules on the donor APC.

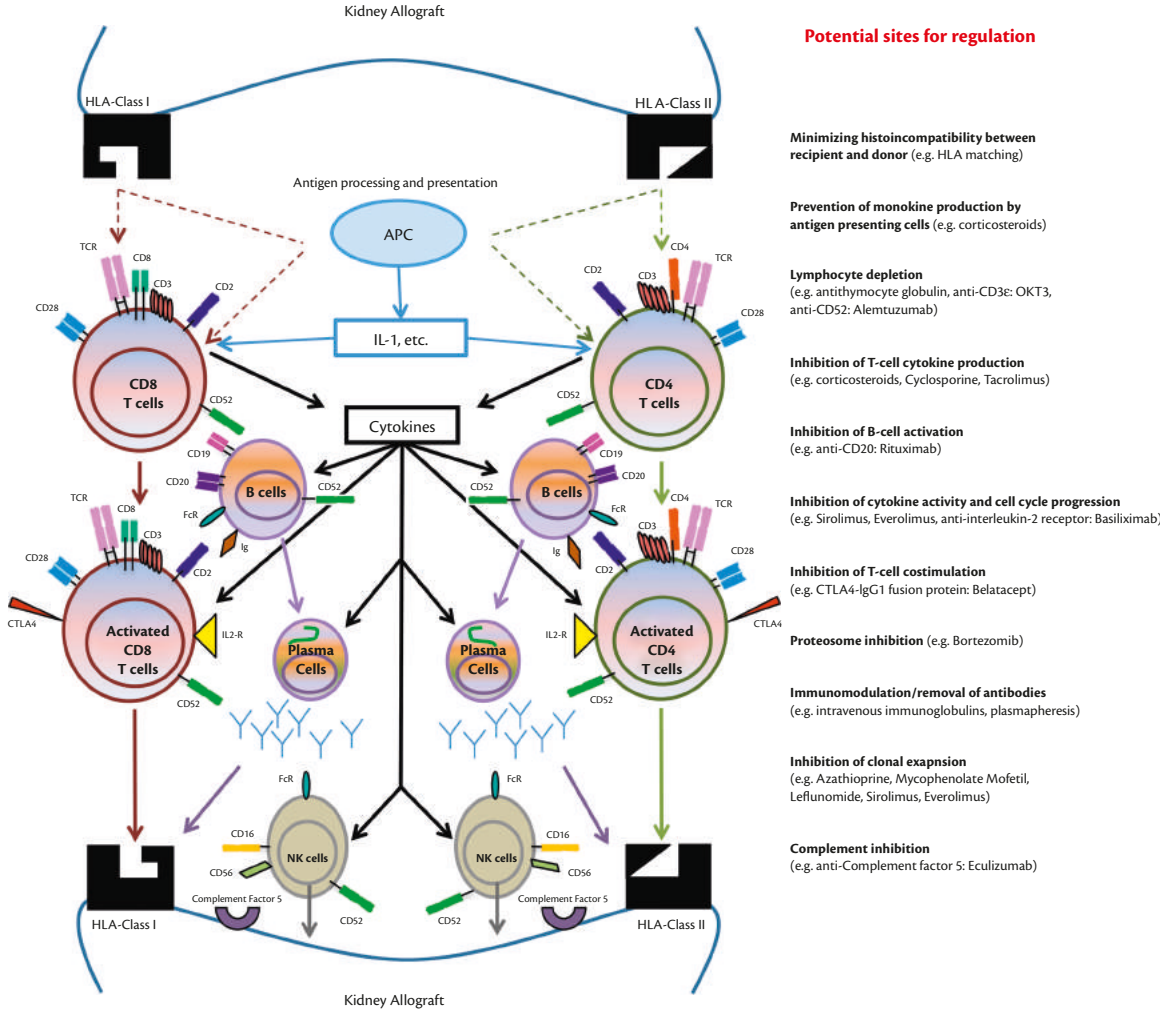

Fig. 279.1 The anti-allograft response. Schematic representation of human leucocyte antigen (HLA), the primary stimulus for the initiation of the anti-allograft response; cell surface proteins participating in antigenic recognition and signal transduction; contribution of the cytokines and multiple cell types to the immune response; and the potential sites for the regulation of the anti-allograft response.

This is contrary to the classical MHC restriction and is fundamentally different from the way the immune system responds to a foreign antigen. Direct recognition is considered to be a cross-reaction between the recipient's TCR (selected to recognize its own MHC molecule plus foreign peptide) and the foreign MHC molecule and a peptide. Thus, a foreign MHC molecule with a bound peptide can sufficiently resemble a self-MHC molecule plus a foreign peptide for binding to occur. Many of the peptides associated with foreign MHC molecules that are involved in direct presentation are derived from proteins that are identical in the recipient and the donor. Both CD4 and CD8 positive T cells directly recognize the donor APCs. Antigen recognition is called indirect when donor MHC molecules are taken up, processed as fragments of approximately 8–16 amino acids, embedded, and presented within the groove of recipient MHC proteins on the recipient's APCs. Indirect recognition resembles the conventional T-cell response to the pathogens. Here CD4 T cells are primarily involved. Transplant-related antigens are acquired by the recipient's APCs through endosomal vesicular pathway due to phagocytosis and are presented by class II MHC molecules on the recipient's APCs (Fig. 279.1) (Jiang et al., 2004; Afzali et al., 2008). The TCR is clonally distributed; clones of T cells with different specificities express different TCRs. Signal transduction in T cells, upon recognition of antigen is not by the TCR itself but proteins CD3 and ζ non-covalently linked to the TCR. The TCR/CD3/ζ complex is composed of clonally distinct TCR-α and -β peptide chains that recognize the antigenic peptide in the context of MHC proteins and clonally invariant CD3 and ζ chains that propagate intracellular signals originating from antigenic recognition (Fig. 279.2) (Suthanthiran, 1990; Dustin and Cooper, 2000; Hennecke and Wiley, 2001; Rudolph et al., 2006). The extracellular portions of α and β chains fold into a structure that resembles the antigen-binding site of an antibody. The regions of greatest variability called complementarity-determining regions are clustered together to form an antigen-binding site. A highly diverse repertoire of TCR variable genes, similar to antibody genes, is generated during T-cell differentiation. In this process of somatic gene rearrangement called variable, diversity, and joining (VDJ) recombination, genes for regions of the clone-specific antigen receptors are

Table 279.1 Cellular elements contributing to the anti-allograft response

Cell type	Functional attributes
T lymphocytes	The T lymphocytes participate in the anti-allograft response. The CD3/TCR complex is responsible for recognition of antigen and generates and transduces the antigenic signal
CD4+ T lymphocytes	CD4+ cells function mostly as helper cells. They secrete cytokines such as IL-2, a T-cell growth/death factor, and IFN-γ, a proinflammatory polypeptide that can upregulate the expression of HLA-proteins as well as augment cytotoxic activity of T cells and NK cells There are three main types of CD4+ T cells: T_H1 cells that produce IL-2 and IFN-γ and are differentiated from T_H-0 cells in the presence of IL-12 and IFN-γ; T_H2 cells that produce IL-4, IL-5, IL-6, and IL-10 and are differentiated from T_H-0 cells in the presence of IL-4; and T_H-17 cells that produce IL-17 and IL-22 and are differentiated from T_H-0 cells in the presence of IL-6 and TGFβ Each cell types regulate the secretion of the other, and the regulated secretion is important in the expression of host immunity
CD8+ T lymphocytes	CD8+ cells function mostly as cytotoxic cells. CD8+ cells can secrete cytokines such as IL-2, and IFN-γ, and can express molecules such as perforin and granzyme that function as effectors of cytotoxicity
Regulatory T lymphocytes	Regulatory T-lymphocytes prevent and suppress immune response. A transcription factor FoxP3 controls the development of these cells. The T-lymphocytes participate in the anti-allograft response. The CD3/TCR complex is responsible for recognition of antigen and generates and transduces the antigenic signal.
B lymphocytes	B-lymphocytes differentiate and produce antibodies directed against donor antigens. The alloantibodies can damage the graft by binding and activating complement components (complement dependent cytotoxicity) and/or binding the Fc receptor of cells capable of mediating cytotoxicity (antibody dependent cell mediated cytotoxicity). B-lymphocytes require the help of T-lymphocytes for differentiation and production of antibodies.
Antigen presenting cells	Monocytes/macrophages, dendritic cells, and B-lymphocytes function as APCs. Under certain conditions, vascular endothelial cells can also function as APCs Donor APCs can process and present donor antigens to recipient's T cells (direct recognition) or recipient APCs can process and present donor antigens to recipient's T cells (indirect recognition) The relative contributions of these two types of recognition to anti-allograft response has not been resolved though it is likely that indirect recognition contributes to chronic rejection
NK cells	The precise role of NK cells (CD3−CD16+CD56+) in the anti-allograft response is not known. They may play a role in rejection as well as in promoting allograft tolerance
Macrophages	The precise role of macrophages (CD68+) in the anti-allograft response is not known. Besides antigen presentation they can also act as effector cells in anti-allograft response

Modified from Suthanthiran M, Morris R. E., and Strom T. B. (1997). Transplantation immunology. In P. C. Walsh, A. B. Retik, E. D. Vaughan Jr., *et al.* (eds.) *Campbell's Urology* (7th ed.) pp. 491–504. Philadelphia, PA: W.B. Saunders Co.

spliced together in a cassette-like fashion during T-cell maturation. Both the TCR-α and -β chains form a single heterodimeric receptor that is responsible for the antigen specificity. A small population of T cells expresses TCR-γ and -δ chains instead of the TCR-α and -β chains. In certain species γδ T cells are abundant in epithelial tissue. These cells do not recognize MHC-associated peptide antigens and may represent an important bridge between innate and adaptive immunity (Born et al., 2006; Scotet et al., 2008; Riganti et al., 2012).

CD4 and CD8 proteins, co-receptors involved in T-cell activation, are expressed on reciprocal T-cell subsets and bind to non-polymorphic domains of human leucocyte antigen (HLA) class II (DR, DP, DQ) and class I (A, B, C) molecules, respectively (Fig. 279.1 and Table 279.2). Class II HLA molecules present peptides derived from extracellular protein antigens whereas class I HLA molecules present peptides derived from intracellular protein antigens. This segregation is due to the specificities of CD4 and CD8 for different classes of MHC molecules. T-cell specific Src family tyrosine kinase Lck is tightly associated with the cytoplasmic tails of CD4 and CD8 and mediates signal transduction function that happens early after TCR-APC interaction. A threshold of TCR to MHC-peptide engagements is necessary to stabilize the immunological synapse stimulating a redistribution of cell surface proteins and co-clustering of the TCR/CD3 complex with the T-cell surface proteins (Brown et al., 1989; Suthanthiran, 1990; Beyers et al., 1992; Lebedeva et al., 2004; Fooksman et al., 2010). The TCR, MHC-peptide, and co-stimulation and signal transduction molecules are segregated in the central region of the synapse, whereas molecules involved in adhesion (CD2-LFA-3, LFA-1-ICAM, CD43, and CD44) localize to the periphery of the synapse. This multimeric complex functions as a unit in initiating T-cell activation. Protein tyrosine kinases (PTKs) catalyse the transfer of a phosphate of ATP to the tyrosine in a substrate protein. In T cells, there are two important families of PTKs: Src and Syk. Following activation by antigen, the TCR/CD3 complex and co-clustered CD4 and CD8 activate Lck, a Src family PTK that is associated with the cytoplasmic tail of CD4 or CD8. Active Lck then phosphorylates the tyrosine in immunoreceptor tyrosine-based activation motifs (ITAMs), the 10-tyrosine containing peptide sequences in the cytoplasmic portions of CD3 chains and ζ chains. Another PTK of the Src family, Fyn, is also associated with CD3 and may play a similar role. The CD45 protein, a membrane bound protein tyrosine phosphatase, activates Src family PTKs by removing autoinhibitory C-terminal phosphates from the Src family kinases and enabling them to assume an active conformation. Once tyrosine is phosphorylated, the ITAMs in the ζ chain become docking sites for ZAP-70, a Syk family PTK. Each ITAM has two tyrosine residues and both must be phosphorylated to dock one ZAP-70. A critical threshold of ZAP-70 is needed prior to downstream signalling and is achieved by recruitment of multiple ZAP-70 molecules.

There are several adapter proteins that are next phosphorylated and are able to bind signalling molecules. Adapter proteins promote activation of multiple signal transduction pathways. The two important early downstream pathways that are activated are the calcium and PKC-mediated signalling pathway and the mitogen-activated protein (MAP) kinase-signalling pathway. Each of these pathways contributes to the expression of genes that are required for clonal expansion of the activated T cells. A key early event in T-cell activation is the ZAP-70-mediated tyrosine phosphorylation of the membrane-anchored adapter protein linker for the activation of T cells (LAT) (Kuhne et al., 2003; Ou-Yang et al.,

Table 279.2 Cell surface proteins important for T-cell activation

T-cell surface	APC surface	Functional response	Consequence of blockade
LFA-1 (CD11a, CD18) ICAM-1 (CD54)	ICAM1 (CD54) LFA-1 (CD11a, CD18)	Adhesion	Immunosuppression
CD8, TCR, CD3 CD4, TCR, CD3	MHC I MHC II	Antigen recognition	Immunosuppression
CD2 CD40L (CD154) CD5	LFA-3 (CD58) CD40 CD72	Co-stimulation	Immunosuppression
CD28	B7-1 (CD80)/B7-2 (CD86)	Co-stimulation	Anergy
CTLA4 (CD152)	B7-1 (CD80)/B7-2 (CD86)	Inhibition	Immunostimulation
ICOS	ICOS-L (CD275)	Co-stimulation	Immunosuppression
OX40 (CD134)	OX40L (CD252)	Co-stimulation	Immunosuppression
PD-1 (CD279)	PD-L1 (CD274)/PD-L2 (CD273)	Inhibition	Immunostimulation

Receptor counter-receptor pairs that mediate interactions between T-cells and APCs are shown. When TCR recognize MHC-associated peptide on the APC an immune synapse is formed. This region of physical contact between the T-cell and the APC has plasma membrane that have a lipid concentration different from the rest of the cell membrane. TCR and costimulatory signalling is initiated in these lipid rafts. Several receptor proteins are rapidly mobilized into the synapse. Some of the receptor proteins are present in naïve cells while several of them are induced following activation. Inhibition of each of these protein-to-protein interactions, except the CTLA4 with B7-1/B7-2 and the PD-1 with PD-L1/PD-L2 interaction, results in an abortive *in vitro* immune response. T-cell activation, proliferation, and cell survival is thought to require three signals; interaction of receptor proteins during antigen binding generates the first signal, interaction of costimulatory proteins generates the second signal, and, inflammatory cytokines generate the putative third signal polarizing the T-cells into various phenotypes. In the absence of signal 2, T-cells that encounter antigen fail to respond and die by apoptosis or become anergic. Signal 2 through CD28 in the absence of signal 1 also makes the T-cell anergic. Of all the costimulation molecules, CD28-B7 interaction is the best characterized. An essential function of CD28-B7 pathway is the generation of regulatory T-cells.

Modified from Suthanthiran M, Morris R. E., and Strom T. B. (1997). Transplantation immunology. In P. C. Walsh, A. B. Retik, E. D. Vaughan Jr., *et al.* (eds.) *Campbell's Urology* (7th ed.) pp. 491–504. Philadelphia, P.A: W.B. Saunders Co.

2012). Bringing together the activated TCR and LAT is a critical event in T-cell signalling and LAT serves to link several components of TCR signalling pathways with their upstream activators. There are two views about the steps involved in the coming together of TCR and LAT. One suggests that TCR and LAT are initially segregated in protein islands in a protein-poor lipid sea on the plasma membrane and these islands tile together during signalling without mixing (Lillemeier et al., 2010). The other suggests that LAT is localized in subsynaptic vesicles that dock with the engaged TCR clusters (Purbhoo et al., 2010; Williamson et al., 2011). Phosphorylated tyrosines of LAT serve as docking sites for Src homology 2 (SH2) domains of other adapter proteins and enzymes involved in several signalling cascades. Phosphorylated LAT directly binds and activates cytosolic enzyme PLCγ1 and triggers a cascade of events that lead to full expression of T cell programmes: hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) and generation of two intracellular messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). The enzyme IP_3, in turn, mobilizes ionized calcium from intracellular stores, while DAG in the presence of increased cytosolic free Ca^{2+}, binds to and translocate protein kinase C (PKC)—a phospholipids/Ca^{2+}-sensitive protein serine/threonine kinase—to the membrane in its enzymatically active form. Sustained activation of PKC is dependent on DAG generation from hydrolysis of additional lipids such as phosphatidylcholine. The depletion of intracellular calcium activates a plasma membrane ion channel CRAC that facilitates the influx of extracellular calcium. Cytosolic free calcium binds to calmodulin and acts as a signalling molecule. The increase in intracellular free Ca^{2+} and sustained PKC activation promote the expression of several nuclear regulatory proteins such as nuclear factor of activated T cells (NFAT), NF-κB, and the activation, and expression of genes central to T-cell growth such as interleukin (IL)-2 and receptors for IL-2 and IL-15.

Phosphorylated LAT also serves as a docking site for Grb-2, another adapter protein, and the complex then generates a small G protein, Ras-GTP, on the plasma membrane. The plasma membrane, in turn, activates one member of the MAP kinases family of enzymes that results in the generation of ERK which is one of the three main MAP kinases in T-cells; the other two being JNK and p38 generated in parallel with the activation of another G protein, Rac-GTP. Both the Ras-GTP and Rac-GTP pathways generate the two components of a transcription factor activation protein 1 (AP-1).

The three transcription factors, NFAT, NF-κB, and AP-1 appear critical for most T-cell responses. NFAT is required for the expression of IL-2, IL-4, tumour necrosis factor (TNF), and other cytokine genes. Calcium–calmodulin complex also activates calcineurin, a serine/threonine phosphatase that in turn dephosphorylates the inactive NFAT thus rendering it active and allowing its translocation into the nucleus. Inhibition by ciclosporin and tacrolimus of the phosphatase activity of calcineurin is considered central to their immunosuppressive activity. Cytosolic NF-κB in the resting T cells is in complex with other proteins called inhibitors of kappa B (IκB). TCR signalling serine phosphorylates and degrades IκB allowing now active NF-κB to translocate into the nucleus. The transcription factor AP-1 physically associates with other transcription factors

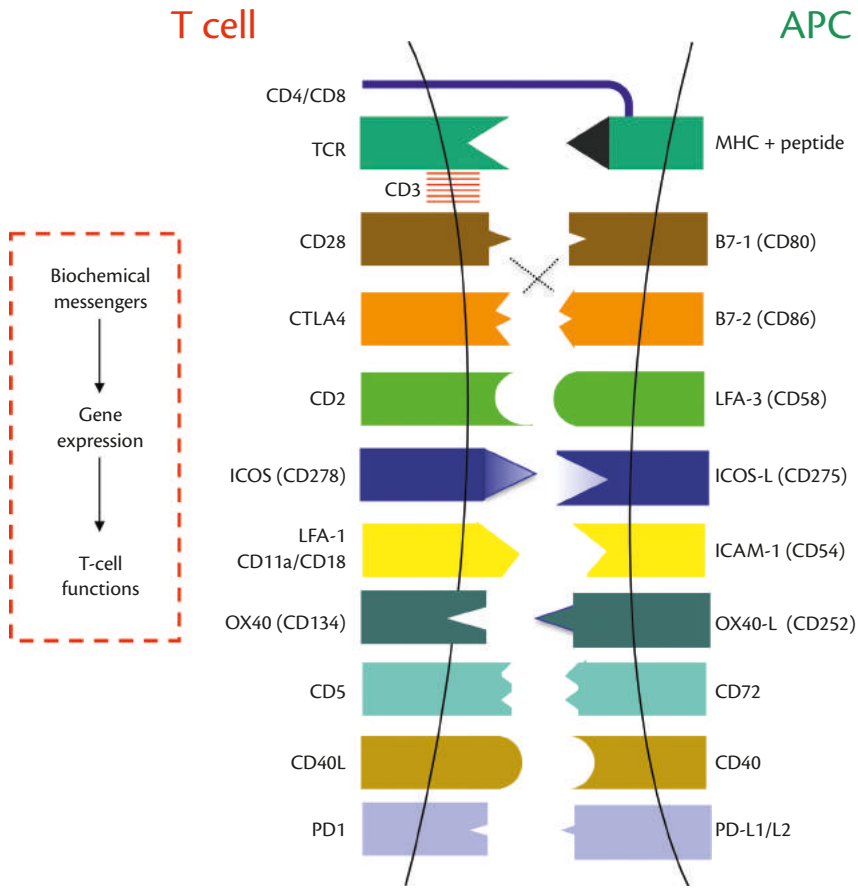

Fig. 279.2 T-cell/antigen-presenting cell contact (APC) sites. In this schema of T-cell activation, the antigenic signal is initiated by the physical interaction between the clonally variant TCR, heterodimer, and the antigenic peptide displayed by MHC on APCs. The antigenic signal is transduced into the cell by the CD3 proteins. The CD4 and the CD8 antigens function as associative recognition structures and restrict TCR recognition to class II and class I antigens of MHC, respectively. Additional T-cell surface receptors generate the obligatory co-stimulatory signals by interacting with their counter receptors expressed on the surface of the APCs. The simultaneous delivery to the T cells of the antigenic signal and the co-stimulatory signal results in the optimum generation of second messengers (such as calcium), expression of transcription factors (such as nuclear factor of activated T cells), and T-cell growth promoting genes (such as IL-2). The CD28 antigen as well as the CTLA4 antigen can interact with both B7-1 and B7-2 antigens. The CD28 antigen generates a stimulatory signal while the CTLA4 generates an inhibitory signal. CD- cluster of differentiation or cluster of designation, the protocol used for identifying cell surface molecules; CTLA4 = cytotoxic T-lymphocyte antigen 4; ICAM = intercellular adhesion molecule; ICOS = inducible T-cell co-stimulator; LFA = lymphocyte function-associated antigen.

Adapted from Suthanthiran, M. (1996). Transplantation tolerance: fooling mother nature. *Proceedings of the National Academy of Sciences USA* 93, 12072–5. Copyright (1996), National Academy of Sciences, USA.

in the nucleus and works best in combination with NFAT. Thus AP-1 represents the convergence of several TCR-initiated signalling pathways.

Signalling of T cells via the TCR/CD3 complex (antigenic signal) is necessary, but insufficient, in itself to induce maximal T-cell proliferation; plenary activation is dependent on both the antigenic signals and the co-stimulatory signals engendered by the physical interactions among the cell surface proteins expressed on antigen-specific T cells and those displayed on APCs (Fig. 279.2 and Table 279.2) (Suthanthiran, 1993). Of all the APCs, mature dendritic cells express the highest level of co-stimulatory proteins. While some of the co-stimulatory proteins are expressed in naïve T cells, several of them are expressed following activation of T cells. Interaction of the CD28 protein on the T cell with the B7-1, B7-2 (CD80, CD86) protein on the APCs, the ICOS protein with the ICOS-L (CD 275), the CD2 protein with the CD58 (LFA-3), CD40L (CD154) with CD40, CD5 with CD72, OX40 (CD134) with OX40L (CD252), and CD27 protein with CD70 contribute to the generation of the obligatory co-stimulatory signal. The best characterized T-cell co-stimulation pathway is the interaction of CD28 protein on the T-cell surface with the B7-1 (CD80) protein expressed on activated APCs (Wang and Chen 2004). CD28 is an enhancer for T-cell activation. In the absence of this second signal, T cells either remain unresponsive or become actively tolerant to antigens. CD28-mediated signals increase the production of cytokines as well as promote the survival of T cells by increasing the expression of anti-apoptotic proteins. Monocytes and dendritic cells constitutively express CD86. Cytokines (e.g. granulocyte-macrophage colony stimulating factor or interferon (IFN)-γ) stimulate expression of CD80 on monocytes, B cells, and dendritic cells. In general, CD86 is the more abundant in terms of expression, and is increased more rapidly upon activation. CD28 binding of B7 molecules activates phosphatidylinositol-3 (PI-3) kinase and facilitates the activation of Ras/ERK MAP kinase pathway and Akt kinase. CD28 also

activates the Ras/JNK pathway. All these pathways promote T-cell survival, cytokine production, and T-cell proliferation. While CD28 is an important co-stimulatory protein, it is not absolutely required for T-cell proliferation. In CD28 knockout mice, response of T cells to antigen is not severely impaired. Interestingly, these mice lack germinal centres, suggesting a gross defect in the ability of T cells to interact with B cells (Ferguson et al., 1996).

The formulation that full T-cell activation is dependent on the co-stimulatory signal as well as the antigenic signal is significant, as it implies that the T-cell molecules responsible for co-stimulation and their cognate receptors on the surface of APCs represent target molecules for the regulation of the anti-allograft response. Indeed, as a confirmation of its importance, transplantation tolerance has been induced in experimental models by targeting cell-surface molecules that contribute to the generation of co-stimulatory signals.

A fine balance between the stimulatory and the inhibitory signals regulate T-cell activation and effector functions. Signalling in T cells is attenuated by the inhibitory receptors, phosphatases, and ubiquitin ligases. Though co-stimulatory pathways were discovered as mediators of T-cell activation, homologous molecules are involved in inhibiting T-cell activation (Acuto et al., 2008). The key inhibitory receptor is the cytotoxic T-lymphocyte antigen 4 (CTLA-4), a member of CD28 family. The mechanism of CTLA-4-induced inhibition is not clear. CTLA-4 can bind to B7 protein on APCs and competitively inhibit the ability of CD28 to bind to B7 or upon binding to B7; CTLA-4 can recruit SHP-2 phosphatase to the synapse and thus block normal phosphorylation of TCR-associated ζ chains. The higher affinity of CTLA-4, as compared to CD28, to B7 may determine the differential binding of stimulatory CD28 and inhibitory CTLA-4 to the same B7 on APCs. The immunosuppressive drug belatacept, a recombinant immunoglobulin–CTLA-4 fusion protein, binds B7 on APCs with a higher affinity than CD28, thus preventing CD28-B7 interaction and thereby inhibiting co-stimulation. The other inhibitory receptor is the programmed cell death 1 (PD-1) protein on the T cells that binds to PD-L1 and PD-L2 on the APCs.

Activated T cells recruit Cbl-b (the prototypical ubiquitin E3 ligase, enzymes that tag ubiquitin to target proteins and mark them for degradation) to the TCR complex where it promotes the ubiquitination of CD3, ZAP-70 and other proteins of TCR complex and their eventual degradation in lysosomes. Co-stimulation through CD28 blocks Cbl-b and augments TCR signals. Ubiquitination is not just a proteolytic recycling system but is thought to have a multifunctional process that can affect protein stability, intracellular trafficking, or functional interactions (Welchman et al., 2005). Cbl-b knockout mice T cells are activated without CD28 co-stimulation and produce high amounts of IL-2. Moreover, the enzymatic function of Cbl-b is required for Foxp3 expression in transforming growth factor (TGF)-β–induced regulatory T cells (Wohlfert et al., 2006).

Interleukin 2/interleukin 15-stimulated T-cell proliferation

Signal transduction by the TCR complex culminates in the transcription of several genes that includes the T-cell growth, survival, and differentiation factor IL-2. Autocrine and paracrine types of T-cell proliferation occur as a consequence of the T-cell activation-dependent production of IL-2 and the T-cell expression of multimeric high-affinity IL-2 receptors formed by the non-covalent association of three IL-2-binding α, β, and γ chains. IL-15 is a paracrine-type T-cell growth factor family member with very similar overall structural and identical T-cell stimulatory qualities to IL-2. The IL-2 and IL-15 receptor complexes share β and γ chains that are expressed in low abundance upon resting T cells; expression of these genes is amplified in activated T cells. The α chain receptor components of the IL-2 and IL-15 receptor complexes are distinct and expressed upon activated, but not resting, T cells. IL-2 is mainly produced by CD4+ T cells at the immune synapse. Secreted IL-2 is a 14–17 kD molecule that folds into a globular protein with four α-helices. In naïve and effector T cells, functional IL-2 receptors are induced on activation whereas regulatory T cells always express IL-2 receptors. The intracytoplasmic domains of the IL-2 receptor β and γ chains are required for intracellular signal transduction. The ligand-activated, but not resting, IL-2/IL-15 receptors are associated with intracellular PTKs. The IL-2R signalling system proceeds through three different pathways: Shc/Ras/Raf-1/MAP kinase pathway, JAK1/JAK3/STAT5 pathway, and PI 3-Kinase/AKT/p70 S6 kinase pathway.

IL-2 was identified first as a T-cell growth factor. However, IL-2 exerts several actions on CD4+ T-cell differentiation that includes promotion of T-helper type 1 cell (Th1) differentiation by inducing IL-12Rβ2, Th2 differentiation by inducing IL-4Rα, Treg differentiation by inducing IL-2Rα, and inhibition of Th17 differentiation by inhibiting gp130 (and IL-6Rα). Besides, IL-2 promotes the development of naïve CD8+ T cells into effector or memory T cells. IL-2 also induces, through cellular degradation of FLIP, a process known as activation-induced cell death, by which T cells undergo apoptosis following repeated antigenic stimulation. It is interesting and probably significant that IL-2, but not IL-15, triggers apoptosis of antigen-activated T cells. In this way, IL-15-triggered events are more detrimental to the allograft response than IL-2. As T cells do not produce IL-15, its expression is not regulated by ciclosporin or tacrolimus (Waldmann, 2006; Liao et al., 2011).

B-cell activation

B cells recognize antigens and are activated in the lymphoid tissues. B cells enter the lymph node follicle 'invited' by the chemokine CXCL13 which is secreted by the lymph node stromal cells and follicular dendritic cells. Activation of antigen-specific B cells is initiated by the binding of the antigen to the membrane immunoglobulin (Ig) molecules. The B-cell antigen receptor complex is made of membrane IgM and IgD associated with the invariant Igα and Igβ molecules that contain ITAMs in their cytoplasmic tails. The Igα and Igβ molecules in B cells function in a similar way to CD3 and ζ proteins in the T cells. Following cross-linking of membrane Ig by the antigen, tyrosine phosphorylation of the ITAMs takes place. Subsequently several calcium-dependent and calcium-independent enzymes are activated culminating in expression of several transcription factors (e.g. Fos, JunB). IgG, IgA, and IgE on B cells that have undergone isotype switching use the same pathways (Batista and Harwood, 2009).

Complement components play an important role in B-cell activation (Carroll, 2004; Dunkelberger and Song, 2010). B cells express CR2 (CD21), which is a receptor for C3d, a degradation product of complement factor 3 (C3). The CD21-CD19-CD81 proteins on the B-cell membrane are termed the B-cell co-receptor complex. Antigen and C3d binding to the co-receptor complex activates several kinases that result in B-cell activation. B-cell

response to protein antigens requires recognition of the antigen by the T-helper cells and antigen-specific T- and B-cell cooperation. Antigen-activated T cells migrate towards the lymph node follicle following a chemokine gradient. The same antigen that activates the T cells also activates the B cells. T and B cells interact physically and the B cells are activated by the binding of the CD40 protein on the B-cell surface with the CD40L on the T-cell surface and cytokines provide co-stimulatory signals. Activated B cells differentiate into antibody-secreting plasma cells. Secreted antibodies form complexes with antigens that simultaneously bind to antigen receptors (antigen-Ig) and Fcγ receptors (Fc portion of antibody-FcγIIB) on antigen-specific B cells, causing inhibition of signalling by the BCR complex and thus inhibiting continued B-cell activation.

Immunobiology of allograft rejection

The net consequence of cytokine production and acquisition of cell-surface receptors for these transcellular molecules by the T cells is the emergence of antigen-specific and graft destructive T cells (Fig. 279.1). With help from T cells, the humoral arm of immunity is activated, resulting in production of donor-specific antibodies. Moreover, IFN-γ and TNF-α can amplify the ongoing immune response by upregulating the expression of HLA molecules as well as co-stimulatory molecules (e.g. B7) on graft parenchymal cells and APCs (Fig. 279.1). CD8+ cytotoxic T-lymphocyte (CTL)-mediated killing of target cells is mainly accomplished by the directed release of perforin and granzyme, as well as by FasL–Fas interaction, all of which lead to the activation of several apoptotic pathways. Antibodies cause target cell destruction by complement-dependent or complement independent mechanisms. Donor antigen-specific CTLs and anti-HLA antibodies are present during or preceding a clinical rejection episode (Strom et al., 1975; Suthanthiran and Garovoy, 1983). Messenger RNA (mRNA) encoding the CTL selective serine protease (granzyme B), perforin, Fas ligand, and immunoregulatory cytokines, such as IL-10 and IL-15, are detected within human renal allografts undergoing acute rejection (Strom and Suthanthiran, 2000). Non-invasive methods for the molecular diagnosis and prognostication of rejection are being developed (Li et al., 2001; Muthukumar et al., 2005). Using either peripheral blood or urinary cells, rejection-related gene expression events evident in renal biopsy specimens are also detected in peripheral blood or urinary sediment specimens. Assays for the measurement of mRNAs in urinary and blood cells for the non-invasive diagnosis and prognostication of acute rejection have been successfully developed and validated (Anglicheau and Suthanthiran, 2008; Hartono et al., 2010; Heidt et al., 2011). Indeed these gene expression events appear to anticipate clinically apparent rejection (Suthanthiran et al., 2013). Sensitive tests for detection of circulating donor specific antibodies have also been developed with the use of solid-phase immunoassay technology (Luminex® assay) (Tait et al., 2013).

Human leucocyte antigen and renal transplantation

The genes that code for the HLA are located within the short arm of chromosome 6. The class I proteins, HLA-A, -B, and -C, are composed of a 41 kDa polymorphic chain linked non-covalently to a 12 kDa β_2 microglobulin chain that is encoded on chromosome 15. The HLA class I molecules are expressed on all nucleated cells including platelets. The HLA class II molecules, HLA-DR, -DP, and -DQ are composed of an α chain of 34 kDa and a β chain of 29 kDa. MHC class II molecules are constitutively expressed on the surface of B cells, monocytes/macrophages, and dendritic cells. T cells and many non-lymphoid cells such as renal tubular epithelial cells express HLA class II proteins upon stimulation with pro-inflammatory cytokines.

HLA matching

The beneficial impact of HLA matching on renal allograft survival has been demonstrated since the early 1990s. Both the United Network of Organ Sharing (UNOS) scientific renal transplant registry data in the United States and the Collaborative Transplant Study, a robust data registry that draws on > 400 transplant centres in > 45 countries, have demonstrated a significant advantage of HLA matching on 1-year and projected long-term renal allograft survival rates (Takemoto et al., 1992). Reassuringly, the actuarial data have been confirmed with the actual survival data. Since the inception of the US national kidney sharing programme in 1987, > 270,000 kidney transplants have taken place in the United States and the 2010 data analyses confirmed the beneficial effect of HLA matching on allograft survival (Amico, 2010). An analysis of UNOS scientific renal transplant registry data for the first deceased donor transplants that occurred from October 1987 to April 2000 demonstrated a 9.8% difference in the 10-year actual graft survival between zero and five to six ABDR mismatches (Sasaki and Idica, 2010). Similarly, an analysis of the first deceased donor transplants from 1987 to 1997 demonstrated a 17% lower 10-year graft survival rate in those with complete HLA-mismatches compared to those with zero HLA-mismatch (Opelz et al., 1999).

The improvement in the graft survival rate following HLA matching is more apparent when matching is based on better-resolved HLA (HLA splits) than when based on broad (parent) HLA, and the improvement in the graft survival rate between the best-matched and the worst-matched grafts increases with time (Cicciarelli and Cho, 1991). Existing molecular methodologies have already helped resolve the ambiguities associated with the serological identification for HLA-DR (Mytilineos et al., 1994; Opelz et al., 1997). The clinical advantage of molecular matching is suggested by the observation that the 1-year deceased renal allograft survival rate is 87% in patients who received kidneys that are HLA-DR identical not only by the serological methods but also by molecular methods (DNA restriction fragment length polymorphism method) but only 69% in patients who received kidneys that are HLA-DR matched by serological methods alone (Opelz et al., 1991). Molecular typing has also been used to detect mismatches at the HLA-A or HLA-B locus. Mismatches that were missed by conventional serological techniques but identified by molecular techniques were found to adversely impact graft survival (Mytilineos et al., 1997).

These molecular techniques became available for clinical practice in the early 1990s. An analysis of the HLA matching effect using UNOS Kidney Registry Data for transplants that occurred from 1995 to 2009 confirmed the beneficial effect of HLA matching in living related donor kidney transplants (Sasaki and Idica, 2010). For living donor transplants, there is a 14% survival difference in the projected 10-year survival between zero HLA-ABDR mismatch and the three or more HLA-ABDR mismatches. In parent to child combination where they share at least one haplotype, the 10-year survival for zero HLA-ABDR is 77% and it is 10% lower for one to two HLA-ABDR mismatchs and 17% lower for three to four

HLA-ABDR mismatches. Similarly, the projected 10-year survival for two-haplotype-matched sibling transplant is 84% whereas for zero-haplotype matched it is only 67%.

The beneficial effect of HLA matching in living unrelated kidney transplants is less apparent. In the UNOS Registry, among unrelated kidney recipients during 1995 to 2009, the 10-year survival of zero HLA-ABDR mismatch grafts was 83% and was 77% for one to two HLA-ABDR mismatched grafts, 74% for three to four HLA-ABDR mismatched grafts and 73% for five to six HLA-ABDR mismatched allografts. These differences, however, were not statistically significant (Sasaki and Idica, 2010). In contrast, beneficial effect of HLA matching was demonstrated in recipients of living unrelated kidney donors in the International Collaborative Transplant Study for kidney transplants performed from 1992 to 1996 (Opelz, 1998). This effect was also demonstrated among Asian recipients with living unrelated kidney donors where a progressive reduction in 3-year graft survival was noted with the number of HLA-ABDR mismatches (Opelz, 2000). The 3-year graft survival of two HLA-DR mismatched kidney grafts was 69% compared to 87% in the zero HLA-DR mismatched recipient-donor combination.

Analysis of the Eurotransplant database of > 39,000 kidney transplant recipients demonstrated that those with zero HLA-DR mismatch had the best survival compared to one or two HLA-DR mismatches (Doxiadis et al., 2007). Furthermore, the beneficial effect of matching at HLA-A and B loci was not seen in those who had zero mismatches at the HLA-DR locus.

Among the deceased donor kidney transplants performed from 1995 to 2009 in the United States, a statistically significant difference in projected 10-year graft survival between zero and five to six HLA-ABDR mismatches was found for both first and second transplants (Sasaki and Idica, 2010). The difference was even greater using death-censored survival measures. For recipients of first deceased donor kidney transplants, the allograft survival difference between the zero and the five to six HLA-ABDR mismatched grafts was 7% using the standard survival measures and 13% using death-censored analyses. For recipients of second deceased donor kidney transplants, the graft survival difference between zero and the five to six HLA-ABDR mismatched grafts was 8% and 10% for standard versus death-censored analysis, respectively.

Analysis of HLA matching at additional loci has also demonstrated incremental benefit in matching for HLA-A, -B, and -C versus HLA-A and -B alone and for HLA-A, -B, -C, and -DR versus HLA-A, -B, and -C alone (Sasaki and Idica, 2010). The 10-year survival difference between lowest and highest mismatched group was 10% for HLA-A and -B, 6% for HLA-C alone and 14% for HLA-A, -B, and -C. Similarly, the 10-year survival difference between the lowest and highest mismatched groups for HLA-DR alone was 8% and 15% for HLA-A, -B, -C, and -DR. Matching for HLA-DQ locus in addition for HLA-A, -B, and -DR loci did not result in a similar beneficial effect.

Sensitized patients are more likely to benefit from a greater degree of HLA matching. HLA-C locus mismatch among deceased donor recipients was found to be a significant risk factor for graft loss in sensitized patients with panel reactive antibody (PRA) > 10% but not in non-sensitized patients (Tran et al., 2011). Similarly, HLA-DP mismatches influenced 1-year graft survival rates of repeat kidney transplants but not that of first-time kidney transplants (Mytilineos et al., 1997). The impact was particular strong among those with repeat transplant recipients with PRA > 50%.

The clinical benefits of HLA matching have been debated over the past decade with the advent of more potent induction and immunosuppressive therapies, lower early acute rejection rates, and improved short-term allograft survival. Since 1988, acute rejection rates have dropped from 25% to 5% in recent years and 1-year graft survival rates have improved by 10% for both living and deceased donor transplants with 1-year survival rate of > 90% for deceased donors and > 95% for living donor recipients (Amico, 2010). Despite the improvement in short-term survival of kidney transplants, the long-term benefits of HLA matching remain.

Sensitization to HLA

Development of anti-HLA antibodies is complex and dependent on the antigenic load and the immunologic memory of the individual. Commonly cited risk factors for development of anti-HLA antibodies are pregnancies, transfusions, organ transplantation, and cell-based therapies. The degree of sensitization is measured in terms of PRA. Traditionally, PRA was ascertained by testing the potential recipient's serum against a panel of lymphocytes obtained from different individuals in the population and selected to represent most of the HLA and by identifying the number of positive reactions with the lymphocyte panel. More recently, the traditional PRA assay based on the lymphocyte cell panel, that does not differentiate HLA from non-HLA antibodies, has been mostly replaced with a more sensitive solid phase antibody screening assays (Tait, 2009). In these assays, the recombinant allele-specific HLA, eluted from HLA-transfected cell lines are immobilized on beads or microtitre plate wells. The patient's serum is incubated with the immobilized HLA and if the patient has an anti-HLA antibody, it binds to the antigen. The bound antibodies are detected using fluorescence signals. Platforms used for solid phase antibody screening include ELISA, the standard flow cytometer, and multiplexing assays on the Luminex® platform. Each well or bead can either have multiple HLA that mimics a cell or can have a single HLA to allow for precise identification of the antibody target. The values for PRA are calculated similar to the method for the multi-HLA-based assays. For single antigen-based assay, a calculated PRA (CPRA) value is obtained by entering the positive reactions into a calculator (<http://optn.transplant.hrsa.gov/converge/resources/allocationcalculators.asp>) that determines the degree of sensitization based on the frequencies of target antigen(s) in the population. At present, a solid phase antibody screening is required for all potential kidney transplant candidates in the United States. The most commonly used platform, the Luminex® platform, is able to provide the relative strength of the antibody in terms of mean fluorescence intensity (MFI) values (Cecka, 2011).

In non-sensitized males, the risk of developing anti-HLA antibodies following multiple blood transfusions has been found to be < 10% and those who developed anti-HLA antibodies tended to have low titres (PRA < 50%) (Opelz et al., 1981). In contrast, among female patients with history of pregnancies, more than half are found to have circulating anti-HLA antibodies following multiple blood transfusions. Furthermore, 29% of these females were found to have a PRA > 50% following the blood transfusions. Although the use of leucocyte-reduced blood transfusions decreases the risk of sensitization in some patient populations, it has not been shown to prevent sensitization and increase likelihood of kidney transplantation in patients with chronic kidney disease (Scornik et al., 1984; Karpinski et al., 2004). Although the load of the HLA will be

less in leucocyte-reduced blood transfusions, approximately 17% of non-sensitized male patients will develop anti-HLA antibodies following leucocyte-depleted blood transfusions (Balasubramaniam et al., 2012).

Kidney transplantation is a major contributor to sensitization, during the life of the transplant kidney as well as after transplant failure (Akalin and Pascual, 2006). The incidence of *de novo* anti-HLA antibodies during the life of the transplant kidney varies from centre to centre. A prospective international multicentre study demonstrated that approximately 20–25% of individuals develop *de novo* anti-HLA antibodies and found an increased risk of graft loss in those that did (Terasaki et al., 2007). Similar findings were reported in a prospective single-centre study in which 17% of 1229 kidney transplant patients developed anti-HLA antibodies and 6% developed donor specific anti-HLA antibodies (DSA) (Hourmant et al., 2005). The presence of both DSA and non-DSA correlated with poor allograft function and survival. The risk of sensitization rises significantly with loss of transplant kidney function and transplant nephrectomy. Among 104 kidney transplant recipients with allograft failure, 70% became sensitized and 38% reacted against multiple HLA and had anti-HLA antibodies with MFI values of >10,000 using the Luminex® single-antigen bead assay and 64% had antibodies with > 5000 MFI (Scornik and Kriesche, 2011). Before transplantation, 89% of these individuals had either weak or no sensitization. In a study of 119 patients who had weak sensitization prior to kidney transplantation and subsequent allograft failure, 56% became highly sensitized with PRA > 80%, 6–24 months after allograft failure (Augustine et al., 2012). In a subgroup of 95 patients who were weaned off their immunosuppression, the percentage of patients with sensitization increased from 21% to 68%. Independent risk factors for sensitization were HLA mismatches and weaning off from immunosuppressive therapy. Matching for HLA may reduce the risk of sensitization following a kidney transplant. Comparison of PRA values prior to the first kidney transplant with PRA values obtained at the time of re-listing for a second kidney transplant in 16,000 patients demonstrated a positive correlation between the rise in PRA values with an increase in the number of HLA-A, -B, and -DR mismatches at the time of transplant (Meier-Kriesche et al., 2009). The mean change in PRA increased from 0.8% for zero HLA-A, -B, and -DR mismatches to 22% in those with six HLA-A, -B, and -DR mismatches. Strategies to avoid sensitization, especially in the setting of an organ transplant, are needed to optimize care of kidney transplant recipients.

Crossmatch

Crossmatch testing of the recipient's serum for antibodies reacting with the donor's HLA must be performed before renal transplantation proceeds. The standard crossmatch test consists of incubating the serum from the recipient with the donor's lymphocytes in the presence of rabbit serum as a source of complement. The sensitivity of the standard complement-dependent cytotoxicity (CDC) crossmatch test has been increased by the addition of sublytic concentration of antihuman globulin (AHG) to the test system (Buabut et al., 1997).

The presence of cytotoxic antibodies directed at the donor's HLA class I (positive T-cell crossmatch) in the recipient's serum is an absolute contraindication to transplantation as 80–90% of kidney transplants performed in the presence of a positive crossmatch will undergo hyperacute rejection (Williams et al., 1968). The presence of pre-formed antibodies significantly affects transplant outcomes and the crossmatch can help define who can be safely transplanted. Of the patients with a positive crossmatch, 80% go on to lose their kidney grafts, whereas only 4% of patients with a negative crossmatch lose their grafts rapidly (Patel and Terasaki, 1969).

The significance of antibodies reacting with the donor's HLA class II (positive B cell crossmatch) is not fully resolved. Analysis of the UNOS Scientific Registry revealed that 4% of 9031 patients were transplanted with a positive B-cell crossmatch during 1994–95 (Mahoney et al., 2002). Those with a positive B-cell crossmatch had increased risk for acute rejection and graft failure within 6 months of transplantation. In a retrospective single-centre study conducted to evaluate the basis for positive B-cell crossmatch (with a negative T-cell crossmatch), 16% had autoantibodies, 23% had HLA class II antibodies, and 61% had neither anti-HLA nor autoantibodies present (Le Bas-Bernardet et al., 2003). Although a positive B-cell crossmatch was associated with an increased rate of acute rejection (36% vs 21%), the difference was not statistically significant and the 3-year survival was similar to those with a negative B-cell crossmatch. Flow cytometry crossmatches (FCXM) permit detection of lower, sublytic concentrations of complement fixing as well as non-complement fixing antibodies and is thus more sensitive than the AHG-CDC crossmatch. Newer technologies are being investigated to identify complement-fixing antibodies using flow cytometry methods (Won et al., 2006). Initial findings of poor kidney transplant survival in patients with a positive FCXM were confirmed using the more recent UNOS registry data (Cho and Cecka, 2001). Kidney recipients from 1995 to 2007 with a positive T- and B-cell FCXM or those with a positive B-cell FCXM had increased risk of graft loss at 5 years compared to those with a negative T- and B-cell FCXM (Lentine et al., 2008). The median graft survival for those with a negative T- and B-cell FCXM was 10 and 17 years for deceased and living donor kidney transplants while those with a positive T- and B-cell FCXM had a median graft survival of 7.9 and 9.5 years respectively.

In the United States, the use of flow cytometry crossmatch prior to kidney transplantation has increased from 2% in 1987 through 1990 to 36% in 2003 through 2005 (Salvalaggio et al., 2009). Data from > 64,000 transplants performed in the United States in 1999 to 2005 were analysed according to the crossmatch technique that was used. Transplants were divided into those with a negative T- and B-cell FCXM, negative T- and B-cell AHG-enhanced CDC XM, and those who used only T-cell AHG-enhanced CDC XM to allow kidney transplantation to proceed. Use of the T- and B-cell FCXM was associated with 15% reduction in the risk of acute rejection and improved 5-year graft survival. However, it is controversial if a positive FCXM is a contraindication for transplantation (O'Rourke et al., 2000; Vlad et al., 2009; Salvalaggio et al., 2009). In a prospective study evaluating the ability of FCXM to predict acute rejection and graft function of 257 kidney transplant recipients with a negative AHG-CDC XM, the presence of positive FCXM at the time of transplant was not an independent predictor of acute rejection or allograft failure (Wen et al., 2006). In the current era, the use of solid phase antibody test results may enhance the ability to interpret FCXM results. Patients who had a positive FCXM and a positive DSA had the highest risk of acute rejection with sensitivity of 87% and specificity of 73% (Couzi et al., 2011).

With the use of solid phase technology, the concept of virtual crossmatch has entered the field of kidney transplantation (El-Awar

et al., 2005; Vaidya et al., 2006). Virtual crossmatch is performed by using a solid phase assay to characterize the anti-HLA antibody profile of the recipient and to evaluate the relative strength of the antibodies to predict the possibility of a positive CDC XM or FCXM based on the HLA type of the potential donor. As of October 2009 in the United States, a recipient's unacceptable HLA must be listed on the UNOS registry to receive points for sensitization (Cecka et al., 2011). The criteria for listing the unacceptable antigens are developed by each centre independently, but they should reflect the recipient's antibodies in a manner that would result in a positive crossmatch at the recipient centre. In effect, a virtual XM is performed each time a potential donor is identified and the donor is eliminated if a positive crossmatch is anticipated. After implementation of this policy, the overall number of positive crossmatches has decreased by 75% and among sensitized patients with a CPRA > 80%, by 84% (Leffell, 2011). Renal allograft outcomes were evaluated in a prospective study of 233 patients transplanted on the basis of a virtual XM; 190 patients with a negative virtual crossmatch (no CDC XM was performed) and 43 patients with a positive virtual crossmatch and a negative CDC XM (Amico et al., 2011). Negative virtual XM group had a lower incidence of clinical/subclinical antibody mediated rejection at 1 year (8% vs 42%) and a better allograft survival at 2 years (98% vs 91%). Virtual crossmatches have also aided the development and execution of kidney paired donation programmes for incompatible recipient-donor pairs (Ferrari et al., 2012; Leeser et al., 2012).

The correlation between solid phase antibody test results and cell-based crossmatch assays is good but not perfect (Lee and Ozawa, 2007; Ellis et al., 2012). Solid phase antibody assays are more sensitive than the cell-based assay and the clinical significance of low-level anti-HLA antibody detected by solid phase assays remains unknown. However, high-titre antibodies are associated with an increased risk of antibody-mediated rejection and chronic graft dysfunction following kidney transplantation (Kaneku, 2010). Guidelines have been developed to assist the transplant professionals on the use and interpretation of data from solid phase antibody assays (Tait et al., 2013). Virtual crossmatches, however, pose a new challenge to the clinicians—how to maximize opportunities for transplantation while minimizing rejection risk and optimizing clinical outcomes following kidney transplantation.

Transplantation tolerance

Transplantation tolerance can be defined as the failure of the organ graft recipient to express a graft-destructive immune response in the absence of immunosuppressive therapy. While this statement does not restrict either the mechanistic basis or the quantitative aspects of immune unresponsiveness of the host, true tolerance is antigen specific, induced as a consequence of prior exposure to the specific antigen, and is not dependent on the continuous administration of immunosuppressive drugs.

A classification of tolerance on the basis of the mechanisms involved, site of induction, extent of tolerance, and the cell primarily tolerized is provided in Table 279.3. Induction strategies for the creation of peripheral tolerance are listed in Table 279.4.

Several hypotheses, not necessarily mutually exclusive and, at times, even complementary, have been proposed for the cellular basis of tolerance. Data from several laboratories support the following mechanistic possibilities for the creation of a tolerant state: clonal deletion, clonal anergy, and immunoregulation.

Table 279.3 Classification of tolerance

A. Based on the major mechanism involved
1. Clonal deletion
2. Clonal anergy
3. Suppression
B. Based on the period of induction
1. Fetal
2. Neonatal
3. Adult
C. Based on the cell tolerized
1. T cell
2. B cell
D. Based on the extent of tolerance
1. Complete
2. Partial, including split
E. Based on the main site of induction
1. Central
2. Peripheral

Clonal deletion

Clonal deletion is a process by which self-antigen-reactive cells (especially those with high affinity for the self-antigens) are eliminated from the organism's immune repertoire. This process is called central tolerance. In the case of T cells, this process takes place early in life in the thymus, and the death of immature T cells is considered to be the ultimate result of high-affinity interactions between a T cell with productively rearranged TCR and the thymic non-lymphoid cells, including dendritic cells that express the self-MHC antigen. This purging of the immune repertoire of self-reactive T cells is termed negative selection and is distinguished from the positive selection process responsible for the generation of the T-cell repertoire involved in the recognition of foreign antigens in the context of self-MHC molecules. Clonal deletion or at least marked depletion of mature T cells as a consequence of apoptosis can also occur in the periphery (Van Parijs and Abbas, 1998). The form of graft tolerance, occurring as a consequence of mixed haematopoietic chimerism, entails massive deletion of alloreactive clones (Wekerle et al., 1998). Tolerance to renal allografts has been achieved in patients that have accepted a bone marrow graft from the same donor (Sayegh et al., 1991; Spitzer et al., 1999). It is interesting that IL-2, the only T-cell growth factor that triggers T-cell proliferation as well as apoptosis, is an absolute prerequisite for the acquisition of organ graft tolerance through use of non-lymphoablative treatment regimens (Dai et al., 1998; Li et al., 1999). Tolerance achieved under these circumstances also involves additional mechanisms, including clonal anergy and suppressor mechanisms (Suthanthiran, 1996; Waldmann, 1999; Li et al., 2000).

Clonal anergy

Clonal anergy refers to a process in which the antigen-reactive cells are functionally silenced. The cellular basis for the

Table 279.4 Potential approaches for the creation of tolerance

A. Cell depletion protocols
1. Whole-body irradiation
2. Total lymphoid irradiation
3. Panel of monoclonal antibodies
B. Reconstitution protocols
1. Allogeneic bone marrow cells with or without T-cell depletion
2. Syngeneic bone marrow cells
C. Combination of strategies A and B
D. Cell-surface molecule targeted therapy
1. Anti-CD4 mAbs
2. Anti-ICAM-1 + anti-LFA-1 mAbs
3. Anti-CD3 mAbs
4. Anti-CD2 mAbs
5. Anti-IL-2 receptor α (CD25) mAbs
6. CTLA4Ig fusion protein
7. Anti-CD40L mAbs
E. Drugs
1. Azathioprine
2. Ciclosporin
3. Rapamycin
F. Additional approaches
1. Donor-specific blood transfusions with concomitant mAb or drug therapy
2. Intrathymic inoculation of cells/antigens
3. Oral administration of cells/antigens

hyporesponsiveness resides in the anergic cell itself and the current data suggest that the anergic T cells fail to express the T-cell growth factor, IL-2, and other crucial T-cell activation genes because of defects in the antigen-stimulated signalling pathway.

T-cell clonal anergy can result from suboptimal antigen-driven signalling of T cells, as mentioned earlier. The full activation of T cells requires at least two signals, one signal generated via the TCR–CD3 complex, and the second (co-stimulatory) signal initiated/delivered by the APCs. Stimulation of T cells via the TCR–CD3 complex alone—provision of signal 1 without signal 2—can result in T-cell anergy/paralysis (Fig. 279.3 and Table 279.2).

B-cell activation, in a fashion analogous to T-cell activation, requires at least two signals. The first signal is initiated via the B-cell antigen receptor immunoglobulin and cytokines or cell-surface proteins of T-cell origin provide the second costimulatory signal. Thus, delivery of the antigenic signal alone to the B cells without the instructive cytokines or T-cell help can lead to B-cell anergy and tolerance.

Immunoregulatory (suppressor) mechanisms

Antigen-specific T or B cells are physically present and are functionally competent in tolerant states resulting from suppressor mechanisms. The cytopathic and antigen-specific cells are restrained by the suppressor cells or factors or express non-cytopathic cellular programmes. Each of the major subsets of T cells, the CD4 T cells and the CD8 T cells, has been implicated in mediating suppression. Indeed, a cascade involving MHC antigen-restricted T cells, MHC antigen-unrestricted T cells, and their secretory products have been reported to collaborate in mediating suppression. A subset of CD4+ CD25+ cells that express FOXP3, the CD4+CD25+FOXP3+ regulatory T cell (Treg) has been shown to mediate potent suppressive activity (Maloy and Powrie, 2001; Sakaguchi et al., 2001).

There are two major types of CD4+CD25+ Treg cells; naturally occurring CD4+CD25+Foxp3+ Treg (nTreg) cells that arise from the thymus (current recommendation: tTreg) and induced or adaptive CD4+CD25+Foxp3+ Treg (iTreg) cells that originate in the periphery (current recommendation: pTreg). IL-2 and TGF-β, a prototypic anti-inflammatory cytokine, are important for the maintenance of nTreg and TGF-β can differentiate CD4+CD25-Foxp3- T cells into CD4+ CD25+Foxp3+ T cells. IL-6, a pro-inflammatory cytokine, inhibits the generation of Treg cells and in the presence of TGF-β induces naïve T cells to differentiate into Th17 cells. Th17 cells are a newly discovered effector T helper cell subset that produces IL-17, a pro-inflammatory cytokine, which activates the NF-κB and MAP kinases pathways (Awasthi et al., 2008). Although not completely proven, Th17 cells may contribute to acute allograft rejection that is resistant to suppression by Treg cells (Burrell and Bishop, 2010).

At least four distinct mechanisms have been advanced to explain the cellular basis for suppression:

1. An anti-idiotypic regulatory mechanism in which the idiotype of the TCR of the original antigen-responsive T cells functions as an immunogen and elicits an anti-idiotypic response. The elicited anti-idiotypic regulatory cells, in turn, prevent the further responses of the idiotype-bearing cells to the original sensitizing stimulus.
2. The veto process by which recognition by alloreactive T cells of alloantigen-expressing veto cells results in the targeted killing (veto process) of the original alloreactive T cells by the veto cells.
3. Immune deviation, a shift in CD4+ T cell programmes away from Th1-type (IL-2, IFN-γ expressing) toward the Th2-type (IL-4, IL-10 expressing) programme.
4. The production of suppressor factors or cytokines (e.g. the production of TGF-β by myelin basic protein-specific CD8 T cells or other cytokines with antiproliferative properties (Miller et al., 1992).

The process leading to full tolerance is 'infectious'. Tolerant T cells recruit non-tolerant T cells into the tolerant state. The tolerant state also establishes a condition in which foreign tissues housed in the same microenvironment as the specific antigen to which the host has been tolerized are protected from rejection. Tolerance is clearly a multistep process (Suthanthiran, 1996; Waldmann, 1999; Li et al., 2000).

It is very likely that more than one mechanism operates in the induction of tolerance. The tolerant state is not an all-or-nothing phenomenon, but is one that has several gradations. Of the mechanisms proposed to explain tolerance, clonal deletion might be of greater importance in the creation of self-tolerance and clonal anergy and immunoregulatory mechanisms might be more applicable to transplantation tolerance. More recent data suggest both

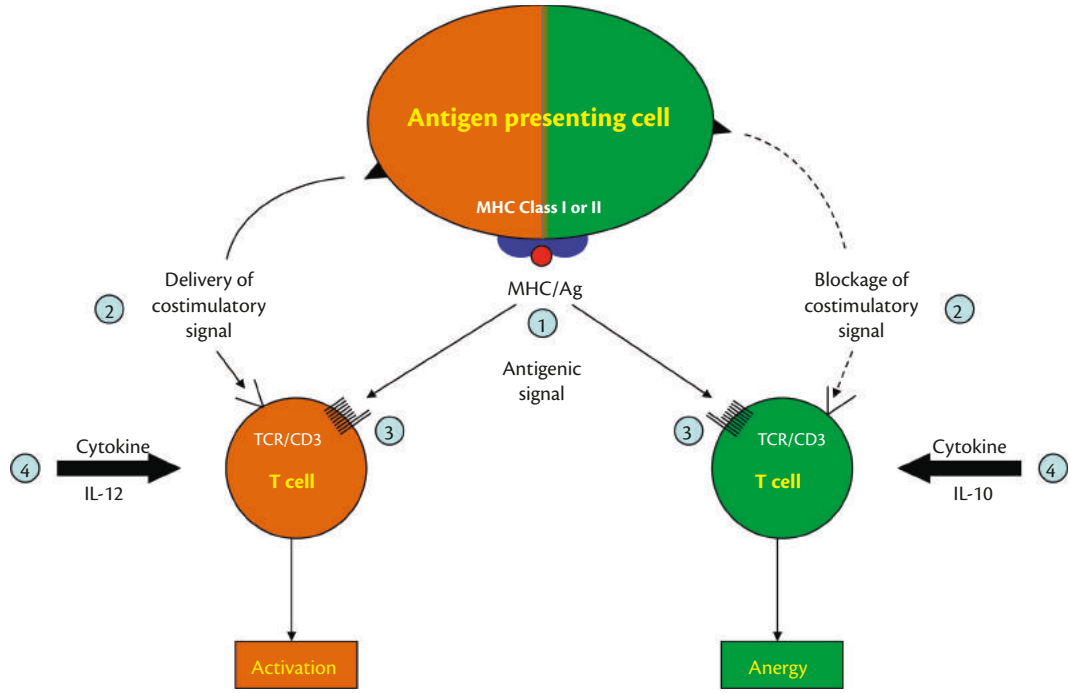

Fig. 279.3 T-cell activation/anergy decision points. Several potential sites for the regulation of T-cell signalling are shown. The antigenic peptide displayed by MHC (site 1), co-stimulatory signals (site 2), TCR (site 3), and cytokine signalling (site 4) can influence outcome. Altered peptide ligands, blockade of co-stimulatory signals, downregulation of TCR, and IL-10 favour anergy induction, whereas fully immunogenic peptides, delivery of co-stimulatory signals, appropriate number of TCR, and IL-12 prevent anergy induction and facilitate full activation of T cells.

Adapted from Suthanthiran, M. (1996). Transplantation tolerance: fooling mother nature. *Proceedings of the National Academy of Sciences USA* 93, 12072–5. Copyright (1996), National Academy of Sciences, USA.

clonal depletion and immunoregulatory mechanisms are needed to create and sustain central or peripheral tolerance. From a practical viewpoint, a non-immunogenic allograft (e.g. located in an immunologically privileged site or physically isolated from the immune system) might also be 'tolerated' by an immunocompetent organ-graft recipient.

Authentic tolerance has been difficult to identify in human renal allograft recipients. Nevertheless, the clinical examples, albeit infrequent, of grafts functioning without immunosuppressive drugs (either due to non-adherence of the patient or due to discontinuation of drugs for other medical reasons) does suggest that some long-term recipients of allografts develop tolerance to the transplanted organ and accept the allografts (Newell et al., 2010). The recent progress in our understanding of the immunobiology of graft rejection and tolerance and the potential to apply molecular approaches to the bedside promises the possibility of creation of a clinically relevant tolerant state and transplantation without exogenous immunosuppressant—the ultimate goal of the transplant physician.

Clinical trials in transplant tolerance

Both small and large animal studies have successfully demonstrated the concept of chimerism in achieving allograft tolerance. In these models, transplanting the donor's haematopoietic stem cells in tandem with the allograft create a bone marrow lymphohaematopoietic chimera in which the donor and recipient haematopoiesis coexist thereby allowing the acceptance of the allograft. In a landmark trial, five patients underwent combined bone marrow and kidney transplants from HLA single-haplotype mismatched living-related donors after a pre-transplant non-myeloablative-conditioning regimen (Kawai et al., 2008). All five patients developed transient chimerism with one allograft failure due to irreversible humoral rejection and four patients achieved tolerance after discontinuation of all immunosuppressive drugs at 240, 244, 272, and 422 days after transplantation. Analysis of kidney allograft biopsy specimens from tolerant patients revealed the presence of high levels of the regulatory T-cell signature, FoxP3 mRNA and the absence of the cellular rejection biomarker, granzyme B mRNA.

In a study designed to circumvent graft-versus-host disease and engraftment syndrome, a conditioning regimen consisting of total lymphoid irradiation and antithymocyte globulin followed by infusion of donor CD34+ progenitor and CD3+ T cells into HLA-matched kidney transplant recipients was used to induce tolerance via chimerism was tested (Scandling et al., 2012). Of the 16 patients in this trial, eight exhibited chimerism for a minimum of 6 months and were free of immunosuppression for a mean duration of 28 months after drug withdrawal. Four patients developed disease recurrence or rejection and did not undergo withdrawal of immunosuppressive medications. At the time of publication, the remaining four patients were in the process of anti-rejection drug withdrawal. The authors noted an increased in the ratio of CD4+CD25+ regulatory T cells and NKT cells versus naïve T cells in the peripheral blood of participants in the study.

In 15 HLA-mismatched kidney graft recipients, a non-myeloablative regimen was evaluated for the induction of

tolerance. Chimerism was achieved in nine participants and six were successfully weaned off immunosuppressive drugs and others remaining on tacrolimus monotherapy (prior to full withdrawal at 1 year) (Leventhal et al., 2013). One of the nine subjects developed bone marrow failure and graft loss as a result of a viral infection. Three participants developed transient donor chimerism and remained on tacrolimus monotherapy while one awaits immunosuppressive drug withdrawal following re-emergence of low-level chimerism at 1 year after transplant. One subject remained on sirolimus monotherapy after failure of donor stem cell engraftment. Taken together, these promising clinical trials illustrate that inducing donor-recipient mixed chimerism is a novel way to achieve tolerance in kidney transplantation.

In a study of 25 tolerant kidney transplant patients who were without immunosuppressive medications for at least a year, unique B-cell signatures were identified from peripheral whole-blood specimens using gene microarrays and urinary cell sediments using real-time quantitative PCR assays (Newell et al., 2010). The predictive genes for tolerance (IGKV4-1, IGLLA, IGKV1D-13) are important for B-cell differentiation and activation. They encode lambda and kappa light chains, which were increased during transition from pre to mature B cells and during class switching and receptor editing. The study also showed that in tolerant patients, there was an increase in transitional B cells (CD38+CD24+) producing the IL-10 cytokine.

Conclusions

In only five decades, improved understanding of transplantation immunology, the administration of potent immunosuppressive drugs, and effective infection prophylaxis have changed organ transplantation from a high-risk experimental procedure to a safe and effective therapy. Deciphering the mechanisms of rejection and tolerance has led to novel and promising tolerance trials. It is likely that many patients in the future will be treated in an individualized fashion and with tailored immunosuppressive regimens. Development of biomarkers, preferably mechanistic, and non-invasively ascertained, should allow the practice of precision transplantation medicine.

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CHAPTER 280

Immediate post-transplant care and surgical complications

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Introduction

Care of the renal transplant recipient in the early post-transplant period is truly multidisciplinary, and all involved must be familiar with both the patient's medical background and the donor's history so that individualized and appropriate care can be provided. Careful attention should be paid to recovery from the surgical procedure, fluid balance, drug therapy and choice of immunosuppression, microbiology (including antimicrobial prophylaxis), and thromboprophylaxis. Patient instruction is an essential part of routine post-transplant care.

Because complications do occur, they must be recognized early and dealt with promptly. The nature of the transplant operation and the need for immunosuppression mean that the complications differ from those of ordinary general surgical patients, and so require specialist medical, microbiological, or radiological input with a narrower time window for correction.

This chapter covers the immediate postoperative care of the renal transplant recipient both as an inpatient and the early period as an outpatient, highlighting the potential complications and their management.

Routine inpatient care

Initial assessment

Knowledge of the recipient's past medical history and current medical state is essential in providing post-transplant care and recognizing complications. Important factors include the primary renal diagnosis (including its speed of onset), the native urine output and the 'dry weight', the dialysis modality, and timing of the last dialysis session. These are needed for assessing post-transplant fluid balance and graft function. If the patient was on haemodialysis prior to transplant, consideration should be given to their vascular access. Comorbidities such as diabetes and heart disease that might affect post-transplant care or increase the risk of complications should also be taken into account. The patient's pre transplant cytomegalovirus (CMV), Epstein–Barr virus, and varicella zoster virus status should be known and documented.

The operation note and anaesthetic chart will contain much useful information for the team caring for the patient on the ward. A well-written operation note will contain vital donor information such as age and donor type, the warm and cold ischaemia times, the mode of preservation (i.e. static cold storage or machine perfusion), and the number of human leucocyte antigen mismatches. These will help predict the risk of delayed-graft function and the need for postoperative dialysis. Anatomical information such as the number of arteries, veins, and ureters including any intraoperative vascular reconstructions will highlight any abnormalities predisposing to a greater risk of technical complication and help interpretation of imaging studies. Documentation of ureteric stent and drain placement should be clear. For those patients on peritoneal dialysis preoperatively, documentation of peritoneal breaches may indicate the need for temporary vascular access (for haemodialysis) if graft function is delayed. The anaesthetic chart will include details of drugs and fluid administered during the operation, including blood transfusion and immunosuppressive drugs.

Fluid balance and fluid management

An appropriate assessment of fluid balance begins with an understanding of the recipient's pre-transplant fluid status and intraoperative fluid administration or losses. On return to the ward, staff will need to interpret clinical examination, vital signs, and urine output in order to determine fluid requirements.

Fluid replacement should compensate for an initial deficit as well as continuing losses. In the euvolaemic recipient, hourly replacement of the previous hour's urine output plus 50 mL to cover insensible losses should be sufficient until the patient resumes oral fluid intake. Replacement is with crystalloid, taking into account the renal function and potassium concentration which should be monitored regularly. Frequency will depend on preoperative concentrations and the presence of an acidosis. The volume of fluid in other intravenous preparations such as drugs should not be ignored in prescribing a fluid regimen. In the living-donor transplant recipient, diuresis can be large with early urine output of > 1 L per hour. In this situation, the fluid input may have to be capped at a maximum rate to prevent driving an artificial diuresis. In the opposite situation of established delayed graft function, fluid administration will need to be reduced to avoid fluid overload.

Fluid balance should be reassessed at least twice a day, and a weight recorded daily to allow changes to be observed and acted upon. Once the patient has an adequate oral fluid intake, intravenous fluids can be discontinued. The patient with delayed graft function may need to return to preoperative fluid restrictions until function is achieved.

Medications

Many of the medications taken pre transplant can be stopped at the time of transplantation. Antihypertensives, phosphate binders,

erythropoiesis-stimulating agents (ESAs), and calcium supplements can all be stopped and reintroduced if required, or if delayed graft function occurs. Exceptions to this are beta blockers, which should continue, and lipid-lowering drugs such as statins (although the preparation and dose may need to change due to interactions with calcineurin inhibitors (CNIs)). Calcium supplementation and vitamin D analogues may need to continue in patients who have had a previous parathyroidectomy, and cinacalcet in those with autonomous hyperparathyroidism.

Immunosuppression and monitoring

Details of immunosuppressive drugs and protocols are given in Chapter 281. The majority of renal transplant recipients will be prescribed a combination of a CNI (ciclosporin or tacrolimus) and an antiproliferative agent (azathioprine or mycophenolate mofetil (MMF)) with or without corticosteroids. They may also require a second dose of an induction agent (e.g. basiliximab) whilst on the ward.

The white blood cell count, along with the plasma levels of calcineurin and/or mammalian target of rapamycin (mTOR) inhibitors must be monitored regularly to prevent toxicity. Both are a particular risk in the early post-transplant period. Accurate timing of dosing and blood sampling are essential. Patient instruction is an important component of this. The date and time of dose adjustments must be clearly documented.

Antimicrobial prophylaxis

The heavy immunosuppression burden in the early stages following a transplant makes it the highest risk period for infection. Although routine broad-spectrum antibiotic prophylaxis will have been administered at the time of operation, prophylaxis for specific opportunistic infections is required (see Chapter 284).

Pneumocystis jirovecii pneumonia (PCP) occurs in about one in five renal transplant recipients not receiving prophylaxis. The risk is increased in those patients receiving intensive immunosuppression for acute rejection and in those with chronic CMV infection (Fishman, 2001). Most transplant units prescribe prophylaxis with low-dose trimethoprim/sulphamethoxazole (480 mg once daily) for 6–12 months post transplant. Infection during the period of prophylaxis is rare, but late infections have been reported. Alternative agents for those allergic to trimethoprim/sulphamethoxazole include dapsone, atovaquone, or intravenous and sometimes inhaled pentamidine.

CMV infection is of concern in all but those patients who are CMV naïve who receive a graft from a CMV-naïve donor. CMV infection can be caused by either donor transmission or reactivation of the latent CMV in the recipient. Two preventative strategies have been suggested—universal prophylaxis of all at-risk recipients, or pre-emptive treatment guided by CMV polymerase chain reaction (PCR) monitoring. There appears to be little difference in efficacy for preventing CMV disease between these strategies, although pre-emptive treatment reduces the risk of leucopenia and may reduce the rates of drug-resistant CMV disease (Owers et al., 2013). Universal prophylaxis may have the additional advantage of preventing herpes simplex and herpes zoster and lowering the risk of rejection.

Where universal prophylaxis is to be used, ganciclovir and valganciclovir are more effective at preventing disease than aciclovir-based preparations (Hodson et al., 2013). Extending the duration of prophylaxis from 3 to 6 months has also been shown to reduce the incidence of disease but at significant cost (Humar et al., 2010). Prophylaxis should commence within 10 days of transplantation.

Tuberculosis prophylaxis should also be prescribed to patients living in an endemic region or those at high risk in non-endemic areas (including previous exposure or residence in an endemic area, or evidence of latent infection). Prophylaxis with isoniazid has been shown to reduce the risk of infection by nearly 70% in endemic areas (Currie et al., 2010).

There is no evidence to support routine antifungal prophylaxis following renal transplantation.

Thromboprophylaxis

Renal transplant recipients, who have had a pelvic surgical procedure, have an increased risk of venous thromboembolism mandating the use of thromboprophylaxis. Prophylaxis is also desirable to reduce the risk of early renal artery or venous thrombosis leading to graft dysfunction or even loss. Various combinations of low-dose aspirin (75 mg once daily), heparin, and mechanical thromboprophylaxis are employed. Either unfractionated heparin or low-molecular-weight preparations can be used. Dose adjustment is not required for renal impairment at the doses used. In some centres, aspirin is reserved for those with a coexisting cardiovascular indication or where an arterial reconstruction has been performed at the time of transplantation (with the associated increased risk of thrombosis). Care must be taken with mechanical thromboprophylaxis as many renal patients have coexisting peripheral vascular disease especially those with diabetes mellitus.

Gastric protection

The concomitant use of corticosteroids and aspirin coupled with surgical stress places patients at risk of gastritis and ulceration. Many transplant units prescribe gastric acid suppression (H_2 antagonists, e.g. ranitidine or proton-pump inhibitors, e.g. omeprazole) in the early post-transplant period to prevent these complications. These agents can be stopped in the outpatient clinic, particularly after steroids and/or aspirin have been withdrawn.

Laboratory monitoring

Monitoring by daily measurement of biochemical and haematology parameters is essential, as the combination of fluid imbalance and drug therapy in the postoperative patient can rapidly lead to abnormalities. Electrolytes should be monitored and corrected to inform the adjustment of intravenous fluids to be administered. Reduction in the plasma creatinine indicates the return of graft function; failure to improve may indicate onset of delayed graft function (DGF), with or without superadded rejection. A rise in serum creatinine may indicate fluid imbalance, drug toxicity, acute rejection, DGF, obstruction, or infection and should be investigated promptly. A fall in haemoglobin concentration should prompt a search for a source of bleeding, but may represent persisting anaemia in the patient with DGF or haemodilution. Leucopenia is common, and may require a reduction in the dose of anti-proliferative agents (MMF or azathioprine). Other agents, such as co-trimoxazole and valganciclovir, can contribute. The serum calcium and phosphate should also be monitored as many patients will have secondary hyperparathyroidism and may also be taking phosphate binders pre transplant. These may need to continue in patients with prolonged DGF. Cinacalcet should be continued in those with autonomous hyperparathyroidism.

Imaging

Standard protocols specify postoperative imaging of the renal transplant either in the recovery room or within 24 hours post transplant. A duplex ultrasound scan will evaluate arterial and venous patency and flow, as well as flow within the kidney parenchyma. It also allows for assessment of significant haematomas or seromas, ureteric stent position, and the presence of ureteric obstruction. Details of post-transplant imaging are covered in more detail in Chapter 282.

Urinary catheters and stents

A urinary catheter is inserted at the time of surgery to facilitate identification of the bladder, allow the postoperative monitoring of urine output, and to protect the ureteric anastomosis. Introduction of a three-way catheter with a large (20–30 mL) balloon has the advantage of ease of filling and drainage of haematuria/clots, but can lead to significant discomfort from bladder spasm. This can be reduced by using a smaller catheter, reducing the amount of fluid in the catheter balloon, or with antispasmodics such as oxybutynin. The catheter should be left *in situ* for 5 days, and be removed before discharge from hospital, unless there has been a urine leak.

It is common practice to insert a double-J ureteral stent at the time of surgery to protect the ureteric anastomosis. This reduces the risk of major urological complications, including leak and stenosis, albeit with an increased risk of infection (Wilson et al., 2005). The stent is removed by flexible cystoscopy between 2 and 6 weeks post transplant. An alternative is the intraoperative insertion of a single-J ureteral stent or paediatric feeding tube that is exteriorized through the bladder wall and skin in a similar manner to a suprapubic catheter (Minnee et al., 2009). This technique has the advantage, particularly in patients with a native urine output or in living donor kidney grafts, by allowing daily volume and quality of the urine from the transplanted kidney to be assessed independently of the native urine output. It also removes the need for cystoscopy for stent removal.

Mobilization and physiotherapy

Patients undergoing abdominal surgery are at increased risk of thromboembolic complications and basal atelectasis which can progress to pneumonia. Early mobilization, with specific chest physiotherapy, helps to prevent these complications, for those most at risk (the elderly, obese, and those with pre-existing lung disease).

Patient education and instruction

Transplantation presents a major change in lifestyle for most recipients, which takes a significant degree of adjustment and support. The whole of the transplant team, including medical staff, nurses, and pharmacists, need to be involved in patient education to ensure compliance with medication and to discuss any anxieties.

They need precise and clear instructions. A particular problem is that of adherence to medication, either intentional or unintentional, usually a consequence of a lack of understanding. Strategies include provision of clear printed medication lists with the reason for the prescription, frequent medication reviews, and dosette boxes (these have the drugs for each day and time in separate compartments) can help to ensure that medications are taken as prescribed. In those groups most at risk of non-adherence (e.g. adolescents) once-daily formulations may help to reduce the number of tablets that have to be taken.

They also need to be told what symptoms to report, when and to whom.

Inpatient complications

Delayed graft function

DGF is defined as the requirement for dialysis in the first postoperative week. Recently, a more functional definition of failure of the serum creatinine to reduce by 10% on 3 successive days within the first post-transplant week has been suggested, irrespective of need for dialysis. The clinical diagnosis of DGF is associated with the histopathological manifestation of acute tubular injury. It is one of the most common immediate complications of renal transplantation and is associated with prolonged hospital stay, increased cost, and reduced graft survival.

DGF manifests early after a transplant with minimal urine output from the transplanted kidney unresponsive to fluid administration. Occasionally there will be initial urine output, which tails off over the hours following reperfusion. It is important to exclude other causes for a reduction in urine output, such as catheter obstruction or graft thrombosis. An urgent graft ultrasound is always performed.

Once normal graft perfusion is confirmed and obstruction excluded, careful attention must be given to fluid balance as fluid overload is a common problem. The serum potassium and bicarbonate concentrations should be monitored. A rising creatinine and urea, rising potassium, worsening acidosis, or clinical evidence of fluid overload are all absolute indications for dialysis. Dialysis should be performed early to avoid these developing. In the patient maintained by peritoneal dialysis a trial of peritoneal dialysis is appropriate but any evidence of leak should prompt a switch to haemodialysis through a temporary vascular catheter.

Failure of DGF to resolve within 7 days following transplant is an indicator for transplant biopsy to exclude concomitant acute rejection, which is associated with worse long-term graft outcomes (McLaren et al., 1999; Lebranchu et al., 2005). In centres using T-cell depleting antibody induction (antithymocyte globulin (ATG) or alemtuzumab) this biopsy is often postponed as early acute rejection with such protocols are rare. The resolution of most episodes of DGF within 14 days of transplantation is usually signified by an increase in urine output followed within 24 to 48 hours by a stabilization and then a fall in the serum creatinine.

Early graft loss

Early graft loss within the first post-transplant month occurs in about 5% of recipients. The causes include (in decreasing order of frequency) allograft vascular thrombosis, acute rejection, death with a functioning graft, and primary non-function (defined as a permanent lack of allograft function starting immediately after transplant) (Phelan et al., 2012). Early graft loss is associated with reduced patient survival.

Allograft vascular thrombosis and accelerated rejection require early surgical re-exploration and graft nephrectomy to reduce the risk of rupture and bleeding. For other causes, the requirement for graft nephrectomy may not be absolute and will be guided by clinical features such as pain, acute inflammatory signs and the presence of haematuria. Following graft nephrectomy, immunosuppression is tapered and then stopped. If the graft is left *in situ*, low-level immunosuppression is commonly continued to prevent the risk of graft rupture and allosensitization.

Early vascular complications

Arterial thrombosis of the renal graft is a rare complication (0.2–7.5%) but important as the risk of graft loss is high. Over 90% of cases of graft arterial thrombosis occur in the first week post transplant. In the recipient with no pre-transplant urine output, it will manifest as a sudden decrease in urine output to zero prompting urgent intervention. The recipient with a native urine output is more problematic as thrombosis is rarely detected before the graft is irreversibly damaged by ischaemia. Management is immediate exploration and thrombectomy.

Risk factors that have been associated with graft thrombosis include paediatric donors and recipients (< 6 years of age), elderly donors and recipients (> 60 years of age), right kidney, haemodynamic instability, preoperative peritoneal dialysis, and deceased donor transplant (Keller et al., 2012). Multiple arteries may also increase the risk, although loss of a polar artery will not necessarily lead to graft loss. Loss of one of the arteries is most problematic when it supplies the lower pole, as the risk of ureteric ischaemia and complications is increased.

It is thought that most arterial thrombosis results from damage to the intima during retrieval, bench preparation or implantation, technical failure at the anastomosis, or damage to the recipient iliac vessel. Concomitant haemodynamic changes in the recipient are also likely to contribute, as reflected by many of the other risk factors.

Renal vein thrombosis occurs in 0.1–8.2% transplants and manifests as a reduction in urine output with graft swelling, tenderness, and haematuria. Causes include technical failure at the anastomosis, prothrombotic conditions, and extrinsic compression from fluid collections or a haematoma. Diagnosis is made on ultrasound with an enlarged graft, absent flow in the renal vein, and reversed arterial flow in diastole. Urgent surgical intervention is required for salvage.

The risk of allograft thrombosis is also increased in the presence of delayed graft function (Bakir et al., 1996). Due to interstitial oedema, renal cortical flow will be sluggish leading to first venous then arterial thrombosis. When discovered, in most cases surgical rescue is not possible and transplant nephrectomy is usually required.

Early urinary complications

The routine use of a ureteric stent has made early ureteric complications rare. The incidence of early major urinary complication rates using either the Politano–Leadbetter or Lich–Gregoir techniques varies between 0% and 17%. An 'acceptable' rate of < 4% is seen with the use of a ureteric stent (Wilson et al., 2005). Disruption of the ureteric anastomosis usually leads to a urinary leak, resulting in swelling, pain, and potential compression of the graft and vessels, which will cause a rise in the serum creatinine. If a retroperitoneal drain is left in the iliac fossa next to the kidney, biochemical analysis of the drain fluid will show a high creatinine concentration. Diagnosis can be confirmed by ultrasound, nuclear renography, or pyelography. Initial management will be percutaneous drainage to relieve pressure and aid diagnosis, but most patients will require surgical re-implantation.

Haematuria is common in the early post-transplant period, and is usually self-limiting. Bleeding usually arises from the bladder wall at the point of ureteric insertion, but can also result from retrieval or intraoperative biopsy damage to the transplant kidney. Heavy haematuria with passage of clots is of more concern as it may cause catheter obstruction. Regular irrigation of the bladder to remove clots is required.

Anaemia and perioperative bleeding

Most of the blood loss following the transplantation procedure occurs at the time of organ reperfusion, that is, when the vascular clamps are released. Many patients with renal failure will have a degree of pre-existing anaemia, which compounds the effects of intraoperative blood loss. Careful bench preparation of the kidney and anastomotic technique, with adequate intraoperative attention to bleeding points will reduce the risk of haemorrhage but there will still be cases in which blood loss will require transfusion. Perioperative transfusion is associated with a worse long-term graft survival, so should be avoided if possible (O'Brien et al., 2012).

Significant postoperative bleeding is uncommon, and can be identified by haemodynamic instability, a falling haemoglobin concentration, swelling, blood in drains, and decreased urine output related to compression. Major arterial or venous bleeding will be obvious, and is a surgical emergency. Full blood count and a clotting screen should be obtained, and blood products (4–6 units of packed red cells) should be available. The wound will be opened and packed to control bleeding. Careful removal of the packs will then allow identification of the bleeding point. Direct suture repair is sometimes possible, but in some cases re-implantation of the vessel or even explant, back table cold-perfusion and bench repair may be required for bleeding from awkward bleeding points. When re-operating for bleeding, the surgeon and theatre staff should be aware of these options and a full range of vascular clamps, along with perfusion fluids and ice, should be available.

Slow venous or kidney surface bleeding can lead to gradual accumulation of a haematoma in the retroperitoneal space. This will manifest as increasing swelling, obstruction, or dysfunction due to pressure or flank bruising. Such bleeding is often related to coagulation dysfunction, which should be sought and corrected with the appropriate blood products, or reversal of anticoagulation. If there is a pressure effect or evidence of infection, the patient should be returned to theatre for exploration, evacuation of clot, and haemostasis, even though active bleeding will often have stopped.

In the majority of patients with primary graft function, ESAs can be discontinued at the time of transplant. The exception is in patients requiring post-transplant dialysis for delayed-graft function, in whom anaemia often worsens. Resistance to ESAs is to be expected so high doses are required. Even in those with primary function, the acute phase response to surgery, immunosuppressive drugs such as mycophenolate, azathioprine, and sirolimus can all contribute to postoperative anaemia.

Infection

Most infections seen in the early post-transplant period are those common to any major abdominal surgical procedure, that is, involving the urinary tract, the wound, or respiratory tract. Opportunistic infections related to immunosuppression are uncommon in the early weeks. Management principles are the same as for any postoperative patient. Obtaining appropriate samples for microbiological examination before starting empirical antibiotics is essential to avoid over treatment.

Most transplant units have a policy of giving broad-spectrum antibiotic prophylaxis as a single perioperative dose (e.g. meropenem

1 g at induction). Others use routine postoperative prophylaxis for urinary tract infection, which may reduce the risk of sepsis but risks the development of resistant organisms (Green et al., 2011). Oral co-trimoxazole given as prophylaxis for *Pneumocystis* pneumonia acts as urinary tract prophylaxis in the majority of patients.

Hypertension

The majority of patients with renal failure will be taking one or more antihypertensive agents. With the exception of beta blockers, it is usual to stop antihypertensive medications at the time of transplantation and reintroduce them in a stepwise fashion as required in the postoperative period. The combination of CNIs and corticosteroids means that many patients will develop recurrent (or *de novo*) hypertension and require therapy. Management of post-transplant hypertension is important to prevent graft damage, and to minimize cardiovascular disease.

The first-choice drugs are calcium channel blockers which are effective and reduce the risk of graft loss, and improve graft function (Cross et al., 2009). Although angiotensin-converting enzyme inhibitors (ACEIs) are effective and reduce proteinuria, they are often avoided in the early post-transplant period for fear of reducing renal perfusion pressure and thereby the glomerular filtration rate. They may also contribute to post-transplant acidosis. Beta blockers appear safe as a second-line agent, although caution should be exercised in patients with a history of airways obstruction or peripheral vascular disease.

Recurrence of primary disease

Recurrence of primary renal disease is an uncommon cause of early graft dysfunction (see Chapter 289). Both focal segmental glomerulosclerosis (FSGS) and haemolytic uraemic syndrome (HUS) can recur in the first post-transplant week and so extra vigilance is required in patients with these diagnoses. Recurrence of FSGS is more likely if the primary disease was of rapid onset and progressive. It is the atypical forms of HUS that are likely to recur.

Monitoring of the urinary protein:creatinine ratio may aid early detection of recurrent disease, and the findings of significant proteinuria with graft dysfunction should prompt early biopsy. HUS is associated with thrombotic microangiopathy with anaemia, reduction in the platelet count, fragmented cells on peripheral blood smear, and high serum lactate dehydrogenase.

Plasmapheresis is the treatment of choice for both conditions, with nine or more treatment sessions required for regression of proteinuria in recurrent FSGS (Ulinski, 2010). Eculizumab has been used in patients with recurrence of HUS (Zuber et al., 2012).

Routine outpatient care

The standard transplant surgery is now such that it is now usual for patients with primary graft function to be discharged from hospital within a week, to be reviewed regularly in dedicated discharge clinics. Such a policy requires the adequate provision of outpatient clinics, with patients being reviewed three times a week or more in the 2 weeks following transplantation. In the absence of complications, the frequency of the visits is gradually reduced to once a week by 1 month. These visits are to allow early detection of rejection (Table 280.1).

Clinical assessment

At each clinic visit, the patient should be questioned about any new issues, with particular attention paid to weight which is a good guide to fluid balance, wound complications, graft tenderness, pyrexia or other evidence of infection, and medication side effects. An up-to-date list of medications and doses should be maintained, with regular review to ensure that prophylactic drugs are stopped at appropriate time-points and medication interactions are avoided. This is best achieved by working with a dedicated transplant pharmacist in the outpatient clinic.

The pulse, blood pressure, and weight should be recorded. The temperature is only measured if indicated. In the presence of hypertension, home blood pressure recordings can be useful to avoid treating 'white-coat' phenomenon. In the early postoperative period the wound should be examined at every visit to exclude the presence of infection or swelling, and the graft should be palpated

Table 280.1 An example schedule for routine post-transplant follow-up in the first 12 months

Time	Clinic Visits	Tac level (ng/mL)	Steroids	Drug changes	Other
Week 1	Inpatient	8–12	15 mg		
Week 2	3×/week	8–12	15 mg		Urine BK
Weeks 3–4	2×/week	8–12	10 mg	Stop aspirin (week 4)	Urine BK (week 4)
Weeks 5–6	Weekly	8–12	5 mg		Urine BK (week 6); stent and peritoneal dialysis catheter out (week 6)
Weeks 7–8	Weekly	8–12	Stop		Urine BK (week 8)
Month 3	Fortnightly	8–12	Stop	Stop ranitidine and CMV prophylaxis (week 12)	Urine BK (week 12)
Month 4	Fortnightly	8–12	Stop		Urine BK (week 16)
Months 5–6	Monthly	8–12	Stop		Urine BK (month 6)
Months 7–12	Monthly	5–10	Stop	Stop co-trimoxazole and TB prophylaxis (month 12)	Urine BK (months 8, 10 and 12)

Adapted from the Oxford Transplant Centre protocol, Oxford, United Kingdom.

for tenderness. The signs of fluid overload include hypertension, an increase in weight, peripheral oedema and eventually signs of heart failure, including pulmonary oedema.

Investigations

At each clinic visit, a full set of laboratory tests including urea and electrolytes, liver function tests, calcium, phosphate, and full blood count should be requested. Calcineurin or mTOR drug concentrations should also be recorded to allow timely dose adjustments.

Routine urine samples should be tested by dip-stick for leucocytes, nitrites, blood and protein. Samples should only be sent for microbiological examination if there is evidence of infection. If proteinuria is detected, a sample should be sent for a urine protein:creatinine ratio.

Screening for BK virus is routine in the outpatient clinic. This includes urine cytology for decoy cells, or blood PCR for BK DNA. Frequency of screening varies, but should be once per month in the early post-transplant period.

Continuing patient education

The importance of patient education and instruction following transplantation should be emphasized. Understanding the restrictions placed by lifelong immunosuppression, and the risks of infection, malignant disease, and cardiovascular disease need to be sensitively explained.

Many patients are concerned about the risk of infection and returning to normal activities and work following their transplant. Most patients should expect to be off work for 6 weeks, but this will vary depending on the postoperative course and the occupation. Gentle exercise should be encouraged from discharge, and activity gradually increased over the coming weeks. Patients should avoid contact with children with transmissible disease like chickenpox and parvovirus, especially if they are not immune. Unpasteurized foods and undercooked meats should be avoided. A healthy diet with modest salt content and sufficient calories should be encouraged. The freedom from the dialysis diet can lead to obesity. Grapefruit juice should be avoided due to the interaction with CNIs.

Patients must be warned about the increased risk of skin malignancy resulting from immunosuppression, and told to avoid sun exposure and encouraged to use high-factor sun cream. Female patients should be advised to avoid pregnancy in the first post-transplant year, and to consult their transplant team before planning to conceive because drugs such as mycophenolate and renin–angiotensin system blockers will have to be switched to alternatives. They should, however, be reassured that with the help of obstetric physicians, pregnancy is usually successful.

Management of graft dysfunction

The most important question to be decided in the outpatient clinic is whether the graft is functioning satisfactorily or whether there has been a significant deterioration. Further investigation should be triggered by an increase in serum creatinine of 10% or more over baseline.

Table 280.2 lists the potential causes of acute graft dysfunction in the early post-transplant period. Many of these occur during the inpatient stay, but some, such as graft thrombosis and recurrence of primary disease, can occur at any point.

Detection of graft dysfunction should prompt urgent investigation. A urine sample should be taken for detection of proteinuria, infection (including decoy cells for BK virus if the test is available locally and urine or plasma PCR) or haematuria. Doppler ultrasound will exclude vascular and ureteric complications, as well as fluid collections causing compression. Drug history should be reviewed with particular attention paid to recent changes and CNI concentrations.

Table 280.2 Causes of early acute graft dysfunction

Immunological:
- Acute rejection

Recurrent disease:
- Focal and segmental glomerulosclerosis (FSGS)
- Haemolytic uraemic syndrome
- Antiglomerular basement membrane disease

Urological:
- Urine leak
- Ureteric stenosis
- Bladder outflow obstruction

Vascular:
- Graft arterial or venous thrombosis
- Renal artery stenosis
- Extrinsic compression (lymphocoele, haematoma)

Infection:
- Bacterial urinary tract infection
- BK virus nephropathy

Drug toxicity:
- Calcineurin inhibitors
- Angiotensin-converting enzyme inhibitors
- Non-steroidal anti-inflammatory drugs

Systemic factors:
- Dehydration
- Sepsis.

If the ultrasound is unremarkable, the essential and most informative investigation is a renal biopsy performed under ultrasound guidance. In the majority of cases this will identify the cause and differentiate between conditions requiring an increase in immunosuppression (i.e. acute rejection) and those requiring a reduction (e.g. infection, BK nephropathy, CNI toxicity).

Acute rejection

Acute rejection is now very uncommon in the first week after transplantation, but can occur at any point thereafter with the highest risk seen in the first few months. Early acute rejection is typically cell mediated, and is managed with high-dose boluses of corticosteroids (e.g. 500 mg intravenous methylprednisolone daily for 3 days). The success of treatment is determined by monitoring of the serum creatinine. Failure to reduce towards baseline within 7 days should prompt another biopsy. Confirmation of steroid-resistant rejection requires treatment with a lymphocyte-depleting agent such as ATG.

Around 90% of acute rejection episodes respond to steroid therapy. After successful treatment, it is recommended that maintenance corticosteroids be administered in those patients in whom they have been previously withdrawn. Patients not receiving azathioprine or mycophenolate should be commenced on mycophenolate,

and those taking azathioprine switched to mycophenolate (Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group, 2009).

Ureteric stenosis

The routine use of ureteric stents means that most ureteric complications do not manifest until after the stent is removed, that is, up to 6 weeks following transplant. Following stent removal, a rise in serum creatinine or graft tenderness, coupled with reduced urine output, may indicate ureteric stenosis which can be confirmed by the detection of transplant hydronephrosis on ultrasound. Stenosis is usually at the level of the ureter–bladder anastomosis or in the distal ureter. This is explained by a compromise to the ureteric blood supply which comes from a single branch of the renal artery, which can be surgically damaged, or thrombosis because of reduced blood flow. Thus distal ureteric ischaemia will result in fibrosis and stenosis. The risk can be reduced by avoiding stripping peri-ureteric tissues and cutting short the ureter before anastomosing it to the bladder.

Emergency management involves insertion of a nephrostomy to relieve calyceal pressure. This is followed by a nephrostogram to determine the site and extent of obstruction, and antegrade stent insertion as a temporary measure (Fig. 280.1).

Successful definitive management with balloon dilatation of the stricture at flexible nephroureteroscopy has been reported, although some patients required additional laser endoureterotomy (Kristo et al., 2003). Failure of dilatation will require surgical intervention, with the procedure of choice dictated by the site and length of the stricture. Distal strictures, especially those short in length, may be managed with simple re-implantation. Longer or more proximal strictures may require reconstruction with a psoas hitch or Boari flap, or ureteroureterostomy to the native ureter.

Renal artery stenosis

True transplant renal artery stenosis occurs in 1.9% of renal transplant recipients (Hurst et al., 2009) and is not an early complication after transplantation except when a stricture at the level of the arterial anastomosis between iliac and renal artery has been created. Risk factors for late stenosis include increasing recipient and donor age, extended criteria donors, delayed graft function, and recipient ischaemic heart disease. A similar clinical picture can also arise from a recipient proximal iliac stenosis—sometimes termed 'pseudostenosis'.

Presentation is with hypertension, fluid retention, oedema and graft dysfunction. High CNI levels and ACEI therapy, both of which potentiate renal hypoperfusion by decreasing afferent blood flow, often worsen symptoms. Initial investigation is by Doppler ultrasound if an appropriately trained sonographer is available, but more usually by computed tomography or magnetic resonance angiogram. If there is doubt as to the significance of a stenosis, formal angiography with pressure measurement can be used; a drop of > 20 mmHg suggests significant stenosis.

Management options for significant stenoses are either percutaneous or surgical. Percutaneous transluminal angioplasty (PTA), with or without stenting, is the treatment of choice but is not without complications. These include arterial dissection and rupture, thromboembolism, and puncture site complications. There is also a long-term risk of restenosis requiring repeat intervention. For those patients in whom PTA fails or is technically impossible, surgical intervention with autologous vein or prosthetic graft bypass of the stenosis is required.

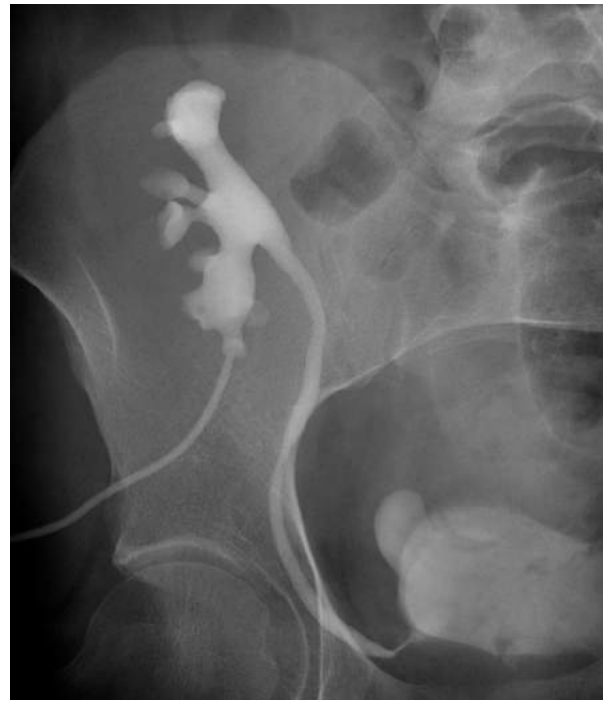

Fig. 280.1 Percutaneous nephrostogram demonstrating stenosis in the transplant ureter.

Lymphocoele

Lymphocoeles usually present as a localized swelling or tenderness over the graft site, often with graft dysfunction due to compression of the renal vein or ureter. Compression of the iliac vein can also cause ipsilateral leg swelling. They are caused by disruption of the lymphatics around the iliac vessels during surgical dissection. Increased incidence is seen with early use of mTOR inhibitors (Pengel et al., 2011). Careful surgical technique with ligation of all lymphatic vessels is essential, and since the use of electrocautery or the harmonic scalpel this complication has significantly decreased.

Diagnosis is confirmed with the detection of a fluid collection on ultrasound, and biochemical analysis will demonstrate a low creatinine (excluding a urinoma) and high triglyceride content. Small, asymptomatic lymphocoeles can be managed conservatively, but larger collections will require intervention. Whilst percutaneous drainage may be successful, the risk of infection and recurrence means that laparoscopic fenestration to the peritoneal cavity is preferred (Lucewicz et al., 2011).

BK virus

Asymptomatic BK virus infection is common in the general population, with the virus persisting in the renal tract. In the post-transplant immunosuppressed state, the virus can reactivate and replicate in the blood (BK viraemia). In a proportion of recipients, this will lead to BK nephropathy or ureteric stenosis associated with graft dysfunction (see Chapter 284).

Routine screening or investigation of graft dysfunction will identify the presence of decoy cells in the urine. BK viraemia can be confirmed by PCR for BK virus DNA. In the presence of graft dysfunction, renal transplant biopsy is essential to exclude

rejection either as an alternative diagnosis or in conjunction with BK nephropathy.

The mainstay of management is reduction of immunosuppression, with monitoring of BK virus copies in the plasma as a marker of response. Addition of leflunomide or cidofovir does not appear to improve results compared to reduction of immunosuppression alone (Johnston et al., 2010).

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CHAPTER 281

Immunosuppression: drugs and protocols

Dirk R. J. Kuypers and Maarten Naesens

Introduction

Immunosuppressive therapy after renal allograft transplantation usually consists of a combination of drugs with different mechanisms of action and side effect profiles in order to enable application of minimal effective doses and reduce drug-related toxicity (Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Working Group, 2009). In the modern era of different classes of available drugs, the choice of an initial or maintenance immunosuppressive treatment protocol after renal grafting is no longer a standard a 'one (drug combination) fits all' but rather a dynamic clinical management strategy trying to accommodate the patient's changing co-morbidity and the different causes of progressive allograft dysfunction (Sprangers et al., 2011). The individualization of the immunosuppressive treatment according to the recipient's profile and taking into account the evolution and causes of allograft (dys)function is termed 'tailoring' of immunosuppressive treatment. The various drug protocols described represent different attempts to 'tailor' immunosuppressive therapy rather than identify an ideal universal drug combination.

To understand the rationale behind the different immunosuppressive drug combinations, the main determinants of patient and graft survival after renal transplantation need to be described. Patient death after renal transplantation is mainly determined by cardiovascular disease, infectious complications, and malignancies (Gill, 2008; Marcén, 2009; Israni et al., 2010; Rama and Grinyó, 2010). Both classical and non-classical cardiovascular risk factors acquired during preceding chronic kidney disease remain important after renal grafting. Arterial hypertension, hyperlipidaemia, and diabetes mellitus represent the most prevalent and clinically modifiable. Although some of the immunosuppressive drugs currently used are characterized by a specific adverse cardiovascular risk profile, all drugs invariably reduce host defences against (opportunistic) infections and malignancies. Death-with-a-functioning-graft remains the most common cause of graft loss while progressive chronic renal dysfunction leads to annual graft attrition rates of 4%. The multiple causes of allograft dysfunction and eventually graft loss are discussed in detail elsewhere (Nankivell and Kuypers, 2011) (see also Chapter 286). Thorough knowledge and regular clinical assessment of these determinants of graft survival are essential for respectively choosing the initial and adapting the maintenance immunosuppressive therapy in the individual transplant patient (Nankivell and Kuypers, 2011).

Quantification of the overall degree of immunosuppression of a particular drug combination would be ideal for clinical monitoring of the individual recipient, enabling practitioners to maintain a fine balance between efficacy and toxicity. Although several (semi-) quantitative methods have been tested in recent years (e.g. interferon gamma (IFN-γ) ELISPOT, mitogen-stimulated CD4 T-cell reactivity), no test has established a place in routine clinical practice, mainly because of considerable diagnostic overlap, limited specificity, and the absence of convincing clinical validation studies (Bestard et al., 2010). As a suboptimal surrogate quantifier of the degree of immunosuppression, clinicians rely on therapeutic drug monitoring, or more correctly, concentration-controlled dosing for those immunosuppressive compounds that have a narrow therapeutic window. Pharmacodynamic monitoring of immunosuppressive drug therapy, potentially in combination with the use of novel biomarkers reflecting immune and graft status, are subjects of recent experimental studies (Barraclough et al., 2010).

Switching transplanted patients who require life-long immunosuppressive treatment from brand name drugs to generic formulations significantly reduces costs. For generic immunosuppressive drugs used in transplantation (calcineurin inhibitors (CNIs), proliferation signal inhibitors (PSIs), and mycophenolic acid (MPA)), strict guidelines for proving bioequivalence with the brand compound have been set by regulatory bodies but these rules do not apply between generics which are therefore freely interchangeable. It is advisable when switching a patient from a brand drug formulation to a generic formulation that: (1) generic formulations are only used if they have fulfilled the strict bioequivalence criteria set by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) (2) drug concentrations are regularly monitored after switching until stable levels have been obtained; (3) the patient is not subjected to repeated substitutions between different generic formulations; (4) the patient is informed and educated about generics; and (5) generic substitutions are only carried out under the supervision of a clinician with experience in transplantation medicine (van Gelder, 2011).

Calcineurin inhibitors

While structurally different, tacrolimus (macrolide) and ciclosporin (cyclic endecapeptide) have very similar mechanisms of action. CNIs reversibly inhibit the calcium-dependent calmodulin-activated calcineurin enzyme, a serine phosphatase that is responsible for dephosphorylation and subsequent translocation

of nuclear factor of activated T cells (NFAT) into the nucleus, leading to transcription of various cytokines including interleukin (IL)-2, IFN-γ, and TNF-α by T lymphocytes amongst many other cell types (Kapturczak et al., 2004). In order to exert their inhibitory capacity (gain of function model), CNIs first need to bind to intracytoplasmatic immunophilins (cyclophilin, FK-BP12) (Kapturczak et al., 2004). Ciclosporin and tacrolimus also share very similar pathways for metabolism, distribution, and excretion. The cytochrome P450 isoenzymes CYP3A4 and -3A5 and the drug-transporter P-glycoprotein (ATP-binding cassette B1), both expressed in the intestinal mucosa and liver, are responsible for the first-pass metabolism and systemic clearance of CNIs and hence are the major determinants of tacrolimus and ciclosporin pharmacokinetics (De Jonge et al., 2009). CNIs are characterized by a high inter-individual variability in dose-normalized drug exposure which, together with a narrow therapeutic window, necessitates the continued use of clinical therapeutic drug monitoring based on pre-dose blood drug concentrations to guide individual dosing (De Jonge et al., 2009). CNIs are usually started preoperatively in an oral loading dose of 0.1–0.2 mg/kg bodyweight for tacrolimus, and 5–10 mg/kg for ciclosporin with further twice-daily dosing after transplantation. Recently, a once-daily extended-release formulation of tacrolimus has been developed, with the objective of improving patient compliance (Krämer et al., 2010). The intricate complex metabolic pathway of CNIs renders them very susceptible for clinically relevant drug interactions. This applies especially to concomitant use of strong inhibitors (e.g. azole antifungals and macrolide antibiotics) and inducers of CYP3A (e.g. rifampicin,) which can respectively cause increased toxicity or reduced drug exposure if concentration monitoring and dose adjustments are not performed (Kuypers, 2009). In some instances, CNIs are purposely combined with strong CYP3A inhibitors like azole antifungal drugs in stable patients to reduce CNI dose requirements and cost. Several interactions with other immunosuppressive drugs (Table 281.1) further complicate the use of CNI. For example, the combination of ciclosporin with a PSI like sirolimus will increase the intrinsic nephrotoxic properties of the former. Finally, the presence of an A allele at position 6986 of the *CYP3A5* gene (CYP3A5*1-allele carriers: CYP3A5 expressers) is the single strongest genetic determinant of tacrolimus exposure, associated with respectively 36% to 59%% reduction in dose-normalized exposure in case one or two A allele(s) are present. These genetically determined higher tacrolimus dose requirements are more often encountered in recipients of African descent who express CYP3A5 more frequently than Caucasians. For ciclosporin, genetic variants that determine clinical drug dosing have not yet been identified (De Jonge et al., 2009).

CNIs are effective immunosuppressive agents, capable of achieving acute rejection rates between 10% and 15%, depending on dose and the combination with other agents (see below). Common adverse effects of CNIs include a negative influence on an already increased cardiovascular risk profile of the renal recipient from end-stage renal disease, particularly arterial hypertension, hypercholesterolaemia, and post-transplantation diabetes mellitus (PTDM) (Table 281.2). Other adverse events are hyperkalaemia and renal tubular acidosis; rarely thrombotic microangiopathy and reversible posterior leucoencephalopathy. In addition, prolonged CNI-based immunosuppressive maintenance therapy in solid organ recipients causes development of histological lesions in the kidney (graft), resulting in progressive arteriolar damage, chronic interstitial fibrosis, and eventually allograft dysfunction. This process is termed calcineurin inhibitor nephrotoxicity (CNIT) (Naesens et al., 2009). The prevalence of CNIT in renal grafts has decreased to between 15% and 25% after 5 years over the last two decades as maintenance doses of CNIs have been substantially reduced. It remains difficult to identify patients at risk for CNIT because very high blood concentrations of CNI will lead to acute nephrotoxicity (with acute rises in serum creatinine) due to vasoconstriction of the afferent arteriole, but CNI concentrations within the therapeutic concentration range do not correlate with their chronic renal toxicity (Naesens et al., 2009). In an attempt to reduce CNI toxicity which compromises long-term patient and graft survival, several novel immunosuppressive regimens have been proposed, aiming at reducing CNI exposure or even withdrawing CNI therapy after transplantation (see below). Switching from ciclosporin to tacrolimus has been proposed in cases where hyperlipidaemia and arterial hypertension have become difficult to manage but this strategy has only limited effect. In situations where hypertrichosis and gum hyperplasia are troublesome, substitute of ciclosporin with tacrolimus will lead to improvement within 3–6 months. Conversely, in transplantation candidates with pre-existing risk factors for developing PTDM (e.g. obesity, older age, or African American descent), the use of ciclosporin instead of tacrolimus is advocated in addition to minimizing corticosteroid doses. In a prospective comparative trial, glucose intolerance occurred in 8.9% of recipients taking ciclosporin and corticosteroids versus 16.8% of patients on tacrolimus and corticosteroids (Vincenti et al., 2007).

CNIs are safe in pregnancy and are extensively metabolized by the placenta which implies higher dose requirements during gestation (Hebert et al., 2013). They can cause transient hyperkalaemia and rises in serum creatinine concentrations in neonates. Infant exposure to tacrolimus is low during breastfeeding (Bramham

Table 281.1 Drug interactions between immunosuppressive drugs

Drug interaction between		Effect	Risk
Tacrolimus	Proliferation signal inhibitors	Exposure ↔ or ↓	Unknown
	Corticosteroids	Exposure ↓	Unknown
Ciclosporin	Proliferation signal inhibitors	Exposure ↑	CNIT
	Corticosteroids	Exposure ↔	–
Mycophenolic acid	Ciclosporin	Exposure ↓	Graft rejection
	Tacrolimus	Exposure ↔	–
	Proliferation signal inhibitors	Exposure ↔	–
	Corticosteroids	Exposure ↓	Unknown
Proliferation signal inhibitors	Ciclosporin	Exposure ↑	CNIT
	Tacrolimus	Exposure ↔ or ↓	Unknown
	Corticosteroids	Exposure ↔	–

↓ = reduced drug exposure; ↑ = increased drug exposure; ↔ = unchanged drug exposure.
CNIT = calcineurin inhibitor nephrotoxicity.

Table 281.2 Specific immunosuppressive drug-related toxicity profiles

	Tacrolimus	Ciclosporin	Proliferation signal inhibitors	Mycophenolic acids	Corticosteroids
CNIT	++(+)	+++	+(+)[a]	–	–
Neurotoxicity	+++	++	–	–	–
PTDM	+++	++	–	–	+++
Bone marrow toxicity	–	–	++	+++	–
Hypertension	++	+++	–	–	++
Lipid disorders	+	+++	+++	–	++
Gastrointestinal intolerance	+	+	+	+++	–
Cosmetic changes	+	++	+	–	+++
Teratogenic	–	–	?	++	–

CNIT = calcineurin inhibitor nephrotoxicity; PTDM = post-transplantation diabetes mellitus.

[a]PSIs in combination with CNIs are nephrotoxic. See text for details.

et al., 2013). Breastfeeding and tacrolimus: serial monitoring in breast-fed and bottle-fed infants is advised.

Antimetabolites

Mycophenolic acid (MPA) is a selective non-competitive reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the *de novo* cell synthesis of guanosine nucleotides. MPA inhibits predominantly IMPDH II which is expressed in lymphocytes that cannot use the salvage pathway as an alternative source of guanosine. MPA is used in combination with CNIs and corticosteroids and has almost completely replaced azathioprine in renal transplantation because of better prevention of acute rejection (relative risk (RR) = 0.62) and better graft survival (hazard ratio = 0.76) (Knight et al., 2009). During pregnancy and for patients intolerant for MPA, azathioprine remains a useful alternative. The metabolism of MPA is complex with formation of active and inactive metabolites, enterohepatic recirculation (EHC) after deglucuronidation in the gut, and finally renal elimination of glucuronide metabolites (Tett et al., 2011). Two formulations of MPA are currently available: mycophenolate mofetil (MMF, Cellcept™, Roche, Swiss) and enteric-coated mycophenolate sodium (EC-MPS, Myfortic™, Novartis, Swiss) which has a delayed absorption peak. Because ciclosporin, in contrast to tacrolimus, interferes with the EHC of MPA, a higher daily dose of MPA is required to obtain similar concentrations as achieved by tacrolimus-treated recipients (Tett et al., 2011). This drug interaction is also relevant when switching between CNIs occurs. Drug interactions with concomitant non-immunosuppressive drugs are less frequent than with CNIs; mostly drugs interfering with the EHC of MPA (e.g. cholestyramine), rifampicin, and certain proton pump inhibitors diminish MPA exposure when combined (Tett et al., 2011). The inter-individual variability in MPA exposure is high and, together with a less clearly defined therapeutic concentration window (the upper limit of exposure indicating onset of drug toxicity is not well established), makes optimal dosing difficult. Whether MPA should continue to be used in a fixed twice-daily oral dosing or should be dosed according to pre-dose trough plasma concentrations, is uncertain. Clinical trials are currently testing whether therapeutic drug monitoring of MPA improves clinical outcome. Transient intensified fixed dosing of MPA initially after transplantation (during 6 weeks) in ciclosporin-treated patients overcomes the early MPA underexposure in these recipients and reduces the risk of acute rejection.

MPA has a toxicity profile which is mostly limited to the gastrointestinal tract and bone marrow (Table 281.2). While anaemia and leucopenia are to a certain extent related to MPA plasma levels, upper gastrointestinal tract symptoms and diarrhoea which occur in 18–27% of patients do not correlate with drug plasma levels but can improve by dose reductions. Because MPA is devoid of any nephrotoxicity, it has been evaluated in several CNI-free, so-called renal-sparing immunosuppressive protocols but often without the expected success (see below). Recently, the use of MPA with belatacept, a novel cytotoxic T-lymphocyte-associated protein (CTLA)-immunoglobulin (Ig) analogue, has demonstrated efficacy in selected renal transplant recipients (see below).

MPA causes cranial and cardiac malformations *in utero* and is contraindicated during pregnancy. In women planning pregnancy and at high immunological risk, MPA can be switched to daily azathioprine 1–1.5 mg/kg bodyweight before conception and reversed to MPA after delivery.

Similarly, recipients with a low to moderate immunological risk who cannot tolerate MPA can be switched to azathioprine dosed at 1.5–2.5 mg/kg/day.

Glucocorticosteroids

Glucocorticosteroids are included as the third compound in the majority of classical triple immunosuppressive regimens used after renal transplantation which contain a CNI and an antimetabolite drug. Corticosteroids are usually started with a high intravenous bolus dose during surgery and continued postoperatively in a tapering oral schedule with gradual reductions towards a maintenance dose equivalent of 5 mg prednisolone (4 mg methylprednisolone) by the third month post transplantation. Glucocorticosteroids are highly lipophilic and readily absorbed after oral ingestion. They interact with the cytosolic glucocorticoid receptor (GR), a member of the nuclear receptor superfamily, which exists in the cell as

a heteromeric complex formed by molecular chaperones. Upon ligand binding, the GR complex is translocated into the nucleus where it binds to glucocorticosteroid-responsive elements (GREs) within the regulatory regions of glucocorticoid-responsive genes and initiates transcription of certain anti-inflammatory genes (e.g. IL-10, inhibitor of nuclear factor kappa B (NF-κB)) and multiple metabolic genes. Corticosteroids mediate their anti-inflammatory effects mainly through downregulation of gene transcription by preventing the action of activators of transcription like activator protein (AP-1) and NF-κB (Heitzer et al., 2007).

Glucocorticosteroids are still the first choice for acute rejection treatment. Many types of corticosteroid antirejection courses exist, varying between 3 and 7 days of intravenous dosing alone and/or followed by an oral tapering scheme. Only when corticosteroids fail to reverse the acute rejection process (so-called steroid-resistant acute rejection), do clinicians resort to the use of T-cell depleting polyclonal antibodies (see below).

Glucocorticosteroid use is associated with arterial hypertension, hyperlipidaemia, diabetes mellitus, impaired wound healing, osteoporosis, osteonecrosis, cataracts, glaucoma, acne, skin fragility, cosmetic changes, depression, and growth impairment in children (Table 281.2). In an attempt to prevent these serious adverse effects, several strategies have been proposed: either withdrawing corticosteroids early after transplantation in eligible patients or even completely avoiding their use (Augustine and Hricik, 2006) (see below). More often corticosteroid discontinuation is attempted later after transplantation by which time side effects have already developed.

Mammalian target of rapamycin inhibitors

Sirolimus and everolimus, the hydroxyethyl analogue of sirolimus with increased solubility, are macrocyclic lactones which, after binding with FK-binding protein, inhibit the mammalian target of rapamycin (mTOR complex 1). mTOR is a 289 kD protein that activates S6K1 (p70 ribosomal S6 kinase). mTOR inhibitors (mTORi) block the mTOR-mediated signal transduction pathways, resulting in the arrest of cell cycle in G1–S phase and hence inhibit proliferation of various cell types, including T and B cells, fibroblasts, and vascular smooth muscle cells but also certain malignant cell types. The term proliferation signal inhibitors (PSIs) has been used to describe their common mechanism of action and has therefore replaced the term mTORi in some places. The antiproliferative action on human vascular smooth muscle cells and the ability to reduce intimal thickening in models of vascular injury, have led to the development of (mTORi) drug-eluting coronary stents. The antiproliferative effects of mTORi have led to their use in certain malignant diseases (with signalling disruption up- or downstream of mTOR) and the development of novel more powerful anti-cancer derivatives (Hartford and Ratain, 2007; Gabardi and Baroletti, 2010).

Similar to CNIs, mTORi are characterized by a narrow therapeutic window, a highly variable absorption, and a large interindividual variability in dose-normalized drug exposure. Therapeutic drug monitoring based on target pre-dose blood trough concentrations is therefore required for use of mTORi in renal transplantation. Sirolimus therapy is usually started with a loading dose (5–8 mg) and followed by a once-daily dose according to pre-dose blood levels. Everolimus is administered twice daily (concentration-controlled), with or without the use of a loading dose (0.75–1.5 mg twice daily). In combination with CNI therapy, pre-dose blood mTORi concentrations of 4–6 ng/mL are sufficient to achieve an acceptable acute rejection incidence. mTORi are the substrate (and weak inhibitors) of CYP3A4 and of P-glycoprotein so clinically relevant drug interactions are comparable to CNIs with respectively CYP3A inhibitors and inducers exerting the strongest effects on mTORi exposure (Christians et al., 2006).

Adverse effects of mTORi commonly lead to clinical intolerance and discontinuation of the drug (20–47%) with prolonged follow-up. Specific reasons for stopping mTORi therapy are mucosal ulceration, oedema, anaemia, new-onset proteinuria, and less frequently wound healing problems and interstitial pneumonitis (Table 281.2) (Kuypers, 2005). mTORi treatment often causes hypercholesterolaemia and hypertriglyceridaemia, requiring statin therapy in approximately 40–50% of patients. While mTORi are not nephrotoxic like CNIs, in some instances the use of mTORi is associated with renal dysfunction (Kuypers, 2005). In situations where CNI therapy is switched to a CNI-free, mTORi-based protocol, proteinuria can worsen, especially in patients with a pre-existing daily urine protein loss > 0.5–1 g (Kuypers, 2005). The combination of mTORi with ciclosporin can also lead to increased CNI-mediated nephrotoxicity because of drug interactions (Table 281.1) (see above). Switching from a CNI to a mTORi is a reasonable strategy in selected patients with either deteriorating graft function due to CNIT or other remediable CNI side effects (Kuypers, 2005). mTORi switch from CNI can also be performed electively early after transplantation in order to prevent the development of CNIT (see below). While the first approach is commonly performed in the setting of individual clinical cases, the latter approach of pre-emptive switching to a mTORi-based immunosuppressive regimen is not common clinical practice. Because of their antiproliferative properties, mTORi are increasingly used in recipients who develop a malignant disease after transplantation (e.g. non-melanoma skin cancer and Kaposi sarcoma) (Gabardi and Baroletti, 2010). The proportion of patients in whom a mTORi-based regimen is started at the time of transplantation is low (≤5%), mainly because of inferior early efficacy (prevention of acute rejection) compared to CNI-based therapy and the risk of wound healing problems and lymphocoele formation.

mTORi can cause reversible gonadal dysfunction and infertility in both male (oligo/azospermia) and female recipients (amenorrhea). Although a few successful cases of pregnancy during mTORi therapy have been reported, caution is advised if a mTORi is to be used during pregnancy. More data are needed.

Induction agents

Under normal circumstances, resting T cells express two IL-2 receptor chains (the β chain (CD122) and the γ chain (CD132)) on their cell surface. T-cell activation rapidly leads to secretion of IL-2 and increased expression of IL-2R with subsequent association of the IL-2R α chain (CD25) with the β and γ chains into a high-affinity receptor complex for IL-2. After IL-2 receptor binding, rapid clonal T-cell expansion ensues, producing different types of effector T cells. Two monoclonal antibodies (mAbs) directed against the IL-2R α chain (anti-CD25) have been developed for use in transplantation: daclizumab and basiliximab (Campara et al., 2010; Webster et al., 2010). Both mAbs suppress the antigen-mediated alloimmune response by selectively blocking activated T cells while resting immune cells are minimally affected. In addition, binding to

the IL-2R α chain induces antibody-dependent cellular cytotoxicity towards activated T cells. These monoclonal antibodies against the IL-2 α chain are currently used as induction therapy (short intravenous infusion) for renal transplantation, especially in patients considered at increased risk for acute rejection (e.g. high panel reactive antibody, those undergoing re-transplantation, and those experiencing delayed graft function) (Campara et al., 2010; Webster et al., 2010). Basiliximab is a chimeric mAb providing approximately 30 days of CD25 receptor saturation when administered twice: one dose of 20 mg preoperatively and again 20 mg on day 4 postoperatively. Daclizumab, a humanized anti-CD25 mAb, is dosed at 1 mg per kg of bodyweight preoperatively and repeated four times every 2 weeks postoperatively, thereby causing up to 90 days of receptor saturation. The latter is no longer available. Both anti-CD25 mAbs are safe and almost completely devoid of cytokine release syndrome in contrast to polyclonal antibodies (see below). Rare incidences of anti-idiotypic IgE formation eliciting anaphylactic reactions after re-exposure to basiliximab have been reported.

Lymphocyte-depleting polyclonal antibodies, like rabbit antithymocyte globulin (rATG), are derived from the purified serum IgG fraction from rabbits immunized with human thymocytes (Deeks and Keating, 2009; Gabardi et al., 2011). rATG is a polyclonal mixture directed against immune response antigens (e.g. CD3/TCR, CD25, CD28, CD40, CD80, and CD86), adhesion molecules and cell trafficking molecules (e.g. CD11a/CD18 (LFA-1), CD54 (ICAM-1), and CD195 (CCR5)), and other pathways mediators like CD2 and CD45. Lymphocyte depleting polyclonal antibodies cause marked depletion of T cells and other immune cells, and are used, like anti-IL-2R mAb, as an induction agent in kidney recipients at greater risk for acute rejection (Deeks and Keating, 2009; Gabardi et al., 2011). In addition, polyclonal antibodies can reverse early acute rejection episodes resistant to corticosteroid treatment ('steroid-resistant acute rejection') but are less effective in late rejection episodes (Deeks and Keating, 2009). The most frequently reported adverse events are fever, flu-like symptoms, leucopenia, thrombocytopenia, and anaemia. Serum sickness is a rare complication of rATG but necessitates prompt discontinuation and plasmapheresis treatment (Deeks and Keating, 2009). Whether the use of polyclonal induction agents is associated with an increased risk for Epstein–Barr virus (EBV)-related post-transplantation lymphoproliferative disease (PTLD) has not been proved. Induction regimens with rATG differ according to centre practice, varying between 2 to 14 days of active treatment using daily intravenous infusions at 1.5–2.5 mg/kg/day. Antirejection treatment is usually given for a minimum of 7 to a maximum of 14 days. Some centre use a subcutaneous test dose before starting therapy with polyclonal antibodies to identify patients with pre-sensitization to rabbit antigens (Deeks and Keating, 2009; Gabardi et al., 2011). Corticosteroids are frequently administered with the first dose to avoid strong allergic reactions.

Previously, muromonab-CD3 was used as induction therapy and treatment for steroid-resistant acute rejection episodes (OKT3, a lymphocyte-depleting monoclonal antibody) but has been replaced by anti-IL-2R mAb because of poor clinical tolerability (cytokine release syndrome, capillary leak syndrome, non-infectious meningoencephalitis) (Gabardi et al., 2011). More recently, alemtuzumab, an anti-CD52 T-cell- and B-cell-depleting monoclonal antibody, has been reintroduced as an induction agent in renal transplantation (see below).

Immunosuppressive protocols

The choice of the initial immunosuppressive drug regimen is based on a pre-transplantation assessment of the immunological risk status of the recipient (Fig. 281.1). A clinical reappraisal of this immunological risk status together with the patient and graft status can arbitrarily be performed at 3 months post-transplantation based on (1) the effect of donor kidney quality on graft function, (2) the damage to the graft sustained from preservation and implantation procedures, (3) the cumulative early and/or persistent acute alloimmune injury to the kidney, and (4) the type and severity of early complications (Fig. 281.1). Clinicians often use surrogate markers for this assessment guided by the clinical course (e.g. delayed graft function, type and severity of acute rejection(s), and infectious episodes), graft function, and histological information obtained from biopsies for cause and/or protocol biopsies. Subsequently, different pre-emptive strategies can be attempted to reduce the global degree of immunosuppressive burden safely, to avoid drug-related adverse effects (e.g. corticosteroids), or to maintain graft function (e.g. prevent CNIT). Which immunosuppressive regimen should be used for these pre-emptive strategies is dependent on the immunological risk status of the patient (Fig. 281.1). Once specific drug-related side effects occur or chronic allograft dysfunction develops, different options for altering immunosuppressive drug therapy are possible and their choice is again weighted between immunological risk and patient morbidity (Fig. 281.1).

Calcineurin inhibitor-based protocols

The vast majority of current immunosuppressive drug protocols in renal transplantation contain a CNI, with tacrolimus replacing ciclosporin as the preferred CNI in almost 90% of all new transplants in the United States (United States Renal Data System, 2010). In so-called standard dosing CNI-based regimens, tacrolimus trough concentrations are usually between 10 and 15 ng/mL (measured by immunoassay) in the first 3 months after grafting and between 7 and 12 ng/mL from 3 months onwards in the first year post transplantation with different further tapering strategies from 1 year onwards depending on the clinical evolution and centre practices (Bowman and Brennan, 2008; Wallemacq et al., 2009). Ciclosporin is usually targeted between 200 and 300 ng/mL in the first 3 postoperative months, between 150 and 250 ng/mL until the end of the first year, and further tapering according to clinical evolution and local practices (Nashan et al., 2005; Schiff et al., 2007). While many strategies have attempted to use lower doses of CNIs (CNI minimization) or to withdraw CNIs (see below), the 'standard CNI dose'-based regimens have proved to be effective in reducing the incidence of acute rejection (10–15%), even more so when IL-2R mAb induction therapy is used, especially in ciclosporin-treated patients (Webster et al., 2005; Opelz et al., 2009; Gralla and Wiseman, 2010). Overall, standard dose tacrolimus-based regimens are more effective (lower acute rejection rates) than standard dose ciclosporin-based therapy but without achieving better renal graft function (Webster et al., 2005). Slightly improved graft survival is achieved with tacrolimus but patient survival is not different during short to medium time follow-up (Webster et al., 2005). The few histological comparative assessments of signs of CNIT have not revealed clear differences between these two CNIs (Solez et al., 1998; Rowshani et al., 2006). Conversely, in comparative trials, tacrolimus use is more often associated with diabetes mellitus and neurotoxicity while

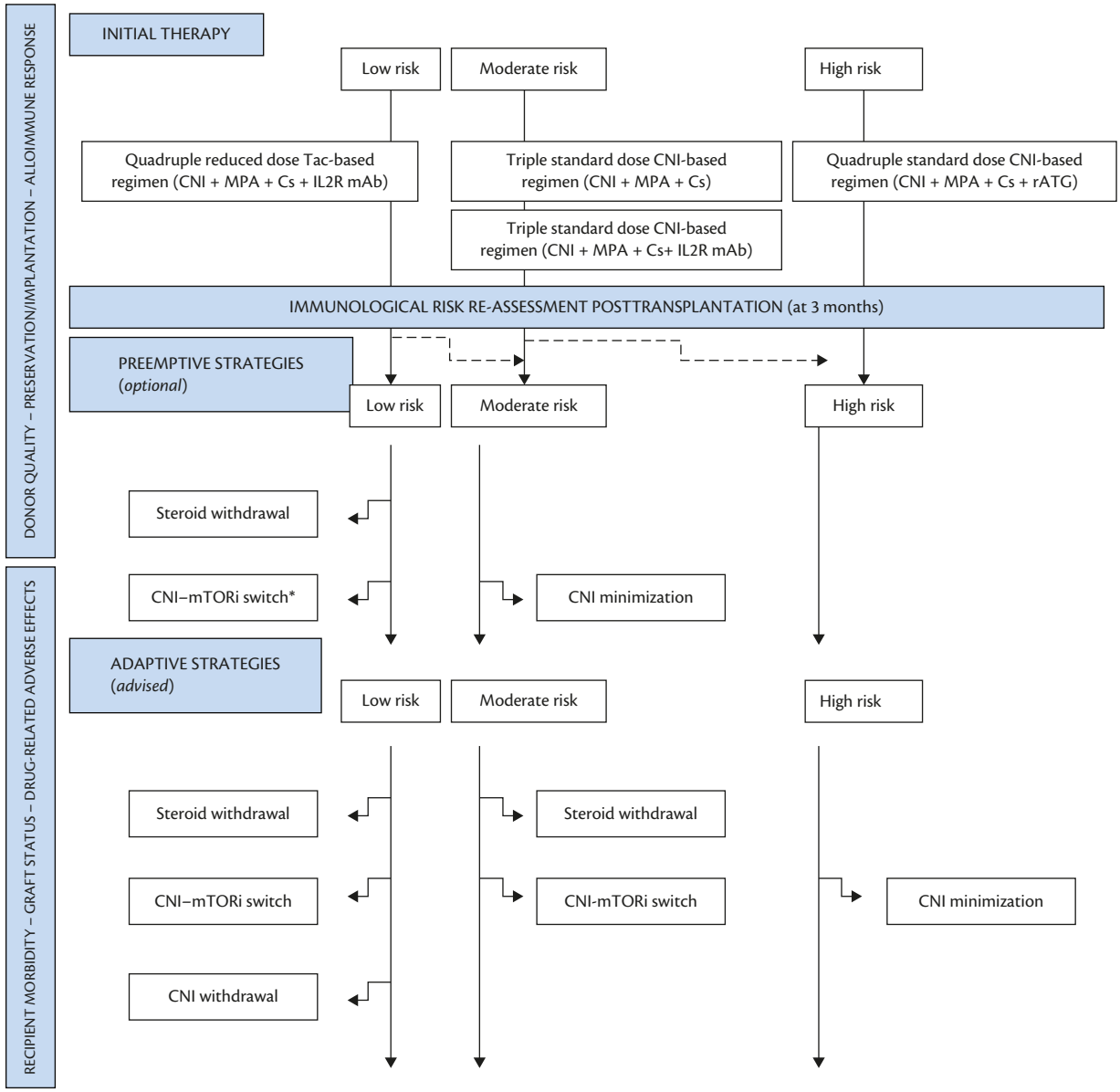

Fig. 281.1 General roadmap for clinical application of immunosuppressive drug regimens.

ciclosporin causes more arterial hypertension, hyperlipidaemia, herpes simplex viral infections, and cosmetic changes (Webster et al., 2005). Part of the differences in efficacy between standard dose CNI-based regimens containing MPA, can be explained by differences in exposure to the latter due to a different interaction between ciclosporin and tacrolimus with MPA (see above, Table 281.1) (Knight et al., 2009). In order to obtain similar and timely early MPA exposure as achieved in tacrolimus-treated patients, ciclosporin-treated patients require a higher MPA dose (Kuypers et al., 2010). With increasing clinical experience and persistence of adequate rejection control by using standard dose CNI-based regimens, attention has shifted towards reducing CNI-related adverse effects without sacrificing efficacy. Notably CNIT (i.e. graft function), PTDM, and cardiovascular risk factors but also infectious complications such as BK polyomavirus associated nephropathy have been selected as new endpoints in comparative clinical trials examining the use of lower doses of CNI (CNI minimization) or attempting CNI withdrawal.

Calcineurin inhibitor minimization

Minimizing CNI exposure has been successfully attempted in many single-centre randomized and non-randomized studies demonstrating that equal efficacy (i.e. acute rejection prevention) can be maintained with lower exposure to CNIs provided MPA was used as the antimetabolite agent and often with the aid of induction therapy in the form of IL-2R mAb (Grinyó and Cruzado, 2009; Moore et al., 2009). Expected improvements in short-term allograft function were inconsistent: especially in ciclosporin minimization trials. No convincing differences in renal function were observed despite lower drug exposure (Grinyó and Cruzado, 2009; Moore et al., 2009). Similarly, few CNI minimization trials have shown clear improvements in the incidence of other CNI-related adverse

events like diabetes, hyperlipidaemia, and arterial hypertension (Grinyó and Cruzado, 2009; Moore et al., 2009). In 2007, the largest randomized study (in 1645 subjects), the ELITE-Symphony Study, confirmed that a combination of low-dose tacrolimus (target trough levels aimed between 3 and 7 ng/mL, levels obtained in the study between 5 and 8 ng/mL) with a fixed daily dose of 2 g of MMF, corticosteroids, and IL-2R blocking mAb resulted in the lowest acute rejection rate (12.4%), the best graft function (65.4 mL/min), and graft survival (94.2%) at 12 months compared to, respectively, standard dose ciclosporin (25.8%, 57.1 mL/min, 89.3%) low-dose ciclosporin (24.0%, 59.4 mL/min, 93.1%), and low-dose sirolimus (37.2%, 56.7 mL/min, 89.3%) regimens with identical concomitant immunosuppression (Ekberg et al., 2007). After 3 years of follow-up, the low dose tacrolimus study group remained superior over the comparator arms in terms of cumulative acute rejection incidence, graft function, and survival (Ekberg et al., 2009). Interestingly, despite lower CNI or mTORi levels achieved in this trial, the toxicity profiles of the respective drug combinations remained unchanged with tacrolimus-treated recipients still having the highest incidence of post-transplantation diabetes and diarrhoea. CNI minimization protocols will steadily progress towards the 'new standard protocols' once widespread clinical experience has reassured practitioners that lower doses of CNI in combination with MPA and IL-2R mAb induction achieve similar or even better efficacy as previous standard protocols. It will be important to balance the beneficial results of CNI minimization against the immunological risk of the patient (e.g. development of donor-specific anti-HLA antibodies) and reserve this approach for recipient considered at low to moderate risk of rejection.

Calcineurin inhibitor-free or avoidance protocols

CNI-free kidney transplantation has been attempted several times in the last 15 years but without achieving acceptable acute rejection rates. Only very recently, a CNI-free drug regimen including the novel immunosuppressive compound, belatacept, has been shown in a randomized study to allow CNI-free transplantation (Vincenti et al., 2005) (see below). In an earlier small randomized study comparing a triple ciclosporin-based with a CNI-free regimen, consisting of a fixed dose MPA, corticosteroids, and intensified daclizumab induction, very high acute rejection rates (52%) were observed in the study arm but renal graft function at 12 months was significantly better in the patients who were able to finish the trial of this CNI-free regimen (Vincenti et al., 2001). Attempts to use PSI as a primary substitute for CNI, together with either azathioprine or MPA, have failed because of high acute rejection incidence and poor tolerance of the CNI-free combinations (Groth et al., 1999; Kreis et al., 2000). Only one randomized study using sirolimus, MPA, corticosteroids, and IL2R-mAb induction demonstrated a clear benefit of CNI-free transplantation in terms of graft function and histology, even after 5 years of follow-up, compared to ciclosporin (Flechner et al., 2007). A similar CNI-free approach using rATG as induction agent and compared to a tacrolimus-based immunosuppressive treatment, produced better renal function in the sirolimus-treated study arm but at a cost of more graft loss, more adverse events, and more premature study withdrawals (Glotz et al., 2010). Unfortunately, no additional randomized controlled studies have confirmed these favourable results on graft function. Finally, in the ELITE-Symphony Study, a low-dose sirolimus-based drug combination with MPA, corticosteroids, and induction therapy had the worst outcome overall compared to CNI-based therapy (Ekberg et al., 2007) (see above). So, except for isolated single-centre experiences, renal transplantation without the use of CNI does not seem to be possible. Belatacept has recently been demonstrated to enable CNI-free transplantation, at least in selected patient study populations. Long-term (5-year) follow-up data on graft (dys)function and safety are encouraging (Vincenti et al., 2005; Rostaing et al., 2013).

Calcineurin inhibitor withdrawal protocols

CNI withdrawal can be executed either as an elective intervention specifically to reduce CNI-related cardiovascular risk factors (i.e. arterial hypertension, hyperlipidaemia) and to prevent the development of CNIT or as a therapeutic strategy once chronic CNI-related renal damage has been diagnosed. Following a series of anecdotal reports, a number of smaller randomized studies have examined the feasibility of withdrawing ciclosporin in stable patients from 6 to 30 months post-transplantation onwards (Guerra et al., 2007). The findings of these studies were quite similar; CNI withdrawal: (1) is only safe with an MPA-based regimen (not azathioprine), (2) causes approximately 10% acute rejection episodes within the first 12 months, (3) is associated with improved graft function, (4) has no impact on graft survival or patient survival up to 5 years of follow-up, and (5) does not consistently improve cardiovascular risk factors (Guerra et al., 2007). In addition, as follow-up increases, cumulative acute rejection rates further increase to approximately 16% (Abramowicz et al., 2005). CNI withdrawal is usually performed following a tapering scheme covering 6–12 weeks and comprising consecutive 25% CNI dose reduction until complete discontinuation. Interestingly, the largest study to date, examining CNI withdrawal between 4–6 months from a quadruple ciclosporin MPA-based regimen, the CAESAR study (N = 536), found not only a high acute rejection rate of 38% in the ciclosporin withdrawal arm but also failed to show the expected improvement of renal allograft function at 12 months, probably a consequence of the excessive early clinical and possibly subclinical alloimmune injury to the graft (Ekberg et al., 2007). By showing that ciclosporin could be stopped when MPA exposure was above a predefined target therapeutic level prior to CNI discontinuation and a pre-withdrawal renal biopsy showed no signs of acute alloimmune injury, Hazzan et al. concluded that concomitant drug exposure and graft status at the time of withdrawal are equally important for achieving success (Hazzan et al., 2005). On the basis of these studies, CNI withdrawal from an MPA-based regimen in recipients with stable graft function is not widely advocated except in very selected cases. A CNI switch to a mTORi is a more feasible strategy in patients who do not tolerate ongoing CNI treatment (see below).

CNI withdrawal in patients with documented renal allograft dysfunction and/or biopsy-proven chronic allograft damage has been successfully attempted in several small uncontrolled studies, resulting in improved serum creatinine concentrations and a delay in allograft lost (Birnbaum et al., 2009). Interestingly, acute rejection rates were low in these trials, suggesting that in the majority of patients, graft dysfunction was mainly of non-immunological causes. A prospective randomized study, the Creeping Creatinine Study in 122 recipients with deteriorating renal graft function, confirmed that by maintaining patients on MMF therapy with corticosteroids after CNI withdrawal, improved graft function could be achieved without acute rejection risk compared to CNI continuation (Dudley et al., 2005). Only six grafts were lost during follow-up

in this study, two in the CNI withdrawal group. Switching CNI to mTORi-based therapy in cases of chronic allograft dysfunction is a more commonly applied strategy that is effective in selected patient groups.

Induction therapy

Induction therapy is defined as a time-limited treatment with a biologic agent, either a lymphocyte-depleting agent or an interleukin 2 receptor antagonist (IL2-R mAb), started prior to, at the time of, or immediately after transplantation. The most commonly used agents are basiliximab/daclizumab (non-lymphocyte-depleting humanized monoclonal antibodies) and rATG (lymphocyte-depleting polyclonal antibodies) (US Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients, 2009). The rationale for induction therapy for prevention of acute rejection in kidney transplantation is that acute rejection is most likely in the first weeks and months after transplantation (Nankivell et al., 2003). In addition, there is evidence—at least in animal studies—that lymphocyte depletion at time of transplantation plays a role in tolerance induction (McCauley, 2005).

Induction therapy is primarily used in renal allograft recipients considered at high immunological risk for acute rejection including high number of human leucocyte antigen (HLA) mismatches, young recipient age, (older donor age) suboptimal organ quality (non-heart-beating donation, expanded criteria donor donors), delayed graft function, African American ethnicity, panel reactive antibodies > 20%, presence of donor-specific antibodies, blood group incompatibility, and cold ischaemia time > 24 hours. In addition, there is ample evidence that the benefits of IL-2R mAb induction therapy outweigh adverse effects also in low-risk patients (Webster et al., 2010). Especially in patients treated with immunosuppressive regimens where corticosteroids are withdrawn early after transplantation or minimal CNI exposure is sought, antibody induction therapy is generally considered to provide an effective safety net against rejection (Woodle et al., 2005).

rATG is usually administered daily over a period of 4–14 days provided that the white blood cell counts and/or platelet counts allow for additional dosing (white blood cell count > 2000/mm^3 or platelet count > 50,000/mm^3). Many clinicians reduce the dose of rATG to 50% when the white blood cell count decreases to 2000–3000/mm^3 or platelet count decreases to 50,000–75,000/mm^3. Alternatively, total lymphocyte counts and CD3(+) cell counts have been successfully applied to guide antithymocyte dosing. Basiliximab is usually given in two doses of 20 mg, at the time of transplantation and at day 4 after transplantation. Daclizumab (which is no longer commercially available) was administered at 1 mg/kg at time of transplantation and then every 14 days until a total of 5 doses.

In comparison with placebo, IL2-R mAb induction produces a 28% decrease of the incidence of acute rejection (RR = 0.72; 95% CI 0.64–0.81) in the first year after transplantation, a lower risk of graft loss (RR = 0.75; 95% CI 0.62–0.90), and a 19% (RR = 0.81; 95% CI 0.68–0.97) reduction of cytomegalovirus (CMV) disease with no effect on patient survival (Webster et al., 2010). Polyclonal antithymocyte globulins also decrease the risk for acute rejection and graft loss compared to no induction, especially in high-risk patients (Deeks and Keating, 2009; Gabardi et al., 2011). In comparison with IL2-R mAb, rATG induction therapy is associated with an additional 30% decrease (RR = 1.30; 95% CI 1.01–1.67) in the incidence of biopsy-proven acute rejection (especially in patients with high immunological risk), but leads to significantly more serious side effects, including a 30% higher incidence of CMV disease (RR = 0.68; 95% CI 0.50–0.93) and 75% higher risk (RR = 0.25; 95% CI 0.07–0.87) of malignancies, while graft and patient survival are similar (Webster et al., 2010). It is suggested that recipients at high immunological risk should receive polyclonal antithymocyte antibodies, while low-to-moderate immunological risk patients should be treated with IL2-R mAb (Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Working Group, 2009).

Induction therapy with alemtuzumab, an anti-CD52 T-cell and B-cell-depleting monoclonal antibody, in low immunological risk patients is associated with a lower acute rejection risk compared to IL2-R mAb (5% vs 17%) and is equally effective as polyclonal antithymocyte globulin in rejection prevention (10% vs 13%) (Hanaway et al., 2011). Alemtuzumab use is associated with an increased risk for serious infections compared to IL2-R mAb (35% versus 22%), similar to that of polyclonal antithymocyte globulin.

Corticosteroid avoidance and withdrawal

The prolonged use of corticosteroids gives rise to a wide array of potentially harmful side effects. The rationale for eliminating corticosteroids from immunosuppressive regimens is largely driven by the desire to prevent or eliminate these side effects and is based on the presumption that other immunosuppressive agents are potent enough to prevent both acute and chronic allograft rejection.

To eliminate the undesired effects of corticosteroid after transplantation, two different strategies are possible: either complete corticosteroid avoidance from time of transplantation onwards ('steroid avoidance protocols') or corticosteroids can be withdrawn at a specific time point in the post-transplantation period ('steroid withdrawal protocols'). Withdrawal of corticosteroids can be performed pre-emptively, before side effects occur, or in response to side effects occurring after prolonged corticosteroid use. Osteoporosis and PTDM are early-onset adverse effects of corticosteroids after transplantation which implies that steroid avoidance protocols should be more effective in reducing these complications.

In the past decades, different patient populations treated with a variety of immunosuppressive drug combinations have been evaluated for eligibility for either corticosteroid withdrawal or avoidance (Pascual et al., 2009; Knight et al., 2010). Most studies were performed in patients on triple maintenance immunosuppressive therapy (a combination of a CNI, MPA, and corticosteroids), often with additional induction therapy. Only few, mostly the earlier studies, have evaluated the effect of corticosteroid withdrawal from dual therapy. Because the majority of corticosteroid avoidance studies or withdrawal studies were performed with antibody induction in the corticosteroid avoidance arm, their results cannot be extrapolated to protocols without induction treatment (Pascual et al., 2012). In addition, corticosteroid withdrawal has only rarely been attempted in patients at high immunological risk (Pascual et al., 2009). Corticosteroid avoidance or withdrawal from CNI-free, PSI-based drug regimens have not been properly studied and so caution is advised when implementing this strategy (Knight and Morris, 2010; Pascual et al., 2010).

Complete corticosteroid avoidance protocols include no steroid administration or the administration of the equivalent of a single perioperative bolus of 250–500 mg methylprednisolone during the transplantation procedure itself. Steroid withdrawal protocols

taper corticosteroids at a given time point after transplantation, either before 14 days post transplantation ('early withdrawal,' which is sometimes categorized with 'steroid avoidance') or after 14 days post transplantation ('late withdrawal,' which ranges from 14 days up to years after transplantation) (Pascual et al., 2009). The tapering of corticosteroids is usually performed over a period of 3–4 weeks to 2–3 months, but many different protocols have been used and no direct comparison between fast or slow tapering has been performed. Abrupt complete discontinuation of corticosteroids in a late withdrawal protocol should be avoided in order to prevent acute hypoadrenalism. When steroid avoidance or withdrawal fails, that is, when acute rejection occurs, corticosteroid antirejection treatment usually reverses the process and steroid withdrawal can be resumed provided graft function has returned to baseline.

The benefits and risks of complete corticosteroid avoidance protocols appear to be similar to protocols with early corticosteroid withdrawal (withdrawal within 14 days after transplantation) (Pascual et al., 2012). In patients treated with ciclosporin as part of the immunosuppressive regimen, the risk for acute rejection is significantly increased by 60% (RR = 1.59; 95% CI 1.01–2.49) in steroid avoidance protocols compared to conventional maintenance therapy with corticosteroids, but this is not the case with tacrolimus-based regimens (RR = 1.06; 95% CI 0.79–1.42) (Pascual et al., 2012). No increased risk for graft loss or patient death with complete corticosteroid avoidance or early withdrawal has been observed. Graft function is similar between withdrawal and control study arms, at least if antibody induction therapy was used (Woodle et al., 2005; Pascual et al., 2012). The only consistent benefit of complete corticosteroid avoidance or early withdrawal is a decreased incidence of PTDM, at least in ciclosporin-treated recipients: a 46% decrease in PTDM risk (RR = 0.54; 95% CI 0.30–0.98), but not in patients treated with tacrolimus (RR = 0.75; 95% CI 0.32–1.77). Intriguingly, the expected changes in lipid levels or blood pressure have not been seen in most studies, illustrating the multicausal origin of these complications (Pascual et al., 2012).

Late corticosteroid withdrawal, after 14 days post transplant, appears to be safe in terms of graft function, graft survival, and patient survival, but is associated with increased biopsy-proven acute rejection rates in patients treated with ciclosporin and MPA (RR = 1.61; 95% CI 1.20–2.17) (Kasiske et al., 2000; Pascual et al., 2010). In patients treated with tacrolimus (and MPA), the acute rejection risk (~ 5%) is much lower after steroid withdrawal (Vanrenterghem et al., 2005; Pascual et al., 2010). Late corticosteroid withdrawal leads to a reduced need for lipid lowering agents and a decreased incidence of PTDM, although this finding did not reach statistical significance (Pascual et al., 2010). In a meta-analysis, cardiovascular risk factors (including hypertension), PTDM, and hypercholesterolaemia were reduced by, respectively, 10% (RR = 0.90; 95% CI 0.85–0.94), 36% (RR = 0.64; 95% CI 0.50–0.83), and 24% (RR = 0.76; CI 0.67–0.87) if steroid avoidance or withdrawal was compared to corticosteroid therapy (Knight and Morris, 2010). A limited effect of steroid avoidance on bone mineral density at 12 months post-transplantation was demonstrated in only a few smaller studies.

There is a growing consensus that the modest benefits of pre-emptive late steroid withdrawal on cardiovascular risk factors do not outweigh the risk of acute rejection in patients treated with a standard combination therapy of a CNI and MPA, especially in patient treated with ciclosporin and/or recipients with moderate to high immunological risk (Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Working Group, 2009). In patients developing harmful corticosteroid side effects, late corticosteroid withdrawal can be considered, after taking account of the surrogate clinical indicators of the immunological status of the patient (Fig. 281.1).

Calcineurin inhibitor conversion to proliferation signal inhibitor

In current clinical practice, there are two main reasons for conversion from a CNI to a mTORi regimen, that is, CNIT and malignancies. Acute CNIT occurs during episodes of increased blood CNI concentrations and is characterized by a transient rise in serum creatinine caused by a strong but reversible vasoconstriction of the afferent arteriole (Naesens et al., 2009). Chronic CNIT is an insidious process leading to progressive vascular, tubular, and interstitial structural damage in the kidney and functional decline (Nankivell et al., 2003). Therefore, modified drug protocols have been proposed with the initial use of a CNI followed by a switch to an mTORi to avoid CNIT. With this strategy, the benefits of CNI therapy in the initial phase after transplantation (i.e. low acute rejection incidences) can be maintained, while avoiding specific undesired effects of mTORi in the immediate postoperative phase (impaired wound healing, lymphocoeles, and acute tubular necrosis).

Secondly, the use of ciclosporin or tacrolimus increases the risk of certain malignancies and accelerated cancer progression (Marcén, 2009; Rama and Grinyó, 2010). As the mTOR pathway also drives tumour genesis, mTOR inhibition plays a role in anti-cancer treatment (Hartford and Ratain, 2007; Gabardi and Baroletti, 2010). Hence, mTORi could have a place in immunosuppressive protocols to decrease the risk of developing certain types of cancer or to improve the prognosis of patients with established malignancies.

If conversion to an mTORi is attempted to avoid the development of chronic CNIT, the switch from a CNI to an mTORi should be performed early after transplantation ('early conversion'). Alternatively, the switch to an mTORi can be carried out when there are clinical and/or histological signs of chronic CNIT which typically increase with time after transplantation ('late conversion'). Likewise, if conversion is considered for malignancies, a pre-emptive approach can be used, before malignant disease is evident, or conversion can be implemented in the treatment algorithm of the kidney transplant recipients with established cancer.

The presence of proteinuria exceeding a threshold of 0.5–1g/24 hours is a specific contraindication to mTORi switch, as it increases the risk of developing overt proteinuria. In addition, substitution of an mTORi for a CNI is futile in patients with poor renal graft function, especially when the GFR is < 40 mL/min/1.73m^2 (Schena et al., 2009). Finally, it should be remembered that the prolonged combination of mTORi and CNI, especially ciclosporin should be avoided because mTORi potentiate CNI-induced nephrotoxicity (Kuypers, 2009).

In practice, there are three main strategies for switching from a CNI to an mTORi: (1) an overnight conversion strategy without overlap, (2) an overnight conversion with overlap, and (3) a CNI tapering regimen. In the overnight conversion strategy without overlap, the last full dose of the CNI is administered in the evening. The next morning, the mTORi is started with a loading dose. For sirolimus, an initial maintenance dose of 4–8 mg once daily is advocated after a single loading dose of 10–12 mg. For everolimus,

starting doses are usually approximately 0.75–1.5 mg twice daily, with a single loading dose of 3 mg. In the overnight conversion with overlap, a similar strategy is followed, but the mTORi is initiated on the last day of CNI use, with one or two combined dosing of the CNI and the mTORi. In the CNI tapering regimen, the CNI is gradually reduced (usually by 25% dose reductions) over a 2–4-weeks period with adjustment of the mTORi dose to obtain target therapeutic trough levels by the time the CNI is discontinued. There are no studies which directly compare these three different strategies.

To assess the benefits of conversion of a CNI to an mTORi, the timing of conversion is an important factor. Late conversion strategies (arbitrarily set at > 6 months after transplantation) have been evaluated in many non-randomized trials and a few randomized studies. In these studies, conversion to sirolimus was associated with an improvement in short-term renal allograft function. However, the most recent and largest randomized study (N = 830) did not confirm this benefit on glomerular filtration rate in an intention-to-treat analysis, but showed a small improvement of glomerular filtration rate in an on-therapy analysis, especially in patients with a baseline glomerular filtration rate > 40mL/min and with absent or minimal proteinuria (urinary protein:creatinine ratio < 0.11) (Oberbauer, 2009; Schena et al., 2009). The risk for certain types of cancers, most notably non-melanoma skin carcinoma, decreased after conversion from a CNI to sirolimus (Alberu et al., 2011). There is no benefit of late mTORi switch on patient or graft survival, and the poor clinical tolerance of late conversion to mTORi therapy often offsets the minor benefits on graft function (Kuypers, 2005). At 24 months post conversion, 20% of patients (110/551) had discontinued mTORi therapy because of adverse events, illustrating the clinical impact of drug-related side effects on drug continuation (Schena et al., 2009). Late conversion is safe in terms of risk for acute rejection. However, late conversion strategies are associated with side effects like hypertriglyceridemia, hypercholesterolaemia, hyperglycaemia, diarrhoea, anaemia, wound healing problems, peripheral oedema, stomatitis or mouth ulcers, pneumonitis, and new-onset or increase of proteinuria (Kuypers, 2005). Some of these side effects are dose dependent and can be partially reversed by dose reductions (Kuypers, 2005; Oberbauer, 2009), others are potentially harmful and necessitate permanent discontinuation of the mTORi. In many patients therefore the small benefits in terms of improvement of graft function after late mTORi conversion and the absence of significant effects on graft and patient survival do not justify tolerating the drug-related adverse effects.

When conversion from a CNI to an mTORi is performed early after transplantation (within the first 6 months, best between 3 and 6 months) the effect on graft function appears to be larger and supported by histological improvement, at least in comparison with continued ciclosporin therapy. However, the benefit on graft function is not clear when the comparison is with tacrolimus (Mota et al., 2004; Budde et al., 2011, 2015; Weir et al., 2011). Although the effects on graft function in the first years after transplantation are promising, the effects in the long term and on graft and patient survival remain to be established. Similar to what is seen in late conversion, early substitution of an mTORi for a CNI is associated with important side effects, leading to discontinuation of the drug in 20–63% of patients (Mota et al., 2004; Kuypers, 2005; Budde et al., 2011; Weir et al., 2011). Of note, most of the early conversion trials have often excluded patients with severe and/or repetitive acute rejection episodes from conversion, indicating that this strategy is only applicable to recipients with a low to moderate immunological risk.

Finally, when conversion of a CNI to an mTORi is performed in the context of a malignant disease developing after transplantation, this is often established in conjunction with cessation of other immunosuppressive drugs in order to maintain patients on dual therapy with an mTORi and low-dose corticosteroids. This strategy has proven effective and safe for inducing complete remission in patients with HHV-6-related Kaposi's sarcoma (Stallone et al., 2005). Given the therapeutic effects of mTORi in other malignancies like renal cell carcinoma, clinicians extrapolate this strategy to renal transplant recipients with different types of malignancies and replace CNI plus antimetabolite combinations by an mTORi and corticosteroids regimen. The effectiveness and safety of this approach has not been evaluated in transplant patients except for ongoing studies examining the use of mTORi as secondary prevention for non-melanoma skin cancer.

Novel approaches

The clinical implementation of different types of immunosuppressive protocols has made transplantation the first-choice treatment for end-stage renal disease (Wolfe et al., 1999). Significant progress has occurred over the last 30 years, mostly seen in improvements in short-term graft and patient survival. Long-term graft and patient survival have increased to a lesser extent (Lamb et al., 2011). Apart from the intrinsic consequences of chronic suppression of the immune system, the current immunosuppressive drugs lack specificity and cause a large number of acute and chronic adverse events. Important efforts have been put into research on methods to overcome alloimmune reactions without the use of the standard immunosuppressive regimens. Two main strategies have been followed: better targeted therapy and tolerance induction (see Chapter 279).

Targeted therapies

The progresses made in molecular biology and immunology have resulted in the development of drugs targeted at interfering with key biological processes. In clinical transplantation, new molecules are being tested in various combination regimens. The most recent agent, which was approved in 2011 by the EMA and FDA for use in kidney transplantation, is belatacept (a fusion protein that inhibits T-cell activation by binding to CD80 and CD86) (Vincenti et al., 2011). Belatacept is used in combination regimens with MPA, corticosteroids, and induction therapy with IL2-R mAb. Patient and graft survival are similar in patients receiving the recommended belatacept regimen (10 mg per kg dose on day 1, on day 5, at the end of weeks 2 and 4, then every 4 weeks through to week 12, followed by the maintenance dose of 5 mg per kg every 4 weeks from week 16 onwards), compared to patients receiving standard ciclosporin, MPA, corticosteroids and induction with IL2-R mAb (Vincenti et al., 2005, 2010). The use of belatacept instead of ciclosporin is associated with significantly better graft function up to 5 years after transplantation (Vincenti et al., 2010) and lower rates of PTDM, less hypertension, and better lipid control up to at least 1 year post transplant (Vanrenterghem et al., 2011). On the downside, there is concern about increased risk for EBV-related PTLD with belatacept, especially in EBV-negative recipients (Vincenti et al., 2005). Its use is not advocated in EBV-negative renal recipients (Vincenti

et al., 2011). Other promising immunosuppressive compounds under investigation for renal transplantation in phase II trials were sotrastaurin (a protein kinase inhibitor) and tofacitinib (a Janus kinase-3 inhibitor). Their further clinical development has been recently halted because of insufficient added clinical value.

Finally, drugs that have been approved for targeted treatment in other medical conditions outside transplantation are being tested for treatment of specific transplant problems. Regimens that include the use of anti-CD20 monoclonal antibody (e.g. rituximab), proteasome inhibitors (e.g. bortezomib), inhibitors of complement activation (e.g. eculizumab), plasma-exchange and intravenous immunoglobulins (IVIG), and other specific inhibitors of B-cell and plasma cell activation are being tested for prevention or treatment of antibody-mediated rejection and for desensitization protocols to overcome the detrimental effects of HLA antibodies or ABO-incompatible transplantation. First clinical experience and short-term outcome of these strategies is promising, but longer-term follow-up and validation of the findings in larger studies is necessary (Knechtle et al., 2010).

Transplantation tolerance

Transplantation tolerance is most commonly defined as prolonged graft survival and persistent graft function in the absence of immunosuppressive drugs. Although data from small animal experiments have been promising, translation into clinical practice has been difficult, and major barriers still need to be overcome (Newell, 2011). First encouraging results in humans are seen with combined kidney and haematopoietic stem-cell transplantation and non-myeloablative conditioning regimens, where it appears to be feasible to maintain good graft function for up to 5 years without immunosuppressive treatment (Starzl, 2008). However, the efficacy of these protocols has yet to reach those achieved by conventional immunosuppression and the procedures present major safety challenges and are unlikely to be used unless these can be overcome. Animal data suggest that lymphocyte depletion at time of transplantation plays a role in tolerance induction, and this is the reason why some clinicians suggest using alemtuzumab or polyclonal antithymocyte antibodies as induction therapy (McCauley, 2005). Others use approved immunosuppressive agents in non-standard combinations (e.g. various combinations of alemtuzumab, belatacept, bortezomib, or sirolimus), infusion of donor lymphocytes, or expansion of regulatory T cells in an attempt to achieve transplantation tolerance (Bishop et al., 2011; Newell, 2011). None of these approaches is ready for large-scale clinical trials.

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CHAPTER 282

Renal transplant imaging

Simon Gruenewald and Philip Vladica

Introduction

The purpose of imaging of the transplant kidney (the graft) is to assess its immediate integrity and to monitor for potential perioperative and later complications. Complications are divided on the basis of their site and timing: immediate (first week), early (1 week to 1 month), and late onset (over 1 month). Perioperative complications can occur in up to 15–20% of grafts and if detected early are readily treatable (Quintela et al., 2009). Early complications include acute tubular necrosis (ATN), acute rejection, haematoma, pyelonephritis, abscess, urinoma, ureteral obstruction, vascular complications, and rarely graft torsion. Late complications are usually related to medical complications arising from immunosuppression, chronic rejection, lymphocoele, cyst, renal artery stenosis, urinary obstruction, and tumours (Box 282.1).

Normal renal transplant ultrasound appearance

Operative information is needed to interpret the first ultrasound (US) scan. This includes the number of main renal vessels and their anastomoses. The superficial position of most kidney transplants allows excellent US resolution of morphology. Compared with native kidneys, the cortex and medulla can be more easily defined as the pyramids are normally hypoechoic relative to the columns of Bertin. The collecting system may be visible but the renal pelvis should be < 10 mm in diameter and the calyces not dilated.

Despite the availability of more sophisticated and expensive tests (Box 282.2), US with colour, power, and spectral Doppler, also called colour Doppler ultrasound (CDUS), remains the mainstay of kidney transplant imaging by virtue of its low cost, safety, rapidity, and portable availability. Together with sonography, radionuclide studies provide important quantitative functional information. Comparing different imaging techniques and performing serial studies is especially useful. Avoiding false-positive or -negative imaging requires knowledge of the operative and clinical information.

CDUS should demonstrate good blood flow throughout the transplant extending close to the capsule but because of decreased Doppler sensitivity with depth and vessel direction, the polar regions of the kidney may appear to have a mild reduction in flow. Flow indices such as the Resistance Index (RI) are measured from Doppler tracings of the arcuate or segmental renal arteries at the upper, mid, and lower poles of the transplant. RI = (peak systolic – end diastolic velocity) / peak systolic velocity.

Immediately post transplantation, RI is variable depending on patient hydration, heart rate, blood pressure, and dose of vasodilators. Immediate post graft RI > 0.8 predicts poor graft function but not its cause, and a single RI result during the first few postoperative weeks is not as useful as the RI trend in reference to clinical findings. Peak systolic velocity should be measured in the main renal artery and vein at the anastomosis and hilum. Surgical technique, however, will influence the length, position, and tortuosity of the vessels that may make accurate measurement difficult.

For well-functioning grafts, CDUS soon after transplantation and before hospital discharge may be all that is required with progress US studies performed routinely at 3 months, 1 year, and yearly thereafter. A routine 3-month radionuclide study and glomerular filtration rate (GFR) serves as a useful baseline for comparison with yearly GFR and further scintigraphy if complications develop. Transplant function at 1 year has been shown to have a good correlation with graft survival (Salvadori et al., 2003).

If, however, on early US the transplant is non-functioning or there is clinical suspicion of a complication, more frequent CDUS examinations will be required with frequency dependant on clinical and laboratory findings. The results will then influence the need and choice of second-line imaging investigation for making definitive and comprehensive assessment (Singh and Sahani, 2008). The relative indications, strengths, and weaknesses of imaging modalities are outlined in Table 282.1.

Vascular complications

Occlusion of an accessory vessel gives rise to segmental infarction, which is not discernible in the acute phase on greyscale US, but CDUS shows a well-defined area of absent blood flow (Fig. 282.1). This hypovascular area tends to become smaller over the course of months. Renal artery (RA) and vein (RV) occlusion are rare occurrences but US findings are specific enough not to require other confirmatory tests before urgent surgery. In RA occlusion, the graft is unchanged in greyscale appearance but CDUS shows no arterial or venous flow. In the early stages of RV occlusion, the graft is also of normal morphology but no venous waveforms are detected in the peripheral venules and the resultant resistance to inflow causes a decrease in arterial diastolic flow and a raised RI. In the later stages of RV occlusion, the kidney becomes enlarged and hypoechoic with loss of corticomedullary differentiation and there is absent or reversed diastolic flow in the renal artery (Fig. 282.2). Although severe acute rejection and tubular necrosis may also cause reversed diastolic flow in early diastole, in RV occlusion it is often during

Box 282.1 Renal graft complications

Vascular:

- Accessory vessel occlusion (Fig. 282.1)
- Renal artery or vein occlusion (Fig. 282.2)
- Renal artery stenosis (Fig. 282.3)
- Renal vein stenosis or compression (Fig. 282.4)
- Torsion

Urologic:

- Non-obstructive dilatation
- Hydronephrosis (Figs 282.5 and 282.6)
- Vesicoureteric reflux (Fig. 282.7)
- Haematoma (Fig. 282.8)
- Infection
- Calculus (Fig. 282.9)
- Urinoma

Procedure related:

- Contusion/haematoma (Fig. 282.10)
- Arteriovenous fistula (Fig. 282.11)
- Pseudoaneurysm (Fig. 282.12)

Perinephric:

- Haematoma/seroma (Fig. 282.13)
- Lymphocoele (Fig. 282.14)
- Urinoma
- Abscess

Parenchymal:

- Acute tubular necrosis
- Acute rejection (Figs 282.15 and 282.16)
- Chronic rejection
- Drug nephrotoxicity
- Infarct (Fig. 282.17)
- Pyelonephritis/abscess (Fig. 282.18)
- Nephrocalcinosis (Fig. 282.19)
- Simple cyst (Fig. 282.20)
- Complex cyst
- Tumours (Fig. 282.21)
- Recurrent native kidney disease

entire diastole (Gao et al 2007). Thrombus will rarely be identified on US in a dilated RV.

CDUS has excellent sensitivity in the detection of graft renal artery stenosis (RAS) but specificity depends on how strict the

Box 282.2 Imaging modalities

- Ultrasound with colour, power, and spectral Doppler (CDUS)
- Radionuclide scintigraphy (DTPA, MAG3, DMSA)
- Computed tomography (CT) and computed tomography angiography (CTA)
- Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA)
- Contrast-enhanced ultrasound (CEU)
- Angiography
- Nephrostogram
- Cystogram.

Doppler criteria are. If the angle corrected (< 60°) PSV in the RA is > 2 m/sec and the ratio of RA to iliac artery peak velocity is > 2, this is suggestive of narrowing which may be due to extrinsic compression, kinking, or stenosis (Fig. 282.3). Using a higher cut off value of 2.5 m/sec and a ratio of 3 improves specificity for a haemodynamically significant RAS and the presence of focal colour aliasing, turbulence distal to the stenosis and a 'parvus-tardus' waveform make RAS even more likely. The parvus-tardus waveform refers to acceleration time (start of systole to peak) of > 0.07 sec and acceleration index (slope of the systolic uptake) of < 3 m/sec^2. When RAS is suspected from US, a diagnostic study such as computed tomography angiography (CTA) or magnetic resonance angiography (MRA) is required. The captopril renal scan does not have the accuracy of the above modalities and is no longer recommended.

In contrast to renal arteries, stenosis of the lower pressured renal vein is most often the result of compression or kinking from adjacent structures or collections (Fig. 282.4). CDUS may demonstrate focal narrowing and turbulence. Although velocity parameters for diagnosis have not been defined, it has been suggested that a three- to fourfold increase in venous velocity from pre-stenotic to stenotic region is highly suggestive (Irshad et al 2008). CT or magnetic resonance imaging (MRI) is once again the most appropriate second-line investigations to elucidate the reason for the raised venous velocity.

Urologic complications

Mild non-obstructive dilatation of the renal pelvis is common on US and imaging should be repeated after the patient has emptied their bladder and any post-void bladder volume noted. If the pelvis and calyces are dilated (Fig. 282.5), the transplant ureter should be carefully followed as ureteric kinking, compression, or stricture (Fig. 282.6) are the commonest causes of urinary tract obstruction. If the lower ureter is more dilated than the rest of the collecting system, vesicoureteric reflux should be suspected (Fig. 282.7). In the diagnosis of obstruction, a raised RI or non-visualization of a ureteric jet are too non-specific. Equally, reliance on progressive collecting system dilatation over time is impractical. Diagnosis is especially difficult in the context of previous obstruction due to an already dilated collecting system (Cosgrove, 2008). Mercaptoacetyltriglycine (MAG3) scintigraphy following good hydration and intravenous frusemide will often confirm obstruction and localize the site (Nankivell et al., 2001).

Table 282.1 Indications, strengths, and weaknesses of each imaging modality

Modality	Indications	Strengths	Weaknesses
Ultrasound ± Doppler	Routine primary test for all indications	Accessible, non-invasive, rapid, cheap, no ionizing radiation	Operator and patient dependant. Raised RI is non-specific. Does not assess function. Poor views of ureters or deeper structures
Contrast ultrasound	Tumour and vascular abnormalities. Allergy to contrast and low GFR	Not nephrotoxic. Superior blood flow detection compared with Doppler US	Operator and patient dependent. Limited to focal lesions due to limited viewing field
DTPA/MAG3	Assessment of transplant function, urinary obstruction, and leaks	Not nephrotoxic. Quantitative study of function and excretion	Low accuracy in early RAS and urinary tract obstruction
DMSA	Suspected parenchymal injuries, e.g. infarcts, scars, infection	High sensitivity for focal parenchymal abnormalities	Poor specificity
CT ± angiography ± intervention	Non-diagnostic US or nuclear studies. Non-invasive vascular study	Large field of view with detailed non-invasive 3D vascular and multiphase images even in large patients	Ionizing radiation. Nephrotoxicity risks. Metal artefacts
MRI ± angiography	Allergy to non-ionic contrast. Morphologic, haemodynamic, and functional imaging	Non ionizing radiation. Low allergic and nephrotoxic risk. MRA with 3D and functional imaging. To characterize masses and collections	Expensive. Poor access. Claustrophobia. Ferro-magnetic objects preclude use. Risk of nephrogenic systemic fibrosis. Flow and movement artefact
Angiography ± intervention	Second line for vascular studies with view to therapy	Most accurate assessment of arterial disease with scope for therapy	Invasive. Nephrotoxic. Contrast allergy. Ionizing radiation
Nephrostogram ± nephrostomy	Suspected urinary obstruction	Useful even in poorly functioning grafts	Invasive. Risk of infection. Ionizing radiation
Cystography	Suspected bladder leak or vesicoureteric reflux	Detects site of leak in poorly functioning grafts	Invasive. Risk of infection. Ionizing radiation

However in cases of low-grade obstruction, or when transplant function is impaired, MAG3 can be equivocal and MR or CT urography correlation indicated. Nephrostomy with assessment of change in renal function may be required to confirm a functionally significant obstruction.

The urothelium is normally a thin echogenic line on US and any fluid in the collecting system echo free. Thick urothelium or echogenic material in the collecting system is usually due to bleeding (Fig. 282.8) or infection but a thick urothelium may also be found in acute rejection. Renal transplant patients have a greater incidence of calculi (Fig. 282.9), which may be painless as the transplanted kidney is denervated (Park et al., 2007). Calculi and tumours have a similar appearance to those in native kidneys. CT and MRI are more accurate than US in diagnosis of small lesions.

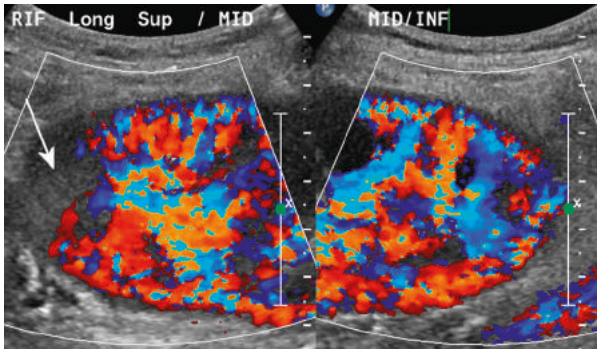

Fig. 282.1 Segmental infarction. Composite longitudinal CDUS which shows (right) normal vascularity at the lower pole and (left) a focal area of absent vascularity (arrow) at the upper pole due to accessory artery occlusion.

Procedure-related complications

Biopsy often causes a contusion which results in abnormal parenchymal echotexture and reduced vascularity (Fig. 282.10). Arteriovenous fistula (AVF) and pseudoaneurysm (PA) are almost always complication of renal biopsy with AVF due to simultaneous arterial and venous wall injury and PA due to arterial wall injury alone. On CDUS, AVF has a focal site of turbulence and aliasing with high PSV and diastolic velocity (low RI) in the feeding artery and pulsatile 'arterialized' flow in the draining vein (Fig. 282.11). PA appears to be a complex cyst on greyscale but CDUS reveals internal swirling blood flow (Fig. 282.12). If these lesions 'steal' blood flow from the rest of the kidney, cause bleeding into the collecting system or perinephric space, or enlarge rapidly, further investigation with CTA or MRA and treatment by angiography may be required.

Perinephric complications

Perinephric collections are common and easily visualized by US. Serial measurements in three axes to estimate volumes which allow progress to be followed are recommended. The time interval of onset from graft transplantation and progress help to determine aetiology, but US-guided aspiration and fluid analysis is required for accurate diagnosis.

Haematomas (Fig. 282.13), seromas, and urinomas are seen in the first few weeks postoperatively. Acute haematomas are echogenic

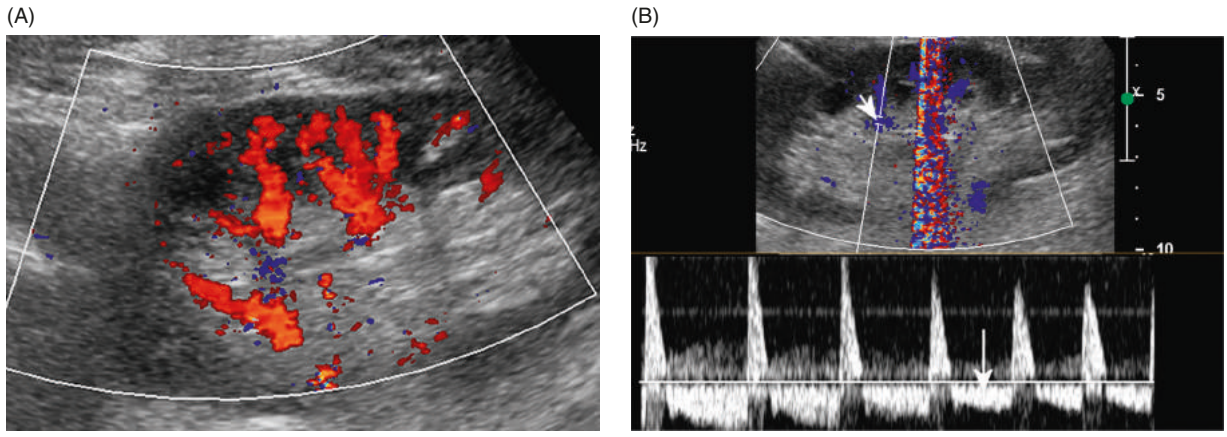

Fig. 282.2 Renal vein occlusion. (A) CDUS shows arterial flow with absent flow in intervening peripheral veins of the renal parenchyma. (B) Pulsed Doppler tracing from an interlobar artery (arrow head) shows reversed diastolic flow (arrow).

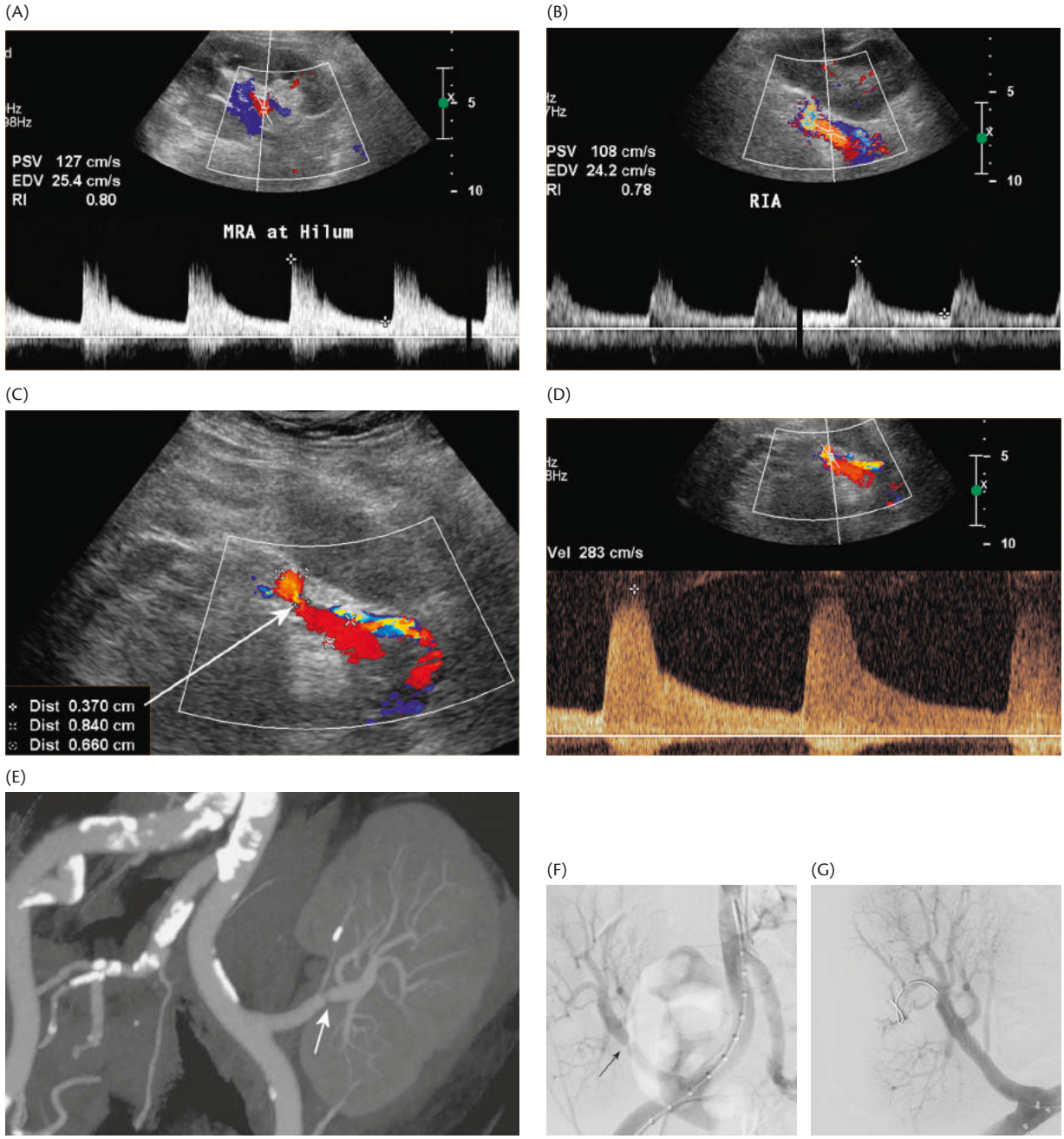

Fig. 282.3 Mid main renal artery stenosis. (A) CDUS shows peak systolic velocity (PSV) at the hilum of 127 cm/sec. (B) PSV in the iliac artery of 108 cm/sec. (C) Focal narrowing in the mid main renal artery to 3.7 mm (arrow). (D) PSV at site of narrowing 283 cm/sec. (E) CTA shows the renal artery stenosis (arrow). (F) Angiogram confirms stenosis before placement of stent (arrow). (G) Angiogram shows normal lumen following stenting.

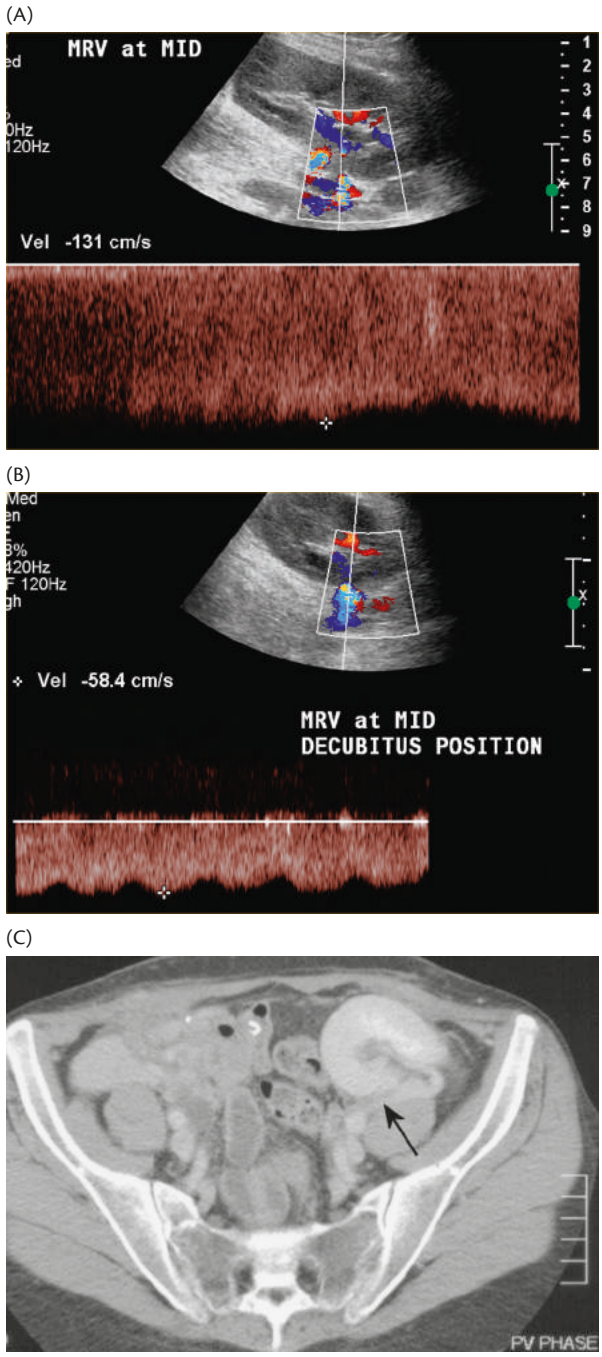

Fig. 282.4 Main renal vein compression. (A) CDUS shows increased velocity in the mid main renal vein of 131 cm/sec with patient supine. (B) Velocity is 58 cm/sec with patient in the decubitus position. (C) CT shows compression of the renal vein (arrow).

but with time become more complex with internal liquefaction and formation of multiple septations. Urinomas, on the other hand, are anechoic with no or few septations and situated close to the ureterocystostomy site. They must not be confused with an ovarian or adnexal cyst in females and a penile prosthetic reservoir in males (Irshad et al., 2009). MAG3 scintigraphy is helpful in proving an active leak of tracer from the urinary tract and single-photon emission computed tomography (SPECT)/CT allows better localization. However, if the leak is intermittent, aspiration of the collection may be required to show it is urine by demonstrating a raised creatinine concentration.

Lymphocoeles (Fig. 282.14) usually occur 1 month or later post transplantation and on US appear anechoic with thin septations. Although generally asymptomatic they may grow so large as to compress the ureter and cause hydronephrosis. Percutaneous drainage will then be required and if not successful, marsupialization into the peritoneum. A perinephric abscess may arise from infection of any collection. It usually has a complex cystic appearance on US, often with a hypervascular thickened or irregular rim on CDUS. Air within an abscess appears as echogenic foci with ring down artefacts.

Parenchymal complications

Transplant imaging is unable to differentiate reliably between ATN, acute rejection, and drug nephrotoxicity. Greyscale findings are totally non-specific and may only show a rapid increase in graft volume due to swelling. Increased RI (> 0.8), absent, or even reversed diastolic flow although sensitive for graft dysfunction, are not specific for any specific cause. The timing of the RI increase and the speed with which it decreases, however, does have diagnostic significance (Fig. 282.15). With ATN, the rise in RI occurs early post transplantation, but interestingly on the first CDUS immediately post surgery, RI is usually high to normal and only reaches a peak within the first few days. A decrease in RI is a good prognostic feature and usually predates a return of function by days. Non-functioning grafts from ATN will require regular CDUS monitoring. If the RI increases further after reaching a plateau then superimposed acute rejection or drug nephrotoxicity should be considered.

Radionuclide scintigraphy using diethylenetriamine pentaacetic acid (DTPA) or MAG3 in the early postoperative period is able to confirm ATN by demonstrating good transplant perfusion but poor uptake function and excretion—however, it is much less convenient than periodic bedside US monitoring. Both acute rejection and calcineurin inhibitor nephrotoxicity give similar scintigraphy findings of reduced transplant perfusion, uptake function, and excretion (Fig. 282.16).

Chronic rejection results in reduction of graft size and increased parenchymal echogenicity. On CDUS there is usually poor peripheral perfusion but RIs are within normal limits.

Biopsy is required for diagnosis, but evaluation of the micro-circulation of the transplant by quantitative Doppler imaging of its vascular area (Nankivell et al., 2002) and contrast-enhanced US analysis of perfusion parameters (Jimenez et al., 2009) have shown promise in non-invasive assessment of chronic rejection.

Biopsy is the most frequent cause of a focal area of abnormal parenchymal echotexture and reduced vascularity as a result of contusion/haematoma. A similar appearance may be caused by an infarct which often is more wedge-shaped in appearance (Fig. 282.17) or by focal infection (Fig. 282.18) which may have a more rounded appearance with a poorly defined border between abnormal and normal parenchyma. US is less accurate than CT in the early detection of focal pyelonephritis and therefore if a patient with urinary tract infection and normal US does not rapidly respond to antibiotics, CT scanning should be performed. Progress US, however, will be helpful to determine whether focal pyelonephritis progresses to abscess, visualized as an area of liquefaction

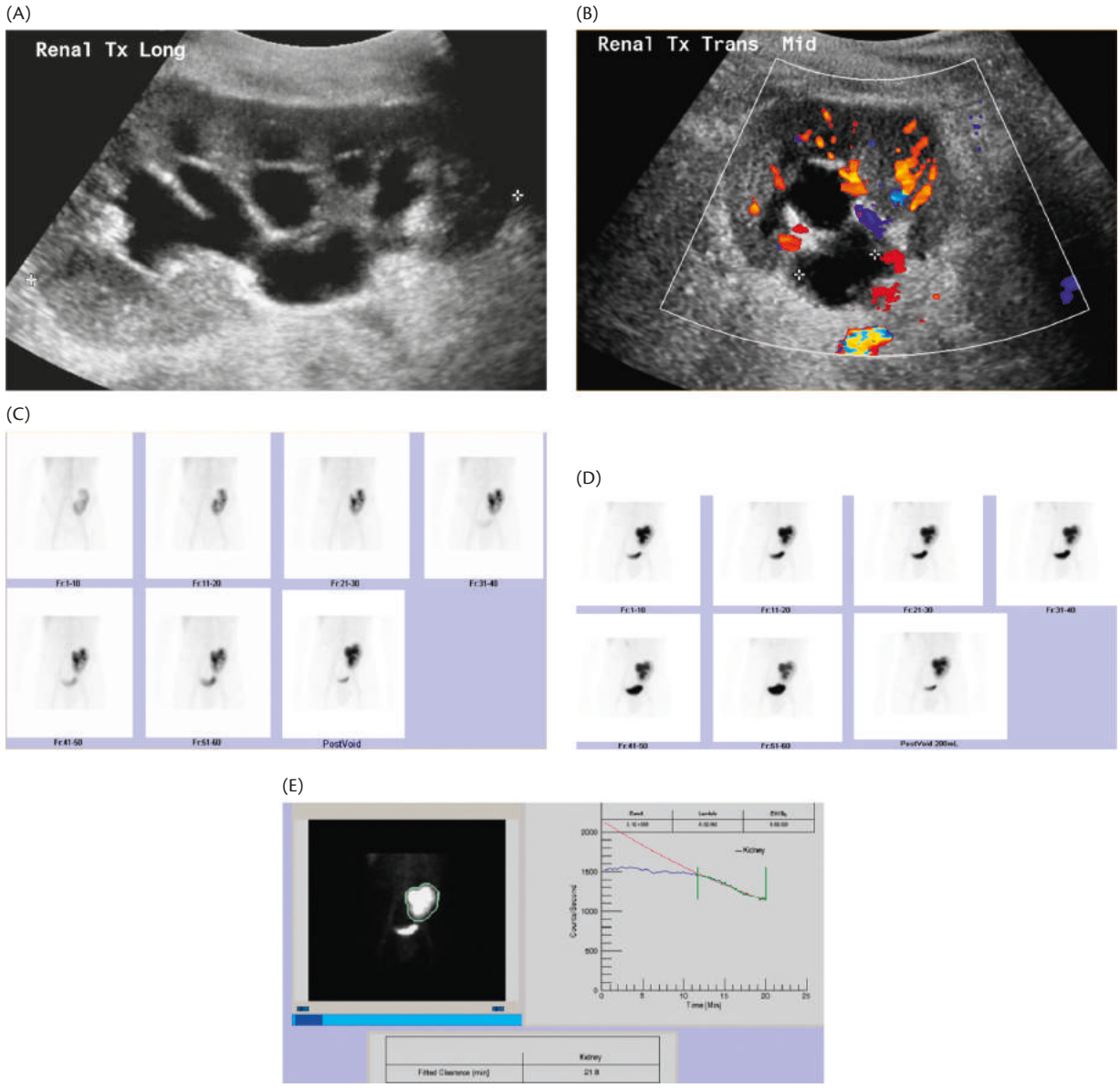

Fig. 282.5 Collecting system dilatation. (A) Longitudinal US shows dilatation of pelvis and calyces. (B) CDUS shows a dilated renal pelvis. (C) MAG3 scan shows reduced transplant function and excretion. (D) MAG3 scan post frusemide injection shows evidence of obstruction with reduced tracer clearance. (E) MAG3 post frusemide prolonged tracer half-time clearance.

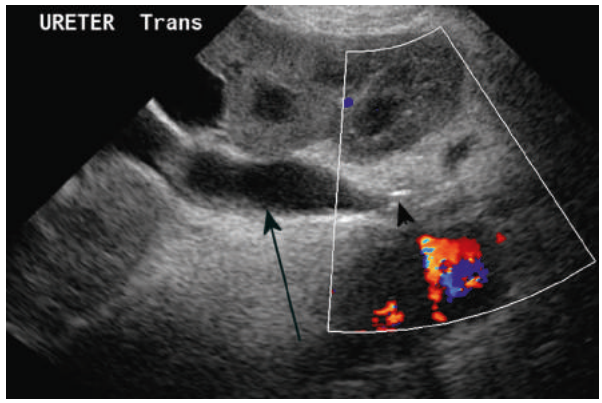

Fig. 282.6 Ureteric stricture. Longitudinal CDUS show a dilated pelvis and upper ureter (arrow) and focal narrowing of the mid ureter (arrow head).

with a surrounding rim of increased vascularity. Over time, these focal abnormalities may heal without sequelae, or result in a focal loss of cortical thickness and increase echogenicity due to scarring. A DMSA scan can be performed to document the size of the focal abnormality in the acute phase or the residual functioning volume after completion of treatment.

Conditions which affect native kidneys may also involve the transplant—if the pyramids are echogenic, especially when there are focal areas of calcification near the apex, nephrocalcinosis is likely (Fig. 282.19). A transplant mass can be divided into a simple cyst, complex cyst, or solid tumour. The simple cyst (Fig. 282.20) has a well-defined thin echogenic wall, no internal contents or vascularity, and does not require further investigation. A complex cyst contains echogenic material and/or septations that suggest bleeding, infection, or neoplasm. Depending on the size of the

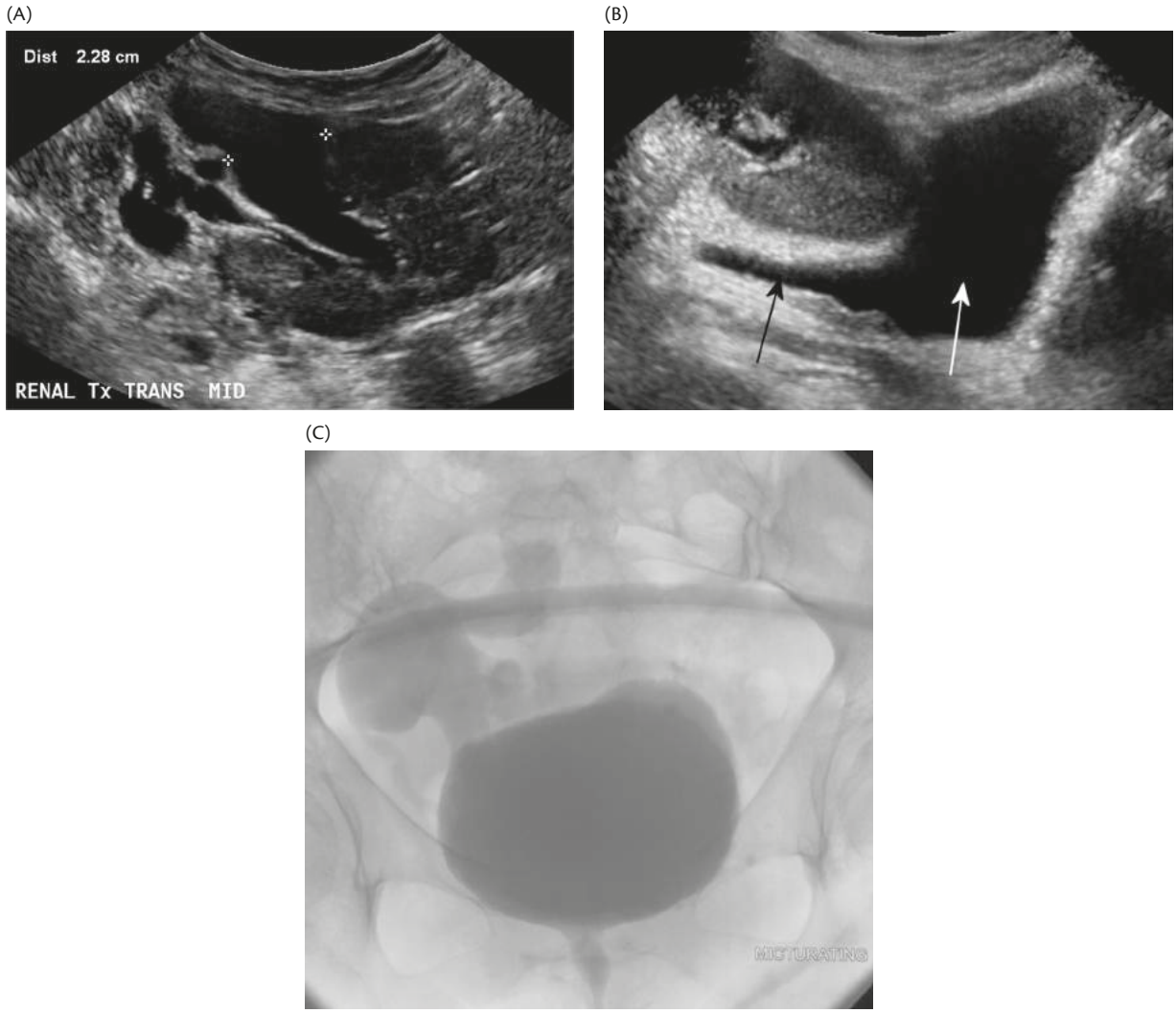

Fig. 282.7 Vesicoureteric reflux. (A) Transverse US image of the transplant hilum shows hydronephrosis. (B) Longitudinal image of the bladder (white arrow) shows a dilated distal ureter (black arrow) posterior to the lower pole of the transplant. (C) Micturating cystogram shows gross vesicoureteric reflux.

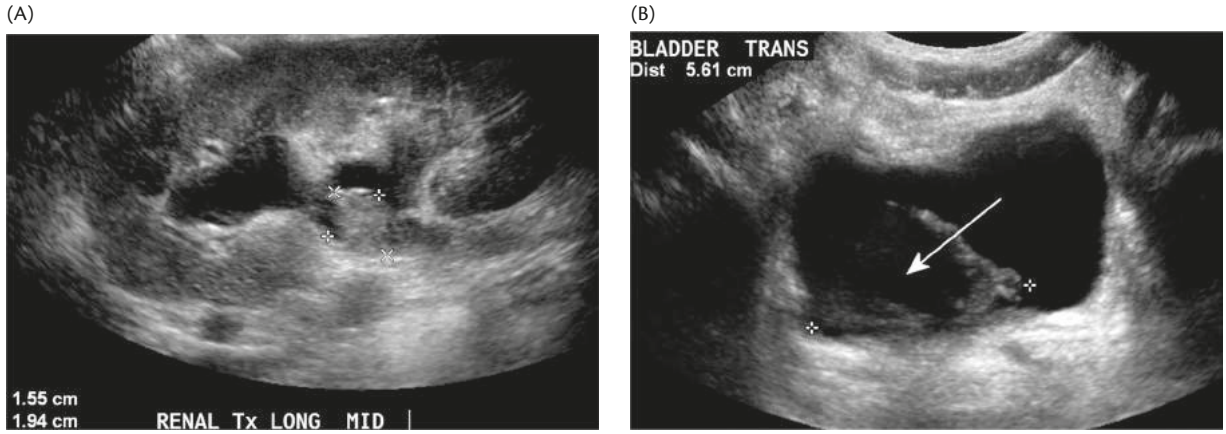

Fig. 282.8 Renal pelvis and bladder clots. (A) US shows an echogenic clot in the renal pelvis and (B) clot in the bladder with liquefaction (arrow).

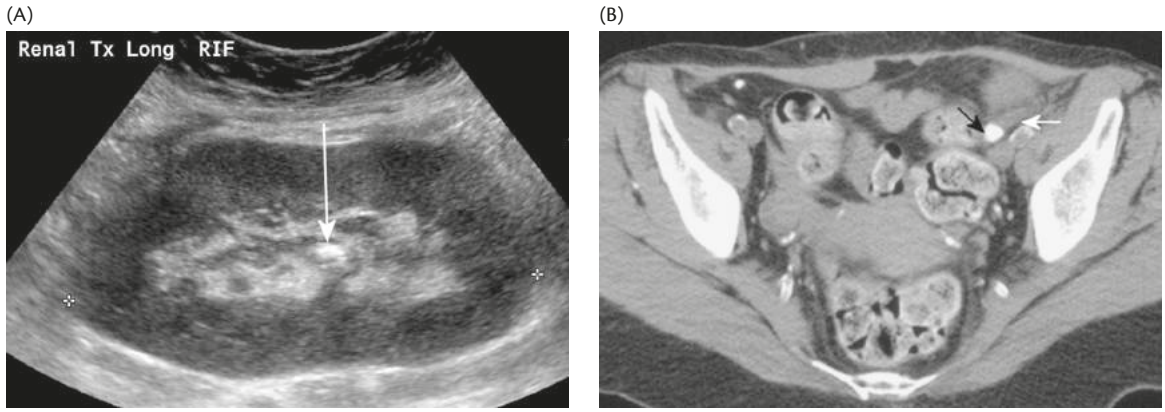

Fig. 282.9 Calculus. (A) Longitudinal view of echogenic shadowing calculus (arrow). (B) Axial CT view of obstructing radio-dense calculus (black arrow) in dilated proximal ureter (white arrow).

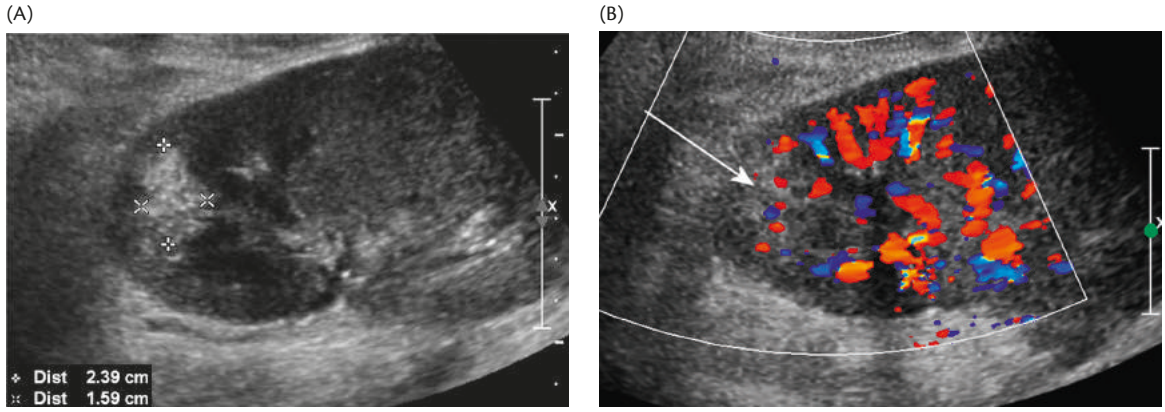

Fig. 282.10 Post-biopsy contusion. (A) US shows irregular hyperechoic wedge-shaped area at upper pole. (B) CDUS shows reduced vascularity in same area (arrow).

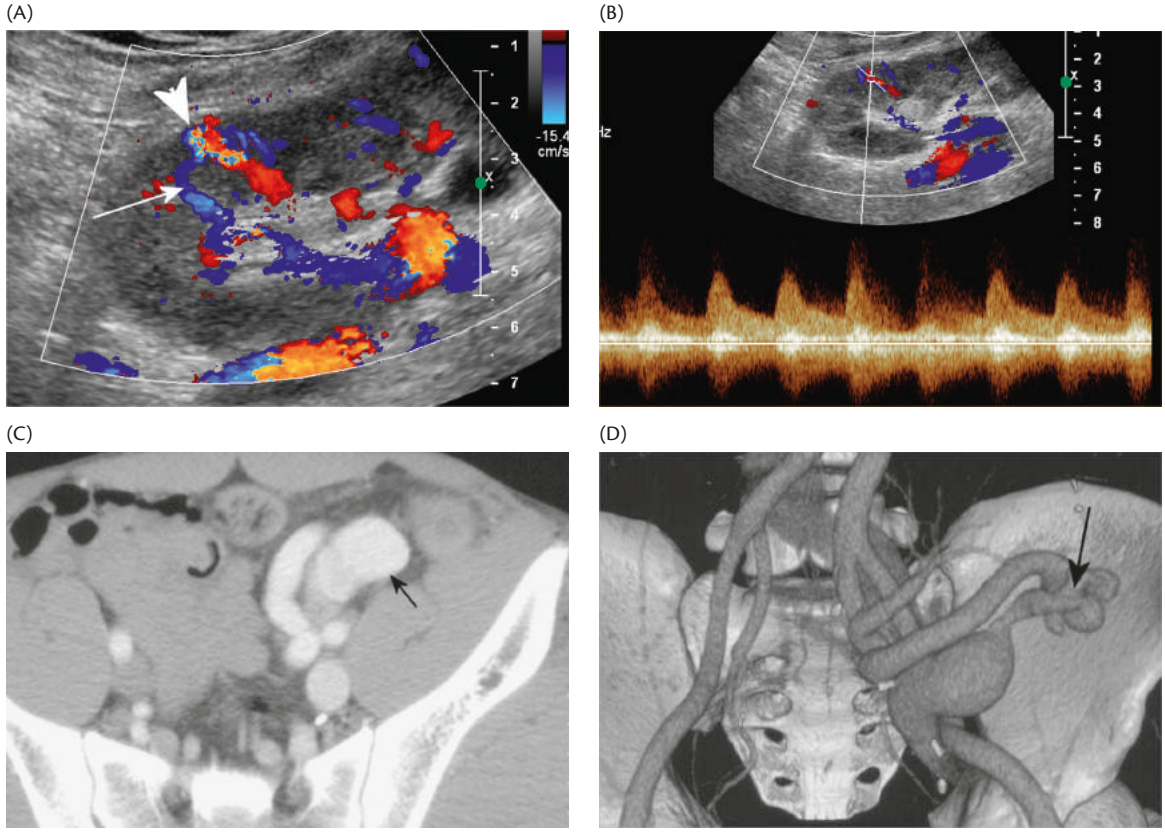

Fig. 282.11 Arteriovenous fistula. (A) CDUS shows a focal area of turbulence at the site of communication (arrow head) and a dilated draining vein (arrow). (B) CDUS shows high diastolic flow in the fistula. (C) Axial arterial CT showing early venous filling in arteriovenous fistula (arrow). (D) CT scan displaying 3D surface-shaded image reconstructions of another patient depicting a large arteriovenous fistula connection, aneurysmal vein and vessel tortuosities.

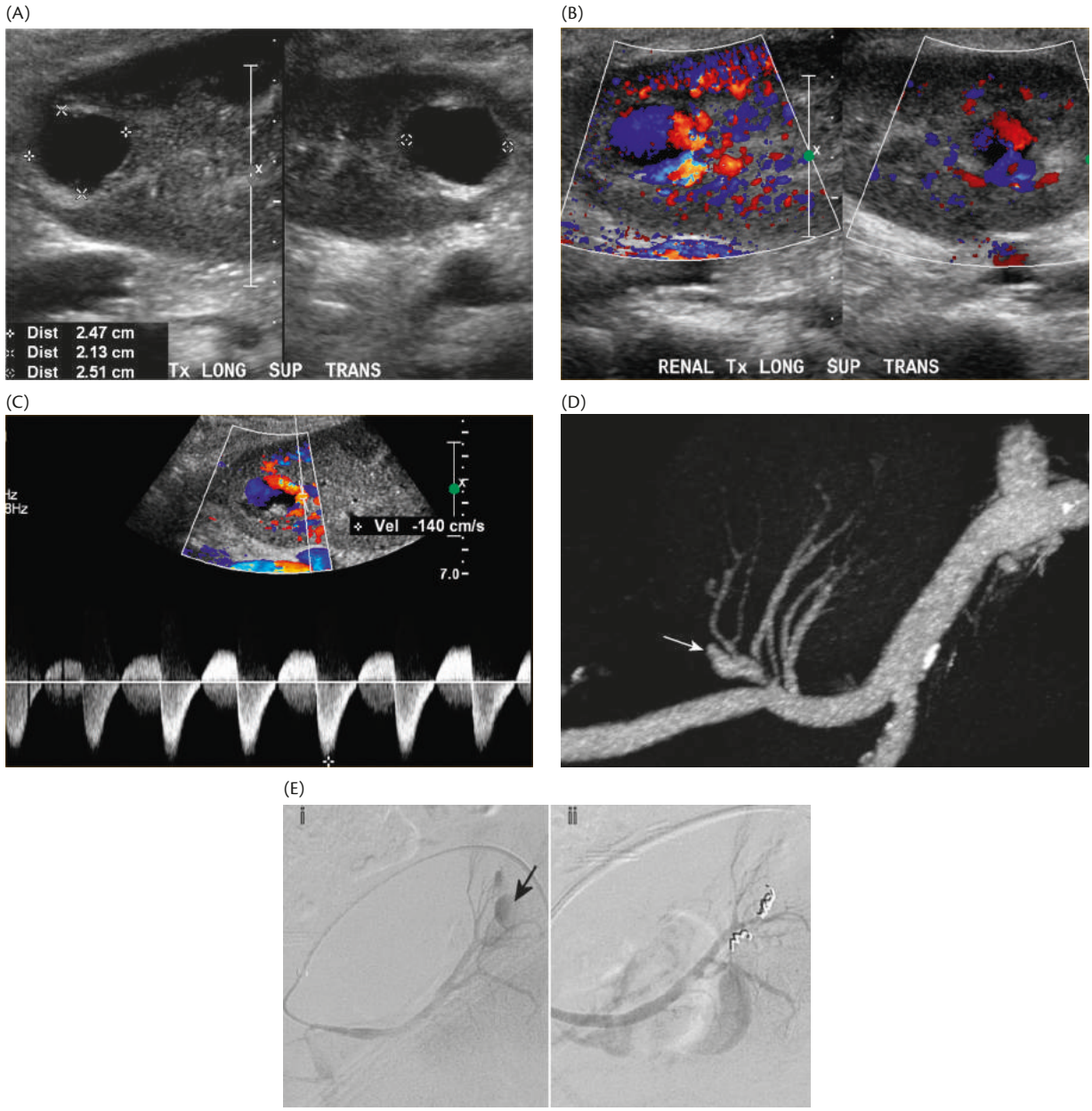

Fig. 282.12 Pseudoaneurysm. (A) Composite US shows a cyst-like structure at the upper pole. (B) Composite CDUS show vascularity within the lesion which has forward and backward flow. (C) CDUS tracing from the neck of the pseudoaneurysm shows bidirectional arterial flow. (D) 3D maximum intensity projection arterial CT scan shows multiple renal arteries and pseudoaneurysm. (E) Angiogram (i) of the pseudoaneurysm (arrow) and (ii) after coiling.

mass (Fig. 282.21), either progress US to measure interval growth or further investigation by contrast-enhanced ultrasound (CEU), CT, or MRI is necessary. Suspicious complex cysts on CEU display enhancement of their walls or septi and should be further categorized with CT or MRI. Solid masses that are echogenic with heterogeneous echotexture are usually renal cell carcinomas while lymphoproliferative disease causes masses of low echogenicity. Other transplant masses include Kaposi sarcoma and metastases. CT, MRI, and positron emission tomography/CT provide the necessary information on the extent of neoplastic disease.

Computed tomography and magnetic resonance imaging

Although modern CTA (Fig. 282.22) and MRA (Fig. 282.23) techniques offer very similar non-invasive and three-dimensional (3D) information, they have different strengths and pitfalls. CT is by far the most readily accessible and cost-effective. Increased safety of modern CT has been achieved through more efficient administration of lower volume, less nephrotoxic, low osmolar, non-ionic intravenous contrast agents coupled with prophylactic patient

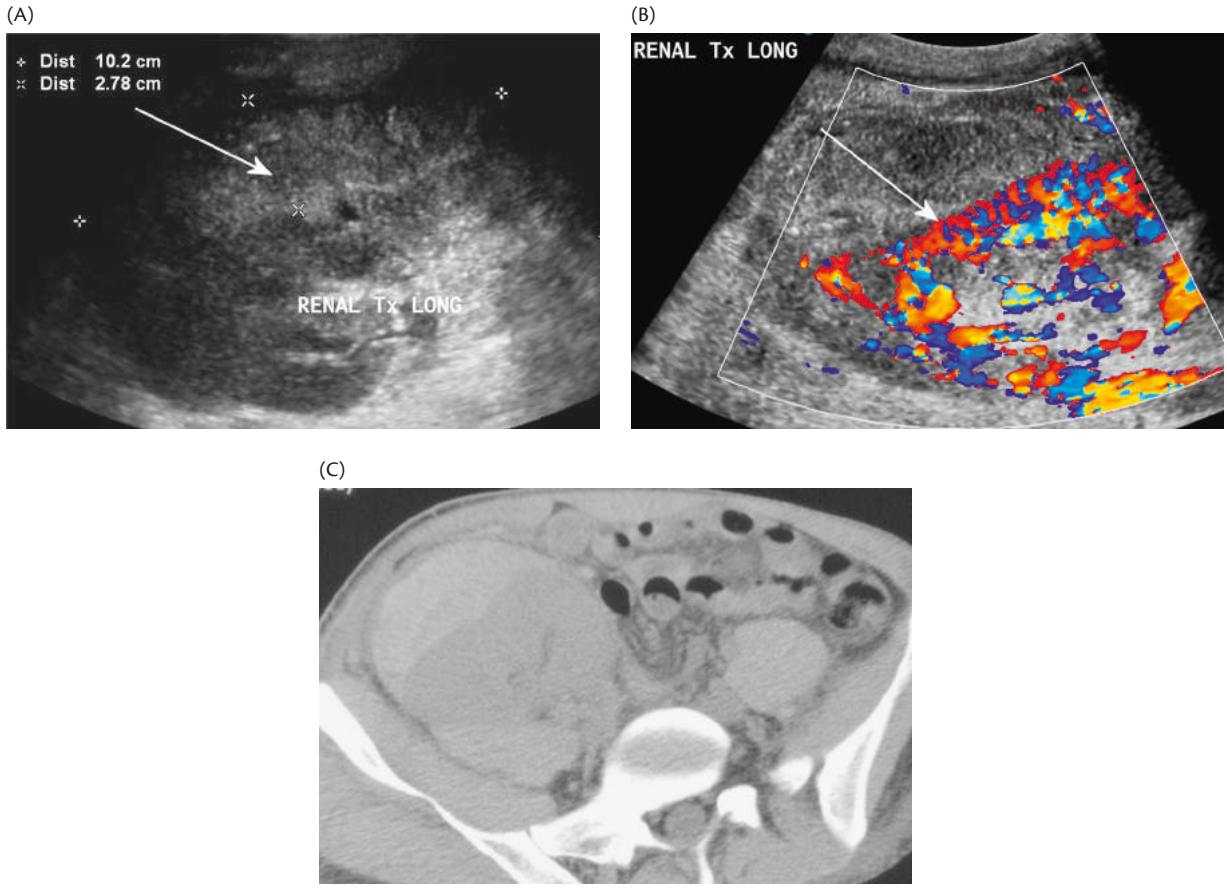

Fig. 282.13 Subcapsular haematoma. (A) US shows echogenic haematoma along the anterior upper pole (arrow). (B) CDUS shows haematoma compressing the vascular parenchyma (arrow) in keeping with a subcapsular haematoma. (C) Unenhanced axial CT of subcapsular haematoma.

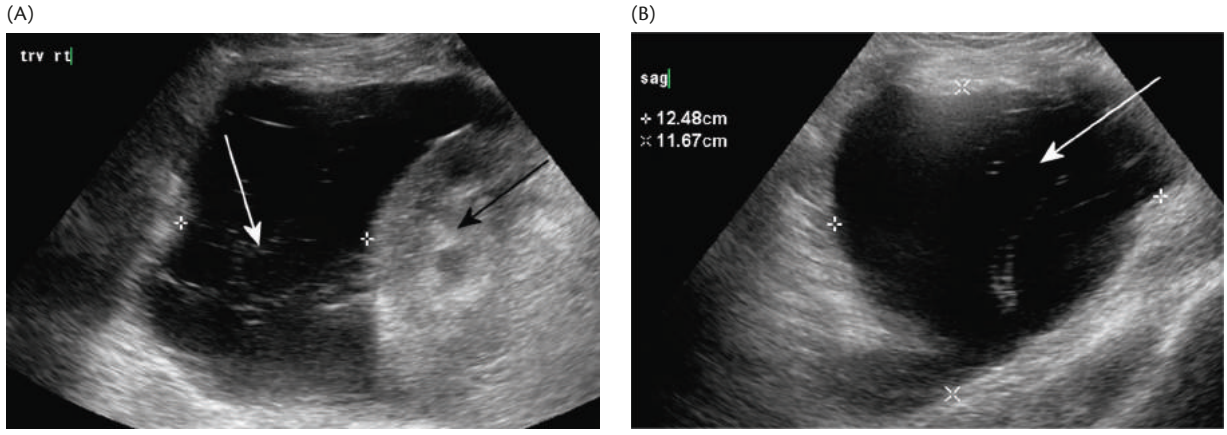

Fig. 282.14 Lymphocoele. (A) Transverse and (B) longitudinal US show large septated perinephric collection (white arrows) adjacent to the transplant (black arrow).

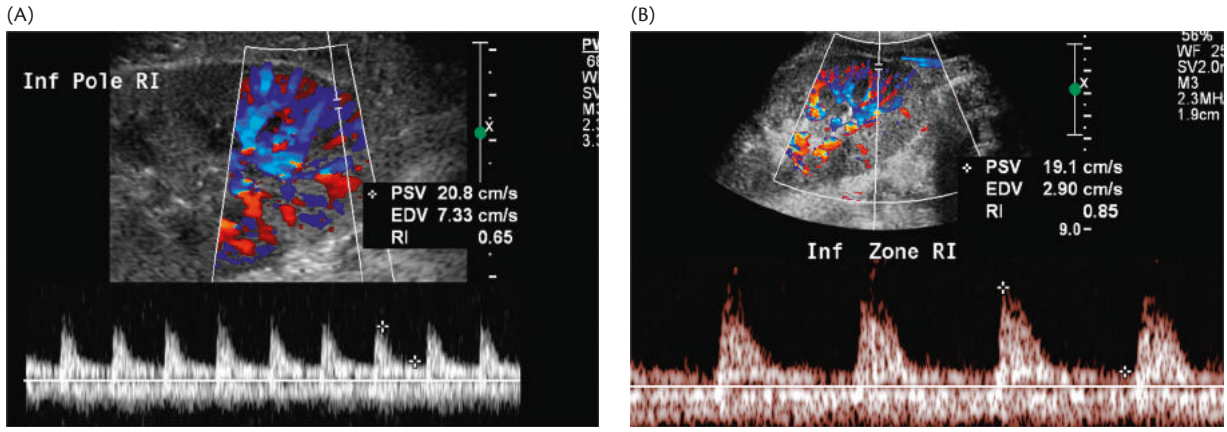

Fig. 282.15 Rise in Resistance Index (RI). (A) Pulsed Doppler tracing from a lower pole arcuate artery 2 days post transplantation shows the peak systolic velocity (PSV), end-diastolic velocity (EDV), and RI of 0.65. (B) Repeat study due to rising creatinine on day 12 post transplantation shows a rise in RI to 0.85 and acute rejection was confirmed on biopsy.

intravenous (IV) hydration. Faster multislice CT scanners equipped with dose modulation deliver lower patient-ionizing radiation. CT and MRI advantages over US are its large field of view, anatomical 3D vascular imaging capabilities, and the ability to depict deeper structures not obscured by bowel gas. Unlike MRI, calcifications and calculi can be clearly and accurately demonstrated on CT. Quantitative analysis of the relative enhancement of lesions using multiphase pre- and post- contrast CT remains the essential means of tumour differentiation.

Modern MRI has the advantages of not having any known adverse radiation effects. The gadolinium IV contrast agents are safer for use in patients with risk of contrast allergy and are less nephrotoxic as they are required in significantly lower volumes. Bolus injection for MRA studies provides qualitative functional information; however, compared with CT its spatial resolution is lower and it can be prone to flow artefacts causing overestimation of the severity of vascular lesions and false negatives in assessing venous abnormalities. Patients with various prosthesis including cochlear implants, pacemakers, and some aneurysm clips cannot be imaged using this technique. In patients with severe renal impairment, gadolinium-based contrast agents have been linked to the development of nephrogenic systemic fibrosis, a debilitating and potentially fatal condition.

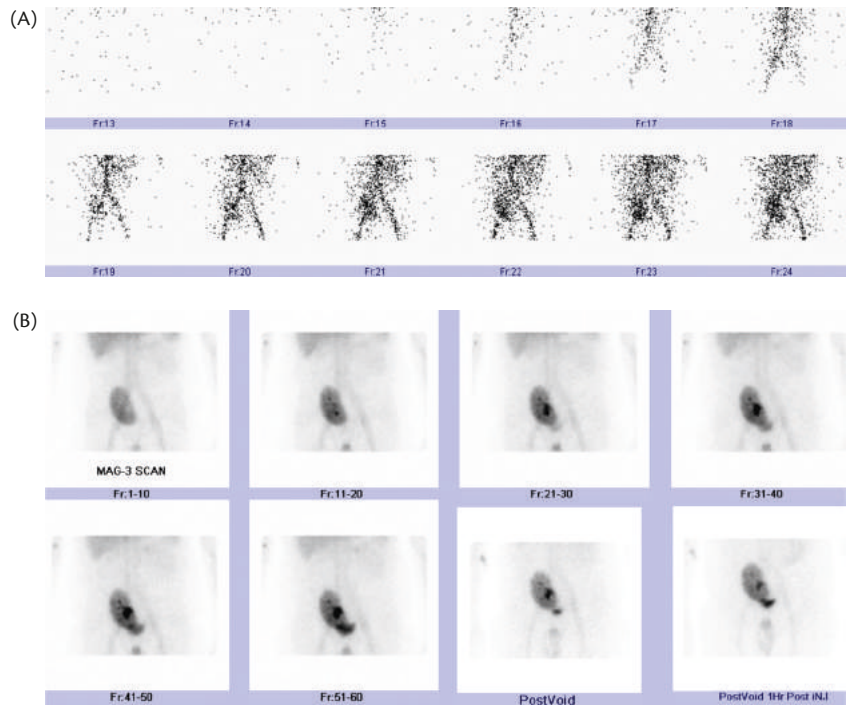

Fig. 282.16 Rejection. (A) MAG3 arterial phase renal scan showing delayed and reduced perfusion. (B) Poor excretion and marked parenchymal tracer retention on later phase MAG3 study.

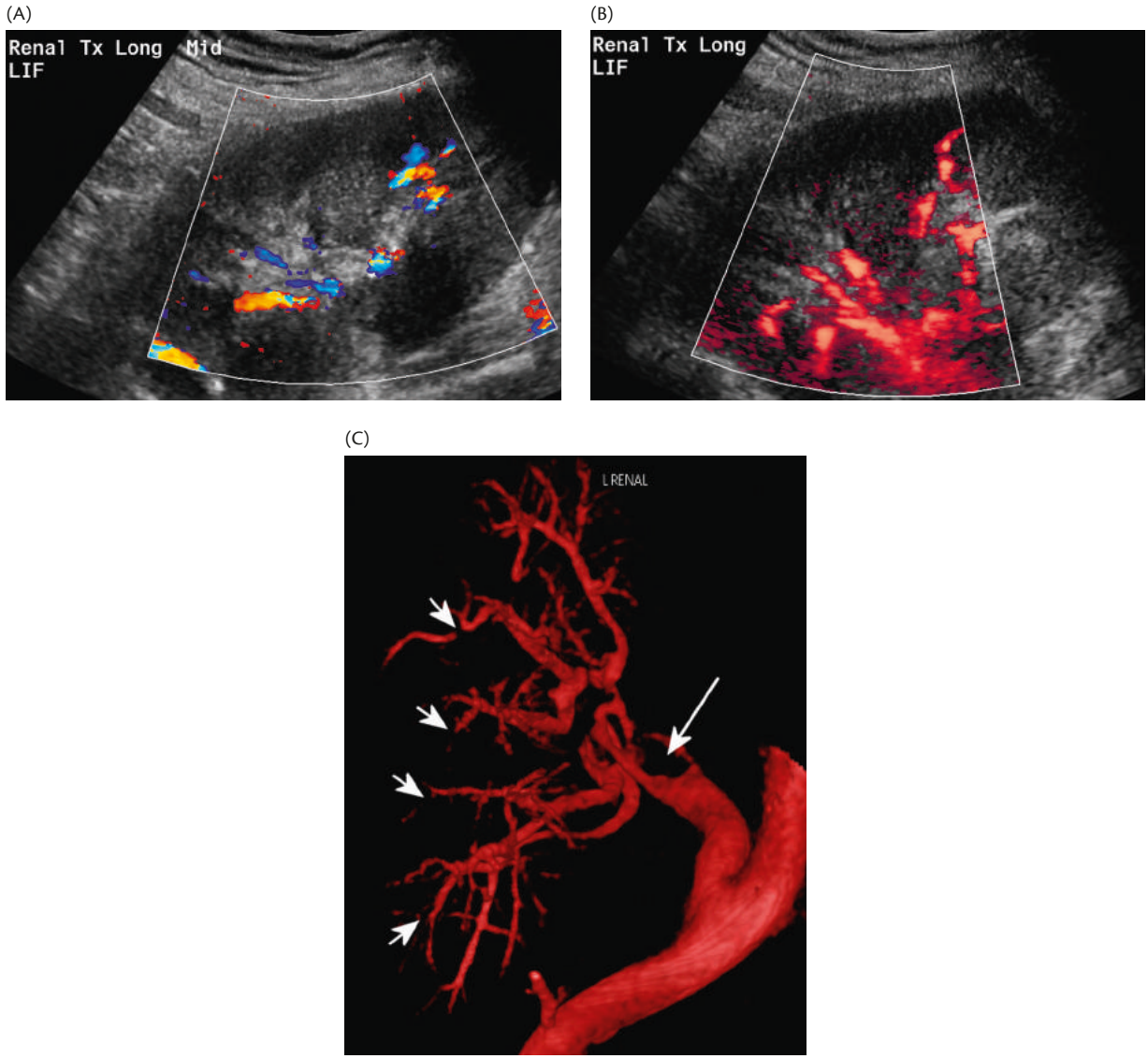

Fig. 282.17 Infarcts. (A) CDUS shows multiple wedge-shaped avascular parenchymal defects. (B) Power Doppler confirms the multiple areas of infarction. (C) Selective catheter 3D angiogram shows a large non-occlusive atheromatous embolus as a filling defect (large arrow) in the distal main transplant artery and multiple small peripheral intrarenal arterial focal embolic defects (small arrows).

Helpful guidelines have been devised to address this safety issue (European Society of Urogenital Radiology, 2014).

Angiography and intervention

Conventional angiography is an invasive technique with significant morbidity and has been largely replaced by modern CTA and MRA. However, in select cases when anticipating intervention or when other imaging has proven non-diagnostic, digital subtraction angiography remains the most accurate means of determining the presence and degree of arterial or venous stenosis (Fig. 282.3F, G), embolus (Fig. 282.17C), AVF, and PA (Fig. 282.12E). Direct measurement of pressure flow gradient is important in confirming the haemodynamic significance of stenotic lesions and the therapeutic endpoint following stenting or angioplasty. False-positive and false-negative results are minimized by performing multiple projections and eliminating movement and misregistration artefacts. Selective angiography using micro-catheters and 3D imaging provides the highest anatomical detail for therapeutic embolization of AVF and PA with minimal collateral renal damage. Various image-guided interventional techniques are now available to obviate the need for repeat surgery by relieving mechanical urinary obstruction through antegrade nephrostomies and ureteric stenting, percutaneous drainage of abscess and cysts, or other collections. They also provide diagnostic certainty through aspiration biopsy of suspected neoplastic lesions and infections. Recent advances in image-guided cryoablation or radiofrequency ablation under CT or US guidance are providing alternative means of prolonging the life and usefulness of the transplant.

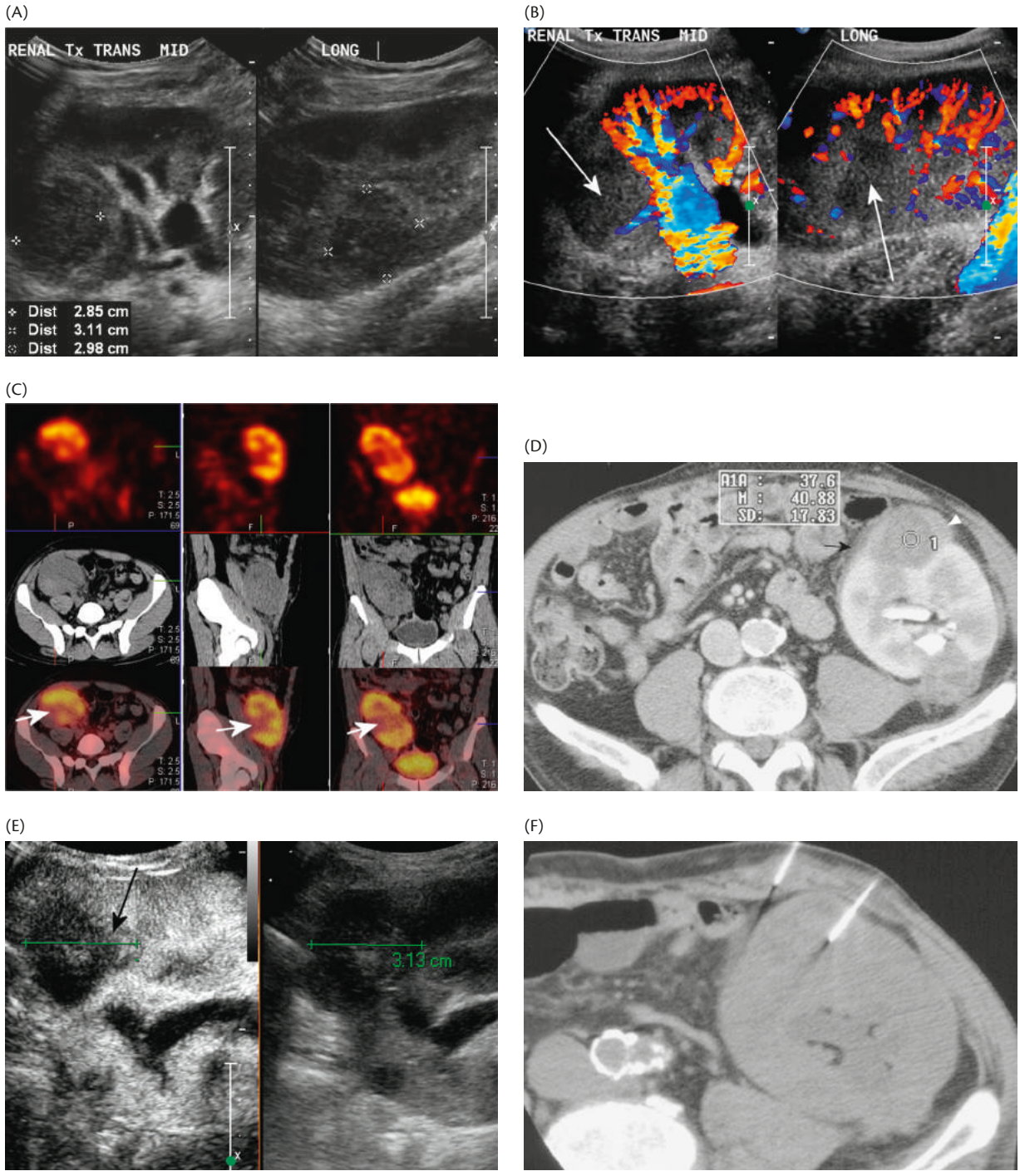

Fig. 282.18 Pyelonephritis with focal nephronia. (A) Composite transverse and longitudinal US shows area of reduced echogenicity in the mid transplant. (B) CDUS show reduced vascularity in same region (arrows). (C) DMSA SPECT/CT show non-specific area of reduced tracer uptake and increased CT density (arrows) in the mid transplant at site of focal infection. (D) Axial corticomedullary-enhanced CT in another patient shows focal abscess with perinephric soft tissue density stranding (arrow) and rim enhancement (arrowhead). (E) Contrast-enhanced CDUS shows abscess with avascular centre and peripheral rim flows (arrow). (F) Axial CT-guided fine-needle aspiration confirmed an abscess.

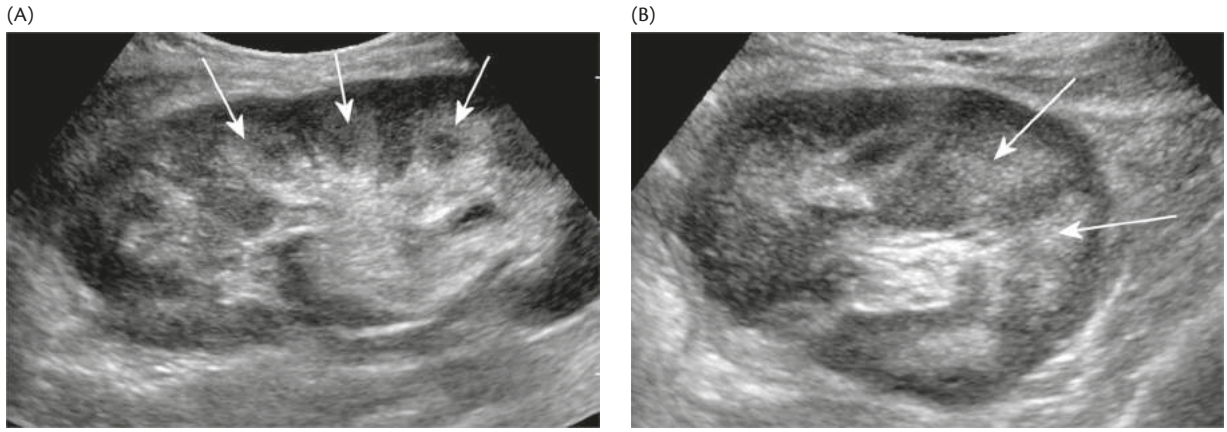

Fig. 282.19 Nephrocalcinosis. (A) Longitudinal and (B) transverse US images show echogenic medullary pyramids (arrows)

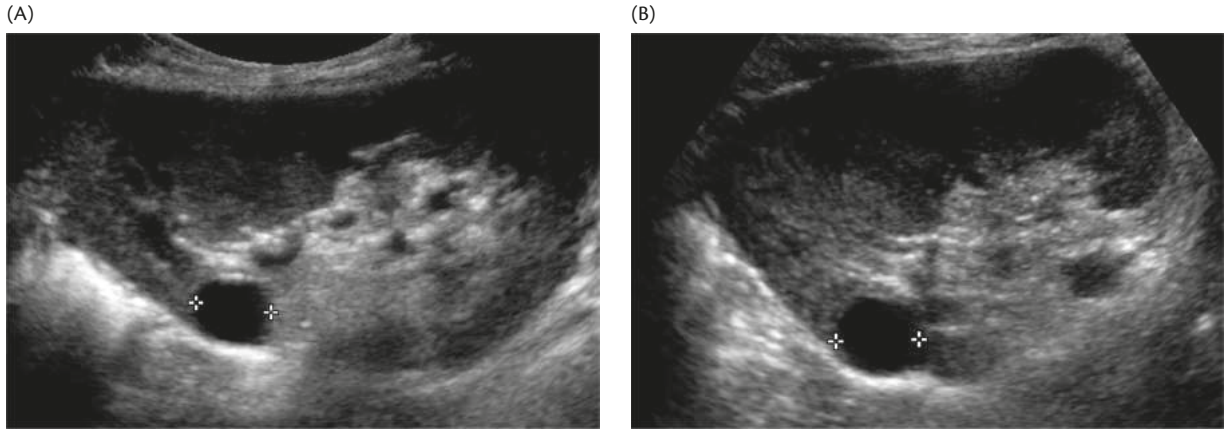

Fig. 282.20 Simple cyst. (A) Longitudinal and (B) transverse US images show anechoic parenchymal cyst.

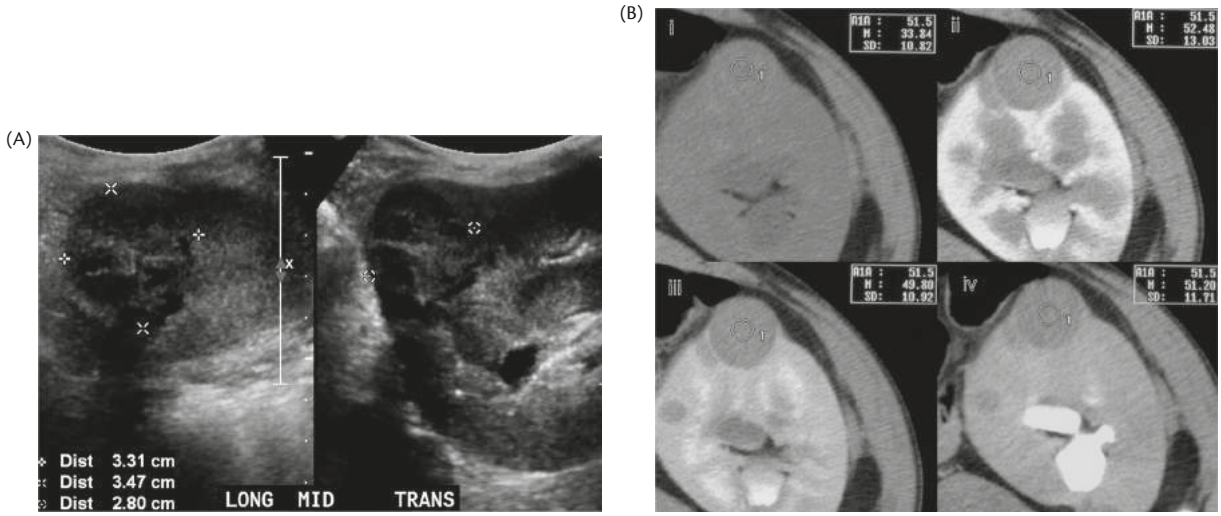

Fig. 282.21 Tumour (renal cell carcinoma). (A) Composite transverse and longitudinal US image shows a solid heterogenous cortical transplant renal cell carcinoma. (B) Axial pre- (i) and multiphase enhanced arterial (ii), equilibrium (iii), and excretory (iv) CT shows tumour-like enhancement in the region of interest measurements comparing Hounsfield densities.

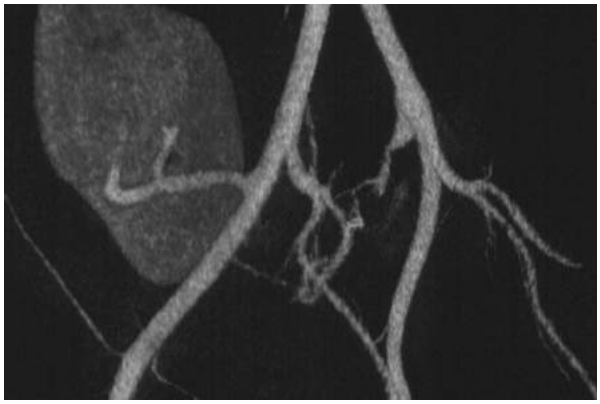

Fig. 282.22 CTA. Arterial 3D-maximum intensity projection reconstruction shows a normal renal artery.

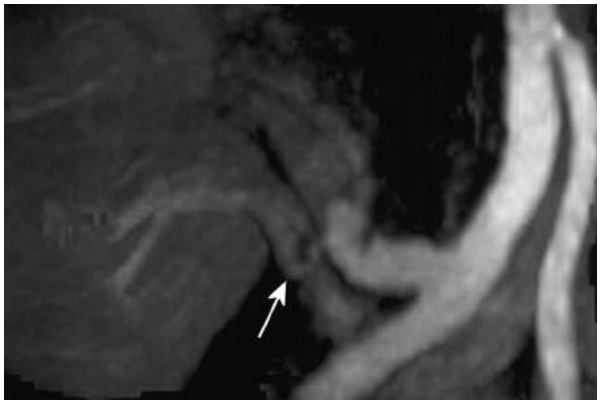

Fig. 282.23 Unenhanced MRA. Tortuous graft renal artery (arrow) without renal artery stenosis.

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CHAPTER 283

Rejection

David N. Rush and Peter W. Nickerson

Introduction

Rejection of the transplanted kidney remains the most important cause of graft loss. The early post-transplant period is generally uneventful, as with modern crossmatching techniques and immunosuppressive agents the incidence of rejection in the first post-transplant year is < 15% in low-risk recipients, and graft survival at 1 year is about 90% in most centres. However, a recent study of > 1300 transplant recipients found that fully one-third of allograft losses over 10 years are due to rejection, despite the fact that the majority of patients had received a kidney from a living donor and had been given induction therapy and a modern maintenance immunosuppressive regimen (El-Zoghby et al., 2009). These rejections occurring after the first post-transplant year may be due in part to injudicious reduction in the dose of immunosuppression or patient non-compliance.

Rejection of a transplant is usually the result of an immune response of the recipient against mismatched human leucocyte antigens (HLAs) in the donor. Furthermore, recipient sensitization to donor HLAs may take place prior to the transplant, for example through blood transfusions, pregnancy, or a previous transplant.

Rejection may occur at any time following transplantation, and it has been classified as hyperacute, if it occurs within minutes of surgery; acute, if it occurs within days or months of the transplant; and chronic, if it occurs months to years after transplantation. Rejection can be further classified as T-cell mediated, or 'cellular', and antibody mediated, or 'humoral', both of which forms can occur simultaneously.

Rejection may present clinically as either abrupt or insidious dysfunction of the graft, or it may be clinically silent, that is, 'subclinical rejection', detected usually by protocol biopsy. The histological features of rejection vary in their location, for example, predominantly tubulointerstitial (T-cell-mediated rejection) or vascular (T-cell- or antibody-mediated rejection), and in the severity of the histological lesions, which can be scored using classification systems such as the Banff schema (Solez et al., 1993; Racusen et al., 1999).

The prevention and treatment of T-cell-mediated rejection is usually successful with current immunosuppressive agents. Antibody-mediated rejection, on the other hand, is not easily treated and is the principal cause of late renal allograft loss.

Pathogenic features of rejection

Acute antibody-mediated rejection

Hyperacute rejection is rarely seen now because modern crossmatching techniques can readily detect donor-specific anti-HLA antibodies in the recipient and such transplants are usually avoided.

Acute antibody-mediated rejection may occur however when these high-risk, pre-sensitized recipients are transplanted knowingly. In these cases, the recipient is often treated pre transplant (e.g. with plasmapheresis to reduce the titre of antibody) and with a more intensive immunosuppressive regimen post transplant. If rejection occurs, the antibody binds to HLAs on the endothelium of the glomeruli and peritubular capillaries in the donor kidney. Activation of the microvascular endothelium results in the release of chemokines such as CCL2 (MCP-1) and CX3CL1 (fractalkine), and cytokines, such as the interleukins 1α and 8, that recruit leucocytes to the target sites resulting in glomerulitis and peritubular capillaritis. Activation of complement by antibody bound to the microvascular endothelium triggers further leucocyte recruitment via the chemoattractant complement components C3a and C5a, while C5b activates the membrane-attack complex that may lead to endothelial cell apoptosis, necrosis, and detachment from the basement membrane. C4d deposition in the peritubular capillaries may occur as a result of complement-dependent endothelial injury. The injured endothelium may also release platelet pro-coagulants, such as von Willebrand factor, which may lead to platelet aggregation, thrombosis, and tissue infarction. The clinical presentation of acute antibody-mediated rejection may be one of abrupt decline in renal function and may result in graft loss.

Acute antibody-mediated rejection has also been reported, albeit infrequently, with antibodies to the major histocompatibility complex class I chain-related gene (MICA) (Zou et al., 2007; Amico et al., 2008), with antibodies to the angiotensin II type I receptor on endothelial cells (Dragun et al., 2005; Reinsmoen et al., 2010), and with antibodies against endothelial cells (Sun et al., 2005). Furthermore, a recent multicentre study reported that transplant recipients that have antibodies against endothelial cells detected prior to transplant have a greater incidence of clinical rejections and worse graft function at 6 months, than recipients without such antibodies (Breimer et al., 2009).

In addition, protein microarray studies have suggested that antibodies against specific (non-HLA) tissue antigens can develop post-renal transplant and result in tissue injury (Li et al., 2009; Dinavahi et al., 2011).

Acute cellular rejection

Cellular rejection is the result of mismatched donor alloantigens that are presented to recipient T lymphocytes causing their activation. The donor antigens are presented to T lymphocytes by antigen-presenting cells (APCs) such as dendritic cells, macrophages, and B lymphocytes of the donor (direct pathway) or by APC from the recipient (indirect pathway). A semi-direct pathway

has also been proposed, in which recipient APC that have acquired donor MHC can present these molecules directly or indirectly to T lymphocytes (Jiang et al., 2004; Ely et al., 2008; Gökmen et al., 2008; Afzali et al., 2008). All three mechanisms may be relevant to clinical rejection. Donor renal tubular epithelial cells can also present alloantigen to recipient T lymphocytes (Frasca et al., 1998).

Activation of T lymphocytes leads to their differentiation into cell subtypes that can injure the graft epithelium by direct cytolysis (e.g. via perforin and granzymes) or indirectly by pathways causing apoptosis (e.g. via Fas and tumour necrosis factor alpha). However, cell death and necrosis are not usually histological features of acute rejection suggesting that non-cytotoxic mechanisms are more commonly involved in causing injury and dysfunction of the graft. Moreover, in experimental animals neither perforin, granzyme (Halloran et al., 2004) nor Fas (Kayser et al., 2008) is required to cause allograft injury, likely an indication of the redundancy of the pathways involved in rejection. Some data suggest that the impairment of graft function in rejection is mediated by macrophages and their secretory products (Grimm et al., 1999; Girlanda et al., 2008) (see below).

Endothelial cells are also activated during T-cell-mediated rejection and promote recruitment of T cells (via chemokines and cytokines), their adhesion to the endothelium (via adhesion molecules such as leucocyte-function associated antigen 1 (LFA-1)) and their eventual migration into the graft interstitium. Some T cells traverse the tubular basement membrane. The infiltration by mononuclear cells of the renal interstitium and of the renal tubules (tubulitis) is the characteristic histological lesion of T-cell-mediated rejection.

Acute T-cell rejection is the most common form of rejection. It can present clinically with a decline in renal function, but the histological criteria for rejection may also be met in early protocol biopsies of well-functioning grafts—'subclinical rejection' (Rush et al., 1994)—the pathogenic potential of which is discussed below.

The cell infiltrates in subclinical and clinical rejection were found to be similar in kidney transplant biopsies that were studied by immunohistochemistry and cell activation markers, with the exception of an increased population of activated macrophages in clinical rejection biopsies (Grimm et al., 1999). Recently a more extensive immunohistochemistry and transcriptome study has confirmed that the cellular and molecular phenotypes of infiltrating cells in subclinical and clinical rejection differ only in terms of the quantity of the gene transcripts related to T-cell maturation (e.g. T-bet and FasL), while other transcripts were equally abundant (e.g. interferon gamma, RANTES, granzyme B, and perforin) (Hoffmann et al., 2005). Furthermore, another study confirmed the lack of difference between clinical and subclinical rejection in terms of the degree of T-cell infiltration, but also reported that clinical rejection differed from subclinical rejection by an increased level of monocyte/macrophage infiltration and tissue HLA-DR expression (Girlanda et al., 2008). Finally, gene transcripts for the inflammatory chemokines CCL3, CCL5, CXCL9, CXCL10, and CXCL11, and the chemokine receptors CCR5, CCR7, and CXCR3, were significantly increased in renal allograft biopsies of patients with either subclinical or clinical rejection compared to those of patients with stable grafts or with calcineurin inhibitor toxicity (Lo et al., 2011).

The above studies suggest that there are specific programmes associated with acute rejection leading to allograft dysfunction that may be unrelated to the extent of T-cell infiltration in the graft. Moreover, the finding of CD4 + forkhead box P3 (FOXP3)+ regulatory T cells in the renal allograft infiltrates indicates that the acute rejection response is likely self-regulated. It has been reported that patients with subclinical rejection in whom the infiltrate has a higher percentage of regulatory T cells may have a better prognosis than those rejections in which the infiltrate has fewer of these cells (Bestard et al., 2008). Similarly, another study has shown that in patients with borderline rejection that is untreated, those with fewer infiltrating regulatory T cells progress to clinical rejection, as compared to those in whom these cells are more abundant (Mansour et al., 2008). In clinically rejecting allografts however, the studies of infiltrating regulatory T cells and graft outcomes after treatment are somewhat contradictory. In one study, the finding of mRNA for FOXP3 in the urine of rejecting renal allografts was associated with a better outcome as the rejection episode was more easily reversed (Muthukumar et al., 2005). In contrast, an immunocytochemical study of FOXP3+ve cells in grafts with acute cellular rejection reported better renal function in those patients with fewer FOXP3+ve cells (Veronese et al., 2007), and similarly, in another study, the finding of an increase in FOXP3 protein or mRNA and increased FOXP3+ve cells was associated with worse outcomes, while there was no correlation between FOXP3 cell counts or FOXP3 mRNA and response to therapy (Yapici et al., 2009).

Other cell subtypes that may be found in acute cellular rejection are B lymphocytes, plasma cells, and eosinophils. B-lymphocyte clusters have been described in renal biopsies with acute cellular rejection, a finding reported to be associated with a less favourable prognosis in children (Sarwal et al., 2003; Muorah et al., 2009). B-cell infiltrates have also been associated with both steroid resistance (Sarwal et al., 2003; Hippen et al., 2005) and with poor renal functional outcomes in adults (Hippen et al., 2005; Hwang et al., 2010). Other studies in adults, however, have not found a significant association between B-cell infiltrates and poor graft outcomes (Bagnasco et al., 2007), including those in which B-lymphocyte clusters are present (Kayler et al., 2007; Scheepstra et al., 2008).

Plasma cells have been described in cellular rejections that may have an associated arteritis, and a worse prognosis (Adrogue et al., 2006; Gärtner et al., 2006). However, in studies of the transcriptome of clinically indicated biopsies obtained after 1 year, B-lymphocyte and plasma cell transcripts were reported to be correlated with time post transplant, but not with rejection (cellular or antibody mediated) or with graft prognosis (Einecke et al., 2008). The presence of eosinophils in cellular rejection has also been associated with arteritis and a worse outcome (Macdonald et al., 1999; Meleg-Smith and Gauthier, 2005).

Late cellular rejection

Tubulointerstitial infiltrates in late biopsies performed for graft dysfunction are often associated with interstitial fibrosis and tubular atrophy as well as with lesions caused by donor specific antibody (see below). These mixed forms of rejection may be the consequence of non-adherence to the immunosuppressive medication regimen (Lerut et al., 2007).

Chronic antibody-mediated rejection

These rejections are usually diagnosed in patients with insidious and progressive dysfunction of the graft that occurs predominantly after the first year post transplant. The antibody is usually a donor-specific anti-class II HLA antibody (DSA) that was either

present prior to transplant or developed *de novo*. Interestingly, a recent paper by Wiebe et al. described three clinical phenotypes associated with *de novo* DSA (acute dysfunction, indolent dysfunction, and stable function) in which the majority of patients had pathologic features of T-cell-mediated rejection (Banff grade II, I, or borderline) as well as antibody-mediated rejection. Risk factors for the development of *de novo* DSA included medication non-adherence, class II HLA mismatching, and early rejection episodes with significant peritubular capillaritis (Wiebe et al., 2012). The typical lesions of chronic antibody-mediated rejection include inflammation of the microvasculature (the glomeruli and the peritubular capillaries) with or without C4d deposition (Einecke et al., 2009; Gaston et al., 2010). The evidence for endothelial injury by antibody in those cases without C4d staining is the finding of increased endothelial cell transcripts by microarray studies (Sis et al., 2009). A putative mechanism of endothelial cell injury in C4d-negative antibody-mediated rejection is cytotoxicity by natural killer (NK) cells, the transcripts of which are enriched in both C4d positive and C4d negative rejection cases. Other Fc-receptor bearing cells such as monocytes may also be responsible for endothelial cell injury in antibody-mediated rejection (Hidalgo et al., 2010).

Another glomerular lesion associated with donor-specific antibody is transplant glomerulopathy that is characterized histologically by subendothelial expansion and duplication of the glomerular basement membrane. The natural history of transplant glomerulopathy has been characterized in a series of studies from the Mayo Clinic, Rochester, Minnesota. In one study, protocol and clinically indicated biopsies were done in 582 renal transplants with a negative pre-transplant T-cell complement-dependent cytotoxicity crossmatch. Transplant glomerulopathy was diagnosed in 55 patients and was associated with anti-HLA antibody, particularly to class II antigens. In 27 patients (49%), the diagnosis was made by protocol biopsy in well-functioning grafts (Gloor et al., 2007). The prevalence of transplant glomerulopathy in a protocol biopsy procured at 1 year post-transplant is 4% (Cosio et al., 2005) and the cumulative incidence of transplant glomerulopathy increases over time to 20% at 5 years (Gloor et al., 2007). It is important to point out that solid phase assays for donor-specific antibody were not done in the above studies; and that it was subsequently shown that many of these patients were in fact sensitized to their donor prior to the transplant. In a subsequent study, the presence and characteristics of anti-HLA antibody were assessed by single antigen bead assays in 598 kidney recipients with a negative T-cell crossmatch. Thirty-nine per cent of patients had anti-HLA antibodies prior to transplant. Transplant glomerulopathy was diagnosed in 73 patients (12%) during 54 ± 19 months of follow-up. The risk of transplant glomerulopathy was greater with higher anti-HLA class II antibody levels, when the antibody was donor specific, and if there was a history of antibody-mediated rejection. Graft survival during the follow-up period was 95% in patients without transplant glomerulopathy and 62% in patients with transplant glomerulopathy. The presence of C4d in peritubular capillaries was an independent risk factor for graft failure in this study (Issa et al., 2008). The prognosis of transplant glomerulopathy diagnosed by protocol biopsy was as poor as that diagnosed by biopsies procured for graft dysfunction, with progressive worsening of histopathological changes and graft survival of 50% at 4 years (Gloor et al., 2007).

The earliest lesions in transplant glomerulopathy may occur as early as 1 month post transplant, but are detectable only by electron microscopy. In a series of seven patients that developed transplant glomerulopathy at a mean of 2.3 years post transplant by light microscopy, endothelial cell vacuolation, hypertrophy, serration, and expansion of the lamina rara interna were found in a protocol biopsy performed at 1 month post transplant. Additional ultrastructural changes found in the endothelial cells were an abundance of mitochondria and ribosomes. Five of the seven of these patients were found to have had donor-specific antibodies either prior to the transplant or at some time postoperatively (Wavamunno et al., 2007).

Other lesions that can be present in chronic antibody-mediated rejection include peritubular capillary basement membrane multilayering, interstitial fibrosis, tubular atrophy, and fibrointimal thickening of arteries. Arteriosclerosis has been reported to be increased in patients with preformed donor-specific HLA antibodies. In a study that looked at protocol biopsies at 3 and 12 months post transplantation in 40 patients with and 59 without donor-specific antibodies, the chronic vascular score almost doubled in patients with antibody, whereas it was unchanged in those without (Hill et al., 2011).

A recent excellent review covers the pathogenesis of the various forms of renal transplant rejection (Nankivell and Alexander, 2010).

Pathology

Standardization of the criteria for renal allograft histopathology started 20 years ago with the first of the Banff conferences held in 1991, in Banff, Alberta, Canada. The Banff conferences have taken place every 2 years since, with the 11th conference having taken place in Enghien-les-Bains, France, in 2011. Significant milestones in the evolution of the Banff conferences as they relate to renal transplantation include the first publication in 1993 (Solez et al., 1993); the amalgamation of the Banff and the CADI ('chronic allograft damage index') systems (Isoniemi et al., 1994) for the scoring of chronic lesions at the 1995 meeting; and the merging of the Banff and National Institutes of Health CCTT systems ('Collaborative Clinical Trials in Transplantation') (Colvin et al., 1997), for the scoring of acute lesions in 1997. In 1999, the Banff 1997 working classification of allograft pathology was published (Racusen et al., 1999). In 2001, the first classification of antibody-mediated rejection was proposed, and in 2003, gene expression in acute and chronic allograft pathology was discussed, as well as tolerance. Since 2005, transcriptomics has been a major focus in Banff conferences; but antibody, C4d staining, and scarring were also topics. After the 2005 meeting, the term 'chronic allograft nephropathy' was eliminated from the Banff schema. In 2007, sessions on regulatory T cells were held. The focus in 2009 was again antibody, and the phenotype of late graft dysfunction from the Deterioration of Kidney Allograft Function (DeKAF) studies was highlighted. In 2011, the focus was acute antibody-mediated rejection in pre-sensitized patients, and the results of studies of Working Groups on BK virus, quantitation of interstitial fibrosis, glomerular lesions, implantation biopsies, and others were presented.

At the Banff 2003 meeting, the histological findings observed in protocol biopsy studies were referred to as being the 'standard of

science'. There have been sessions on protocol biopsy studies at all subsequent Banff meetings.

Specific Banff diagnoses

A useful table for acute T-cell-mediated and acute chronic antibody-mediated rejection can be found in the Banff 2009 meeting report (Sis et al., 2010).

Acute T-cell-mediated rejection

Acute T-cell-mediated rejection has three types (or grades). The threshold for the diagnosis of type I acute rejection is interstitial mononuclear cell infiltration in > 25% of the normal (non-atrophic) parenchyma (i2) and greater than four lymphocytes (t2) within the tubular basement membrane in the most affected (non-atrophic) tubule ('tubulitis'). In type II rejection there is mild or moderate intimal arteritis, while in type III acute rejection there is 'transmural' arteritis and/or fibrinoid change and smooth muscle cell necrosis with inflammation. The category of borderline changes refers to interstitial and/or tubular inflammation that is below the threshold for acute rejection, and is considered 'suspicious' for acute T-cell-mediated rejection. At the 2005 Banff meeting, t2 and t3 lesions with i0 or i1 were included in the borderline category.

At the 2007 Banff meeting, a new lesion, the 'total inflammation' score (ti) was proposed for addition to the Banff schema. This score refers to inflammation in areas of interstitial fibrosis and tubular atrophy, subcapsular cortex and perivascular tissue that were not considered in the original Banff classification. The 'ti' score was subsequently shown to correlate better with graft survival and with inflammation and parenchymal injury gene transcripts than the original Banff 'i' score (Mengel et al., 2009a).

The term 'subclinical rejection' was coined outside of the Banff schema to describe the finding of Banff histological criteria for acute T-cell-mediated rejection in protocol biopsies of well-functioning grafts (Rush et al., 1994). Two randomized studies in ciclosporin-treated patients showed a beneficial effect of corticosteroid treatment of subclinical tubulointerstitial infiltrates (improved renal function in both studies, less interstitial fibrosis in one) (Rush et al., 1998; Kurtkoti et al., 2008). In these studies, the prevalence of subclinical tubulointerstitial infiltrates in the first 3 months post transplant was between 15% and 30%. However, a more recent randomized study in low immunological risk patients treated with tacrolimus and mycophenolate mofetil showed a very low prevalence of subclinical rejection (< 5% in the first 3 months; and up to 9% at 6 months), the treatment of which, a 2-week course of corticosteroids, did not result in either functional or histological benefit (Rush et al., 2007). Conversely, borderline inflammation in a protocol biopsy at 1 or 4 months post transplant that was treated with a single dose of methylprednisolone was associated with increased fibrosis at 1 year in a steroid withdrawal study in which the baseline immunosuppression included induction with thymoglobulin, tacrolimus, and mycophenolate mofetil (Heilman et al., 2010). Furthermore, the finding of borderline inflammation and fibrosis in protocol biopsies at 4 or 12 months has been reported to be associated with decreased graft survival as compared to that of grafts with fibrosis alone (Cosio et al., 2005; Moreso et al., 2006). Lastly, the persistence of any degree of inflammation in sequential protocol biopsies has been reported to correlate with decreased graft survival (Mengel et al., 2007). These findings suggest that subclinical inflammation of any degree, if not treated or treated inadequately, may be detrimental to graft survival. It is likely that the use of protocol biopsies should be considered in patients with high immunological risk, for example, in those that are pre-sensitized to their donor (Haas et al., 2007) and in those patients in whom the potency of the immunosuppression regimen is reduced through minimization or avoidance protocols (Heilman et al., 2010).

Acute antibody-mediated rejection

The 2005 Banff conference defined three types (grades) of acute antibody-mediated rejection. These types required the demonstration of anti-donor antibody, and were considered 'suspicious' if antibody was not demonstrated; a positive stain for C4d was required for all types. Type I had features of acute tubular necrosis with little inflammation, type II had capillary margination and/or thrombosis, and type III had v3 arteritis.

C4d detection in peritubular capillaries appears to be a reliable feature of antibody-mediated rejection, and can be detected by monoclonal antibody and immunofluorescence on frozen tissue or by polyclonal antibody with immunoperoxidase detection on paraffin sections. Focal or diffuse staining can be observed, with 10% of peritubular capillaries staining defining the threshold for positivity of the test. The margination of inflammatory cells (neutrophils and/or mononuclear cells) in peritubular capillaries is also a reliable marker of antibody-mediated rejection. However, peritubular capillaritis can also be found in T-cell-mediated rejection and in glomerulonephritis (Gibson et al., 2008). Scoring of peritubular capillary inflammation (Banff ptc score) was added to the Banff schema in 2007. Antibody-mediated rejection was the major focus of the 2009 Banff meeting. The recognition of a new entity, that of C4d-negative acute antibody-mediated rejection, was highlighted. This entity is associated with donor-specific antibody and increased expression of endothelial cell transcripts by microarray studies, and has a poor graft outcome (Sis et al., 2009). C4d-negative antibody-mediated rejection has been described more recently in a cohort of 54 kidney transplant recipients that had donor-specific antibodies at the time of transplant. Protocol biopsies were procured in these patients at 3 and 12 months. At 3 months, subclinical inflammation of the microvasculature and positive staining for C4d, consistent with antibody-mediated rejection, was found in one-third of the patients, almost 50% showed microvasculature changes without C4d staining, while the remaining patients had no lesions attributable to antibody. Both groups with microvascular inflammation showed progression of the histological lesions and decreased renal function at 12 months compared to the group without antibody-mediated inflammation (Loupy et al., 2009).

Evolution of the Banff schema: integration with transcriptomics and results of the 'DeKAF study'

Transcriptomic studies and Banff

Since 2007, the studies of the transcriptome in 'for cause' renal allograft biopsies performed by the Edmonton group have been a central feature of the Banff conferences, and have had a profound influence on both the science and recent clinical practice in renal transplantation. Selected findings of interest from these studies include the demonstration that gene transcript disturbances are

stereotyped and continuous across rejection and non-rejection biopsies, similar in cell-mediated and antibody-mediated rejection and proportional to their severity, and inversely related with gene transcripts of tubular transporters (Mueller et al., 2007); the demonstration of C4d-negative antibody-mediated rejection through an increase in endothelial cell transcripts (Sis et al., 2009); and the finding that mast cell transcripts are present in areas of scarring and portend a poor outcome (Mengel et al., 2009b), among many others.

Prior to the transcriptome series of studies, Halloran proposed that study of late deterioration of function of the renal allograft required a new approach that looked for specific entities and required the elimination of the concept of chronic allograft nephropathy (Halloran, 2002). This concept resulted in the creation of the DeKAF ('Deterioration of Kidney Allograft Function') Consortium.

DeKAF

The DeKAF study was undertaken at five centres in the United States and two in Canada, with the goal of defining specific clinicopathological entities that cause late graft dysfunction and loss. One of the central hypotheses in DeKAF is that the term 'chronic allograft nephropathy' (CAN) is likely an aggregate of many distinct entities that if characterized by clinical, pathological, and laboratory features, may lead to specific interventional trials. Prevalent (cross-sectional) and incident (prospective) patient cohorts in DeKAF are biopsied for new-onset graft dysfunction or proteinuria, with central laboratories reporting on renal pathology, HLA (and other) antibodies, and magnetic resonance spectral analysis of the urine obtained at the time of biopsy (Gourishankar et al., 2010). Findings of interest from the DeKAF study to date include the very poor prognosis of patients with late graft dysfunction, in whom death-censored graft loss is 30% at 2 years; the lack of prognostic significance of the diagnosis of CAN (versus no CAN); the high prevalence of inflammation in late biopsies for cause (50%); and the importance of inflammation in areas of fibrosis and atrophy found to be independent predictors of death-censored graft loss (Gourishankar et al., 2010; Mannon et al., 2010). Furthermore, cluster analysis performed in a subset of the cross-sectional cohort of biopsies performed at a median of 6 years after transplant, identified six distinct patient groups that had different graft survival, based exclusively on their histology. All patients had tubular atrophy and virtually all had interstitial fibrosis (and therefore CAN). Two major and robust biopsy clusters were identified. One cluster has very little inflammation, and the diagnosis rendered for this group is frequently calcineurin inhibitor toxicity. These patients have an excellent prognosis at least in the short-term follow-up of 2 years. Another robust cluster has acute inflammation (Banff acute i and t scores), and has a worse prognosis that can potentially be improved with additional immunosuppression (Matas et al., 2010). The finding of these clusters is important. First, because it is demonstrated for the first time that distinct histological entities can be found within CAN; second, because the poor prognosis that is attributed to calcineurin inhibitor toxicity is not supported, at least in the first 10 years post transplant; and third, because inflammation was found to be frequently associated with late graft dysfunction in previously stable grafts. In addition, the DeKAF study demonstrated the very bad prognosis of late graft dysfunction where the biopsy is positive for peritubular capillary C4d staining, irrespective of whether or not there is donor-specific antibody detectable in the circulation (Gaston et al., 2010).

Non-invasive renal allograft monitoring and biomarkers of rejection

The recent findings that suggest that alloimmune injury is the major cause of late allograft loss has provided new impetus for the development of non-invasive biomarkers of graft rejection. The ideal biomarker and monitoring tool would identify allograft rejection at its earliest stage, at a time when tissue injury can be reversed and permanent damage is therefore prevented. Protocol biopsies have identified subclinical inflammation in all compartments of the kidney that have later been correlated with permanent fibrotic or atrophic sequelae. Moreover, the cellular and molecular phenotypes identified in tissue biopsies of acute and chronic inflammation have led to the study of a number of candidate gene transcripts and proteins in urine and blood as potential biomarkers in the clinic.

Cytotoxic T-lymphocyte biomarkers

Gene transcripts for granzyme B, perforin, and FasL in peripheral blood mononuclear cells were shown to be increased in acute clinical rejection (Vasconcellos et al., 1998). Moreover, the levels of granzyme B and perforin were reported to rise prior to the diagnosis of acute rejection and decrease following therapy (Simon et al., 2003). Similarly, granzyme B and perforin transcripts were reported to be elevated in the urine of patients with acute clinical rejection but not in patients with chronic allograft nephropathy or stable transplants (Li et al., 2001). Increased urine transcripts for granzyme B, perforin, and FasL were also reported in acute clinical rejection by others, but these did not differentiate acute rejection from cytomegalovirus (CMV) infection, urinary tract infection, and delayed graft function (Yannaraki et al., 2006). More recently it has been reported that urine granzyme A mRNA is elevated in subclinical rejection (van Ham et al., 2010).

Urine chemokines

An increase in urine transcripts for the chemokine IP-10 was shown in acute rejection patients compared to controls (Tatapudi et al., 2004), and CXCL9, CXCL10, and CXCL11 protein levels in the urine were shown to be elevated in acute rejection, acute tubular injury, and in polyoma viral infection but not in chronic rejection or stable grafts. Moreover these chemokines decreased after treatment of acute rejection (Hu et al., 2004). An increase in both CXCL10 transcripts and protein in patients with acute rejection but not those with CMV or stable grafts has also been reported (Matz et al., 2006). More recently, urine CXCL9 and CXCL10 protein levels were shown to correlate with the degree of acute interstitial inflammation and tubulitis, being significantly higher in subclinical and clinical acute rejection compared to levels found in borderline, normal, or interstitial fibrosis (Schaub et al., 2009). These findings were validated in a separate cohort (Ho et al., 2011). Finally, a cross-sectional analysis of 110 adults and 46 children has been reported that included healthy volunteers, stable renal transplant recipients, and recipients with clinical or subclinical acute rejection or BK virus infection, calcineurin inhibitor toxicity, or interstitial fibrosis in whom urinary CXCL9 and CXCL10 was analysed using a solid phase bead-array assay. Chemokine levels were markedly elevated in adults and children with either rejection or BK infection

(P = 0.0002), but not in stable allograft recipients or recipients with calcineurin inhibitor toxicity or interstitial fibrosis. The sensitivity and specificity of these chemokine assays exceeded that of serum creatinine. Neither chemokine distinguished between acute rejection and BK virus infection (Jackson et al., 2011).

Tissue injury biomarkers

In a series of studies, Schaub et al. used both proteomic approaches and quantitative protein assays, to demonstrate that intact and digested forms of beta-2 microglobulin increased in the urine of patients with acute clinical rejection and decreased with treatment of the clinical rejection episode. Other tubular injury markers (retinol-binding protein, alpha-1 microglobulin, and neutrophil gelatinase-associated lipocalin) also increased in acute clinical rejection. However, these proteins did not distinguish subclinical rejection from normal histology, and were also increased in interstitial fibrosis and polyoma virus nephropathy (Schaub et al., 2004, 2005, 2007). The prognostic significance of continuous proximal tubular injury has been suggested in a recent study in which increased urinary retinol-binding protein excretion was associated with poor long-term allograft function despite no histological changes at biopsy (de Matos et al., 2010).

HLA antibody as a biomarker

It has been increasingly apparent that the development of *de novo* donor-specific HLA antibodies post transplantation is associated with significantly higher graft failure rates (Martin et al., 1987; Worthington et al., 2003; Hourmant et al., 2005; Hidalgo et al., 2009; Wiebe et al., 2012), suggesting that they represent both a mechanism of repetitive injury and a potential prognostic biomarker (Terasaki and Ozawa, 2004).

Furthermore, Terasaki et al. conducted a multicentre, cross-sectional, prospective study of renal allograft recipients in which patients were divided into those with *de novo* HLA antibodies and those without (Terasaki et al., 2007). Four years later those who had HLA antibodies detectable using sensitive solid phase flow-based assays had a graft survival rate of 58% versus 81% in those who did not. This study raises the concept that *de novo* HLA antibody detection post transplant portends a poor prognosis. However, there were no biopsies performed to determine whether or not the *de novo* HLA antibody was the cause of the graft failure or that it was associated with tissue injury at the time of initial detection.

While clearly in the early stages of development, the sensitivity and donor specificity afforded by the new solid phase assays makes HLA antibody surveillance an appealing biomarker to be further investigated for its utility in clinical practice, as its early detection can be used to determine the state of graft injury and introduce therapies to modify the natural history (Archdeacon et al., 2011).

Additional biomarker candidates

FOXP3—a marker of regulatory T cells (Muthukumar et al., 2005), Tim-3—a marker of T-helper 1 differentiated cells (Renesto et al., 2007), and serine protease inhibitor (PI)-9—a marker of cytotoxic T cells and natural antagonist to granzyme B (Muthukumar et al., 2003), have been studied as candidate biomarkers. In each of these studies the urine transcripts were elevated in acute rejection as compared to stable allografts. Recently, it has been reported that urine fractalkine, a chemokine capable of recruiting T cells, monocytes, and NK cells, increases in acute clinical rejection and can be used to differentiate rejection from acute tubular injury or interstitial fibrosis (Peng et al., 2008). In addition, urinary microRNA-210 has been shown to be increased in patients with acute clinical rejection compared to patients with stable function or urinary tract infection (Lorenzen et al., 2011).

Management of rejection: prevention and treatment

Prevention of rejection

The better understanding of the mechanisms of T-cell activation has resulted in the development of a variety of potent immunosuppressive agents that are currently used in the clinic setting. Moreover, the development of assays that are capable of identifying high immunological risk patients (i.e. memory) prior to transplantation has allowed for the appropriate tailoring of the intensity of the initial immunosuppressive regimen to some extent. However, the beneficial results of these advances have been limited primarily to the early post-transplant period, for example, 1 year. Most grafts continue to be lost at later time points at rates that are similar to those observed in earlier decades.

In most centres in the United States, the initial immunosuppressive regimen consists of a calcineurin inhibitor (usually tacrolimus), an antiproliferative agent (usually mycophenolate mofetil), and corticosteroids. Induction therapy with either polyclonal or monoclonal antilymphocyte preparations is common in many centres (US Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients, 2009). The incidence of early (e.g. before 6 months) clinical rejection episodes in unsensitized patients with the above or similar protocols is now in the single digits in many institutions. However, potential side effects of immunosuppression such as arterial hypertension, nephrotoxicity, diabetes, infections, and malignancy, have led to the reduction in the doses of these medications or in their discontinuation at later times post transplant. Furthermore, the frequency of patient visits to the clinic decreases over time, resulting in fewer opportunities for patient counselling and potentially in a decrease in patient compliance with the immunosuppressive regimen. The lack of reliable tests to assess the adequacy of the immunosuppressive regimen at any given time further compounds these problems.

The balance between efficacy and side effects (nephrotoxicity, diabetes, infection—see Chapter 281) of tacrolimus- and mycophenolate mofetil-based immunosuppression have been highlighted in recent studies, some of which have used protocol biopsies. Comparing two different eras of exposure to tacrolimus, Cosio et al. reported that in the earlier era of higher exposure, the incidence of polyomavirus nephropathy, renal interstitial fibrosis on protocol biopsy, and fasting blood sugar at 1 year were increased, as compared to a more recent era of low exposure to tacrolimus. However, there were more cases of subclinical borderline cellular rejections and humoral rejections in the lower exposure tacrolimus era. The difference in tacrolimus trough levels between the high- and low-exposure groups, starting at 12–15 micrograms/L for the first month in the high exposure era and at 10–12 micrograms/L in the low exposure era, was approximately 2 micrograms/L throughout the 2 years of follow-up. Induction with thymoglobulin was used in both eras (Cosio et al., 2007).

Conversely, Naesens et al. reported that the independent correlates of chronic histological lesions on protocol biopsy at 12 months were clinical rejection episodes and low levels of exposure to tacrolimus between 3 and 12 months post transplant (mean trough 9.95 ± 1.76 micrograms/L), whereas lower chronicity scores were obtained with higher exposures to tacrolimus (mean trough 11.3 ± 1.43 micrograms/L). The immunosuppressive regimen used included mycophenolate mofetil, but induction therapy, which consisted mostly of anti-CD25 antibody, was given to only one-quarter of the patients. The incidence of polyoma virus was not reported (Naesens et al., 2007). Moreover, in a recent multicentre protocol biopsy Canadian study using full-dose tacrolimus and mycophenolate mofetil without antibody induction, the mean calculated creatinine clearance was approximately 74 mL/min at 24 months (Rush et al., 2007), whereas the creatinine clearance at 12 months was 65 mL/min in the Symphony study, which used a reduced dose of tacrolimus, mycophenolate mofetil, and induction with anti-CD25 antibody (Ekberg et al., 2007). The incidence of acute clinical rejection episodes in the above studies, most of which were T-cell mediated was between 8% and 12%. A more recent similar randomized trial used daclizumab induction for all patients and compared low-dose tacrolimus plus mycophenolate against low-dose tacrolimus or low-dose ciclosporin plus sirolimus. This study reported fewer rejections with tacrolimus and mycophenolate (14%) versus the other two groups (~30%) and better renal function at 2 years in the tacrolimus mycophenolate group (estimated glomerular filtration rate (eGFR) ~62.5 mL/min) versus the other two groups (eGFR~ 52 mL/min). There were no differences in graft function or graft losses at 5 years between the groups (Guerra et al., 2011).

In aggregate, the above studies suggest that thymoglobulin with high doses of tacrolimus provides excessive immunosuppression, but that high exposure to tacrolimus is probably best if the induction agent is anti-CD25 antibody.

Newer immunosuppressive agents

The notion that progressive calcineurin inhibitor toxicity is the major cause of late graft losses has been challenged by the DeKAF study (Gourishankar et al., 2010) and others (El-Zoghby et al., 2009), and recent findings from several groups suggest that most graft losses are due to an alloimmune response involving donor-specific antibodies that target the microcirculation in the allograft (Einecke et al., 2009; El-Zoghby et al., 2009; Gaston et al., 2010). To what extent calcineurin inhibitor toxicity is a cause of later graft loss in patients who do not develop donor-specific antibodies remains to be determined. However, the new immunosuppressive agents under investigation are non-nephrotoxic drugs that attempt to replace calcineurin inhibitors, and drugs that target B-lymphocytes.

The monoclonal antibody that targets the CD28-CD80/86 pathway (belatacept) has recently been approved by the FDA for initial immunosuppression in renal transplant patients. In the original study, two belatacept regimens (more intensive and less intensive) were compared to ciclosporin in *de novo* low immunological risk kidney transplant recipients that received anti-CD25 antibody induction, mycophenolate mofetil, and prednisone. Rejection rates between zero and 6 months were similar for the three groups (<10%), and at 12 months renal function was better in both belatacept groups compared to ciclosporin (creatinine clearances of 66.3 mL/min and 62.1 mL/min for the more intensive, and less intensive belatacept regimens, and 53.5 mL/min per for the ciclosporin regimen), and chronic histological changes were less common with both regimens of belatacept than with ciclosporin (29% for the more intensive and 20% for the less intensive belatacept regimens, and 44% for the ciclosporin regimen) (Vincenti et al., 2005). A subsequent study showed that the difference in renal function favouring the belatacept-treated patients persisted at 2 years in recipients of both standard criteria (N = 493) and extended criteria (N = 347) donors (Larsen et al., 2010). Moreover, at 5 years, 78 of 102 patients on belatacept and 16 of 26 on ciclosporin had an identical incidence of neoplasms (12%), while infections were less frequent in the belatacept treated patients (16%) than in those treated with ciclosporin (27%) (Vincenti et al., 2010). Post-transplant lymphoproliferative disorder was more common in the belatacept-treated patients if there was an Epstein–Barr viral mismatch between donor and recipient.

Agents under development that target B lymphocytes are the monoclonal antibody belimumab and the recombinant fusion protein, atacicept. Both agents interfere with differentiation, survival, and activation signals for B lymphocytes that are delivered by ligands of the tumour necrosis factor superfamily BlyS (or Baff) and April. Binding of BlyS and April to their specific receptors results in enhanced B-cell survival through the increase in antiapoptotic factors, as well as in B-cell activation and immunoglobulin production (Webber et al., 2011).

Treatment of rejection

T-cell-mediated rejection

T-cell-mediated rejection is usually responsive to corticosteroids and renal function may return to baseline within a few days to weeks after treatment. These rejections are typically early type I (Banff) rejections and if not recurrent, may have no effect on long-term graft survival (Famulski et al., 2010).

However 'steroid-resistant' rejection episodes occur, defined as those in which corticosteroids are replaced by other agents such as anti-T-cell antibodies because of a lack of improvement in renal function with corticosteroids alone. These rejections may be characterized by more severe tubular epithelial injury (e.g. tubular basement membrane rupture; Banff t3) or with arteritis (Minervini et al., 2000). Renal function may not return to baseline in such cases, a finding that may correlate with later graft dysfunction and loss.

A large multicentre study randomized patients with steroid-resistant rejection to either of two antilymphocyte agents, thymoglobulin (1.5 mg/kg/day; N = 82) or Atgam® (15 mg/kg/day; N = 81). The randomization was stratified by renal histology and > 90% of the biopsies had arteritis. Reversal of rejection, defined as return of the serum creatinine to baseline, occurred in 88% of thymoglobulin-treated patients, but in only 76% of those that received Atgam®. Similarly, rejection recurrence was 7.3% with thymoglobulin and 15% with Atgam®, whereas graft survival at 1 year was 83% versus 75%, respectively (Gaber et al., 1998). An additional two studies in patients with steroid-resistant rejection compared low-dose thymoglobulin (0.75 or 2 mg/kg/day) to low doses of the anti-CD3 antibody. In both studies, rejection reversal, recurrence rates, and side effects favoured the use of thymoglobulin (Mariat et al., 1998; Midtvedt et al., 2003). Finally, in a small randomized study of 30 patients, Casadei et al. reported that intravenous immunoglobulin 500 mg daily was almost as efficacious as

anti-CD3 antibody in reversing steroid-resistant rejections, and was associated with less recurrences and fewer side effects (Casadei et al., 2001).

Another clinical feature of T-cell-mediated rejection that may correlate with graft dysfunction and loss is the time of its occurrence, particularly those rejections that occur after 6 months or 1 year post-transplant. However, the poor prognosis associated with these late rejections may be due to the fact that they are occurring in previously injured grafts, are the result of patient non-compliance with the immunosuppressive regimen (Lerut et al., 2007), or because they may be associated with donor-specific antibody (Einecke et al., 2009). Interestingly, late rejections were less frequent in patients randomized to the biopsy arm in a protocol biopsy study that treated patients with early subclinical rejection (Rush et al., 1998).

Antibody-mediated rejection

The treatment of antibody-mediated rejection involves many measures that attest to both its complex pathogenesis and the lack of controlled studies. In fact, there are no therapies for antibody-mediated rejection that are approved by the Food and Drug Administration (Archdeacon et al., 2011). In general, the interventions for antibody-mediated rejection are centred on the following concepts.

1. Circulating antibody can be removed by such measures as plasmapheresis and immunoadsorption.
2. The effect of antibody or its production may be modulated by the use of intravenous immunoglobulin.
3. B-lymphocyte suppression can be attempted with the use of mycophenolate mofetil, steroids, thymoglobulin, rituximab, and corticosteroids.
4. T-cell suppression can be achieved with calcineurin inhibitors, in addition to some of the above agents.
5. Plasma cell depletion may be achieved with proteasome inhibitors such as bortezomib.
6. Terminal complement activation can be inhibited with the use of eculizumab.

Many of these interventions are applied simultaneously.

Plasmapheresis

Plasmapheresis is the fastest way of removing donor-specific antibodies but a rebound in antibody production may occur. A useful endpoint in many centres that utilize plasmapheresis for 'desensitization' is the achievement of a negative T-cell cytotoxicity crossmatch after a certain number of plasma exchanges. The large number of exchanges needed in patients with high titres of antibody limits this approach mostly to the recipients of living-donor transplants. The most often used replacement fluid is 5% albumin, although fresh frozen plasma may also be used. Immunoadsorption is used infrequently because of its cost.

In the case of pre-sensitized patients with low titres of antibody, plasmapheresis can be performed briefly prior to transplant, and the effects of antibody may be inhibited with intravenous immunoglobulin that is given post plasmapheresis.

Intravenous immunoglobulin

Intravenous immunoglobulin is a commercial product obtained from pooled human plasma of several thousand healthy blood donors. It is composed largely (90%) of IgG. The mechanism of action of intravenous immunoglobulin is unclear, but suppression of antibody synthesis, anti-idiotypic antagonism of the pathogenetic HLA antibody, blockade of Fc-receptors, inhibition of complement activation, and anticytokine antagonism have been suggested. An example of combined therapies for the prevention of acute renal transplant antibody-mediated rejection is a study that compared the combination of plasmapheresis/intravenous immunoglobulin, and anti-C20 antibody versus high-dose intravenous immunoglobulin alone. The combined treatment resulted in 91.7% graft survival at 36 months, whereas graft survival was only 50% in the intravenous immunoglobulin group alone. The study was, however, not randomized, and the patients treated with combination therapy were of a more recent epoch (Lefaucheur et al., 2009).

Rituximab

Anti-CD20 antibody (rituximab) is a chimeric monoclonal antibody that is used for the treatment of lymphoma. The antibody targets the CD20 antigen on B lymphocytes. Profound B-cell depletion occurs, presumably due to antibody or complement-dependent cytotoxicity or the induction of B-cell apoptosis. The effect of anti-CD20 administration can be prolonged, with B-cell populations reaching pre-treatment levels a year after antibody administration. The first use of rituximab for the treatment of antibody-mediated rejection in kidney transplantation involved 27 patients treated with a single dose of rituximab (375 mg/m^2) in addition to corticosteroids (in 24), antithymocyte globulin (in 22), and plasmapheresis (in 22). Twenty-four patients recovered normal graft function and there were three graft losses (Becker et al., 2004). A more recent study used rituximab (375 mg/m^2 weekly) for 3–5 consecutive weeks, in addition to plasmapheresis, steroids, mycophenolate mofetil, and tacrolimus, in eight consecutive renal transplant patients presenting with acute antibody-mediated rejection. After a mean follow-up of 10 months (range 7–23), patient and graft survival were 100% and 75%, respectively. Renal function improved in six cases and there were two grafts lost. At last follow-up, the donor-specific antibody had disappeared or decreased in four cases. Four patients had infectious complications (Faguer et al., 2007). Adverse reactions to rituximab have included fever, cytopenias, and leucoencephalopathy.

Bortezomib

Bortezomib is a boronic acid dipeptide derivative that is used for the treatment of multiple myeloma. Bortezomib causes a reversible inhibition of the chymotrypsin-like activity of the 26s proteasome, which results in decrease proteolysis and accumulation of unfolded proteins in the endoplasmic reticulum. Because of its effect on malignant plasma cells, bortezomib was tested on normal CD 138+ cells *in vitro* obtained from patients sensitized to HLA in whom it induced apoptosis of plasma cells and inhibition of alloantibody production (Perry et al., 2009). Clinical experience with bortezomib in renal transplant patients was first reported by the Cincinnati group in six patients that had eight episodes of mixed antibody-mediated rejection and acute cellular rejection. Bortezomib was given at labelled dosing. Monitoring was done by serial donor-specific anti-HLA antibody levels and repeated allograft biopsies. In all cases bortezomib reversed the rejection episode and deceased antibody levels by 50% within 2 weeks for up to 5 months (Everly et al., 2008). In a subsequent study, two adult kidney transplant recipients with antibody-mediated rejection received a bortezomib-based regimen as the primary therapy. Plasmapheresis was used immediately before each bortezomib

dose, and a single rituximab dose (375 mg/m^2) was given with the first bortezomib dose. The rejections occurred within the first 2 weeks after transplantation. High DSA levels and positive C4d staining of peritubular or glomerular capillaries were present at the time of diagnosis. Both patients experienced prompt rejection reversal and elimination of detectable donor-specific antibody within 14 days of bortezomib administration. Renal function was excellent with normal urinary protein excretion at 5 and 6 months after the rejection diagnosis. One patient experienced a repeated elevation of donor-specific antibody (including the development of antibody against two new HLA specificities) 2 months after initial bortezomib therapy, but without C4d deposition or histologic evidence of antibody-mediated rejection. Re-treatment with bortezomib provided prompt, complete, and durable elimination of antibody (Walsh et al., 2010). More recently, 10 consecutive patients with antibody-mediated rejection were treated with one cycle of bortezomib (1.3 mg/m^2) intravenously (on days, 1, 4, 8, and 11), and compared to a historical control group of nine patients treated with a fixed single dose of rituximab (500 mg). All patients received plasmapheresis, intravenous immunoglobulin (30 g), and methylprednisolone. Patient survival in both groups was 100%. However, at 18 months, graft survival was 6/10 in the bortezomib group as compared to 1/9 in the rituximab group (P = 0.071) (Waiser et al., 2011).

Eculizumab

Eculizumab is a humanized $IgG_{2/4k}$ monoclonal antibody that blocks the terminal activation of complement by high-affinity binding to C5. Binding of C5 prevents the activation of the chemoattractant C5a and of C5b halting the formation of the membrane attack complex. Eculizumab was first used for the treatment of paroxysmal nocturnal haemoglobinuria.

In renal transplantation, eculizumab has been used in combination with other agents (plasmapheresis, intravenous immunoglobulin, anti-CD20 antibody, and bortezomib) for the treatment of acute antibody-mediated rejection (Locke et al., 2009; Lonze et al., 2010; Stegall et al., 2011). Eculizumab has also been used for the prevention and recurrent of haemolytic uraemic syndrome after renal transplantation (Larrea et al., 2010; Zimmerhackl et al., 2010).

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CHAPTER 284

Infection: prophylaxis, diagnosis, and management

Camille Nelson Kotton

Introduction

Infections are the most common complication after transplantation, increasing both morbidity and mortality, and decreasing graft survival. Infections may be acquired in the hospital (i.e. nosocomial infections), from the organ transplant itself, the blood product donor, or in the community. Reactivation of latent host infection is the most common cause. In general, the intensity of immunosuppression is at its highest for a year after solid organ transplant (Jong and Freedman, 2012). Guidelines on diagnosis, treatment, and prevention of many infections after solid organ transplant have been provided by the Infectious Diseases Community of Practice of the American Society of Transplantation (2013).

A timeline of infection risk after transplantation has been described by Fishman (Fig. 284.1) (Fishman, 2007). In the first month after organ transplant, infections tend to be related to the surgical procedure and hospital environment, and include wound infection, consequences of anastomotic leaks and ischaemia, aspiration pneumonia, catheter infection, and *Clostridium difficile* colitis. In this population, repeatedly exposed to healthcare settings, such infections are more likely to be due to resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), and non-albicans *Candida* spp. Donor-derived infections and recipient-derived infections, due to prior colonization with agents such as *Aspergillus* or *Pseudomonas*, may present in this phase.

From months 1 to 5 after transplant the classic opportunistic infections occur. Their risk can be mitigated or delayed by prophylaxis, and increased by intensified immunosuppression, leucopenia, or immunomodulatory viral infections.

The stable and relatively healthy organ transplant recipient who is > 6 months out from transplant tends to develop community-acquired or ordinary infections, including urinary tract infections, upper respiratory infections and pneumonia, gastroenteritis, and varicella zoster. Infections with unusual and opportunistic pathogens such as *Aspergillus*, unusual moulds, *Nocardia* (Fig. 284.2), and *Rhodococcus* are still seen. In the era of effective prophylaxis with valganciclovir, an increased risk of 'late cytomegalovirus (CMV)' (occurring > 6 months after organ transplant) has been noted, particularly in the few months after prophylaxis has been discontinued (Kotton et al., 2013). Other late viral infections include polyomavirus infections (from BK, causing nephropathy primarily in renal transplant recipients, or JC, causing progressive multifocal leucoencephalopathy) and Epstein–Barr virus (EBV)-related post-transplant lymphoproliferative infections (Kotton and Fishman, 2005).

Pre-transplant evaluation can mitigate the risk of some infections, especially latent ones. Knowledge of serostatus for CMV, EBV, hepatitis B virus (HBV), and hepatitis C virus (HCV) can improve post-transplant management. Potential transplant recipients and donors are screened for latent tuberculosis, by history, chest X-ray, skin testing, or use of an interferon gamma release assay-based blood test such as the T.SPOT®.TB or QuantiFERON® TB Gold. Recipients from or in endemic regions should be screened for latent infections such as *T. cruzi, Coccidioides*, and *Strongyloides*. Those subjects seronegative for measles, mumps, rubella, hepatitis A and B, and varicella should be vaccinated pre transplant. Some vaccines are live so cannot be given after transplant when the recipient is immunosuppressed (Jong and Freedman, 2012).

Atypical presentation of infection is more common in immunosuppressed hosts. Clinical presentations may be subtle, and the patients more ill than normal. For example, transplant recipients infected with West Nile virus are much more likely to have clinical illness and succumb. Clinicians need to consider a broad differential diagnosis in transplant recipients. The diagnosis of emerging, novel, and atypical pathogens is especially challenging in this vulnerable population, as has been seen with cases of lymphocytic choriomeningitis virus, tuberculosis, Chagas disease, and strongyloidiasis.

The importance of donor-derived infections is now recognized. Such infection occurs in up to 1% of deceased donor organ transplants (Ison and Nalesnik, 2011). While transmission of some infections is expected, such as CMV and EBV, others have been a surprise to clinicians caring for patients. Such unanticipated donor-derived infections range from viruses such as rabies, lymphocytic choriomeningitis and West Nile virus, to bacteria including tuberculosis, fungi including cryptococcosis and histoplasmosis, and parasites such as *Trypanosoma cruzi* (causing Chagas disease) and *Strongyloides stercoralis* (Ison and Nalesnik, 2011). Enhanced appreciation of donor-derived infections has resulted in better screening and diagnosis.

The risk of post-transplant infection can be mitigated by preventative measures such as routine vaccination, consumption of clean food and water, preventative measures during times of outbreaks (as with severe acute respiratory syndrome (SARS) and H1N1 influenza), safe sexual practices for non-monogamous recipients, visits to travel medicine specialists prior to visiting high-risk regions, and guidance on safer tattoo acquisition.

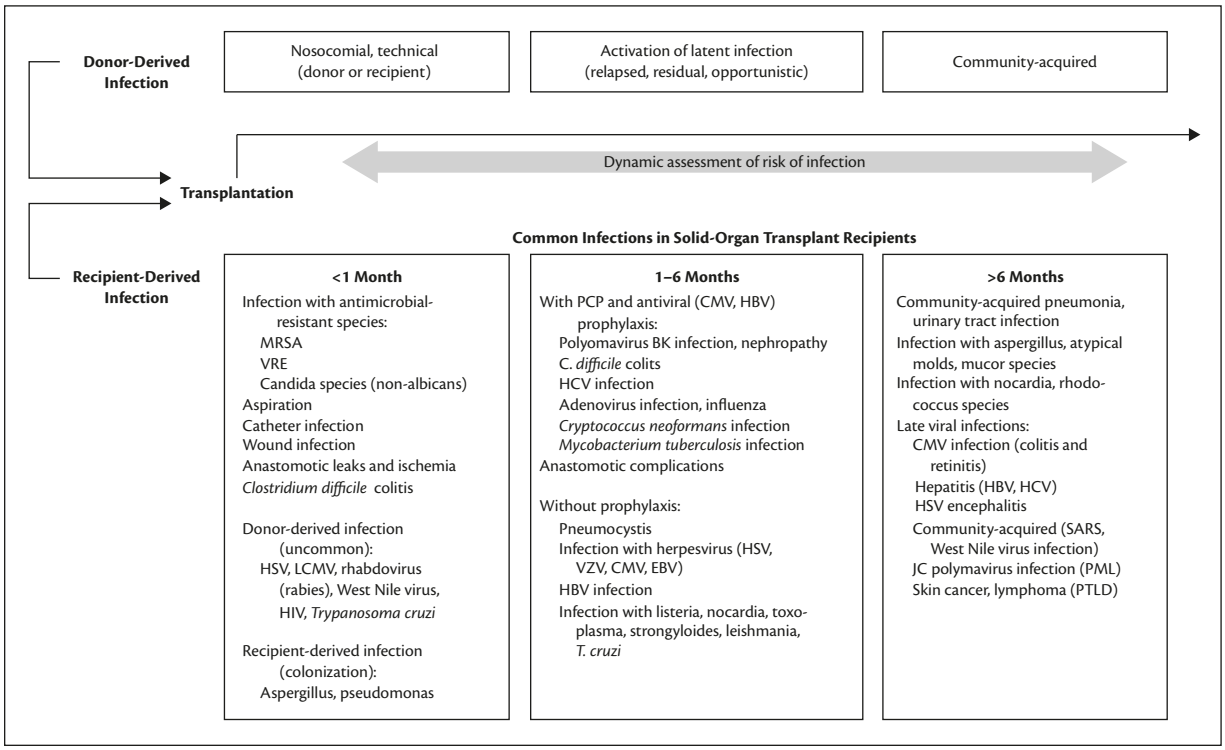

Fig. 284.1 Trends in the timings of infection after organ transplantation. Infections tend to occur in fairly predictable phases after solid organ transplant. While many of the classic opportunistic infections occur in the first six months, the period of most intense immunosuppression, the risk of such infection is indefinite and still exists for a period after discontinuation of immunosuppressive drugs. The risk of infection is decreased by the use of prophylaxis, and increased by the use of more potent immunosuppression (both in the induction and maintenance phases, and after treatment of rejection), allograft rejection itself, concomitant infections, leucopenia, and technical complications of surgery. HBV = hepatitis B virus; HIV = human immunodeficiency virus; HSV = herpes simplex virus; LCMV = lymphocytic choriomeningitis virus; MRSA = methicillin-resistant *Staphylococcus aureus*; PCP = *Pneumocystis jirovecii* (formerly *carinii*) pneumonia; PML = progressive multifocal leucoencephalopathy; PTLD = post-transplantation lymphoproliferative disorder; SARS = severe acute respiratory syndrome; VRE = vancomycin-resistant *Enterococcus faecalis*; VZV = varicella zoster virus.

Reproduced from Fishman, J. A. (2007). Infection in solid-organ transplant recipients. *N Engl J Med*, 357, 2601–14.

Viruses: prophylaxis, diagnosis, and management

Viruses are the most common cause of infection after transplantation. Viruses encompass a broad array from herpes to respiratory to hepatitis. In addition to the direct effects (i.e. clinical syndromes) caused by viruses, they can be immunomodulatory, especially CMV, HCV, or EBV, resulting in both inflammation (potentially mitigating graft tolerance) as well as increased immunosuppression, further increasing the risk of infection from other opportunistic pathogens. Since many of the important viruses after transplantation are latent (i.e. the herpes viruses), their prevention and management needs a fine balance between optimal levels of immunosuppression and reactivation of infection.

Viruses of the human herpes virus family are the most common viral pathogens after transplantation. The family includes eight viruses: herpes simplex type 1 and type 2 (HSV-1, -2), varicella (VZV), EBV, CMV, the roseola-like human herpes virus 6 and 7 (HHV-6, -7), and human herpes virus 8 (HHV-8, the aetiologic agent of Kaposi sarcoma). The alpha herpes virus family (HSV-1, -2, VZV) establishes latent infections primarily in sensory ganglia, while the beta herpes viruses (CMV, HHV-6, -7) maintain latency in leucocytes, endothelium, and other tissues. The gamma herpes viruses (EBV and HHV-8) are latent in lymphoid tissue. Disseminated infection from any of the human herpes viruses can be life-threatening. Recipients who acquire *de novo* infection from their donors, who do not have prior immunity to these viruses, are at highest risk for severe infection.

Numerous other viruses cause disease in transplant recipients. Respiratory viruses such as influenza, respiratory syncytial virus (RSV), adenovirus, parainfluenza, and human metapneumovirus are common and may present more subtly or with fulminant disease. Hepatitis viruses (primarily B and C) are common reasons for liver transplantation and commonly complicate transplantation, predominantly as reactivation of latent infections. The primarily zoonotic hepatitis E has been reported as an emerging pathogen in transplant recipients. Most adults have latent infection with the polyomaviruses BK and JC. While BK is predominantly a pathogen in kidney transplant recipients, it can cause disease in recipients of other organs. Risk of BK reactivation relates directly to the intensity of the immunosuppression, and early diagnosis of BK replication and subsequent reduction in the immunosuppressive regimen largely abrogates the risk of BK nephropathy, which generally has poor outcomes in kidney transplant recipients with high rates of graft loss. JC virus causes progressive multifocal leucoencephalopathy (Fig. 284.3), which though often fatal is fortunately rare. Numerous other viruses have been shown to cause disease in transplant recipients, including parvovirus B19, West Nile virus, and lymphocytic choriomeningitis.

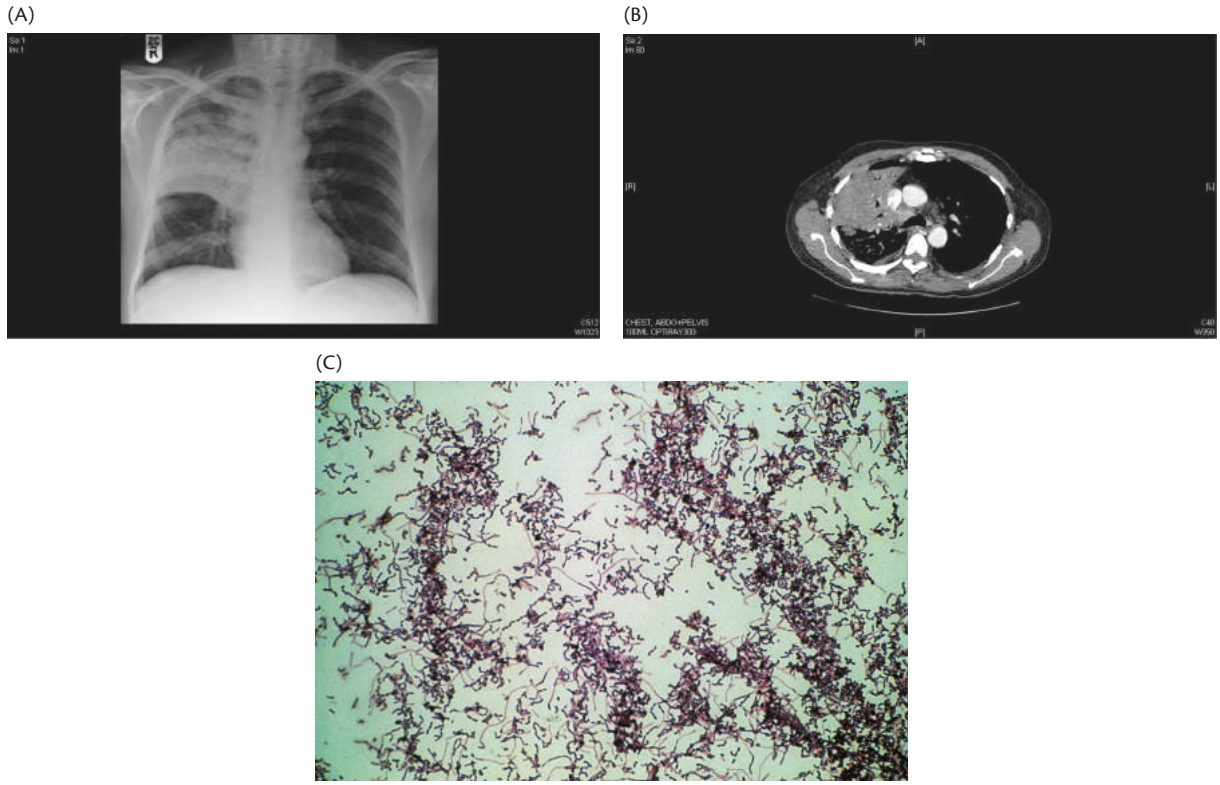

Fig. 284.2 Examples of dense consolidation seen with *Nocardia* and Gram stain. (A) Chest roentgenogram demonstrating dense consolidation in right upper lobe from *Nocardia* pneumonia. (B) Chest computed tomography with dense consolidation from *Nocardia* pneumonia. (C) Gram stain of *Nocardia* showing weakly Gram positive, rod-shaped bacteria.

Courtesy of Dr C. G. Winearls, Oxford University Hospitals, Oxford, UK.

Hundreds of patients with HIV infection have undergone organ transplantation, primarily kidney but also liver and other organs. In a large multicentre trial between November 2003 and June 2009, a total of 150 HIV-positive patients underwent kidney transplantation. Patient survival rates at 1 year and 3 years were 94.6 ± 2.0% and 88.2 ± 3.8%, respectively, while the corresponding graft-survival rates were 90.4% and 73.7% (Stock et al., 2010). These outcomes fall somewhere in the national database between kidney transplant recipients who are > 65 years old and those reported for all kidney transplant recipients. Multivariate analysis showed that the risk of graft loss was increased among patients treated for rejection and those receiving antithymocyte globulin induction therapy, while living-donor transplants were protective. A higher-than-expected rejection rate was observed, with 1-year and 3-year estimates of 31% and 41%, respectively. HIV infection remained well controlled, with stable CD4+ T-cell counts and few HIV-associated

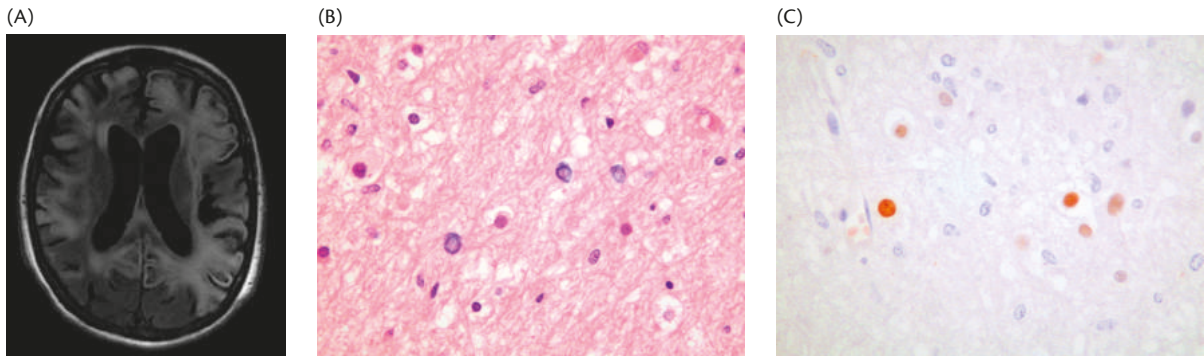

Fig. 284.3 (A) Brain MRI with axial FLAIR sequence showing confluent white matter (to include subcortical U fibres), T2 hyperintensity, and associate cavitation of the left temporal lobe, classic findings of progressive multifocal leucoencephalopathy. (Kindly interpreted by Roderick Borgie, MD, Massachusetts General Hospital, Boston, MA, USA.) (B) White matter showing demyelination and intranuclear inclusions within glial cells by haematoxylin and eosin stain. (C) There is nuclear positivity for SV40 T antigen (c; immunoperoxidase method), confirming the diagnosis of JC virus-associated progressive multifocal leucoencephalopathy.

Images (B) and (C) supplied courtesy of Professor Ian S. D. Roberts, Oxford University Hospitals, Oxford, UK.

complications. Such data suggest that in some HIV-positive candidates, renal transplant is appropriate.

Prophylaxis

Viral infections can be prevented by the use of antiviral drugs, prudent use of immunosuppression, administration of immunoglobulins, careful monitoring, and vaccination. The most frequently used antiviral drugs include the aciclovir family (including famciclovir and valganciclovir) primarily for HSV and VZV prophylaxis; ganciclovir (with the oral prodrug, valganciclovir), which is used primarily for CMV infection prevention but may also decrease the rates of other herpes virus infection; as well as anti-hepatitis B agents. Prophylaxis against hepatitis C is not usually given, because toxicity outweighs the benefit. The duration of prevention varies among transplant units, but many programmes prescribe antivirals for 3–6 months after the organ transplant. This is standard in high-risk situations where the donor is CMV seropositive and the recipient seronegative (Kotton et al., 2013). Aciclovir-type drugs are used to prevent disseminated VZV infection, as well as HSV. Some organ transplant centres use antiviral agents in certain cohorts of patients at risk for CMV (termed 'universal prophylaxis'); others use 'pre-emptive therapy', this treatment is begun only when monitoring of CMV copy numbers shows evidence of active infection. There are guidelines for optimal management of CMV after solid organ transplant (Kotton et al., 2013), with an updated version soon to be published. CMV immunoglobulin and HBV and VZV hyperimmune globulins are effective in preventing infection in certain settings, and repleting hypogammaglobulinaemic recipients with intravenous immunoglobulin can reduce their risk of infection.

Cytomegalovirus

Universal prophylaxis and pre-emptive therapy comprise the two main methods for CMV prevention. Universal prophylaxis involves giving antiviral medication at prophylactic doses for a defined period of time to a cohort (i.e. when either donor and/or recipient are seropositive for CMV) or defined subset of a cohort (i.e. given only to the highest risk subset, e.g. when donors are seropositive and recipients are negative for CMV (D+/R−)). Pre-emptive therapy is defined as use of treatment dose antivirals only once a certain test threshold is achieved. In patients subject to serial testing or after treatment of infection, some units elect to use neither of these methods of prevention and treat only when there are signs and symptoms of active CMV. This should be strongly discouraged, as this is likely to result in high rates of symptomatic CMV infection, ranging from CMV syndrome to systemic disease (colitis, hepatitis, pneumonitis, encephalitis, retinitis, and others). Clinical outcomes are inferior to those of programmes using either pre-emptive therapy or universal prophylaxis because of higher rates of graft dysfunction and loss, more opportunistic infections, and a great risk of post-transplant lymphoproliferative disorder (PTLD) (Fishman, 2007).

The two methods of prevention have their merits and disadvantages. Although large, randomized trials have not been conducted, numerous studies suggest that universal prophylaxis results in better outcomes than those achieved with pre-emptive therapy, especially in the higher risk D+/R− population (Kotton et al., 2013). Benefits of universal prophylaxis include lower drug costs, fewer opportunistic infections (including Kaposi sarcoma and PTLD), improved graft and patient survival, lower rates of rejection, easier logistics, and lower monitoring costs. The downsides include higher rates of late CMV infection, emergence of resistant strains, and higher drug costs and toxicities. Advantages of pre-emptive therapy include lower drug costs, reduced drug exposure, lower rates of late CMV (possibly due to enhanced immunologic priming (Abate et al., 2010)), lower drug costs, reduced drug exposure, and theoretically lower risk of resistant CMV due to lower rates of drug exposure. Disadvantages include lower rates of graft and patient survival, higher rates of opportunistic infections, and more complex logistics (organizing and managing the results of weekly testing for several months after transplant). Whether to initiate secondary chemoprophylaxis or viral monitoring after treatment of active CMV infection has not been well studied. Experts vary in their practice (Kotton et al., 2013). Institutions should develop local protocols, based on clinical outcomes, use of cytolytic induction therapies, the overall state of immunosuppression, costs, and ability to do periodic testing.

Outcomes with 'pre-emptive therapy' may not be as good as with universal prophylaxis, especially in high-risk transplants (i.e. CMV D+/R−). Compelling data comes from a randomized clinical trial of valganciclovir prophylaxis (N = 74) versus pre-emptive therapy with intravenous ganciclovir (N = 74). Prophylaxis significantly improved long-term graft survival 4 years after transplant (92.2% vs 78.3%; P = 0.0425) (Kliem et al., 2008). The lowest rate of graft loss following prophylaxis (0.0% vs 26.8%; P = 0.0035) was in the D+/R+ group, suggesting that perhaps the prophylaxis strategy can be tailored according to serostatus.

There has long been concern about the 'indirect' effects of CMV on transplant recipients (Kotton, 2013). These are more insidious and may have an adverse impact on both organ outcome and the recipient, and include increased rates of bacterial, viral, and fungal infections; more aggressive recurrent HCV after liver transplantation; higher rates of acute rejection and increased graft dysfunction and failure; chronic allograft nephropathy; vascular disease (coronary, aortic, and transplant); cancer (especially PTLD); and diabetes (Freeman, 2009).

Costs of serial testing (including personnel and laboratory costs) may be similar to the costs of medications (i.e. with 'universal prophylaxis') in some settings, although the net costs to the patient (i.e. co-payments for medication) or to the transplant programme or healthcare system may be different. It is, however, better to focus on long-term outcomes and overall cost and benefit to the patient and to the programme. In a recent study, the incidence of CMV infection in seropositive kidney transplant recipients within the first year after transplant was 4.1% and 55.5% when under universal prophylaxis and pre-emptive therapy, respectively (Luan et al., 2011). Universal prophylaxis incurred $1464 more direct costs compared with pre-emptive therapy, while saving $7309 in indirect costs, and resulted in a net gain of 0.209 in quality-adjusted life years per patient over a 10-year period. Thus, universal prophylaxis resulted in a cost saving of $27,967 for one quality-adjusted life year gained when compared with pre-emptive therapy. This supports the view that universal prophylaxis in CMV seropositive kidney transplant patients is clinically effective and cost saving.

The 'Improved Protection Against CMV in Transplant' (IMPACT) trial demonstrated that prolonged prophylaxis for 200 days with valganciclovir compared with 100 days significantly reduces the incidence of CMV in high-risk kidney transplant (D+/R−) recipients. Subsequent researchers developed a cost-effectiveness model

to evaluate prolonged prophylaxis for 200 days with valganciclovir in D+/R− kidney transplant recipients and its long-term economic impact from the US healthcare payer perspective (Blumberg et al., 2010). They found that for the 5-year time horizon, the incremental cost-effectiveness ratio of US $14,859/quality-adjusted life year suggests that 200-day valganciclovir prophylaxis is cost-effective over the 100-day regimen considering a threshold of US $50,000 per quality-adjusted life year. The 10-year analysis revealed the 200-day prophylaxis as cost saving with a 2380 quality-adjusted life year gain (per 10,000 patients) and simultaneously lower cost. They concluded that prolonged prophylaxis with valganciclovir reduces the incidence of events associated with CMV infection in high-risk kidney transplant recipients and is a cost-effective strategy in CMV disease management. Another single-centre, retrospective study reached the same conclusion, that is, 6 months of prophylaxis in those who are CMV D+/R− was more cost effective than 3 months, with an incremental cost of $34,362 and $16,215 per case of infection and disease avoided, respectively, and $8304 per one quality-adjusted life year gained (Luan et al., 2009).

Polyomavirus

Polyomaviruses, especially BK virus, can cause significant graft dysfunction and even loss. Numerous studies show that routine monitoring for BK for the first 12–18 months after transplant, with early detection of viral replication and subsequent reduction of immunosuppression provides the best clinical outcomes. Once BK nephropathy has become established, salvage of the kidney is less likely to be successful. BK viraemia and nephropathy are the consequences of potent immunosuppression. The primary method of prevention is screening, and the primary method of treatment is reduction in immunosuppression. Antiviral therapy, including cidofovir and leflunomide, has an uncertain effect with significant toxicity. Limited data suggest that fluoroquinolones (such as ciprofloxacin or levofloxacin) after renal transplant may decrease the risk of BK viraemia. While generally considered as antibacterial agents, fluoroquinolones seem to have some activity against large T-antigen helicase activity in polyomavirus, and may also inhibit cellular enzymes, inhibiting but not stopping BKV replication.

Hepatitis B

Hepatitis B is a common cause of cirrhosis and the need for liver transplant. Post-transplant management may include antiviral agents such as lamivudine, entecavir, adefovir, and others, as well as the use of hyperimmune hepatitis B globulin. Other organ transplant recipients may have latent hepatitis B, which can reactivate after induction of immunosuppression, especially in those who have hepatitis B surface antigen, and much less commonly in those who have a negative surface antigen but a positive core antibody. If they are non-immune, all patients undergoing dialysis who are candidates for organ transplantation should undergo vaccination in the pre-transplant period. Some patients may need a higher dose of vaccine, and accelerated vaccine series especially if they will be undergoing organ transplantation soon.

Epstein–Barr virus

EBV is mainly an issue for subjects who are seronegative before transplant, and for those who are very heavily immunosuppressed (lung, intestinal, composite tissue transplants). EBV replication increases the risk of PTLD, 90% of which is EBV mediated. Some centres screen periodically in the first year after transplant in EBV D+/R− recipients, and reduce the immunosuppression when there is significant viraemia. There are no data to suggest that antiviral agents can prevent or decrease EBV viraemia. Ganciclovir only works in the very small percentage of virus that is in the lytic phase. Belatacept, one of the newer immunosuppressive medications, is approved only for use in EBV-seropositive recipients, because of the higher risk of PTLD in seronegative recipients.

Miscellaneous

Mosquito-borne infections such as West Nile virus, dengue fever, eastern equine encephalitis, chikungunya, and others can cause significant disease in transplant recipients. Avoidance of insect bites by wearing protective clothing, using insect repellent, and screens or sleeping nets will decrease the risk of transmission.

Vaccination against influenza, hepatitis A and B, human papillomavirus, varicella zoster, and other viral pathogens can provide protection. This is best given prior to transplant, as the immunologic response is likely to be augmented. Certain viral vaccines have live attenuated virus and cannot be used after transplant, such as varicella zoster, measles, mumps, rubella, and yellow fever. In general, transplant centres are much more inclined to administer vaccines to transplant patients than previously, and influenza vaccine is recommended by numerous experts (Kumar et al., 2011). Surveys in 1999 and 2009 of United Network for Organ Sharing-certified kidney and kidney-pancreas transplant centres in the United States regarding their influenza vaccination practices established that the 2009 respondents, compared with 1999, were more likely to recommend vaccination for kidney (94.5% vs 84.4%; $P = 0.02$) and kidney-pancreas recipients (76.8% vs 48.5%; $P < 0.001$), and family members of transplant recipients (52.5% vs 21.0%; $P < 0.001$) (Chon et al., 2010). While there has been some concern that vaccines could disrupt tolerance or increase the risk of rejection, this has not so far been borne out in trials. When possible, it is recommended that transplant recipients avoid the adjuvants in some vaccines, and be given vaccines without adjuvants, which are immunostimulatory molecules (Kumar et al., 2011).

Diagnosis

Viral infection diagnosis has been improved hugely by the availability of molecular techniques. Viral culture is being replaced by more rapid and specific molecular assays. Within a matter of hours, various amplification methods can precisely identify active replicating viral infections. In the era of quantitative assays, trends in viral load can be followed over time, as is seen with serial assays for response to CMV treatment, or for BK viraemia/viruria, or EBV viraemia. Molecular diagnostics have provided powerful assays for infections that were previously difficult (or even impossible) to diagnose in this population, for example, parvovirus B19, HHV-6, and -7. Knowledge of pre-transplant serostatus (i.e. antibody titre) for some viruses, such as CMV, EBV, and the hepatitis viruses, can be helpful in guiding diagnosis and management. In general, serology is much less helpful in the immunosuppressed population, as they are much less likely to seroconvert in response to the acute illness, and molecular diagnostics have a much higher yield. Immunohistochemistry on biopsy specimens is very helpful for various herpes infections, including HSV, VZV, EBV, CMV, and HHV-8. For other viruses including BK virus; the appropriate diagnosis of BK virus nephropathy can only be made by tissue biopsy, as viraemia alone is not diagnostic of nephropathy.

The recent development of an international standard by the World Health Organization for CMV viral load testing may revolutionize our ability to diagnose and manage CMV, enabling development of multicentre protocols. Assays for cellular immunity, such as interferon-gamma enzyme-linked immunospot assay (ELISpot), intracellular cytokine staining, major histocompatibility complex (MHC)-multimer-based assays, and QuantiFERON®-CMV, are emerging as technologies that may be able to predict an individual's risk of developing viral diseases.

Management

Effective treatment of viral infections involves a multipronged approach: use of antiviral agents, reduction of immunosuppression when possible, and augmentation of immunity through the use of immunoglobulins and sometimes adoptive infusions of CMV-specific T cells. Common antiviral drugs include the aciclovir family (including famciclovir and valaciclovir, primarily for HSV and VZV infections), ganciclovir (with the oral prodrug, valganciclovir, for CMV and other infections), foscarnet (predominantly for resistant CMV), cidofovir (for resistant CMV, BK virus, and others), and ribavirin (for RSV and other less common infections). There are numerous antiviral agents for treatment of Hepatitis B. Hepatitis C is primarily treated with ribavirin and interferon; although there are newly released protease inhibitors with anti-HCV activity. Data in transplant patients is lacking, and drug interactions are profound. Reducing the intensity of immunosuppression (even transiently) may allow for more rapid clearance of a viral infection. Although not well evidence-based, repleting recipients who have hypogammaglobulinaemia with intravenous immunoglobulin may help clear infection. Some centres use CMV immunoglobulin in seronegative recipients with active disease. The novel use of adoptive infusions of CMV or EBV-specific T cells has been shown to be effective especially in haematopoietic stem cell recipients and increasingly in organ transplant recipients(Savoldo et al., 2006; Brestrich et al., 2009).

Bacteria: prophylaxis, diagnosis, and management

Bacterial infections occur at increased frequency in the vulnerable transplant recipient. They range from ordinary infections such as urinary tract infections, pneumonias, and bacteraemias to more exotic infections with *Nocardia, Rhodococcus, Listeria*, and other pathogens. Their more frequent exposure to healthcare settings increases the risk of resistant pathogens, including MRSA and vancomycin-intermediate *Staphylococcus aureus* (VISA), VRE, *Pseudomonas, Stenotrophomonas*, and others. Latent infections such as *Mycobacterium tuberculosis* reactivate at much higher rates in those with renal and hepatic failure, as well as in the post-transplant period. For management of urinary tract infections see Section 7 in this book.

Prophylaxis

Prevention of bacterial infection requires review of the risk factors in the individual patient, that is, recurrent urinary tract infections, prior pneumonias or episodes of cellulitis, and poorly drained collections (ascites, pleural fluid). The use of trimethoprim-sulfamethoxazole after transplant to prevent *Pneumocystis* has the additional advantage of preventing other bacterial infections, including *Streptococcus, Listeria*, and *Nocardia*. Chemoprophylaxis is encouraged for those with latent tuberculosis, or who may have exposure via their donor (Morris et al., 2012), either before, during, or after transplant (cautiously in those with cirrhosis before transplant, or when using rifamycins after transplant due to drug interactions). Such chemoprophylaxis usually does not have to delay the transplant. Vaccination against *Streptococcus pneumoniae, Clostridium tetani* (tetanus), *Corynebacterium diphtheriae, Bordetella pertussis* (whooping cough), and other bacterial pathogens will provide some additional protection.

Diagnosis

Diagnosis of bacterial infections relies on cultures. To optimize the diagnostic yield of cultures, clinicians should notify the laboratory when unusual organisms are suspected, such as *Listeria, Rhodococcus*, mycobacteria, and *Nocardia*. Expanding the standard panel of antibiotic sensitivity at the time of initial diagnosis may help with subsequent therapy, especially given the increased risk of drug interactions and side effects, partly due to concomitant use of multiple medications (i.e. increased risks of leucopenia, nephrotoxicity, etc.) Molecular and/or rapid diagnostics are increasingly available for bacterial infections. Serologic techniques tend to yield diagnoses less frequently in this population due to more muted immunologic responses. Histopathology, especially with special stains for microorganisms, can sometimes be helpful in achieving a diagnosis; examples include the Fite stain for mycobacteria, the May–Grunwald Giemsa stain, and the Warthin–Starry or Steiner stain for spirochaetes.

Management

Treatment in febrile or ill transplant recipients is with empiric antibacterial therapy, which should be chosen based on local epidemiology. This approach is justified by the significant incidence of bacteraemia in the post-transplant period and by the concomitant high mortality rate when treatment is delayed. Transplant patients are at higher risk for resistant pathogens, and the empiric antibiotic choice should reflect this. Once a culture diagnosis has been made and antibiotic sensitivities are available, the antibiotic regimen may be modified. Optimal duration of therapy has usually not been well studied in this population, but is often longer than in normal hosts. Certain antibiotic classes should be avoided when possible due to toxicities and side effects. Examples include aminoglycosides (which can increase the risk of renal toxicity) and rifamycins (rifampin/rifampicin or rifabutin, which have profound interactions with tacrolimus and ciclosporin).

Because of the increased rates of resistance resulting in decreased susceptibility to oral antibiotics, intravenous therapy is often needed in this population. This requires prolonged intravenous access, sometimes through peripherally inserted central catheters (PICC or PIC lines). In general, arm veins should be avoided to preserve them for future haemodialysis access in those at higher risk for chronic kidney disease. Small-bore tunnelled central venous catheters have become the access of choice. Drainage of collections by radiographically or surgically placed drains helps clear infection and prevent recurrence. Appropriately drained infections do not necessarily need long-term antibiotics while the drain stays in place. Preventative measures include eliminating any nidus of infection (such as intravascular catheters, indwelling urinary catheters, stents, skin defects that encourage abscess formation,) and dealing with anatomical problems (e.g. in the urinary tract). Treatment of complex bacterial infections, such as those from mycobacteria

(including *Mycobacterium tuberculosis*), *Nocardia, Rhodococcus*, and others, should be done with guidance from an experienced transplant infectious disease expert, as there may be a need for prolonged therapy and/or therapeutic drug monitoring, management of drug interactions, and secondary prophylaxis.

Fungi: prophylaxis, diagnosis, and management

Infections with *Candida* spp. are manageable, but those such as invasive aspergillosis and zygomycosis (due to *Rhizopus, Absidia, Rhizomucor, Mucor*, and *Cunninghamella*) have very high mortality rates and are a dreaded infectious complication (Fig. 284.4). Although they tend to occur in the early post-transplant period, *Candida* infections in particular may also occur years later. For example, *Cryptococcus neoformans* is the most common cause of meningitis in organ transplant recipients. *Pneumocystis jirovecii* (formerly *P. carinii)* is also a fungus (having previously been classified as a protozoan). It causes a severe pneumonitis at any time after transplantation (Fig. 284.5).

Prophylaxis

Preventing *Candida* and other yeast infections requires precise use of antibiotics and immunosuppression. Spontaneous candidal infections (without risk factors) are quite rare on their own. They often follow broad antibiotic exposure, decreasing normal flora, and increasing *Candida* colonization of the gut, urinary system, and upper respiratory tract, which increases the risk of translocation from a non-sterile site to a sterile site, such as the bloodstream, pleural or peritoneal spaces. Urinary catheters greatly increase the risk of urinary *Candida* colonization and subsequent invasive infection. *Cryptococcus neoformans* spores live in bird droppings (especially pigeon droppings) and in soil contaminated with bird droppings; humans can get cryptococcal infection by inhalation of airborne fungi from such sources, and it is recommended that transplant patients avoid bird contact. There are multiple cases of transmission from donors (Sun et al., 2010).

Preventing mould infections involves a combination of avoidance measures, including filtered air systems in hospitals, recognition of existing infection or colonization, and targeted antifungal prophylaxis. Mould spores are ubiquitous in the environment and it is rarely possible to distinguish community-acquired from nosocomial aspergillosis. Transplant recipients should wear gloves while gardening, or touching plants or soil, and they should avoid inhaling or creating soil or dust aerosols that may contain mould spores. They may wish to wear N95 masks if exposure is unavoidable. They should always wash their hands after such contact, and care for skin abrasions or cuts sustained during soil or plant contact. They should not have birds as pets. Airway colonization with mould in organ recipients may blossom into a full infection after transplant, thus knowledge of culture data at or before the time of transplant may help target therapy. Policies of antifungal prophylaxis vary among transplant centres. Most renal centres would not give prophylaxis against *Aspergillus* and other moulds. *Pneumocystis jirovecii* infection is easily prevented using trimethoprim-sulfamethoxazole, which the majority of transplant patients take, at least in the first year after transplant. Alternative agents (if patients are intolerant of sulphas) include dapsone, atovaquone, and pentamidine. Trimethoprim-sulfamethoxazole has the broadest spectrum of prevention of infection, and is the agent of choice.

Infections with *Coccidioides* or *Histoplasma* are also more common in transplant patients. Interestingly, coccidiomycosis occurs more commonly than histoplasmosis in transplant patients, a result of reactivation of latent infection. Travelling to endemic areas increases the risk of acquisition of *de novo* infection, so patients should be counselled about this. Rare cases of donor-derived infection from demographic fungi have also been described. In some cases, these infections were not considered in the non-endemic regions where the transplant occurred. Diagnosis can be delayed, increasing the risk of death.

Diagnosis

Diagnosis of fungal infection requires use of dedicated fungal stain, culture, and detection of fungal antigens in blood, urine, and other fluids. Fungi may be more difficult to grow in culture and harder to diagnose than other pathogens. A high level of suspicion, as well as multiple diagnostic approaches, is imperative in the diagnosis of these more elusive pathogens. Some pathogens such as *Candida*

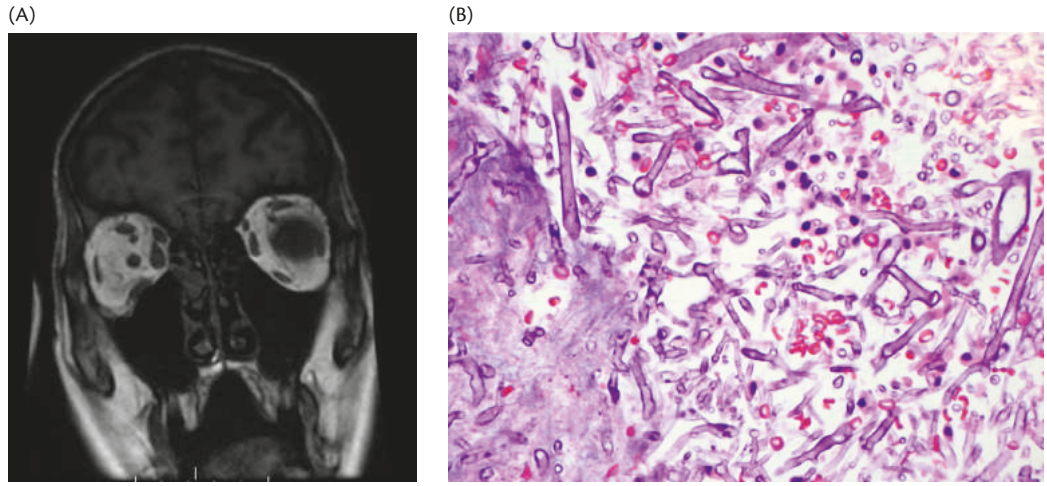

Fig. 284.4 (A) MRI of head showing right eye muscle oedema and right sinus involvement from invasive mucormycosis. (B) Angioinvasive mucormycosis seen on sinus biopsy by haematoxylin and eosin stain.
Courtesy of Camille Nelson Kotton, Massachusetts General Hospital, Boston, MA, USA.

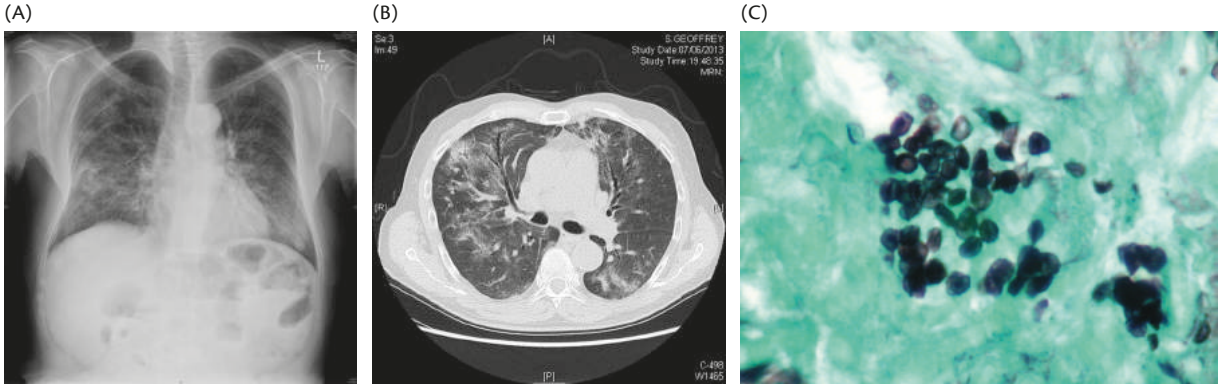

Fig. 284.5 Examples of infiltrates seen with *Pneumocystis jirovecii*. (A) Chest roentgenogram of *Pneumocystis jirovecii* pneumonia showing diffuse, bilateral disease. (B) Chest computed tomography of *Pneumocystis jirovecii* pneumonia showing multifocal disease with ground-glass opacities. (C) Grocott–Gomori methenamine silver stain (GMS) demonstrating *Pneumocystis jirovecii*.

Radiographic images courtesy of Dr C. G. Winearls, Oxford University Hospitals, Oxford; pathology courtesy of Professor Ian S. D. Roberts, Oxford University Hospitals, Oxford, UK.

will grow on routine culture, while others require dedicated fungal culture media. *Candida* will grow from regular blood cultures, while filamentous fungi (such as *Aspergillus*, very rarely found by blood culture, i.e. in < 1% of cases of aspergillosis) need fungal isolators.

Fungal antigens, including the 1,3 β-D-glucan, galactomannan, and cryptococcal assays, have been increasing the diagnostic capacity in recent times. The 1,3 β-D-glucan assay, tested in blood, can be positive with a variety of fungal pathogens, ranging from *Candida* to *Aspergillus* and numerous others including *Fusarium* spp., *Trichosporon* spp., *Saccharomyces cerevisiae, Acremonium, Coccidioides immitis, Histoplasma capsulatum, Sporothrix schenckii, Blastomyces dermatitidis*, and *Pneumocystis jirovecii*. The galactomannan antigen has been used on a variety of specimens and is relatively specific for *Aspergillus*; both blood and body fluids can be tested. Cryptococcal antigen testing of blood or spinal fluid is both very sensitive and specific for cryptococcosis. For pulmonary lesions that may be fungal in nature, bronchoscopy with bronchial alveolar lavage and transbronchial biopsy, or radiographically guided transthoracic biopsy, or open lung biopsy is often imperative in making the diagnosis. Delays in diagnostic procedures in patients on empiric antifungal regimens greatly decrease the diagnostic outcomes of such procedures, and should be avoided. Galactomannan antigen testing on bronchial alveolar lavage fluid can be diagnostically helpful for *Aspergillus* when it is positive. Special stains and polymerase chain reaction (PCR) testing for *Pneumocystis jirovecii* should be included. *Pneumocystis jirovecii* may also have an elevated lactate dehydrogenase (LDH) and a positive 1,3 β-D-glucan assay.

Serology and urinary antigen testing may sometimes be helpful (i.e. *Coccidioides*). When biopsy material or tissue is available, histopathology can also provide diagnostic input, especially with special stains for fungi, including Gomori's metanamine silver, Periodic acid–Schiff staining, and mucicarmine for *Cryptococcus*, and immunohistochemistry for *Pneumocystis jirovecii*. Some centres use PCR testing for *Pneumocystis jirovecii*.

Management

Treatment of fungal infections involves use of one or more antifungal agents, as well as surgical debulking (especially with mucormycosis and sometimes aspergillosis). *Candida* infection may be treated with an azole (primarily fluconazole) or with an echinocandin (i.e. micafungin, caspofungin, or anidulafungin). Depending on the individual pathogen, the filamentous mould infections are treated with an amphotericin B product, often a lipid based one for better tolerability (such as Ambisome® or Abelcet®), or with a higher level azole such as voriconazole or posaconazole. The echinocandins are sometimes used in salvage regimens, or as part of a multidrug regimen, albeit with very little available data for multidrug regimens in this setting. Antifungal susceptibilities are increasingly being used to guide treatment, as is therapeutic drug monitoring, especially for the higher-level azoles, voriconazole and posaconazole. There are important drugs interactions between the immunosuppressive agents (especially tacrolimus, ciclosporin, and sirolimus) and the azoles (especially the higher-level ones), necessitating reductions in doses of the immunosuppressive agents. Most of the endemic fungal and cryptococcal infections respond to treatment with an amphotericin product or fluconazole. *Pneumocystis jirovecii* is treated (and prevented, using lower doses) with agents such as trimethoprim-sulfamethoxazole, clindamycin, primaquine, and atovaquone.

Parasites: prophylaxis, diagnosis, and management

Parasitic infections are much less common than the previously mentioned pathogens. The clinically significant parasites in transplant recipients include *Toxoplasma gondii, Strongyloides stercoralis, Trypanosoma cruzi* (the aetiologic agent of Chagas disease), *Leishmania*, and intestinal parasites (*Cryptosporidium, Giardia*, and others). The incidence of parasitic infection is expected to increase in transplant recipients for a number of reasons, including increases in active organ transplant programmes in places where parasitic infections are endemic; increases in travel and migration of donors and recipients from endemic areas (with latent or asymptomatic infections), as well as patients from developed countries undergoing transplantation in endemic areas (transplant tourism); increases in leisure tourism to endemic regions by transplant recipients; and decreases in ciclosporin-based immunosuppressive regimens as they are replaced by newer drugs that lack the antiparasitic

effects of ciclosporin metabolites. Specific guidelines regarding parasitic infections in transplant recipients have been published (Kotton and Lattes, 2009).

Prophylaxis

Parasitic infections can be prevented by avoiding ingestion of contaminated food and water (predominantly for intestinal pathogens and *Toxoplasma gondii*), by avoiding skin contact with soil harbouring pathogens (*Strongyloides*), and by avoiding insect bites (*Plasmodium* (malaria), *Babesia, Trypanosoma cruzi*, and *Leishmania*). In addition, recipients with epidemiologic risk factors should be screened for latent infection prior to transplant, as should organ and blood product donors in endemic regions (i.e. *T. cruzi*/Chagas disease, malaria, babesiosis, and *Leishmania*). Preventative medications such as trimethoprim-sulfamethoxazole (used to prevent *T. gondii* infection, both *de novo* and reactivation disease) or ivermectin (to treat active or latent *Strongyloides*) are effective methods of prevention. Toxoplasmosis, once a more common infection after solid organ transplant, has become a largely preventable disease in the era of trimethoprim-sulfamethoxazole (or atovaquone, or dapsone) prophylaxis. Use of antimalarial prophylaxis in endemic regions is recommended for all transplant recipients travelling to such regions.

Diagnosis

Diagnosis of parasitic infections in solid organ transplant recipients is complex. Depending on the parasite suspected, a variety of techniques are used, ranging from rapid diagnostics on stool by microscopic examination for ova and parasites, peripheral blood smears (*Babesia*, malaria, *T. cruzi*), special stains and microscopic examination of various specimens or tissues (blood, stool, biopsy), culture, serology (which may be less helpful in this population, as they are less likely to seroconvert), and histopathology. Molecular diagnostics can be quite helpful. Examples include rapid malaria diagnostics and PCR testing for *T. cruzi* and *Toxoplasmosis*. Clinical markers such as eosinophilia may be suppressed in this population, where the immunosuppressive regimen (especially steroids, for eosinophilia) may cause false-negative results.

Certain diseases may require monitoring after transplant, or after treatment of infection. For example, pre-transplant treatment of Chagas disease has not been shown to decrease the risk of reactivation disease after transplant. Because the minority of infected patients will experience reactivation with immunosuppression, and the medications are toxic, many experts recommend monitoring in the post-transplant period, and treating if there is evidence of parasitaemia or clinical disease. Similarly, treatment of donor or recipients with positive *Leishmania* serology is not necessarily indicated in the absence of clinical disease. Some parasites such as *Schistosoma* spp. die after several years, so the recipients may have a positive serology for much longer. It is not known whether they need treatment.

Treatment

Treatment of parasitic infections involves medications with significant potential side effects, toxicity, and the propensity to interact with transplant medications. Immunocompromised hosts are more likely to have relapses of certain parasitic infections (i.e. *Babesia, T. cruzi*, and *Strongyloides*) and should be monitored after treatment. Clinicians may wish to lengthen the treatment course in certain infections, especially with more readily tolerated antiparasitic medications and for diseases at higher risk for relapse, that is, with treatment for *Babesia, Strongyloides*, and others. Whether or not reduction of immunosuppression is helpful in clearing such infections is unknown.

The pre-transplant infectious disease evaluation

Pre-transplant evaluation by an infectious disease specialist familiar with organ transplantation provides an opportunity to minimize the risk of infection. Epidemiology and medical history should be evaluated for risk of latent infections (tuberculosis, histoplasmosis, coccidiomycosis, cryptococcosis, Chagas disease, hepatitis B, and others); if testing is positive or history strongly suggestive, centres may wish to initiate prophylaxis or screening for reactivation. Potential transplant recipients and donors are typically screened for latent tuberculosis, by history and sometimes by chest X-ray and either by skin testing or use of an interferon gamma release assay based blood test such as the T.SPOT®.TB or QuantiFERON® TB Gold. Those with latent tuberculosis should be given chemoprophylaxis. Although the optimal timing around transplant has not been determined, it usually does not have to delay the transplant, as it could be given after transplant. Patients with *Staphylococcus aureus* colonization should undergo a decolonization protocol shortly before surgery, which can decrease their risk of surgical site infection; such protocols may include the use of intranasal mupirocin, chlorhexidine washes, oral doxycycline, and rifampin/rifampicin.

Vaccination status should be reviewed and updated, both for routine vaccines and for more exotic vaccines if the recipient is expected to have high-risk exposures (i.e. vaccinating a veterinarian against rabies, a Brazilian native who plan to return home, against yellow fever). Those seronegative for measles, mumps, rubella, hepatitis A and B, and varicella should receive pre-transplant vaccinations, as some are with live viral vaccines (varicella/zoster, measles, mumps, rubella, yellow fever, BCG) that cannot be given after transplant (Jong and Freedman, 2012). When live viral vaccines are given, a minimum of 1 month should elapse before the recipient undergoes organ transplant. This is to allow the live virus to be cleared from the system.

An optimal prophylaxis regimen for each recipient after transplant should be developed. Recipients with possible trimethoprim-sulfamethoxazole allergies (or other significant antibiotic allergies, especially when multiple) could be seen by an allergist to determine whether such agents could be used after transplant. Antituberculosis prophylaxis may be needed in those who did not get pre-transplant treatment, or who are at higher risk of reactivation. While histoplasmosis does not usually require chemoprophylaxis, many clinicians in endemic regions do give it to those recipients with evidence of coccidiomycosis.

Key points

- Infections are among the most common complications after transplantation, and greatly increase the morbidity and mortality of transplantation.
- Improved understanding of various infections, diagnostics, therapeutics, and prevention has improved outcomes of infection in transplant recipients.

- Prophylactic measures and medications can significantly decrease the risk of infection after transplantation.
- Pre-transplant evaluation for latent infections and optimization of vaccination can minimize the risk of infection after transplant.

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CHAPTER 285

Cardiovascular disease: prophylaxis, diagnosis, and management

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Introduction

The risk of cardiovascular disease (CVD) in renal transplant recipients (RTRs) is approximately one-fifth of that of patients receiving maintenance haemodialysis (Baigent et al., 2000) and therefore, transplantation should be the main means to reduce CVD in patients with end-stage renal disease (ESRD). Despite this large reduction in risk, RTRs remain at significant cardiovascular (CV) risk, having a three- to fivefold increased risk of premature CVD compared to the general population (Fig. 285.1). CVD is the leading cause of death and graft loss in RTRs.

It has become clear that the pathogenesis of CVD in transplant recipients differs from that in the general population and as such, both manifestations and management are different. By the time a patient comes to transplantation, many of the multiple risk factors accumulated during the period of their progressive renal disease will have become irreversible. Furthermore, transplantation itself carries with it specific risks which have an adverse impact upon CV risk. These include immunosuppressive therapies, progressive transplant dysfunction, and episodes of acute rejection.

Clinically, the manifestations are of conventional atheromatous disease such as acute myocardial infarction (MI), but these patients are also at increased risk of arrhythmias and sudden cardiac death.

Cardiovascular disease in renal transplant recipients

Epidemiology and nature

CVD is the leading cause of death in RTRs, which in turn is the leading cause of kidney graft loss. This problem will increase with the trend towards transplanting older recipients, higher risk recipients, and the use of extended criteria donors. This has to be balanced by the fact that transplantation still improves survival over dialysis.

CVD and atherosclerotic coronary artery disease (CAD) are terms which are used interchangeably. However, the assumption that CVD in RTRs is purely due to CAD is flawed. This can be seen in studies of determinants of CVD in RTRs. Kasiske and colleagues reported longitudinal follow-up of over 1000 RTRs in a single US centre (Kasiske et al., 1996, 2000) and have provided important epidemiological data relating to CVD. Firstly, they demonstrated the high prevalence of CV event and CV mortality in RTR. Secondly, they confirmed the role of conventional atherosclerotic CV risk factors (age, gender, smoking, and diabetes mellitus) in determining CV risk. As such, for each year of life the risk of a CV event is increased by 3–5%, with male gender or diabetes (either pre-existing or post-transplant diabetes) associated with an approximate doubling of overall risk. Furthermore, these studies confirmed that much of the risk for post-transplant CV events predates transplantation, with major risk factors for post-transplant CV events being pre-existing CAD, peripheral vascular disease, or cerebral vascular disease.

A recent prospective multinational study—the Patient Outcomes in Renal Transplantation (PORT) study (Israni et al., 2010)—followed 23,575 adult RTRs for a median of 4.5 years. Using a composite CV outcome (proven MI, coronary intervention, and cardiac death), the overall cumulative incidence of CVD was 3.1%, 5.2%, and 7.6% at 1, 3, and 5 years after transplantation. The risk of individual events differed depending on time from transplant. In the first year, the distribution of events was non-fatal MI (49%), coronary intervention (38%), and cardiac death (13%). Beyond 1 year the corresponding values were 39%, 38%, and 23%. Correspondingly, risk factors varied with time after transplantation. Early events were predicted by age, male sex, history of cancer or diabetes, obesity, pre-existing CVD, deceased donor transplant, and time on dialysis prior to transplantation. Conventional risk factors such as smoking, hypercholesterolaemia, and hypertension were not significant although they did correlate with a past history of CVD. Later events were dependent on poor graft function (low estimated glomerular filtration rate (eGFR); factors that adversely influence graft function such as acute rejection, delayed graft function, and post-transplant lymphoproliferative disease (PTLD); the development of diabetes; and race.

Follow-up of clinical trial participants has provided valuable information because external validation of study endpoints makes the data more robust than registry data. The Assessment of LEsecol in Renal Transplantation (ALERT) (Holdaas et al., 2003) and Folic

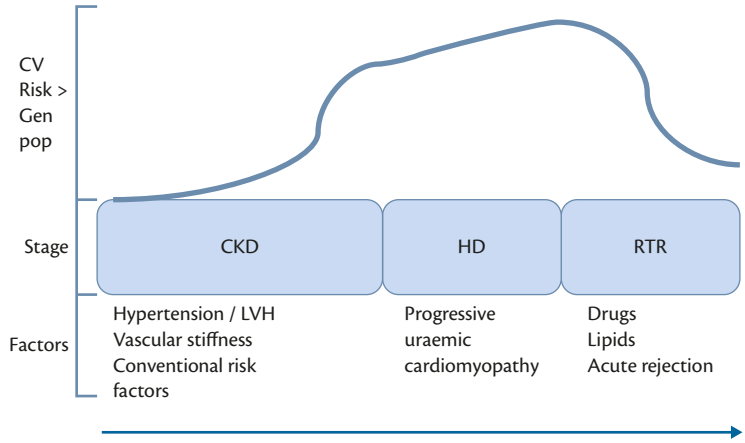

Fig. 285.1 Figure illustrating the cardiovascular burden that patients carry with them to transplantation. Although transplantation significantly reduces CV risk in these patients, it does not reduce to background population risk. CV risk > gen pop = cardiovascular risk increase as compared with background age adjusted risk. Stage = stage of renal career: CKD = chronic kidney disease; HD = haemodialysis; RTR = renal transplant recipient. Factors = stage-specific risks: LVH = left ventricular hypertrophy; drugs = immunosuppressive agents.

Acid for Vascular Outcome Reduction (FAVORIT) (Bostom et al., 2011) studies are the two good examples. ALERT was a study of stable RTRs randomized to placebo or fluvastatin and followed for up to 6 years (with an additional 2-year extension) (Holdaas et al., 2005a). Comparing the endpoints reached by patients in this study with three comparable studies of lipid reduction therapies in different high-risk study populations (Table 285.1), it can be seen that the patterns of outcomes differ. Patients with ESRD are at higher risk of sudden cardiac death than non-fatal acute MI, compared with the general population. As expected, RTRs are at a risk that is intermediate between patients receiving dialysis and the general population, with the increase in CV events reflecting an equal risk of cardiac death and non-fatal coronary events. Around 10% of RTRs suffered a cardiac event during 5 years of follow-up, emphasizing the significance of this cause of morbidity and mortality.

The ALERT study also confirmed that the determinants of traditional atheromatous outcomes (specifically acute myocardial infarction (aMI)) differ from the determinants of sudden cardiac death, supporting the concept that these two presentations have a different pathogenesis. In a multivariate analysis, the leading determinants of aMI, in addition to the irremediable (age, gender, and pre-existing diabetes), were lipid levels. All commonly measured lipid subfractions were associated with aMI, as they are in the general population (Jardine et al., 2004, 2005). In contrast, no lipid subfraction was significantly associated with cardiac death, the main determinants of which were renal dysfunction and left ventricular hypertrophy (LVH), particularly when associated with subendocardial ischaemia (LVH with 'strain'). These observations strongly support the notion of the existence of a 'uraemic cardiomyopathy' (Jardine et al., 2006).

In FAVORIT, 4110 stable RTRs were studied. They had been randomized to high-dose folic acid (Bostom et al., 2011). The primary endpoint was a composite measure of MI, CV death, revascularization procedures, and stroke. The intervention showed no benefit, perhaps unsurprisingly given the pooled endpoint. The main determinants of outcome were age, pre-existing CVD, diabetes, systolic blood pressure, and low eGFR. Low-density lipoprotein (LDL) cholesterol had no relationship with outcome.

Other studies, including those of Abbott et al. (2002) and Rigatto et al. (2002), support these findings and confirm that novel risk factors including graft dysfunction (specifically graft failure) are associated with an approximately threefold increase in CV events including heart failure.

Thus, RTRs do suffer from CAD (fatal and non-fatal MI), the determinants of which are the same as the general population but cardiac death is a more important and less well-understood problem, the determinants of which are LVH, vascular stiffness, and hypertension.

Table 285.1 Patient event rates for pre-determined endpoints in the placebo arm of four trials of statin therapy in different study populations. 4S (Scandinavian Simvastatin Survival Study, 1994) (patients at conventional risk of IHD), ALERT (Holdaas et al., 2005a) (RTRs), 4D (Wanner et al., 2005), and AURORA (Fellstrom et al., 2009) (maintenance haemodialysis). The trials were of comparable size and had around 5 years of follow-up

Endpoint	4S	ALERT	4D	AURORA
Sudden cardiac death	8.5%	5.1%	23%	23.4%
Acute myocardial infarction (non-fatal)	22.6%	6.3%	12%	7.7%
Non-cardiac death	2.2%	6.2%	25%	19.4%

Specific risk factors and management

Hypertension, LVH, vascular stiffness, and uraemic cardiomyopathy

Hypertension in RTRs is a consequence of both the pre-existing hypertension, particularly due to vascular stiffness and calcification, and the effects of immunosuppressive agents (corticosteroids and calcineurin inhibitors (CNIS)), which cause hypertension even in patients without primary renal disease.

The mechanisms by which corticosteroids cause hypertension are incompletely understood but the two principal components are firstly, retention of sodium and water due to actions of corticosteroids on the

kidney, involving to some extent the mineralocorticoid receptor; and secondly, via enhanced sympathetic activity causing increased vascular tone (Walker, 2007). CNIs cause hypertension through direct renal sodium retention and increased vasoconstrictor tone, as well as indirectly via renal impairment (Zhang et al., 2003). Overall, the majority of patients require antihypertensive agents (Tutone et al., 2005) with most requiring more than one.

Epidemiological studies and placebo arms of interventional trials confirm that hypertension is associated with CV events in RTRs (Kasiske et al., 2000). Blood pressure was the strongest determinant of cardiac death in the ALERT study (Jardine et al., 2005). The most significant blood pressure parameters in these studies were systolic blood pressure and pulse pressure, both markers of vascular stiffness (secondary to calcification or vascular hypertrophy). Vascular stiffness is also independently linked to adverse CV outcomes in RTRs and provides a potential short-term surrogate endpoint for interventional trials (Hornum et al., 2011; Ignace et al., 2011).

LVH is present in up to 50% of patients starting dialysis and is associated with poorer outcomes (Foley et al., 1995; Mark et al., 2006). Hypertension is the main determinant of LVH, which in the context of uraemia then leads to subendocardial ischaemia and myocardial fibrosis (uraemic cardiomyopathy). Fibrosis leads to aberrant conduction and is associated with a prolonged QT interval and abnormal T-wave alternans (Stewart et al., 2005; Patel et al., 2008a), which predisposes to fatal arrhythmias and sudden cardiac death. The arrhythmias may be spontaneous or complicate otherwise minor ischaemic episodes. The less common manifestation of dilated cardiomyopathy (with systolic dysfunction) may be a consequence of LVH or CAD which may be asymptomatic.

Measurement of LVH is commonly performed using echocardiography, however it should be emphasized that the echocardiogram findings depend on the patient's intravascular volume status and can result in aberrant measurements in patients with ESRD. Electrocardiography is useful for assessing rhythm and the presence of ischaemia, but is unreliable at detecting LVH. Cardiac magnetic resonance imaging is the gold standard method of measuring LVH. Using this method, transplantation was not shown to be associated with regression in LVH (Patel et al., 2008b), emphasizing the importance of addressing CV risk early in the course of patients' chronic kidney disease and preventing the development of end-organ damage.

In registry data, Opelz and colleagues (1998) examined the impact of blood pressure measurements recorded at outpatient clinics in patients with a functioning transplant, 1 year after transplantation. These data show that blood pressure, albeit not independent from graft function, is a major determinant of long-term patient and graft survival. Furthermore, the data suggested that aggressive blood pressure control may be of benefit, as patients with a systolic blood pressure of 130 mmHg had a substantially worse graft outcome than patients with a systolic blood pressure of 120 mmHg.

At present, no trials of therapy or treatment targets exist for hypertension in RTR. The one large-scale trial of angiotensin receptor blockade, was stopped early due to the low event rate (Philipp et al., 2010). Short-term studies have confirmed the effectiveness of individual antihypertensive agents, with angiotensin receptor blockers, angiotensin-converting enzyme inhibitors (ACEIs), and calcium channel blockers all shown to be of comparable benefit to that seen in other populations (Mangray and Vella, 2011; Ponticelli et al., 2011).

Dihydropyridine calcium channel antagonists—such as amlodipine—may attenuate the nephrotoxic effects of CNIs (Walker, 2007; Mangray and Vella, 2011) and have been favoured in the early phases following transplantation. Concerns exist over usage of blockers of the renin–angiotensin system because of the possibility of 'functional' transplant artery stenosis (Gaston et al., 2009). Exclusion of transplant renal artery stenosis is often performed before starting ACEIs, but intervention in renal artery stenosis is of no proven benefit in this population, nor in any other population when atheromatous disease is the underlying cause (Wheatley et al., 2009). These drugs may have specific benefits in patients with proteinuria (Philipp et al., 2010) and LVH. A carefully conducted retrospective analysis by Oberbauer and colleagues showed that patients treated with ACEIs or angiotensin receptor blockers had better graft function and patient survival (Heinze et al., 2006) (Fig. 285.2). Clinicians should exercise caution in patients with hyperkalaemia who are prescribed ACEIs and CNIs.

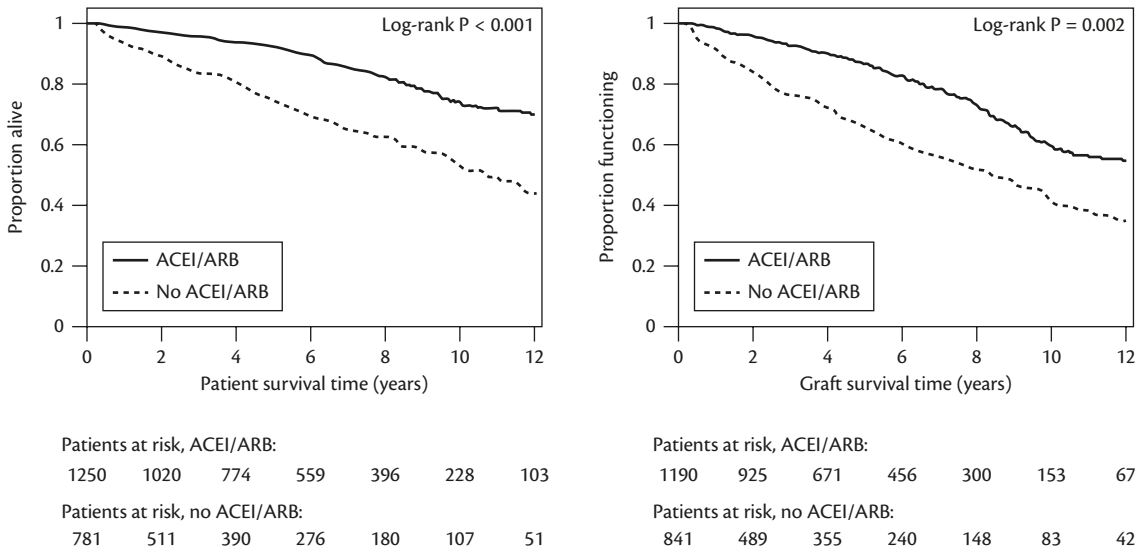

Fig. 285.2 A retrospective analysis of patient and graft survival in RTR patients taking and not taking angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), adjusted for covariates (Heinze et al., 2006). Patient and graft survival was better in patients prescribed ACEI/ARB.

Table 285.2 Effects of immunosuppressive agents on cardiovascular risk factors

CV risk factor	Steroids	Ciclosporin	Tacrolimus	Azathioprine/ mycophenolate mofetil	Target of rapamycin inhibitors
Cholesterol	↑	↑	↑	→	↑
LDL	↑	↑	↑	→	↑
Triglycerides	↑	↑	↑	→	↑
NODAT	↑	↑	↑	→	↑
Hypertension	↑	↑	↑	→	→
LVH	↑	↑	↑	→	→
Renal function	→	↓	↓	→	→

↑ = increases risk; ↓ = reduces risk; → = no effect. LDL = low-density lipoprotein; LVH = left ventricular hypertrophy; NODAT = new-onset diabetes after transplantation.

Consideration should be given to the modification of immunosuppressive therapy in RTRs with hypertension (Table 285.2). Strategies include minimization or withdrawal of steroids (Walker, 2007), minimization of CNIs, switching from ciclosporin to tacrolimus (Srinivas et al., 2008), or stopping CNIs and switching to sirolimus (Johnson et al., 2001). All have shown a substantial reduction in blood pressure, similar to that achieved by antihypertensive therapy. One intriguing observation is that the use of sirolimus, in place of CNI, is associated with regression of LVH (Paoletti and Cannella et al., 2010). Similarly, encouraging early data from the BENEFIT trial (belatacept versus high dose ciclosporin), looking at blood pressure, lipids and NODAT showed patients on belatacept had an improved metabolic risk profile at 12 months (Belatacept-based regimens are associated with improved CV and metabolic risk factors compared with ciclosporin in kidney transplant recipients (BENEFIT and BENEFIT-EXT studies). However, clinicians and patients may be reluctant to modify immunosuppression to achieve blood pressure control, because of the perceived immunological risk and possibility of jeopardizing graft function.

More radical approaches to the treatment of hypertension, such as embolization or laparoscopic removal of the native kidneys, have been tried in extreme cases and may be effective. However, they appear to do little to improve blood pressure in patients with long-standing hypertension. Nephrectomy before transplantation may be associated with improved long-term blood pressure control.

The best choice of agents and target blood pressure remain to be defined. Citing targets and published guidelines in other populations, the recently published KDIGO guidelines suggest a target of 130/80 mmHg, and the use of blockers of the renin–angiotensin system when patients have significant proteinuria (> 1 g/day in adults) or diabetes (Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group, 2009). In practice, these targets may be difficult to achieve and the majority of patients require multiple agents.

Dyslipidaemia

Dyslipidaemia is almost an invariable accompaniment of renal transplantation. It is a consequence of impaired renal function and effects of immunosuppressive agents. The pattern is typically elevated total and LDL cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol. There are also increased concentrations of intermediate—highly atherogenic—lipoproteins, including small, dense LDL (Holdaas et al., 2008).

Individual immunosuppressive agents have variable, but often synergistic, effects on serum lipids (Table 285.2). Corticosteroids increase total and LDL cholesterol, triglycerides, and HDL cholesterol; CNIs increase total and LDL cholesterol; and the mTOR inhibitors increase total, LDL cholesterol, HDL cholesterol, and triglycerides in a dose-dependent manner (Holdaas et al., 2008). Immediately post transplantation, immunosuppression, normalization of renal function, and increased appetite are associated with an average 1.5 mmol/L increase in total cholesterol, 1 mmol/L increase in LDL cholesterol, and increased triglyceride and HDL cholesterol (Holdaas et al., 2001).

Statin therapy is one of the few interventions to be tested in a large interventional study in transplant recipients. The ALERT trial studied 2100 stable, ciclosporin-treated RTRs followed for up to 6 years, and randomized initially to fluvastatin 40–80 mg daily or placebo (Holdaas et al., 2003). The primary endpoint was a composite of MI, cardiac death, stroke, and coronary intervention. Statin therapy was associated with a 35% reduction in MI. A 2-year extension, where all patients were offered fluvastatin 80 mg/day, increased follow-up to 8 years (Holdaas et al., 2005a) and showed a significant reduction in a variety of composite CV endpoints (Fig. 285.3). Fluvastatin reduced LDL cholesterol by 1 mmol/L and was well tolerated. Post hoc analysis of this study revealed that early introduction following transplantation was associated with additional benefit (Holdaas et al., 2005b).

It should be noted that in patients receiving CNIs, the concentration of statins metabolized by cytochrome P450 (CYP)-3A4 (specifically simvastatin, lovastatin, and, to a lesser extent atorvastatin) is increased resulting in increased efficacy and side effects (Manitpisitkul et al., 2009). Fluvastatin and pravastatin (which are not metabolized by CYP3A4) excepted, statins should be started at very low dose and monitored cautiously in CNI-treated RTRs.

Guidelines now recommend the use of statins minimizing CV risk in RTRs (Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group, 2009). The guidelines have tended to adopt lipid targets from the general population (LDL cholesterol for adult patients of 2.6 mmol/L), although the recent KDIGO guidelines did not specify a target, as there are inadequate data on targets specific for the transplant population. Despite this,

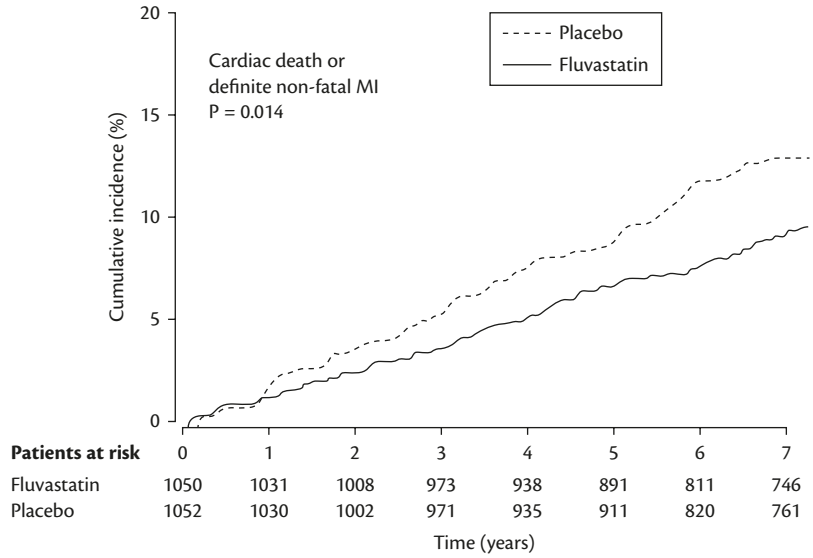

Fig. 285.3 Kaplan–Meier curves of time to cardiac death and non-fatal acute myocardial infarction in the ALERT study (with extended follow-up). Statin therapy was associated with a reduction in cardiac death and non-fatal MI during 8 years of follow-up (P = 0.014, log rank test) (Holdaas et al., 2005a).

a recent report suggests that despite mounting evidence, the use of statins (and CV risk management in general) is poor in transplant recipients (Gaston et al., 2009).

One reason for the slow adoption of statin therapy and CV risk management in RTRs is the expectation that post-transplant reduction of immunosuppression will correct dyslipidaemia. Wissing et al. directly compared modification of immunosuppression with the initiation of lipid-lowering therapy (Wissing et al., 2006). In this study, patients were switched from ciclosporin-based therapy to tacrolimus-based therapy, and this was compared to the addition of atorvastatin. Although tacrolimus-based therapy was associated with a reduction in total, LDL cholesterol, and triglycerides, patients on ciclosporin and atorvastatin had lipid levels comparable to those on tacrolimus and atorvastatin combined. Thus, modification of the CNI provided no additional benefit to statin therapy. Additionally, the fact that some components of the dyslipidaemia—for example, hypertriglyceridaemia—are insensitive to statin therapy, and that both atherogenic and potentially protective lipid subfractions (HDL cholesterol) are increased with immunosuppression has increased the reluctance to prescribe statins. Most clinicians and patients remain reluctant to change immunosuppression because of dyslipidaemia, without data to support long-term outcomes with this strategy.

Fibrates and nicotinic acid derivatives are not recommended for primary use and only with caution as add-on therapy in transplantation (Holdaas et al., 2008).

New-onset diabetes after transplantation

Diabetes is a common cause of end-stage renal failure requiring transplantation, however, post-transplant diabetes mellitus (new-onset diabetes after transplantation (NODAT)) is an increasingly common complication of transplantation with significant negative impact on CV risk (Balla and Chobanian et al., 2009;Wilkinson et al., 2005). It occurs in 3–20% of patients, usually in the first few months post transplantation, and is more common in older patients, those who are overweight, patients of African or Asian origin, and in patients who have experienced stress-induced diabetes previously, for example, after surgery, steroids, or pregnancy (Woodward et al., 2003). It is likely that transplantation merely exposes and accelerates the underlying predisposition to develop diabetes. The main contributory factor is the use of corticosteroids, which cause insulin resistance. CNI can also contribute to the development of diabetes; tacrolimus being considerably more diabetogenic than ciclosporin (Vincenti et al., 2007). This is explained by the effect of tacrolimus specific, intracellular FK-binding proteins on insulin secretion (Knight and Morris, 2010).

Emerging evidence suggests that NODAT has a greater impact on patient outcomes than acute rejection, and is associated with a two- to threefold increase in all-cause mortality and CV events (Revanur et al., 2001; Cole et al., 2008). Thus, strategies to limit the incidence and impact of NODAT have emerged as a major target in the fight against CVD (Cole et al., 2008). Minimization of corticosteroids reduces the risk of post-transplant diabetes mellitus and may reverse the diabetes, and restore insulin sensitivity (Wilkinson et al., 2005; Knight and Morris, 2010). An alternative strategy is to avoid tacrolimus and/or steroids in patients at high risk for the development of NODAT; or switching from tacrolimus to ciclosporin. This strategy may be of particular relevance in older patients where rejection is less of an issue (Joss et al., 2007). Such an approach, however, has few advocates, largely as a consequence of the availability of management strategies for diabetes and the failure to recognize the long-term consequences of NODAT. Treatment of NODAT is similar to management of type 2 diabetes in the general population, with many patients requiring insulin or oral hypoglycaemic agents.

Graft dysfunction and acute rejection

A further potentially remediable CV risk factor in RTRs is renal allograft dysfunction, the effect of which is similar to the impact of reduced eGFR in the general population (Zoccali, 2006). Renal impairment is likely to be associated with other factors which contribute, directly and indirectly, to CV risk. Post hoc analyses of the

two largest CV outcome trials in RTR—FAVORIT and ALERT (Jardine et al., 2005; Weiner et al., 2012) have shown that renal function predicts the risk of graft loss and of patient outcomes. Graft failure is associated with an established increased risk of sudden cardiac death, heart failure and all-cause mortality failure (Abbott et al., 2002; Fellstrom et al., 2005; Soveri et al., 2006). Achieving and preserving good graft function is an effective means of reducing CVD in RTRs (Abbott et al., 2002). Acute rejection has also been shown to be an independent risk factor for CVD in a retrospective worldwide cohort study (Israni et al., 2010).

Smoking

In the general population, cigarette smoking is highly associated with the development of CVD. Studies in RTRs have shown that smoking is associated with all-cause mortality, with CV events, with graft loss, and with more rapid progression of chronic transplant glomerulopathy (Kasiske and Klinger, 2000; Israni et al., 2010). Due to competing risk, the impact of smoking on CV events is likely to be under-represented, for example, due to the concurrent associated increased risk of malignancy. RTRs should be strongly advised to stop smoking.

Other risk factors

Exercise and weight loss are part of the general advice recommended in guidelines for post-transplant management (Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group, 2009; Heemann et al., 2011). Several studies have shown the association of poor exercise capacity with adverse CV outcomes following transplantation (Painter et al., 2003); however, a small interventional study showed potential benefits (Painter et al., 2002) and improvements in exercise capacity (such as VO_2 max) can be expected in transplant recipients. Engagement in exercise may require some encouragement post transplantation as patients often find barriers (physical and psychological) relating to protracted ill health and disability. In addition to exercise there is also interest in functional factors, such as depression, which are associated with outcome, and are potentially remediable (Zelle et al., 2012).

The use of antiplatelet agents appears to be reasonable as a strategy to reduce atheromatous coronary artery endpoints. However, at present there is no evidence to support the empirical use of antiplatelet agents to prevent CVD in RTRs. In patients who have established ischaemic heart disease, the use of antiplatelet agents is advised.

Recent studies have shown associations between a wide variety of biomarkers (including circulating inhibitors of nitric oxide (Abedini et al., 2010), elevated phosphate (Stevens et al., 2011), fibroblast growth factor 23 (Wolf et al., 2011), oxidative stress (Turkmen et al., 2012), endothelin (Raina et al., 2012), and hyperuricaemia (Chung et al., 2011)) and CV and related outcomes in RTRs. Whether these are independent markers and therapeutic targets remains to be established.

Other cardiac conditions

RTRs are also at increased risk of the common valvular conditions, although there are limited data on the incidence and management. Calcific aortic stenosis is common in patients with ESRD and progresses more rapidly in parallel with the development of vascular calcification (Bakri and Goldsmith, 2003). The incidence of infective endocarditis is increased, reflecting the higher prevalence of valvular abnormalities and concomitant immunosuppression (Shroff et al., 2008). The management of valvular disease and endocarditis in RTRs is the same as in the general population. The risk associated with valve replacement is higher in RTRs but better than that of patients requiring maintenance dialysis (Sharma et al., 2010). Recent data suggest that the risk of developing atrial fibrillation is increased in chronic kidney disease, including RTRs, and associated with CV risk (Nelson et al., 2012). RTRs are likely to benefit from rate control and anticoagulant therapy and there is no reason to withhold this proven therapy.

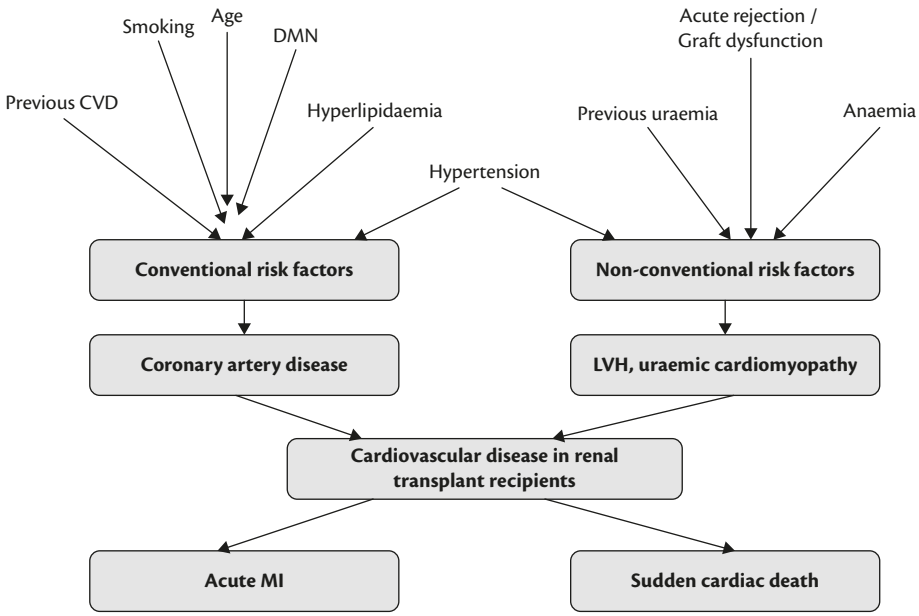

Fig. 285.4 Flow diagram demonstrating the main risk factors for atheromatous and uraemic cardiomyopathy and their respective presentations.

Pre-transplant cardiovascular management—screening

Screening patients for CVD pre transplantation, with the aim of identifying patients for whom the CV risk of transplantation outweighs the risks of maintenance dialysis, is an important topic. The difficulty arises first from the fact that the majority of screening tests are designed to detect obstructive CAD and not uraemic arrhythmogenic abnormalities. Second, disagreement exists about the prognostic benefits of intervening in this population, particularly if abnormalities are detected in asymptomatic individuals (Boden et al., 2007). As such, the intervention rate in screening programmes is low, with only around 5% of all those screened ultimately undergoing revascularization (Patel et al., 2008a). No unified approach to CV screening exists, with some centres undertaking invasive coronary angiography as standard pre-transplant workup. In others, selected non-invasive stress testing is performed in high-risk patients and further investigation only undertaken if this is positive.

In the absence of a randomized controlled trial, it is reasonable that patients who are at high risk of underlying CVD, such as diabetics with peripheral vascular disease, undergo non-invasive stress testing preoperatively. They should receive advice regarding risk factor optimization and any invasive intervention should be a joint decision between the transplant team, cardiologist, and patient. Furthermore, benefits of screening should be balanced against the risk of delaying treatment proven to reduce their CV risk and overall mortality, viz. transplantation.

Conclusion

RTRs carry with them the risk factor burden associated with their preceding progressive chronic kidney disease. Intervention and prevention should start long before transplantation. CVD in RTRs differs from the general population so the approach is directed at the specific risk (Fig. 285.4). There are, however, very few interventional trials assessing treatment targets and therapeutic strategies, so the present approach remains pragmatic. CV risk should be in the forefront of the mind of the transplant physician from the outset. Management is necessarily multifactorial, including lifestyle approaches, tailored immunosuppression, and targeted medication.

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CHAPTER 286

Chronic allograft dysfunction

Lorna K. Henderson, Brian J. Nankivell, and Jeremy R. Chapman

Overview

The major causes of renal transplant loss include death with a functioning graft predominantly from vascular, infectious, or malignant disease, and failure of the graft from progressive renal dysfunction associated with glomerulosclerosis. Interstitial fibrosis and tubular atrophy (IF/TA), formerly described as chronic allograft nephropathy or CAN, is a histological definition that includes atrophy, fibrosis, glomerulosclerosis, and vascular damage in renal allografts, and represents a non-specific pathophysiological outcome of a variety of injuries to the graft over time.

Mechanical causes of graft dysfunction, immune-mediated injury resulting from acute, subclinical, and chronic rejection, and non-immune injury such as donor disease, ischaemia-reperfusion injury, nephrotoxicity from calcineurin inhibitors (CNIs), BK viral infection, and recurrent disease, all pose potential threats to the graft. Clinical programmes typically rely on monitoring serum creatinine to identify allograft dysfunction; however, the change in creatinine often occurs late in the course of disease and underestimates the severity of pathological damage. Serial monitoring of renal function, together with regular urinalysis and measurement of immunosuppressive drug concentrations, allows early recognition of graft dysfunction and should prompt renal imaging and diagnostic biopsy before irreversible nephron loss has occurred. Surveillance biopsy yields a high incidence of subclinical pathology that may allow early intervention before graft dysfunction is clinically apparent and should be considered, particularly for high immunological risk recipients.

Specific interventions targeting the cause of dysfunction are influenced by clinical and histopathological information and include strengthening immunosuppression for chronic cell-mediated or humoral rejection, CNI minimization, elimination or substitution for CNI nephrotoxicity, and lowering immunosuppression with consideration of antiviral agents for BK virus-associated nephropathy (BKVAN). Control of hypertension, proteinuria, dyslipidaemia, diabetes, and helping with smoking cessation and other co-morbid conditions are also important.

Late identification of IF/TA, with failure to improve long-term graft survival, suggests that current approaches to identify graft damage are insufficient to prevent chronic allograft dysfunction and graft loss. Strategies to strengthen surveillance and protect transplant function to prolong graft survival remain a major challenge in transplantation and an important form of research.

Introduction

Chronic allograft dysfunction is a prelude to the majority of graft failures. Advances in transplant immunosuppression and infection prophylaxis have improved short-term graft survival with early acute rejection rates < 15% and 1-year graft survival rates > 90% (Meier-Kriesche et al., 2004). Despite this, impact on long-term graft survival has remained unchanged with graft loss reported at 4% graft loss per year (McDonald et al., 2007) (Fig. 286.1).

This chapter will outline assessment of renal dysfunction following transplantation, define the causes of chronic allograft failure, and their pathophysiology, and evaluate current therapeutic strategies used to improve or stabilize chronic allograft dysfunction.

Summary of major points:

- Major causes of graft loss include death with a functioning graft (with cardiovascular death the most common cause) and loss of graft from progressive fibrosis and tubular atrophy.
- Chronic allograft damage results from the summation of numerous immune and non-immune insults over time.
- Interstitial fibrosis, tubular atrophy, and glomerulosclerosis represent the histological endpoint of chronic allograft dysfunction arising from multiple pathologies.
- Overall graft survival is predicted by baseline glomerular filtration rate (GFR) and rate of decline.
- Some patients show rapid decline late after transplantation with the onset of new pathology.

Evolution of chronic allograft dysfunction

Injury to renal allografts is a consequence of both immune and non-immune-mediated injury, where damage may be initiated in the donor during organ retrieval and after transplantation. Chronic allograft damage results from the summation of insults over time, which, in combination with the kidney's healing response to injury, is influenced by alloimmunity and immunosuppression. Response to insults is variable, and may manifest within different anatomical compartments of the graft (tubules, interstitium, glomeruli, and vessels). Multiple mediators of nephron damage and fibrosis may operate, often simultaneously, and with varying rates of progression. Thus, the histological findings of interstitial fibrosis and tubular atrophy, glomerulosclerosis, and vascular abnormalities that ultimately cause graft failure, are the endpoints of an often complex

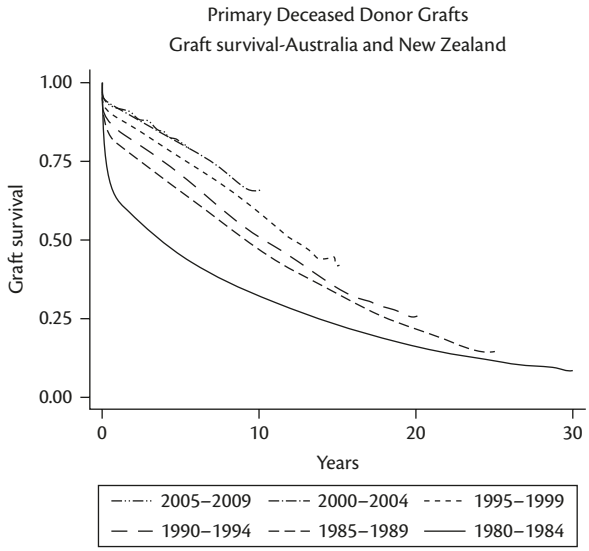

Fig. 286.1 Long-term graft survival in primary deceased donor grafts in Australia and New Zealand.
Data courtesy of Australia and New Zealand Dialysis and Transplant Registry (ANZDATA). Available at <http://www.ANZDATA.org.au>.

sequence of events, posing a major challenge to the physician considering appropriate therapeutic intervention (Fig. 286.2).

Clinical scenario of chronic graft loss

For many years, kidney allograft damage and failure was attributed simply to chronic rejection. Although frequently described in the era of prednisolone and azathioprine, this became less common with the introduction of more potent immunosuppressive regimens that typically incorporated CNIs. Although acute and chronic rejection remain clinically relevant, especially in non-adherent patients, long-term graft survival is little improved despite lower acute rejection rates and use of potent immunosuppression, suggesting that additional mechanisms must contribute to graft injury and loss.

The baseline GFR achieved in each renal transplant is influenced by a variety of factors including donor characteristics (age, comorbidity, and type of donor, i.e. living or deceased); ischaemia reperfusion injury; prolonged cold ischaemic time (CIT) with the potential for delayed graft function; and early post-transplant events such as acute rejection and nephrotoxic insults. It is therefore not surprising that renal allograft recipients achieve very different levels of baseline GFR after transplant. Subsequent progression and decline in graft function also follows a variable course and was first described using the concept of 'intercept' and 'slope' by Hunsicker and Bennett (1999). The intercept (i.e. GFR achieved by 6 months) and the slope (rate of decline in GFR) combine to predict time to eventual graft failure. The model is influenced by baseline GFR, acute injury after transplantation, and the kidney's response to injury including the extent of graft hyperfiltration before chronic damage to the renal tubules, interstitium, and glomeruli occurs. For example, a kidney from an extended criteria donor with a prolonged CIT and early acute rejection may only achieve a baseline GFR of 30 mL/min. If the subsequent decline in GFR is 2 mL/min/year, it will reach 10 mL/min by 10 years after transplant. Alternatively, a kidney from a young live donor with no early acute

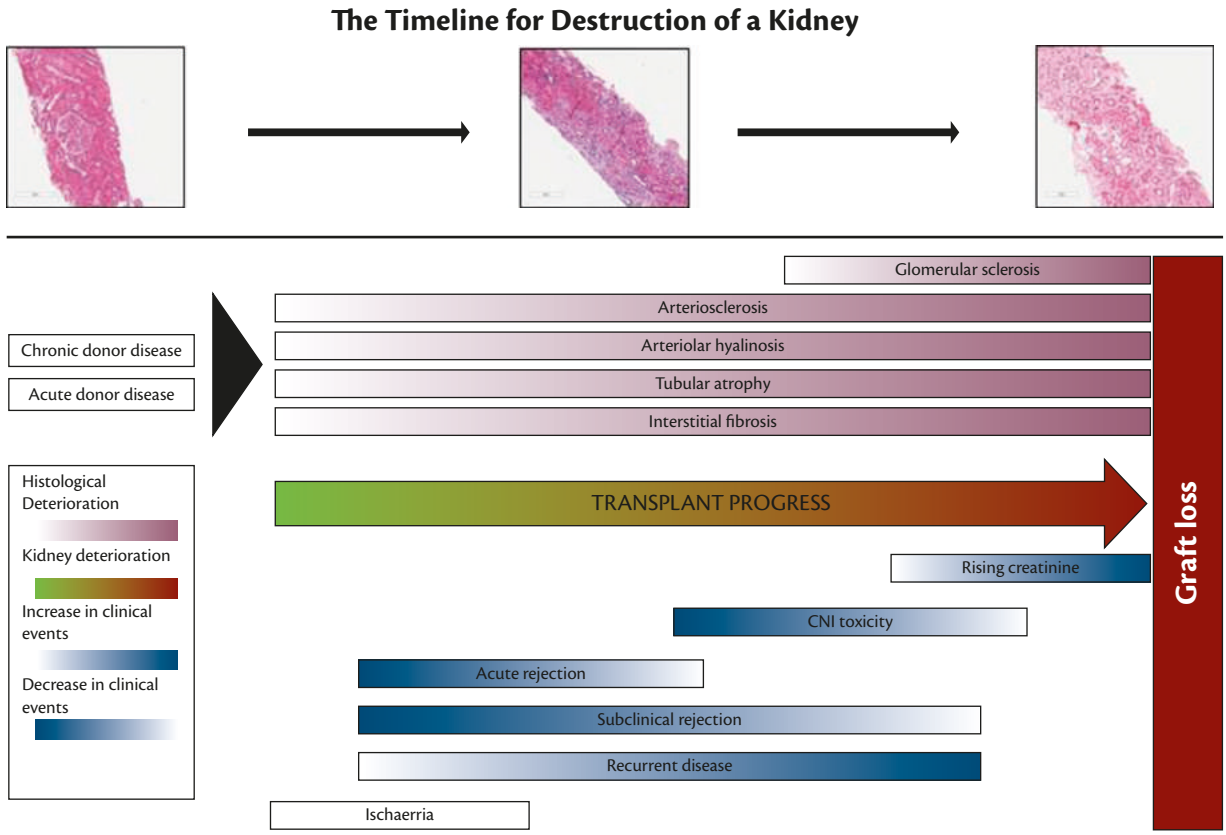

Fig. 286.2 The relationship between factors leading to chronic allograft dysfunction in the majority of grafts lost from chronic allograft dysfunction.

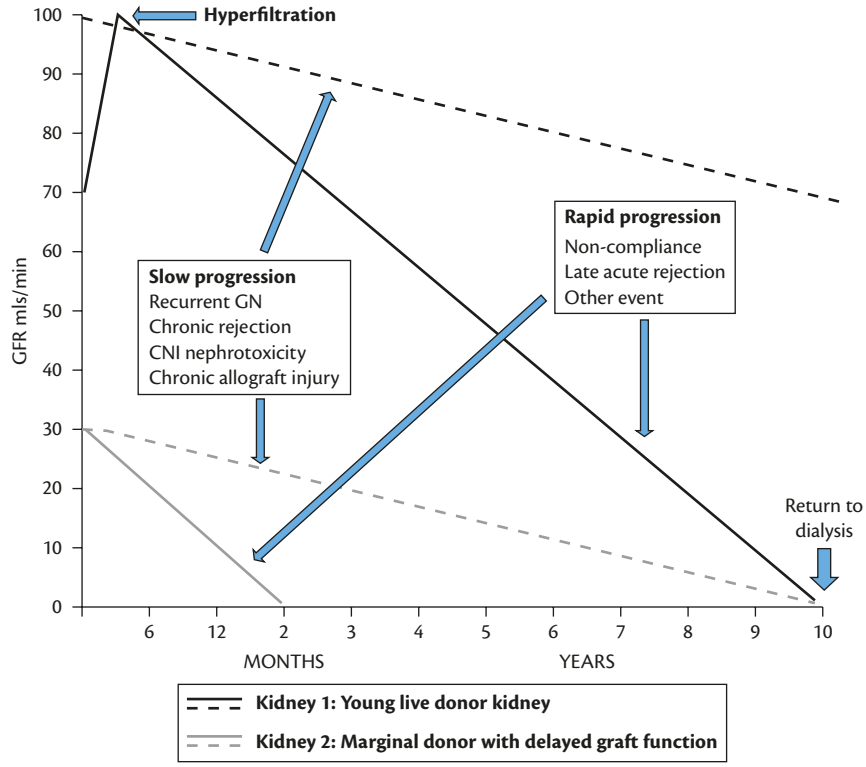

Fig. 286.3 Model of the concept of 'intercept' and 'slope' showing two different kidneys, and their respective time to graft failure according to rate of progression and decline in GFR. Time to graft failure can be predicted from the baseline rate (intercept, determined by donor quality and early events) and rate of decline in GFR (slope, determined by ongoing injury).

rejection may yield a baseline GFR of 70 mL/min, rising to 100 mL/min after several months due to glomerular hyperfiltration. If this kidney also declines at a rate of 2 mL/min/year, graft GFR will reach 10 mL/min at 45 years (Chapman et al., 2005). However, a rapid decline in GFR over time (e.g. 10 mL/min) will cause example one to fail in 2 years and example two in 10 years (Fig. 286.3).

The predictors of poor 6-month baseline GFR are shown in Table 286.1. As with the remaining native kidney in live donors following transplant, the graft initially retains the capacity to increase and decrease GFR in response to external stimuli such as a protein load. Subsequent decline in GFR is associated with early acute rejection episodes, female recipients, and hypertension at 2 years (Bertolatus et al., 1985).

Pathophysiology of chronic allograft damage

Allograft injury is mediated by a combination of ischaemic, inflammatory, and alloimmune stimuli. Several unifying hypotheses and specific mechanisms have been proposed to best explain chronic allograft injury and are summarized below.

Models of progressive damage

The 'input stress model' describes the interaction between the starting 'input' of the renal allograft, that is, overall quality of the kidney (determined by donor age and pre-existing disease) combined with events surrounding procurement, preservation, and implantation, followed by a series of immune stresses such as cell-mediated or humoral rejection and non-immune 'load'-related mechanisms (which include hypertension, hyperfiltration, proteinuria, dyslipidaemia, nephrotoxic drugs, and infection). This theory postulates that these stressors ultimately deplete the graft tissue's finite ability to repair, driving inflammation which in turn promotes further stress to tissue parenchyma, vessels and immune response, promoting fibrosis and ultimate graft failure (Halloran et al., 1999).

The 'cumulative damage model' assumes that chronic damage is the end product of a series of immune and non-immune injuries, imposed over time, which ultimately results in irreversible damage to the nephron. Given that nephron number is finite and although hypertrophy of remaining nephrons may compensate initially, the graft will eventually fail from the incremental loss of nephrons and internal structural damage. Alloimmune and non-immune ischaemic and inflammatory factors are again responsible for tubular injury and the resultant pro-fibrotic healing response leads to nephron loss.

In addition to these unifying theories, several additional specific, though not mutually exclusive, mechanisms of injury have been proposed. Degradation of internal structure can occur at the level of the individual nephron or the intact kidney. Damage may affect any component along the length of the nephron from glomerulus (glomerular sclerosis, transplant glomerulopathy, or atubular glomeruli) to tubules (apoptosis of tubular cells, tubular atrophy, or luminal obstruction). Structural failure may result from disruption of internal architecture, with loss of tubular capacity to concentrate and acidify urine and misdirection of glomerular ultrafiltrate (Kriz et al., 2001). Resultant inflammatory necrosis and fibrosis with structural compromise and breach of the tubular basement membrane leads to a 'leaky' kidney and ultimate reduction in functional efficiency (Bonsib et al., 2000).

Table 286.1 Factors reported to be associated with chronic allograft dysfunction

Non-immune donor risks	Non-immune recipient risks
◆ Deceased vs live donor kidney ◆ Non-heart beating donor kidney ◆ Extended criteria donor ◆ Female sex ◆ Donor vascular disease ◆ Ischaemia reperfusion injury ◆ Prolonged cold ischaemic time ◆ Delayed graft function	◆ Age ◆ Female sex ◆ Size mismatch ◆ Obesity ◆ African American ◆ Cause of renal disease ◆ Hypertension, hyperlipidaemia, smoking ◆ Proteinuria > 500 mg/24 hours ◆ Pre-existing or post-transplant diabetes ◆ Compliance with treatment ◆ Creatinine at 1, 6, and 12 months ◆ GFR at 6 and 12 months ◆ Change in serum creatinine or GFR between 6 and 12 months
Alloimmune factors	**Renal events**
◆ HLA matching ◆ Recipient pre-sensitization (panel reactive antibodies or donor-specific antibodies) ◆ Acute rejection (especially steroid resistant, vascular rejection, antibody-mediated rejection, and late rejection) ◆ Subclinical rejection, chronic T-cell mediated rejection, late *de novo* anti-HLA antibody formation (donor-specific antibodies), chronic antibody-mediated rejection	◆ CMV disease ◆ BK virus nephropathy ◆ Ascending urinary tract infection ◆ Doppler ultrasound resistive index > 0.80 ◆ Renal histology in first year; nephrocalcinosis, arteriolar hyalinosis, interstitial fibrosis, tubular atrophy, recurrent or *de novo* glomerulonephritis

Cortical ischaemia

Metabolically active tubular cells are vulnerable to ischaemia resulting from glomerulosclerosis, CNI-induced vasoconstriction, arteriolar hyalinosis, and small vessel fibrointimal hyperplasia. Injury and attenuation of the peritubular capillary (PTC) network supplying the tubules parallels tubulointerstitial damage, leading to allograft dysfunction and proteinuria (Ishii et al., 2005).

Failure to resolve chronic inflammation

Repeated episodes of acute injury result in partial or incomplete resolution of inflammation. Persistent non-specific injury and inflammation strengthens allorecognition which perpetuates further injury, with chronic inflammation ultimately leading to graft fibrosis and functional impairment (Halloran et al., 1999).

Epithelial-mesenchymal transition and fibrosis

Transformation of tubular epithelial cells into spindle-shaped cells that resemble mesenchymal or myofibroblast type cells has been reported to follow tubular injury. Ultimately cells migrate into the interstitium, with production of matrix proteins, collagen and fibronectin. Epithelial-mesenchymal transition (EMT) may be reversible, with surviving cells repopulating injured tubules with new functional epithelia (Carew et al., 2012). Although evidence for EMT is increasing, contribution to allograft fibrosis remains unclear.

Donor age and replicative senescence

This process has been postulated as an explanation for the inferior graft survival observed with older kidneys (Halloran et al., 1999; Naesens, 2011). It describes the ageing process of normal cells that ultimately leads to cellular exhaustion and irreversible growth arrest. Alternative explanations for poor outcomes include a differential response to injury and a limited ability to repair with age, impaired ability to tolerate stress, and amplification of external insults by pre-existing structural abnormalities.

Persistent pathological stressors

Hyperfiltration, proteinuria, hypertension, smoking, hyperlipidaemia, reactive oxygen species (ROS), and cytokines have all been proposed as potential mediators of interstitial fibrosis and tubular atrophy. Although all are plausible candidates, detailed human mechanistic studies are lacking and much evidence remains circumstantial. In contrast, CNI-mediated nephrotoxic injury constitutes the most important and likely constant pathological stressor to the transplant kidney (Solez et al., 1998; Davies et al., 2000; Pilmore and Dittmer, 2002; Nankivell et al., 2004).

Epidemiology

Summary of major points:

- Death with a functioning graft is responsible for up to 50% of all graft failures.
- Interstitial fibrosis and tubular atrophy is present in up to 25% of allograft biopsies at 1 year from transplant and 90% at 10 years.
- Ten-year adjusted patient survival is < 40% after graft failure and return to dialysis.

Impact of graft loss

Major risks that face patients following transplantation include death with a functioning graft and graft failure with consequent increase in morbidity and mortality associated with a return to dialysis.

Death with a functioning graft is responsible for up to 50% of all graft failures, with cardiovascular disease the leading cause, accounting for approximately 30% of all deaths followed by infection (21%) and malignancy (8%) (United States Renal Data System, 2011). For those who return to dialysis or receive a further transplant, interstitial fibrosis and tubular atrophy is the most common pathology of graft failure, followed by acute rejection and recurrent primary disease (Briganti et al., 2002; El-Zoghby et al., 2009). Moderate to severe interstitial fibrosis is present in at least 25% of allografts at 1 year and prevalence rises to approximately 90% by 10 years (Nankivell et al., 2003; Meyers and Kirk, 2005; Nankivell and Chapman, 2006).

For those who return to dialysis after graft loss, adjusted patient survival is extremely poor, with < 40% of patients surviving 10 years compared with > 75% survival with a functioning transplant (Kaplan and Meier-Kriesche, 2002) (Fig. 286.4).

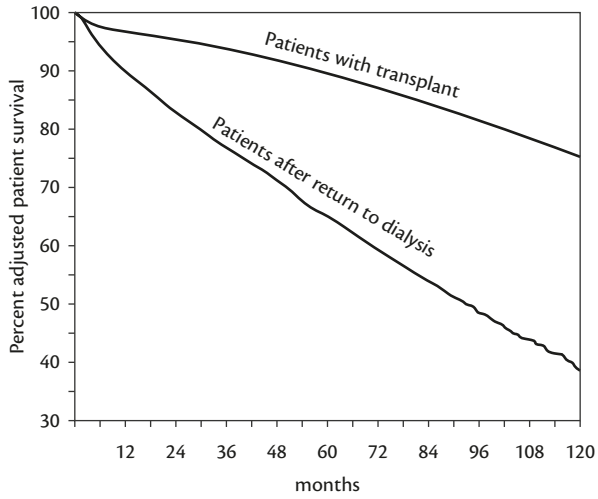

Fig. 286.4 Adjusted patient survival for patients with a transplant and for patients after loss of transplant. * Months post transplant for patient death during transplant and months after graft loss for patients returned to dialysis.
From Kaplan, B. and Meier-Kriesche, H. U. (2002). Death after graft loss: an important late study endpoint in kidney transplantation. *Am J Transplant*, 2, 970–4. Data courtesy of Wiley publishers.

Clinical features

Summary of major points:

- Chronic dysfunction typically presents with rising or persistently elevated creatinine.
- Serum creatinine will only rise appreciably once significant damage has occurred in the graft.

Clinical approach to chronic allograft dysfunction

Transplant recipients with deteriorating graft function typically present with a rising or persistently elevated serum creatinine. Patients should be assessed for obvious acute reversible causes such as volume depletion, sepsis, drugs that are nephrotoxic (e.g. non-steroidal anti-inflammatories), or that may affect serum creatinine (e.g. angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) in the context of renovascular disease), acute CNI nephrotoxicity (by CNI levels), obstruction, renovascular disease (by imaging), and evidence of glomerular disease (by urinalysis for haematuria or proteinuria). Biopsy should be considered both to provide a diagnosis and inform prognosis.

Primary causes of chronic graft dysfunction in the absence of a mechanical cause, should be classified as (a) immune-mediated acute or chronic cell-mediated and/or antibody-mediated rejection; (b) non-immune mediated-donor disease, ischaemia-reperfusion injury, recipient hypertension, renal artery stenosis (RAS); or (c) a specific and new pathology such as CNI nephrotoxicity, BKVAN, or recurrent or *de novo* glomerulonephritis (GN). Rejection should always be considered irrespective of time from transplant due to problems with late non-adherence or iatrogenic under-immunosuppression.

The patient's history may raise clinical suspicion as to the likely cause of dysfunction, for example, known sensitization, non-adherence, or previous rejection. The histology may yield transplant glomerulopathy or positive C4d staining, peritubular capillaritis, fibrointimal hyperplasia of small arteries, tubulitis, or interstitial infiltration, which can guide intervention.

Serum creatinine is an imprecise measure of graft function, and thus renal function and significant histological damage will occur before the serum creatinine rises noticeably. This is, in part, due to the log-linear relationship of creatinine and GFR and partly due to hyperfiltration of remaining preserved nephrons maintaining GFR despite significant injury. While declining reciprocal creatinine and increasing serum creatinine may both correlate with graft failure, both are poor predictors of graft failure in prospective studies (Kaplan et al., 2003).

Investigations

Summary of major points:

- Patients should be assessed for reversible causes of graft dysfunction such as volume depletion, RAS, ureteric obstruction, effects of nephrotoxins, and sepsis before considering glomerular disease.
- Serum creatinine is sensitive early after transplantation and for comparative changes in allograft function but becomes less sensitive for changes in graft function in the long term.
- Persistent proteinuria is a major indicator of renal disease and is associated with increased risk of graft failure.
- Transplant biopsy usually provides a clear diagnosis but should be performed early to guide timely intervention.

Measurement of renal function

Serum creatinine is cheap, readily available, and frequently used after transplantation to recognize acute tubular necrosis (ATN), acute rejection, infection, or urinary obstruction. The relatively consistent daily rate of creatinine generation means that this measure is sensitive, because comparative changes in allograft function, with a 25% rise above baseline, are significant. As monitoring becomes less frequent with time from transplant, these measurements become less sensitive to changes in graft function and are not an accurate measure of function when taken in isolation (particularly at levels of GFR between 30 and 70 mL/min). GFR is a better measure of renal function and may be calculated from variables including weight, height, gender, and serum creatinine. Numerous equations have been developed in recent years to estimate GFR in normal individuals (Cockcroft and Gault, 1976), those with chronic kidney disease (Walser et al., 1993; Levey et al., 1999), and transplant recipients (Nankivell et al., 1995). Although more accurate than a serum creatinine alone, each have advantages and disadvantages but all perform poorly against formal GFR measurement because of variations in muscle mass, tubular secretion of creatinine, the non-linear relationship of creatinine with GFR, and differences in creatinine assay. The more expensive isotopic GFR measurement using iodine-labelled iothalamate, ^{51}chromium ethylene-diamine-tetra-acetic acid (EDTA), and ^{99m}technetium diethylene-triamine-penta-acetic acid (DTPA), provide the most accurate measurement of GFR but are not practical for repeated monitoring and still have their own systemic bias. (See Chapter 282.)

Tubular function

The renal tubules are responsible for the majority of metabolic functions of the kidney, and sustain the greatest injury from nephrotoxins and allograft rejection. Despite this, assessment of tubular function is not routine. The failure to develop assays of tubular function in the past is probably explained by tubular capacity to maintain functional reserve, together with expense and inconvenience. Renewed interest has emerged with the development of urine genomic (Schaub et al., 2004) and proteomic approaches (Li et al., 2001). Although preliminary results are encouraging from some centres examining urine biomarkers such as granzyme and perforin, the techniques require validation and are not yet available for application in routine clinical practice.

Urinalysis

New-onset and persistent proteinuria, or haematuria and proteinuria (in the absence of infection or mechanical cause) are suggestive of *de novo,* or recurrent GN and should prompt further investigation, in particular allograft biopsy.

Persistent proteinuria is present up to 45% of transplant recipients. Quantified by either spot or 24-hour protein measurement, it is a powerful independent risk marker for graft loss and patient survival (Cherukuri et al., 2010). It represents a composite endpoint of many possible pathologies including transplant glomerulopathy, non-specific IF/TA, and tubular proteinuria or glomerular proteinuria from a *de novo* or recurrent GN. Urinary protein excretion also increases with hyperfiltration, obesity, hypertension, and from iatrogenic causes such as use of mammalian target of rapamycin inhibitor (mTORi). Conversely, proteinuria may be reduced by renin–angiotensin blockade, CNIs, and through reduced GFR.

Some centres consider a threshold of 0.5 g/24 hours or higher as abnormal, while others have shown that any proteinuria exceeding the renal range of 0.15 g/24 hours is associated with worse outcome (Keane and Eknoyan, 1999).

Mounting evidence from protocol and diagnostic biopsy studies suggests a specific disease is responsible for proteinuria in most patients (Nankivell et al., 2003). Early allograft biopsy is recommended to inform management.

Renal imaging of the failing graft

Ultrasound

Ultrasound is non-specific and therefore uninformative for the diagnosis of either acute rejection or CAN. Chronic parenchymal changes such as loss of differentiation between cortex and medulla, increased cortical echogenicity, and irregular cortical outline with reduced width are late features of significant and irreversible damage.

Vascular flow

The resistance index (RI) is a non-invasive measure of intrarenal blood flow and compliance, determined by averaging RI measurements in the segmental arteries, which branch off the main renal artery. It is now a routine investigation in acute transplantation and may also be informative in the long-term assessment of allografts where higher RI values correlate with intra-renal compliance and interstitial fibrosis. Although features are non-specific, an RI > 0.80 is associated with ninefold increased graft failure (Radermacher et al., 2003). Magnetic resonance imaging (MRI), gadolinium MRI perfusion, and blood oxygen level-dependant (BOLD) MRI have been shown to be sensitive for parenchymal disease, distinguishing between acute rejection, CNI nephrotoxicity, and ATN. Although sensitive, these techniques are expensive and not routinely used in clinical practice.

Transplant biopsy

Chronic allograft damage is best described from transplant histology and biopsy should be considered after other obvious causes of allograft dysfunction have been excluded.

Transplant biopsy can provide a clear diagnosis that will guide treatment. However, chronic tubulointerstitial damage may be the end result of various prior insults and identification of a single aetiology is difficult. Treatment strategies are therefore limited and if the graft is already severely damaged, response to therapy is poor. Renal biopsy should therefore be considered at an early stage where morphological features are more likely to yield a specific diagnosis, allowing timely intervention and greater probability of response to therapy.

Samples should contain at least 10 glomeruli and two arteries, and should also contain arterioles to exclude CNI-induced hyalinosis and small muscular arteries for evidence of fibrointimal hyperplasia. Some pathological features are patchy, and two cores of cortex are recommended. Histology should be processed similarly to native biopsies, where light microscopy will help define the extent of chronic allograft damage, together with specific diagnosis such as BKVAN, glomerulitis, CNI nephrotoxicity, and hypertensive nephropathy. Periodic acid–Schiff stain defines the basement membrane and arterial hyalinosis, silver stain detects the double contours of transplant glomerulopathy, while a trichrome stain detects collagen deposition and determines the extent of fibrosis. Immunofluorescence or immunoperoxidase is invariably negative or non-specific, but is useful in the diagnosis of recurrent or *de novo* GN, viral nephropathies (BK virus and cytomegalovirus stains), and antibody-mediated rejection (peritubular C4d deposition). Although infrequently assessed, electron microscopy (EM) can detect early transplant glomerulopathy before light microscopy appearances, and is useful to detect electron–dense deposits to confirm transplant GN.

Many units undertake surveillance biopsies at fixed time-points after transplant, irrespective of graft function, in order to detect subclinical pathology such as rejection, recurrent GN, or early CNI toxicity. Biopsies in this setting are best carried out within the first 3 months to allow early detection of pathology at a time when intervention may improve outcome and prevent or minimize progressive injury. Consideration of surveillance biopsies or at least maintaining a low threshold for indication biopsy is advisable in patients at greater risk of immunological graft loss, or in patients with proteinuria and possible early recurrent disease such as those with focal segmental glomerulosclerosis (FSGS) or mesangiocapillary GN.

Aetiology and pathogenesis

Causes of chronic graft dysfunction

Pathophysiologic causes of chronic graft injury should be distinguished from factors associated with progressive graft dysfunction. Factors associated with chronic *injury* are shown in Table 286.1, while differential diagnosis of chronic renal *dysfunction* is outlined in Table 286.2. As the kidney allograft response to injury is relatively restricted, histological description of injury may fail to differentiate

Table 286.2 Differential diagnosis of renal allograft dysfunction

Structural	◆ Ureteric obstruction ◆ Lower urinary tract obstruction ◆ Renal artery stenosis
Infection	◆ Recurrent pyelonephritis ◆ Vesico-ureteric reflux ◆ Polyoma (BK) virus nephropathy
Alloimmune injury	◆ Late/recurrent acute rejection (non-compliance or iatrogenic) ◆ Chronic cellular rejection ◆ Chronic antibody-mediated rejection with transplant glomerulopathy
Other pathology	◆ Chronic calcineurin inhibitor nephrotoxicity ◆ Recurrent or *de novo* glomerulonephritis ◆ Non-specific sclerosing tubulointerstitial damage (formerly designated chronic allograft nephropathy) ◆ Thrombotic microangiopathy ◆ Hypertension ◆ Nephrotoxic drugs (ACEIs, ARBs, non-steroidal anti-inflammatories, COX-2 inhibitors) ◆ Pre-renal acute kidney injury associated with renal hypoperfusion

the cause. Longitudinal studies have assisted our understanding of the pathophysiologic processes contributing to chronic allograft damage over time, identifying potential therapeutic strategies to prevent or abrogate injury.

Ureteric obstruction

Obstruction of urinary flow is a reversible cause of chronic graft dysfunction. Acute and complete obstruction is uncommon, but is clinically obvious, presenting with oligoanuria and acute renal impairment. Hydronephrosis is invariably present on ultrasound in a well-hydrated patient. The source of obstruction may be identified by antegrade or retrograde nephrostogram. Diagnosis of chronic partial obstruction is a greater challenge primarily because mild hydronephrosis is common after transplant and may not be clinically relevant. Therefore, assessing functional significance is key to determining management. Diuretic isotope renography utilizes ^{99m}technetium mercaptoacetyltriglycine (MAG3), which is secreted by renal tubules despite poor function. With a sensitivity of 92% and specificity of 87% for functional ureteric obstruction (Nankivell et al., 2001a), it is the investigation of choice to resolve diagnostic uncertainty. (See Chapter 282.)

Renal artery stenosis

The incidence of transplant RAS ranges from 1.5% to 7%. This disparity is likely to reflect different policies for investigating and ultimately diagnosing RAS. Some studies, describing results of arteriography on all functioning transplants report an incidence of 23% (Lacombe, 1975), indicating that 'radiological stenosis' may not necessarily represent a functional stenosis. RAS typically occurs from damage to the transplant renal artery at retrieval, during perfusion, or anastomosis. A long transplant renal artery may be prone to kinking and subsequent stenosis, but chronic rejection may be a late cause.

Presentation typically occurs between 3 months and 2 years after transplant with a peak incidence at 6 months. The diagnosis should be considered in any patient with deteriorating graft function, and strongly suspected with new-onset, unstable, or resistant hypertension. With ACEI or ARB use, an acute, reversible deterioration of graft function may occur and is diagnostically helpful. This mandates imaging of the renal artery. Other less common presentations include new polycythaemia and sudden-onset left ventricular failure. A bruit over the graft may be present but it is not a reliable clinical sign, and a significant stenosis may be present without a bruit. Femoral pulses should be examined for evidence of aorto-iliac disease, which may produce a transmitted bruit. Doppler ultrasound, by an experienced sonographer, has a sensitivity of 100% and specificity of 75%, but a positive predictive value of 56%. For many years, digital subtraction angiography (DSA) was considered the gold standard for diagnosis but recent improvements in imaging techniques such computed tomography angiography and MRI now provide an alternative, non-invasive approach to diagnosis but must be weighed up against the risks of exposure to contrast or gadolinium in the setting of renal dysfunction (see Chapter 282).

Recurrent and *de novo* glomerulonephritis

Reported as the third most common cause of graft failure (after IF/TA and death with a functioning graft), recurrence of primary disease is an important cause of graft dysfunction (Briganti et al., 2002) (see Chapter 289). Clinical suspicion is raised by new-onset haematuria and/or proteinuria, or renal dysfunction. Reported recurrence rates range from 10% to 20%, but are likely to be an underestimate. Introduction of rapid steroid withdrawal or complete avoidance may have resulted in a threefold increase in the incidence of recurrent GN (Kukla et al., 2011). Surveillance biopsies confirm recurrence rates between 42% and 55% reported for membranous nephropathy and lupus nephritis respectively (Dabade et al., 2008; Norby et al., 2010).

In a US study, median graft survival with recurrent disease was 1360 days versus 3382 days without recurrence ($P < 0.0001$) (Hariharan et al., 1999). Timing of recurrence and impact on graft outcome varies according to the primary disease. While FSGS may recur within days to weeks after transplantation, immunoglobulin A (IgA) nephropathy may not recur until many years from transplant, with little or no deleterious effect on graft outcome (see Chapter 289).

Screening for recurrent FSGS requires early and frequent monitoring for proteinuria, and haemolytic uraemic syndrome requires monitoring for microangiopathic haemolysis, while urine should be assessed for microscopic haematuria and casts in addition to proteinuria in patients with a primary diagnosis of IgA, membranoproliferative GN, and anti-glomerular basement membrane (GBM) disease. Transplant biopsy should be examined by immunofluorescence and EM, in addition to light microscopy, to aid diagnosis (Golgert et al., 2008).

BK virus nephropathy

BKVAN, caused by the BK human polyoma virus subtype, has emerged in the last decade as an increasingly important cause of chronic dysfunction in renal allografts. (See also Chapter 284.)

BK viruria provides the earliest evidence of BK virus replication and is detectable in 35–57% of kidney transplant recipients. A subset of these patients will develop BK viraemia (Brennan et al., 2005) with only a proportion of these patients progressing

to biopsy-proven BKVAN (estimates from 1.1% to 10.3%) (Hirsch et al., 2005). Over-immunosuppression and use of potent combination therapies such as tacrolimus and mycophenolate mofetil (MMF) have been postulated as being responsible for the recent increased incidence (Hirsch, 2002; Lipshutz et al., 2004). Although overall prevalence is low, BKVAN can lead to early graft loss in up to 45% of affected individuals (Hirsch, 2002).

Incidence is highest within the first year from transplant with 95% occurring within the first 2 years and 50% within the first 3 months from transplant. The disease typically presents with evidence of graft dysfunction, but ureteric ulceration and stricture and cystitis are less common manifestations. However, detection of BK virus in the blood by polymerase chain reaction and allograft by surveillance biopsy may occur with stable graft function (Brennan et al., 2005). BKVAN, when detected using staining for SV40 present in many polyoma viruses, is often associated with renal allograft nephropathy and early graft loss despite current therapeutic interventions. For this reason, prevention of BKVAN is a better strategy, with early screening of urine or blood, allowing prompt intervention as described below.

Late or recurrent acute rejection and the role of non-adherence

Late acute rejection is a strong predictor of chronic allograft dysfunction and late graft loss (Nankivell et al., 2001b). A few cases may relate to a late switch or reduction of immunosuppression for other cause, but the majority of cases are due to non-adherence and are especially common during the transition from paediatric to adult nephrology programmes. Now acknowledged as a common problem following transplantation, the incidence increases over time, with reports of up to 25% non-adherence after transplant (Butler et al., 2004). The degree of non-adherence correlates with clinical outcomes and is associated with early and late acute rejection, which in turn, impacts on graft function and survival (Vlaminck et al., 2004) where graft loss is sevenfold more likely in non-adherent compared to adherent patients (Gaston et al., 1999). Risk factors include lack of pre-transplant education, poor communication, lack of social support, and long duration of treatment. The patient and their environment are central to this, where side effects, complexity of drug regimens, cost, and poor access, along with lack of medication knowledge and negative beliefs in medication, all contribute.

Recent consensus recognition of this problem as a medical syndrome has resulted in the classification of non-adherence, encompassing timing and severity of non-adherence (partial and/or total) as well as timing of medication. These definitions have helped guide strategies to prevent, detect, and treat this still under-recognized problem. A multidisciplinary approach to include education, behavioural, and social support with careful monitoring, and early recognition and intervention are crucial. Measures such as simplifying drug regimens, pillboxes to organize medication, coordinating medication with daily routine activities and electronic devices have all been shown to improve adherence. Sadly, in some situations it is simply a matter of the cost of the drugs.

Chronic allograft nephropathy

The term chronic allograft nephropathy (CAN) was first used in the Banff schema in the early 1990s. It was coined in preference to the misleading term, 'chronic allograft rejection' and describes a histological endpoint of fibrosis and tubular atrophy caused by multiple pathologies. It remains the most commonly reported histological change in chronic graft failure, occurring in 27–45% of late graft losses (El-Zoghby et al., 2009; Gourishankar et al., 2010) and is usually accompanied by vascular changes and glomerulosclerosis (Nankivell et al., 2003). The Banff schema has been refined over the years with the incorporation of alternative classification systems including the Chronic Allograft Damage Index (CADI) and the Collaborative Clinical Trials in Transplantation, (CCTT) (Solez et al., 1993; Racusen et al., 1999). In 2005, the term CAN was eliminated in favour of 'interstitial fibrosis and tubular atrophy not otherwise specified' (IF/TA nos) (Solez et al., 2007).

Although Banff has provided a system to standardize histological criteria, the term can be confusing. Implantation biopsies have shown that IF/TA lesions may be present in the donor kidney where interstitial inflammation and fibrosis, tubular atrophy, and basement membrane thickening can occur in up to 40% of grafts. Furthermore, the attribution of some immune-mediated involvement to the description of CAN may obscure histological diagnosis, for example, vascular changes with elastic disruption, inflammatory cells within the fibrotic intima, and proliferating myofibroblasts in the intima. As a result, criteria have been refined to identify lesions supporting evidence for one aetiology over another, for example, the association of fibrosis and tubular atrophy with nodular arterial hyalinosis, suggesting CNI toxicity (Colvin, 2003).

Surveillance biopsies have revealed interstitial fibrosis to be a two-stage process, where two-thirds of the fibrosis at 10 years from transplant was already present by 1 year. The likelihood is that early interstitial fibrosis is linked to factors such as ischaemia-reperfusion injury and direct immune-mediated mechanisms in addition to early tubular damage. Beyond the first year after transplant, interstitial fibrosis and tubular atrophy appear to progress simultaneously, with additional features of CNI toxicity, progressive arteriolar hyalinosis, glomerulosclerosis, and a still to be quantified role from chronic antibody-mediated rejection.

Chronic cellular rejection

Chronic active T-cell-mediated rejection causes continued immune-mediated transplant injury. It is less common in compliant patients receiving CNI-based immunosuppression, and is usually the result of a failure of maintenance immunosuppression to suppress residual alloimmune reactivity sufficiently. There is T-lymphocyte infiltration into the graft, often accompanied by B lymphocytes and macrophages. Banff criteria define chronic cellular rejection using arterial and capillary changes as discriminating features. Vascular changes include fibrointimal hyperplasia, focal destruction of the internal elastic lamina, and infiltration of smooth muscle cells into the neointima of the small muscular arteries, and can lead to vascular occlusion. Though characteristically classified as T-cell mediated, some vascular changes may also reflect donor specific antibody, while small muscular artery changes, expressed as chronic fibrointimal thickening or 'cv' by Banff criteria, may be modulated by non-immune factors such as donor disease, hyperlipidaemia, hypertension, and smoking.

Calcineurin inhibitor nephrotoxicity

CNIs have been the cornerstone of maintenance immunosuppression in transplantation for the last three decades. The introduction of ciclosporin in the 1980s and tacrolimus in the 1990s reduced the incidence of early rejection and subclinical rejection, modifying

the development of IF/TA and increasing early graft survival to > 90% (McDonald et al., 2007). However, the anticipated improvement in long-term graft survival has not yet been realized perhaps due to their nephrotoxic effects. Chronic nephrotoxicity was first recognized in cardiac transplant recipients, and similar renal histological findings were subsequently described in renal transplants and in native biopsies of patients treated for autoimmune disease. Evidence that CNI nephrotoxicity contributes to allograft injury, is surmised from unchanged rates of graft loss despite reduced incidence of rejection, the recognition of characteristic histological lesions from longitudinal studies, and clinical trials of early CNI withdrawal or avoidance demonstrating structural or functional improvement (Abramowicz et al., 2005).

CNIs may cause dose-dependent acute and usually reversible nephrotoxicity, by increasing afferent arteriolar resistance, leading to a reduction in GFR. Prolonged CNI exposure, however, leads to chronic vascular and tubulointerstitial changes, which if detected late, may be irreversible (Nankivell et al., 2004; Abramowicz et al., 2005). Pathological hallmarks of chronic CNI toxicity on light microscopy include glomerular sclerosis, 'striped' tubular atrophy and fibrosis, afferent arteriolar hyalinosis, and isometric tubular vacuolization or microcalcification. Other reported diagnostic lesions such as peritubular and glomerular capillary congestion, diffuse interstitial fibrosis, toxic tubulopathy, and juxtaglomerular hyperplasia are non-specific and unreliable. Although 'striped fibrosis' has been associated specifically with CNI injury, this pattern has also been described with other insults and so with the exception of peripheral nodular arteriolar hyalinosis, most lesions are non-specific (The Canadian Multicentre Transplant Study Group, 1986). When arteriolar hyalinosis occurs in a failing graft, the diagnosis of CNI nephrotoxicity is substantiated if hyalinosis is *de novo* or progressive compared with baseline histology and the presence of nodularity. Other diagnoses should be excluded including donor arteriolar hyalinosis (by implantation biopsy), dyslipidaemia, ischaemia arteriolar injury, hyperglycaemia, and hypertensive nephrosclerosis, which is histologically distinguishable, by subendothelial hyalinosis, elastic lamina reduplicationm and medial hyperplasia in small arteries.

Chronic humoral rejection and transplant glomerulopathy

Antibody-mediated injury has long been recognized as a form of rejection, but was thought to have been largely solved through the identification of the human leucocyte antigen (HLA) system and the use of sensitive crossmatch tests prior to transplantation. Improvements in the techniques used to detect HLA antibodies and the evolution of histological criteria defining antibody-mediated rejection, has resulted in an increased awareness and better understanding of this form of rejection. Chronic antibody-mediated rejection arises from unrecognized pre-existing donor specific antibodies (DSA) or following acute rejection episodes, especially late acute rejection resulting from non-adherence. Clinical manifestations are non-specific and include progressive graft dysfunction, hypertension and proteinuria.

Chronic transplant glomerulopathy is the major histological expression of chronic antibody-mediated rejection. It incorporates a spectrum of abnormalities, but typically involves a triad of:

1. Light microscopy features of thickening or duplication of the glomerular and PTC basement membranes, double contour formation and mesangial matrix expansion, with widening of the subendothelial space and PTC basement membrane multi-lamination on electron microscopy.
2. Endothelial C4d deposition in glomeruli and/or peritubular capillary loops reflects classical complement pathway activation by antibody. This finding is relatively insensitive, with prevalence of C4d deposition varying from 36% to 91% on biopsy specimens with transplant glomerulopathy. It is present in up to 61% of biopsies with chronic rejection and 2% of clinically stable protocol biopsies.
3. The presence of circulating donor specific antibodies to donor HLA or endothelial antigens.

Mononuclear cell infiltrates within the PTCs, transplant glomerulitis, chronic arteriopathy with fibrointimal thickening of the elastic lamina, and plasma cell interstitial infiltrate also support the diagnosis of chronic antibody mediated rejection. The differential diagnosis includes thrombotic microangiopathy, secondary to infection, recurrent haemolytic uraemic syndrome or anticardiolipin antibody thrombotic microangiopathy, and hepatitis C mesangiocapillary GN that can be distinguished by standard immunofluorescence and electron microscopy.

Despite successful treatment of acute antibody-mediated rejection, > 40% of patients with antibody-mediated rejection will go on to develop transplant glomerulopathy, which once established, carries a 50% 5-year graft survival rate (Stegall and Gloor, 2010). Surveillance biopsies have shown that early transplant glomerulopathy may be detected by electron microscopy as early as 1 month after transplantation (Wavamunno et al., 2007). It is not yet known whether the deleterious effect of antibody-mediated rejection is a result of prolonged endothelial injury or persistent exposure to DSA, or both.

Treatment strategies and outcome

Therapeutic approaches

Both preventative and therapeutic interventions should be considered when managing chronic allograft dysfunction. Before discussing specific interventions, a number of basic principles should be highlighted to guide management following transplant:

1. Chronic damage is the end result of multiple pathophysiological injuries. Therefore, it is unlikely that a single intervention will be sufficient. Several therapies may be required, informed by clinical and histological data. These include specific antagonists to target fibrogenic mechanisms, or indirect therapies to treat hypertension, hyperlipidaemia, infection, and smoking.
2. Experimental and clinical data suggest that different pathologies may have different time frames within which response to treatments is effective. Some interventions may only provide benefit early after transplant, and others may even be detrimental if used late.
3. Prevention is always better than a cure. Chronic allograft dysfunction associated with interstitial fibrosis and tubular atrophy reflects the endpoint of numerous pathogenic insults. Strategies need to be pre-emptive, to prevent permanent nephron damage and graft loss.
4. Therapy should be tailored according to the individual, depending on their co-morbidities, immunological risk, and in response

to their evolving clinical progress, including tolerance of immunosuppressive agents.

5. Molecular methods to allow minimal or non-invasive monitoring of allograft pathology offer great promise. Gene complementary DNA microarrays, proteomics, and metabolomics have contributed to the understanding of mechanisms underlying chronic allograft damage, with potential to improve diagnostics and predict relevant pathology before histologically or clinically apparent (Perkins et al., 2011; Roedder et al., 2011).

Screening strategies

Screening for pathology at a stage where injury may be reversed is key to preserving graft function. Data from surveillance biopsies in several groups have helped clarify the time frame of evolution of changes and correlated events with histological changes. Damage, regardless of cause, results in histological injury and repair that precedes any basic biochemical marker of function such as measured serum creatinine or GFR. This is explained by glomerular hypertrophy compensating for loss of nephrons so that the total GFR is either maintained or declines relatively little compared to the histological changes. GFR itself has to decrease considerably before a rise in serum creatinine is apparent. Not surprisingly, renal transplant biopsies in patients with high serum creatinine values invariably show non-specific and usually irreversible changes.

Treatment approach for specific diagnosis

Interstitial fibrosis and tubular atrophy

Therapeutic strategies to halt IF/TA have focused on avoiding or reducing the dose of CNI. Long-term agents relied upon as alternative immunosuppression have included azathioprine, MMF, and/or mTORi, usually all in combination with steroids. Meta-analysis of randomized controlled trials (RCTs) examining CNI-sparing regimens supplemented with MMF, showed an improvement in GFR but no clear benefit in graft survival (Moore et al., 2009). The incidence of acute rejection increased with CNI elimination but not avoidance. Conversion from CNI to an mTORi has been shown in some studies to be effective and non-nephrotoxic when compared with a CNI and an anti-metabolite (Moore et al., 2009). Some controlled trials with CNI elimination with or without mTORi substitution, have demonstrated an improvement in vascular and tubulointerstitial damage (Mota et al., 2004; Flechner et al., 2008). However, the largest RCT examining conversion from CNI to mTORi, failed to show a difference in calculated GFR and substitution was futile when calculated GFR was < 40 mL/min or proteinuria > 0.5 g/day (Schena et al., 2009). Moreover, meta-analysis has shown no difference in hard endpoints of patient and graft survival with mTORi when compared with other immunosuppressive interventions (Webster et al., 2006).

In the absence of a clear cause but with histological evidence of chronic injury such as IF/TA, management is challenging. It is axiomatic that antihypertensive medication, antiproteinuric, and lipid-lowering agents are beneficial and should be included as part of long-term management to help prevent or slow progression of IF/TA. The roles of CNI toxicity, chronic antibody-mediated rejection and other immune and non-immune causes are less clear and appropriate treatment is controversial.

Isolated CNI toxicity, either acute CNI-mediated vasoconstriction or chronic nephrotoxicity, without rejection, has a relatively good prognosis when treated with CNI-sparing regimens (Gourishankar et al., 2010). If CNI toxicity occurs in a low immunological risk recipient with no evidence of subclinical rejection on biopsy, CNI withdrawal in favour of MMF and corticosteroid maintenance therapy can be considered. In higher immunological risk recipients, CNI may be minimized or replaced with mTORi, providing careful monitoring is ensured to manage the risk of rejection, which may be as high as 10% following withdrawal. mTORi are often poorly tolerated due to adverse effects of acne, mouth ulcers, oedema, proteinuria, anaemia, and thrombocytopenia and discontinuation rates are usually 30–45%.

Alternative non-nephrotoxic agents are now available. Newer immunosuppressive agents such as belatacept, a T-cell co-stimulatory blocker targeting the CD28-CD80/86 pathway, and tofacitinib, a Janus kinase inhibitor. Long-term clinical data is awaited but initial results are promising. Voclosporin is being trialled as a CNI with reduced nephrotoxicity, while alefacept, targeting the LFA3-CD2 pathway and memory T cells, and sotrastaurin, a protein kinase C inhibitor reducing T-lymphocyte activation and cytokine release, are in early phase clinical trials.

Chronic antibody-mediated rejection

Earlier detection of chronic antibody-mediated rejection is now possible but the persistent problem is the lack of evidence supporting effective treatment. Current data is from small, uncontrolled, non-randomized cohort studies of approaches adapted from regimens used to treat acute antibody-mediated rejection. Suggested strategies include raising baseline immunosuppression, for example, increasing tacrolimus and/or MMF dose (to suppress T- and B-cell expansion) and introducing or increasing corticosteroids. More aggressive intervention such as plasma exchange, with or without intravenous immunoglobulin (IVIg) (to remove circulating DSA), rituximab, bortezomib, and eculizumab have also been trialled. Results are variable and the impact on long-term graft survival remains uncertain. Concurrent non-immunomodulatory therapy includes control of blood pressure and proteinuria using conventional drugs.

Chronic active T-cell mediated rejection

A persistent interstitial T-cell infiltrate and tubulitis, often accompanied by B cells and plasma cells, is characteristic of chronic active T-cell-mediated rejection and represents persistent alloimmune activity. Treatment usually involves increasing maintenance immunosuppression. Options include switching CNI therapies from ciclosporin to tacrolimus, exchanging azathioprine for mycophenolate, and the introduction of corticosteroids. Maintaining therapeutic drug levels and adherence should be ensured, and any interfering agents such as cytochrome P450 enzyme inducers should be removed.

Recurrent disease

Therapy in many recurrent diseases is limited to optimizing blood pressure and renin–angiotensin system blockade. In the patient with FSGS, early detection and intervention with plasma exchange to remove circulating protein and antibodies, switch to ciclosporin in combination with corticosteroids, and angiotensin system blockade have been shown to induce remission in 80–90% of cases (Canaud et al., 2009). Immunosuppression usually controls most other immune-mediated GN such as ANCA vasculitis, lupus GN, anti-GBM, and membranous nephropathy. IgA commonly recurs

but clinical impact is usually mild. Dense deposit disease recurs in 50–90% with graft failure but recent case reports suggest that eculizumab may be beneficial. (See also Chapter 289.)

Viral nephropathies

Current antiviral therapies targeted against polyoma virus, lack a good evidence base and are relatively unsuccessful once BKVAN is established. BK viruria and viraemia before development of nephropathy is best treated with a reduction in immunosuppression. Once BK viral allograft nephropathy is established, therapy is less likely to be successful, particularly if concomitant interstitial inflammation is also present. Ciprofloxacin, cidofovir, exchange of MMF for leflunomide which is a weak antiviral agent, or azathioprine, and reduction in dose or conversion of tacrolimus to ciclosporin, or conversion to an mTORi, and IVIg have all been used to variable effect but with limited benefit in small uncontrolled studies (Kasiske et al., 2010).

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CHAPTER 287

Cancer after kidney transplantation

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De novo cancer

The fact of the increased risk of cancer in kidney transplant recipients is now established from observational and registry data. This pattern of increased risk is consistent worldwide and appears to be associated with the intensity and the level of immunodeficiency. A recent meta-analysis of six observational studies in Europe, Australia, and North America which assessed the incidence of cancer in kidney transplant recipients has shown that the pattern of increased risk is often associated with a known or suspected infectious cause, such as Kaposi sarcoma and cervical cancers (Grulich et al., 2007). The risk of other non-infectious, common epithelial cancers such as colorectal and lung cancers is also increased, but the magnitude of effect is much less pronounced than that of the virus-related neoplasms. Table 287.1 shows the standardized incidence ratio of the common solid organ and melanocytic cancers in the Australian and New Zealand kidney transplant cohort. Cancers related to viral infections such as human herpes virus 8 (HHV8), Epstein–Barr virus (EBV), and hepatitis B and C viruses have been found to occur at a markedly increased rate, but the risk of non-infectious solid organ cancers such as breast and prostate cancers are not increased compared to the non-transplanted populations (Webster et al., 2007).

Skin cancers

Non-melanocytic skin cancer is the most common cancer type to occur after kidney transplantation. Ultraviolet radiation, long-term immunosuppression, human papillomavirus (HPV) infections, and genetic susceptibility such as that associated with green eyes and fair skin are thought to be the major risk factors. Australia has the world's highest incidence of skin cancers among recipients of kidney transplants (Carroll et al., 2003). The cumulative incidence of skin cancers among Australian transplant recipients increased from 7% after 1 year of immunosuppression to 45% and 70% after 10 and 20 years of immunosuppression exposure, respectively. In contrast to the general population, the incidence ratio of squamous cell (SCCs) and basal cell carcinomas (BCCs) is reversed at 4:1. The most frequently encountered skin cancer in transplant recipients is SCC, occurring at least 65–200 times as frequently as in the general population. BCC, the second most common type of skin cancer in kidney transplant recipients, is increased by at least 10-fold greater than in the general population (Webb et al., 1997; Ramsay et al., 2003). SCCs are also more aggressive in kidney transplant recipients than in the general population. Unlike cancers in the general population, SCCs in kidney transplant recipients have a greater propensity to metastasize and invade surrounding structures such as bones and regional lymph nodes. Survival of recipients with invasive SCC is poor, with a 1-year disease-specific survival of 87%, 67%, and 30% for patients who received surgical treatment, non-surgical treatment and no treatment, respectively, for their metastatic disease (Bouwes Bavinck et al., 1991; Parrish, 2005).

Melanocytic skin cancers are also common in kidney transplant recipients, but the risk is increased to a much lesser degree than SCCs and BCCs. Kidney transplant recipients are at least 1.5–4 times more likely to develop melanomas than people in the general population. Similar to SCCs and BCCs, melanomas tend to occur in recipients who have phenotypic characteristics of blue/green eyes, blonde/red hair, fair complexion, and those who have an inability to tan. The intensity and types of immunosuppression also appear to have a significant impact on the overall incidence. On average, the mean time between cancer development and time of transplantation is at least 3.5–5 years, but the cumulative risk of developing melanoma increases with the duration of immunosuppression exposure. Having a prior history of non-melanocytic skin cancers such as BCC or SCC is also a significant risk marker for melanomas (Parrish, 2005). The predictive prognostic features of cutaneous melanomas in transplant recipients include the depth of cancer invasion, the Breslow thickness, the presence of tumour ulceration, and lymph node involvement. The 5-year survival of early-stage melanoma is similar to that in the general population. However, the prognosis for more advanced stage cancer is significantly worse than patients without kidney transplants, with a 5-year survival of < 20% among those with stage T3 and T4 tumours (Le et al., 2006; Vajdic et al., 2009).

Prevention and screening

In view of the higher incidence, the aggressiveness, and the poorer prognoses of skin cancer among kidney transplant recipients, prevention and screening may play important roles in improving the outcomes of these at-risk patients. Skin cancer prevention with sun protective behaviours such as using sun-screen (sun protection factor 15+), sun hats, avoidance of exposure to ultraviolet radiation during peak-sun hours, and covering up with long sleeves, trousers, and tops are effective measures to reduce the incidence of skin cancers and should be encouraged for all transplant recipients (Griffith and Fulton, 1996; Autier et al., 1999; Helfand et al., 2001;

Table 287.1 Standardized incidence ratio of common cancers in the Australian and New Zealand kidney transplant recipients

Cancer types	SIR	95% confidence interval
Kaposi sarcoma	25.50	16.2–38.3
Gynaecological	18.00	15.7–20.4
Lymphoma	11.4	10.1–12.9
Kidney	9.76	8.10–11.7
Bladder	6.19	5.00–7.58
Thyroid	4.82	3.25–6.89
Liver	4.43	2.67–6.91
Melanoma	3.11	2.67–3.60
Multiple myeloma	2.48	1.39–4.09
Colorectal	1.72	1.43–2.04
Breast	1.40	0.90–2.17
Prostate	0.95	0.47–1.89

Neale et al., 2002; US Preventive Services Task Force, 2002; Ramsay et al., 2003). Transplant follow-ups combined with regular skin surveillance by experienced dermatologists should be available in all transplant units.

Management

Surgical resection and radiotherapy remain the preferred treatments for early-stage and locally invasive tumours. More recently, randomized controlled trials conducted in Europe and Australia have reported immunosuppression reduction and/or switch from calcineurin inhibitor-based to mammalian target of rapamycin inhibitor (mTORi)-based therapy may be effective in recipients with a prior history of non-melanocytic skin cancers (Alberu et al., 2011; Campbell et al., 2012; Euvrard et al., 2012). Other novel treatment strategies for metastatic melanomas such as the antiangiogenic and immunomodulatory drugs, the proteasome inhibitors, and the specific targeted molecular therapies have been applied in the general population (Guida et al., 2012; Simeone and Ascierto, 2012). However, the efficacy and safety of these newer agents are unclear and unproven in the transplant population.

Post-transplant lymphoproliferative disease

Post-transplant lymphoproliferative disease (PTLD) is a well-recognized complication among solid organ transplant recipients. The overall incidence of PTLD among kidney transplant recipients is 1–5%, with an excess risk of at least 10–15-fold greater than that of the general population. EBV infection is the most important risk factor for the majority of PTLD in the transplant population. When the host EBV T-cell-specific immunity is impaired after transplantation, unopposed proliferation of B cells may occur, leading to malignant transformation of haemopoietic cells (Opelz and Dohler, 2010). Although 80% of all PTLD are of B-cell lineage (most of which are EBV related), the remaining 10–15% are of T-cell lineage, with approximately 30% related to EBV infection.

Pre-transplant EBV seronegativity is an important predictor for the development of PTLD, particularly when the recipient receives a graft from an EBV-positive donor. Transmission of EBV infection from such donors to EBV-naïve patients is common among kidney transplants. Primary EBV infection increases the risk of early PTLD post transplant by at least 10–76-fold compared to those without a history of primary infection (Quinlan et al., 2011). Intensive and aggressive immunosuppression is also an important risk factor for PTLD. Retrospective studies have suggested a greater incidence of PTLD among those who have received tacrolimus-based immunosuppression compared to those on ciclosporin-based maintenance immunosuppression, with a greater risk in the paediatric population. Other significant risk factors include prior use of antithymocyte/antilymphocyte globulins as antirejection therapies, the number of human leucocyte antigen (HLA) mismatches, older recipient age (> 65 years), and patients on higher doses of triple and quadruple immunosuppression therapies. The newer immunosuppressive agents such as the monoclonal antibodies, alemtuzumab, and the selective co-stimulatory blocker, belatacept, have been associated with a greater risk of fatal PTLD among transplant recipients (Vincenti et al., 2010). The risk of PTLD also appears to be the greatest during the first 12–36 months post transplant (Larsen et al., 2010; Muzaffar et al., 2010). The risk of PTLD is 25% in the first 3.5 years after transplantation, declining to < 4.4% in the subsequent 5 years. In Australia and New Zealand, the pattern of increased risk appears to be bimodal, with the greatest increase in the first 2 years and peaks again 5–10 years after transplantation (Faull et al., 2005).

The prognosis of PTLD is poor with an overall 1-year mortality rate of at least 40%. Although the incidence of PTLD among the paediatric transplant population is higher than their adult counterparts, the overall outcomes are much more favourable than adults transplant recipients with PTLD, with a 1- and 5-year patient survival of 90% and 87%, respectively (Opelz and Dohler, 2010). On the contrary, the outcomes of adult transplant recipients are poor, with an overall 5-year survival rate of < 69%. The disparate outcomes may be explained, in part, by the temporary nature of the EBV infections in children, disappearing with the reduction in immunosuppression, and antiviral treatment. In the adult population, many of the cases are EBV negative, and therefore less responsive to immunosuppression reduction and chemotherapeutic treatment.

Prevention and screening

Evidence to promote effective preventive and screening strategies for PTLD among transplant recipients are lacking. Many centres have advocated that only EBV-negative grafts should be transplanted to EBV-naïve recipients, but others have argued against such a policy because of equity and allocation issues with prolongation of waiting time on the deceased donor list among EBV-seronegative transplant recipients. Given that high viral loads have been reported in patients with PTLD, some have proposed measuring EBV DNA on peripheral blood as a means of early PTLD detection in immunocompromised patients (Tsai et al., 2002). However, conclusive recommendations for screening for PTLD cannot be made because the ideal screening assay, the defined thresholds where the increased risk of PTLD occurs, and the treatment benefits of early detection are unknown.

Management

Reduced immunosuppression has been the empiric treatment for the treatment of PTLD in kidney transplant recipients.

Other proposed strategies such as surgery, limited field irradiation, antiviral treatment such as aciclovir, interferon (IFN) alpha, and other cytotoxic chemotherapy had all been reported as potentially useful agents in inducing remission in patients with PTLD. The newer monoclonal anti-B cell therapy, rituximab, has resulted in complete remission rates of 30–60% in the treatment of this disease. In patients who are refractory to immunosuppression reduction, cytotoxic chemotherapy such as the cyclophosphamide–doxorubicin–vincristine–prednisone (CHOP) or a reduced intensity regimen may be of some benefit (Svoboda et al., 2006; Trappe et al., 2009, 2012). However, most patients experience toxic side effects such as pancytopenia and the treatment efficacy is poor, with an overall 1-year mortality rate ranging from 33% to 70%.

Genitourinary cancers

There are three groups of genitourinary cancers—anogenital carcinomas such as cancers of the vulva, cervical cancers, and cancers of the urinary tract—the incidence of all of which is increased in kidney transplant recipients compared to those without kidney transplants. The HPV and herpes simplex virus (HSV) both play an important aetiological role in the development of cervical and anogenital cancers. The four major oncogenic stains of HPV infections—HPV genotypes 6, 11, 16 and 18—account for > 70% of all HPV-related cervical malignancies (Castellsague, 2008; Lowy et al., 2008). The risk of developing cervical cancers in transplant recipients is at least 10–15-fold greater than the matched general population, and it contributes 11% of all post-transplant cancers in women (Vajdic et al., 2006). Although the increased risk related to HPV infections and subsequent cervical cancers is well established in the transplant population, the natural history and the rate of disease progression appears to vary. Post-transplant malignancies are thought to be a late complication of long-term immunosuppression, but cervical cancers can occur as early as 2 years after kidney transplantation.

Although the overall prevalence of anal cancer in the general population is low, accounting for < 1.5% of all cancers of the gastrointestinal tract, it is a significant and important disease in men with kidney transplants. The overall prevalence of anal intraepithelial carcinoma, the precursor of anal cancer, in the kidney transplant population is at least 20%, with the prevalence of HPV infections among prevalent and incident transplant recipients being 47% and 23%, respectively. The relative risk of anal cancer among recipients of kidney transplants is at least 10-fold greater than the age- and gender-matched general population. Vulval cancer, which is a rare disease in the general population, is increased by at least 15–100 times compared to the age- and gender-matched general population. Similar to cervical and anal cancers, vulval cancers also appeared to be HPV related (Ozsaranet al., 1999).

Cancers of the urinary tract are the most common solid organ cancers in kidney transplant recipients. The risk of developing urinary tract cancers in kidney transplant recipients is high, particularly among those with a history of analgesic nephropathy, a prior history of urinary tract cancer, and acquired cystic disease of the kidneys (Stewart et al., 2003; Zavos et al., 2007). The relative risk is approximately 7 and 300 times higher for cancers of the kidneys and the ureters, respectively, in the kidney transplant population compared to people without kidney transplants. The average age of initial diagnoses is also younger (by at least 10 years) than in the general population, with a mean age of 50 years compared to 63 years in patients without a kidney transplant. The median time to cancer diagnoses is 77 months after transplantation. Compared with the general population, disease- and stage-specific survival for cancers of the urinary tract is also poor, with an average survival of < 40%, among those with more advanced-stage disease (stages III and IV) (Reinberg et al., 1992).

Prevention and screening

Better understanding of the casual relationship between HPV infection and cervical cancer has led to the development of HPV vaccines and the initiation and funding of national HPV vaccination programmes for the primary prevention against oncogenic HPV 6,11, 16, and 18 infections for cervical dysplasia in Australia and worldwide. Despite proven benefits in the general population, the effects of HPV vaccination for the prevention of cervical dysplasia after transplantation are unknown. Under the influence of long-term immunosuppression, B-cell suppression is expected, so a decline or a reduction in vaccine-induced immunity is predicted in transplant recipients.

Screening for cervical cancer using conventional or liquid-based cytology is now standard practice for adult women in the general population. Previous modelled analyses have reported that the current recommendation of annual cervical cancer screening using Pap conventional cytology is effective in reducing cancer-specific mortality and should be recommended to all adult women with kidney transplants (Wonget al., 2009).

Screening for urinary tract cancers using ultrasonography may be useful for high-risk patients such as those with a history of analgesic or aristolochic acid use, or with a history of acquired cystic disease of the kidneys. A major concern associated with ultrasonographic screening is the test performance characteristics of the screening tool. The accuracy of ultrasonography is an important determinant of screening efficiency, but is uncertain in recipients of kidney transplants. Ultrasonography is not only operator dependent but performance varies with the size and shape of the patient, the kidneys, and the tumour. The difficulties associated with ultrasonographic screening in people with chronic kidney disease include the effect of multicystic diseases and small scarred native kidneys on the overall test accuracy and poor reliability in differentiating small hyperechoic renal cancers from lesions such as adenomas, oncocytoma, and angiomyolipomas (Wong et al., 2011).

Management

Other than judicious immunosuppression reduction in transplant recipients with a prior history of cancer, conversion to mTORi-based immunosuppression may be indicated in recipients with a prior diagnosis of renal cancer. mTORi had been shown *in vitro* to possess antioncogenic and antiangiogenic properties that may inhibit angiogenic-stimulated tumour growth by interference with the vascular endothelial growth factor-related pathways (Gutierrez-Dalmau and Campistol, 2007). Clinically, there is emerging evidence showing potential anticancer effects in patients with advanced stage renal cancer. A recently published phase III trial that compares everolimus, a type of mTORi, against placebo for the treatment of advanced stage renal cancer in patients without kidney transplants have shown a reduction in the risk of cancer progression and deaths associated with the renal cancer (Amato

et al., 2009). mTORi, however, should be used with caution and consideration of all other potential adverse side effects such as poor wound healing, dyslipidaemia, pneumonitis, proteinuria, and infectious complications.

Other solid organ cancers

Lung and colorectal cancer are also common in kidney transplant recipients, but the magnitude of the increased risk is much less than that of the virus-related neoplasms. Transplant recipients are at increased risk of lung and colorectal cancer by 3.5- and 2.5-fold, compared to the age- and gender-matched general population (Vajdic et al., 2006). Apart from the increased cancer risk, a few recent reports have shown that solid organ cancers after transplantation are generally more aggressive and have much poorer outcomes than those without transplants. A recent case–control study evaluating the characteristics of colorectal cancer in kidney transplant recipients reported a significantly higher incidence of cancer recurrences compared to those with colorectal cancer alone (35.2% vs 15.2%; P = 0.025). In addition, the 2-year patient survival rate of the transplant group was also significantly worse than those without a transplant but with advance staged colorectal cancer (stages III–IV; 45.7% compared to 71.6%, P = 0.023) (Kim et al., 2011). Lung cancers in transplant recipients are often aggressive, present at a much later stage, and are not amenable to surgical treatment. In addition, there is also an increased incidence among non-smoking transplant recipients compared to non-smokers in the general population. The mean duration of patient survival after the diagnosis of lung cancer is 14 months, but is stage dependent and varies from <1year to 5 years.

Prevention and screening

Recommendations for screening colorectal and lung cancers are not standardized across the different transplant guideline groups. For example, the American Society of Transplantation (AST) recommends annual faecal occult blood testing (FOBT) and flexible sigmoidoscopy every 5 years. In Australia, biennial screening using immunochemical faecal occult testing (iFOBT) is the screening strategy recommended by the National and Medical Research Council (NHMRC) of Australia. In Europe, the European Best Practice Guidelines (EBPG) suggested annual screening for all transplant recipients using iFOBT (Wong et al., 2008a) followed by diagnostic colonoscopy. Although there is no trial-based evidence for screening in this at risk population, previous modelled analyses using information from the general and transplanted population have shown that screening for bowel cancer is probably effective. However, uncertainties exist over the costs, the test specificity, and patient preferences for the specific screening modalities and frequencies (Wong et al., 2008b). A recent diagnostic test accuracy study conducted in an Australian cohort of transplant recipients suggested the test sensitivity of iFOBT screening may be as low as 36% compared to the expected test sensitivity of at least 75% in the non-transplanted populations (Collins et al., 2012).

Donor transmission of cancer

The potential for donor transmission of malignancy was recognized early in the history of kidney transplantation, following early reports that cancer occurred in recipients, either in the transplanted kidney or at other sites, in subjects who received apparently normal kidney transplants from donors with cancer (Martinet al., 1965). However, the frequency of donors with malignant tumours and the risk of transmission of malignant diseases from donors to recipients are not known. Considering transmission risk using registry data, between 1994 and 2000, the United Network for Organ Sharing (UNOS) described six donor-transmitted malignancies from deceased donors (4 per 10,000 donors) and two from living donors (Myron et al., 2002). The deceased donors transmitted their tumours to 13 of the 108,062 recipients (1 transmitted tumour for each 8312 transplanted organs) and two recipient cases arose from the two living donors. Of the total 15 transmitted tumours, histology was declared as follows: adenocarcinoma (1), breast cancer (1), lung cancer (2), melanoma (4), neuroendocrine tumour (1), non-differentiated squamous carcinoma (1), oncocytoma (1), pancreas cancer (1), papillary tumour (1), prostate cancer (1), and small cell carcinoma (1). During this same time period, there were also six document cases of 'donor-derived' cancer in recipients which arose in donor tissue after transplantation (PTLD and leukaemia). Including the donor-transmitted and the donor-derived cancers, the interval from transplantation to diagnosis was 3–40 months (mean 14.2 months), and the mortality rate 38%.

In 2011, the World Health Organization, the Italian National Transplant Centre (CNT), and the European Union-funded Project 'Vigilance and Surveillance of Substances of Human Origin' collaborated to organize a global initiative aimed at raising the profile of vigilance and surveillance of 'substances of human origin'; the initiative was called Project NOTIFY (<http://www.notifylibrary.org/>). As part of this initiative, a malignancy working group focussed on transmission of malignancies, and this included consideration of malignancy transmitted with solid organ transplantation. After systematically reviewing the published literature (including registry data, cohort studies, and case reports) the key messages that the group generated were that data derived from transplant registries have to be interpreted with caution, because of their voluntary nature, variations in reporting rates, epidemiological differences between donors populations, and disparities in the design and the quality and accuracy of the information recorded. They concluded that donors with a previous history of malignancy were not rare (approximately 1.7% of all donors), but donor-transmitted malignancies were infrequent (approximately 2 cases per 10,000 organs transplanted). Rates of malignancy transmission varied depending on the histological type of tumour, stage, and grade, with recorded donor-transmitted malignancies generally involving more clinically aggressive cancers. The conclusion was that the observed risk of transmitting cancer was very small when appropriate standards of donor selection were applied and an individualized risk:benefit analysis performed.

To rate a donor as a standard risk is difficult because there is no consensus on the waiting interval following successful treatment (to define the donor as clinically cancer free). Project NOTIFY made the general statement that waiting time depends upon tumour type, grade and stage, and the situation of the recipient. There have been no documented cases of transmission of malignancy from low-grade central nervous system tumours, *in situ* (non-invasive epithelial tumours which have not crossed the basal lamina) lesions of the cervix and colon (after treatment), and non-melanoma skin cancers. Tumours regarded as high risk of transmission even after significant (10 years) disease-free intervals and apparent curative

treatment in a donor are malignant melanoma, sarcoma, and breast cancer diagnosed beyond grade T1b. For most other cancer sites, the suggested waiting interval after treatment is completed before considering donation to be 'standard risk' is between 5 and 10 years. For more details, the Project NOTIFY documents (<http://www.notifylibrary.org/>) should be consulted.

Transplantation in people with pre-existing cancer

An increasing number of potential transplant candidates have a history that includes a previous malignancy. This is in part because the prevalence of treated end-stage kidney disease has increased markedly over recent decades, and because a growing proportion of people treated with dialysis are > 65 years of age. Dialysis patients have an increased risk of cancer compared to the general population, overall and at most cancer sites. Assessing a potential transplant candidate with a prior cancer creates an ethical and clinical dilemma at both patient and population level. Transplantation has survival and quality of life advantages over dialysis for most patients, but the demand for cadaveric donor organs outstrips availability, so clinicians have to direct this scarce resource to reap maximum population benefit. At the patient level, a clinician must judge when the risk of transplantation, which increases risk of cancer recurrence and of *de novo* malignancy, outweighs the risk of remaining on maintenance dialysis, which would leave cancer recurrence risk unaltered, but may potentially offer decreased overall survival.

Current clinical practice guidelines suggest that potential transplant recipients with a history of cancer should wait a period of 2–5 years (depending on cancer site and characteristics) without recurrence before undergoing transplantation (Kasiske et al., 2001). The evidence base for these guidelines is limited because there are few reports of clinical experience in contemporary transplantation and based on this practice.

An analysis of the Cincinnati Transplant Tumour Registry in 1997, examined the recurrence rate in 1258 people with 1297 pre-existing malignancies transplanted predominantly in the 1980s and early 1990s, when clinical decision-making and immunosuppression protocols were quite different to the present (Penn, 1993). For those patients diagnosed and treated before transplantation, 239 (21%) of the 1137 recipients subsequently experienced cancer recurrence or *de novo* cancer of the same histological type, 67 of these were non-melanoma skin cancers. Although recurrence generally was less likely to occur with a longer time from treatment of the cancer to transplantation, 13% of the recurrences occurred in patients who had been treated > 5 years before transplantation. Tumours with high recurrence rates were breast cancer, where of 90 patients, half of whom had been treated > 5 years prior to transplantation, showed a 23% recurrence rate; renal cancers, diagnosed when symptomatic (22% recurrence rate from 222 recipients); and multiple myeloma which showed a 67% lapse rate. However, the majority of myeloma patients were not in complete remission when transplanted (9 of 11).

An analysis of a similar era (1963–1999) from the Australian and New Zealand Dialysis and Transplant Registry (ANZDATA) showed different findings. Cancer recurred in 11 of 210 (5%) recipients with a known non-skin cancer diagnosis prior to transplantation (Chapman et al., 2001). The differences in these findings are likely to be largely due to the different reporting nature of the two registries; ANZDATA is population based and incorporates all subjects transplanted in Australia and New Zealand, where the Cincinnati Transplant Tumour Registry relies on voluntary reporting.

In clinical decision-making, the impact of a history of cancer on patient management is likely to be determined by the availability of alternative options, including dialysis experience and the possibility of a living kidney donor transplant. The various clinical practice guidelines, although relying on sparse contemporary evidence, are fairly consistent in their recommendations. To summarize, no waiting interval is necessary for a tumour at low risk of recurrence, such as incidentally discovered renal carcinoma < 2 cm, *in situ* carcinoma, basal cell and squamous cell skin carcinomas, and low-grade bladder cancer. For most other tumours the recommended waiting interval after completion of treatment is at least 2 years, except for cancers regarded as high risk for recurrence diagnosed beyond the *in situ* stage, where at least a 5-year wait is suggested. High-risk tumours include melanoma, breast, colorectal, and renal tumours of > 5 cm (Kasiske et al., 2001). All patients proceeding to transplantation after a prior cancer should be counselled that their risk of *de novo* non-skin malignancy at any site is increased by 40% above other recipients (Webster and Wong, 2008).

Survival after post-transplant malignancy

The impact of cancer on post-transplant survival has not been widely studied. Investigation of survival in transplant recipients must consider the competing risks of death from other causes such as cardiac disease, vascular disease, and other comorbidities. Analysis of the United States Renal Data System (1990–2004) looking specifically at cancer deaths, showed that when compared to the general population, risk of death for younger recipients with cancer was very high, but that this effect reduced with age, such that for older recipients the death rates were similar or lower, suggesting these recipients were dying of causes other than cancer (Webster and Wong, 2008; Kiberd et al., 2009). Consistent with this hypothesis, older age, diabetes, and prior history of congestive heart failure and stroke were independently associated with lower cancer mortality.

Analysis from Australia and New Zealand using the ANZDATA registry used a different approach, and instead of attributing cause of death, compared death rates among four groups: those with a transplant but no cancer, those with a transplant and cancer, those with cancer but no transplant, and those with neither transplant nor cancer (i.e. the general population) between 1988 and 2005 (Webster and Wong, 2008). After standardizing for differences in age, sex, and calendar year, death rates were compared (Fig. 287.1). As expected, there were differences in survival for men and for women. Death rates for people with a transplant and no cancer were similar to the general population with cancer (but no transplant), and approximated the death rates of people 30 years older from the general population. Within the transplant population, a cancer diagnosis increased the risk of early death more than fourfold.

An alternative way to consider cancer-related mortality in the transplant population is to examine relative survival. This is calculated as the ratio of observed cancer in the transplant population compared to expected survival in the general population of the same age and sex, over the same time period, thus standardizing for any differences. A ratio of 1 indicates survival equivalent to the

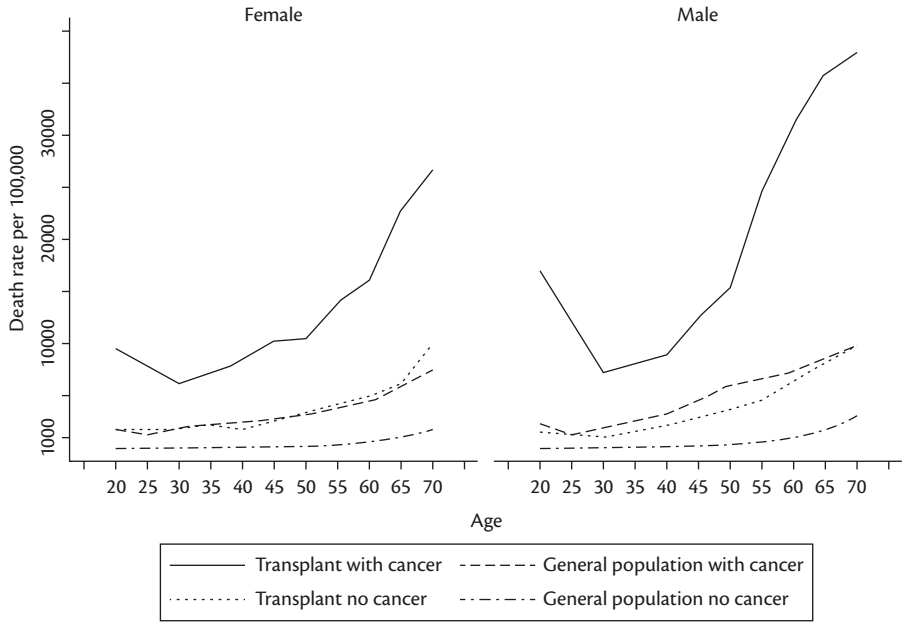

Fig. 287.1 Mortality with and without cancer after kidney transplantation and for the general Australian population; indirect standardization by age, sex, and calendar year.

general population and ratios < 1 lower survival (higher mortality). An analysis from the ANZDATA registry considered expected survival for transplant patients with breast or colorectal cancer compared to transplant recipients without cancer, and compared it with people in the general population diagnosed with breast or colorectal cancer. Results are shown in Fig. 287.2. The effect of co-morbidity with a kidney transplant and cancer was pronounced overall and for all age subgroups, with poorer relative survival compared with the transplant alone and the cancer alone groups. For example, a woman aged 50–59 with breast cancer experienced 14% excess mortality compared with expected background mortality in the general population, a woman of the same age with a transplant experienced 16% excess mortality, and a woman with both a transplant and a breast cancer experienced 48% excess mortality. For men with colon cancer aged > 55 years, the 5-year relative survival was 0.79 with a transplant alone, 0.57 with colorectal cancer alone, but 0.27 with transplant plus colorectal cancer (73% excess mortality compared to general population expectations).

Conclusion

Cancer is a major cause of mortality and morbidity in kidney transplant recipients. Despite established evidence to suggest cancer incidence increases after transplantation, information about screening, treatment, and monitoring strategies for this at-risk population is limited and is largely extrapolated from information in the general population. The newer immunosuppressive agents such as mTORi may have antioncogenic and antiproliferative properties,

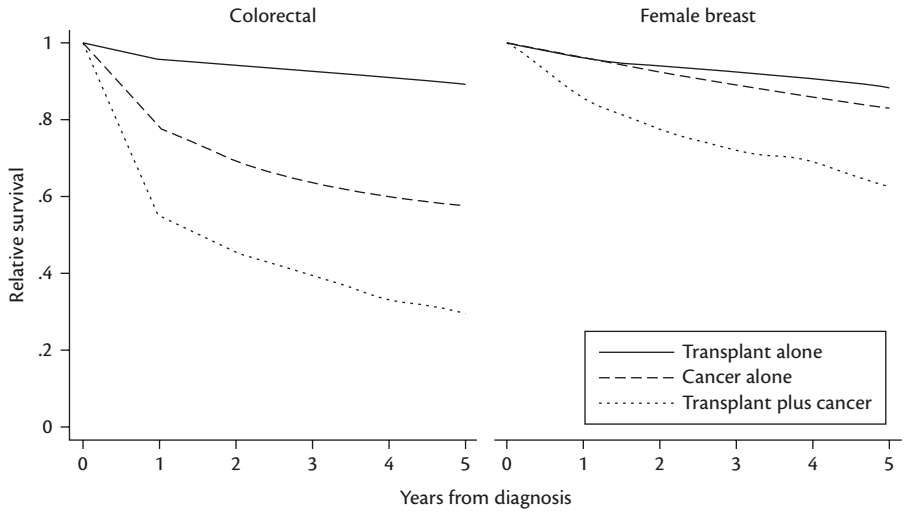

Fig. 287.2 Relative survival estimates for people with breast (females) or colorectal cancer, comparing people with transplant, with cancer, or with both transplant and cancer in Australia.

but evidence from well-powered randomized controlled trials assessing the longer-term cancer outcomes in transplanted patients is necessary to inform clinicians and healthcare professionals about the risks and benefits of these agents as standard maintenance immunosuppression.

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CHAPTER 288

Metabolic bone disease after renal transplantation

Grahame J. Elder

Introduction

All patients who undergo kidney or simultaneous pancreas-kidney (SPK) transplantation have components of chronic kidney disease mineral and bone disorder (CKD-MBD), a cluster of biochemical and bone abnormalities, often accompanied by vascular and soft tissue calcification (Kidney Disease Improving Global Outcomes (KDIGO) CKD-MBD Work Group, 2009). These changes may be superimposed on skeletal abnormalities such as osteoporosis that develop independently of CKD. Following transplantation, persisting hyperparathyroidism, vitamin D deficiency, glucocorticoid therapy, immobility, hypogonadism, and reduced renal function can contribute to abnormal bone turnover and mineralization, reduced bone volume, and loss of structural integrity (Fig. 288.1). Common consequences are bone or muscular pain that reduces mobility, fractures that are associated with increased mortality, and avascular necrosis of bone that may require joint replacement. These complications are likely to be reduced by good pre-transplant management and evaluation in the early post-transplant period to identify patients at highest risk.

Background

CKD-MBD develops as early adaptive responses to maintain mineral homeostasis become maladaptive, when regulatory feedback loops fail with advancing CKD. As nephron loss occurs there is an increase in the requirement for remaining nephrons to excrete phosphate, so that phosphate homeostasis is maintained (Bricker et al., 1960). This is achieved by increased secretion of the phosphaturic hormone fibroblast growth factor 23 (FGF23) from osteocytes and osteoblasts, with levels rising progressively as renal function declines (Isakova et al., 2009). FGF23 also reduces synthesis of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) from its substrate 25-hydroxyvitamin D (25(OH)D) and increases its catabolism. As $1,25(OH)_2D$ values fall, parathyroid hormone (PTH) levels rise in many patients, although this response may be modified by drugs such as calcium-based phosphate binders, calcitriol or its analogues, and the dialysate prescription.

Interwoven with these laboratory changes, most patients on dialysis have renal osteodystrophy (ROD), defined by abnormalities of bone turnover, mineralization, and volume. ROD reduces the quality and strength of bone by impairing its structural integrity and altering its material properties. Fracture risk and fracture-related mortality increase with progressive CKD (Dooley et al., 2008; Nitsch et al., 2009), but once on dialysis this risk increases dramatically. Patients under age 45 are estimated to have a fracture risk of 90:1 compared to the general population, and the risk is estimated to be 22:1 from age 45 to 54 (Coco and Rush, 2000; Ott, 2009). These patients share traditional risk factors for fracture with the general population, such as older age, female gender, low body mass index (BMI), previous fracture and the use of psychoactive medications. However, additional risk factors include the length of time on dialysis and elevated or suppressed values of PTH and alkaline phosphatase (ALP), indicating an increased risk of microarchitectural change and abnormal bone turnover (Toussaint et al., 2010). Other biochemical markers used to assess bone turnover in patients with CKD include bone-specific ALP (b-ALP) derived from osteoblasts, procollagen type 1 N-terminal propeptide (P1NP) indicating type 1 collagen synthesis, tartrate-resistant acid phosphatase 5b (TRAcP 5b) reflecting osteoclast numbers, and biomarkers of collagen breakdown indicating osteoclastic resorption of bone.

Based on bone biopsy studies, around 62% of Caucasian on dialysis have low bone turnover, whereas around 68% of African American patients have normal to high turnover (Malluche et al., 2011). These changes are often associated with abnormalities of bone volume and less frequently mineralization.

Laboratory changes after transplantation

When patients with CKD-MBD undergo successful transplantation, tubular targets for PTH and FGF23 are suddenly restored, but these hormones are inappropriately raised for their new environment. Simultaneously, drugs used to control PTH levels such as the calcimimetic cinacalcet or calcitriol and its analogues are often withdrawn, and most patients commence glucocorticoid treatment. Longer term, post-transplant disturbances of bone and mineral metabolism correlate strongly to transplant kidney function (Ambrus et al., 2009).

Parathyroid hormone

Values of PTH measured by intact-PTH (iPTH) assays fall immediately as renal function improves, and by 4–6 weeks are approximately 40% of pre-transplant values (Fig. 288.2A). However, this reduction may not be mirrored by corresponding changes in PTH activity, because 'intact' assays measure not only biologically

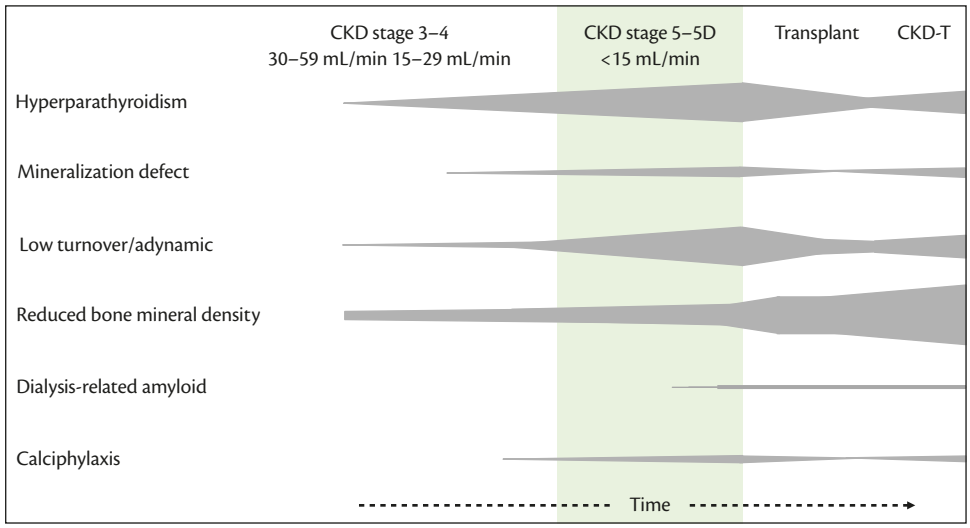

Fig. 288.1 Development of bone and mineral disorders with progressive CKD and following transplantation.
Adapted with permission from Elder G. Pathophysiology and Recent Advances in the Management of Renal Osteodystrophy, *Journal of Bone and Mineral Research*, pp. 2094–2105, Copyright © 2002.

active PTH 1-84, but also long C-terminal fragments that accumulate in CKD and are cleared once renal function improves. By contrast, when cinacalcet treatment is stopped at the time of transplantation, early post-transplant iPTH values may be stable or even rise, This 'rebound hyperparathyroidism' can lead to an increase in post-transplant values of calcium and calcitriol and lower phosphate values (Evenepoel et al., 2012). Although PTH values measured by intact and 'biointact' PTH 1-84 assays are generally lower 3 months post transplant, hyperparathyroidism is slow to regress if glandular hyperplasia has developed, because cell turnover is low and the lifespan of parathyroid cells is approximately 20 years. Levels of iPTH often remain above the normal range 12 months post transplant (Fig. 288.2A), and persistent hyperparathyroidism is predicted by more severe pre-transplant

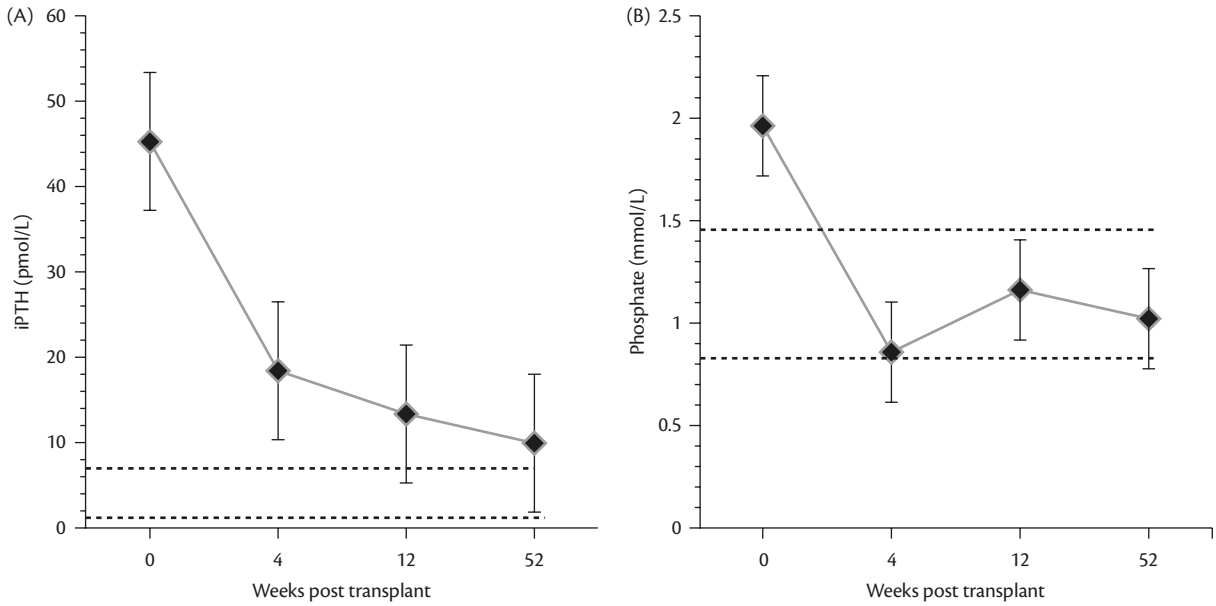

Fig. 288.2 Laboratory values (mean ± standard error) immediately before and at 4, 12, and 52 weeks after transplantation. Patients were treated according to the algorithm (Fig. 288.3). Dashed lines indicate upper or lower values of the normal range. Shaded histograms: kidney transplants; unshaded histograms: simultaneous pancreas-kidney (SPK) transplants. Note x-axes are not to scale. (A) Serum intact parathyroid hormone (normal range 1.0–6.8 pmol/L). (B) Serum phosphate (normal range 0.81–1.45 mmol/L). (C) Male calculated free testosterone levels (normal range 120–470 pmol/L). (D) Serum 25-hydroxyvitamin D (adequacy ≥ 50–75 pmol/L). Levels are lower in patients undergoing SPK than kidney transplants. Patients with inadequate levels were supplemented from 4 weeks. (E) Serum $1,25(OH)_2D$ (in-house normal range 36–120 pmol/L). By 12 weeks, levels approach or exceed the assay's normal upper range. (F) The bone resorption marker urinary deoxypyridinoline to creatinine ratio (urine DPD/creatinine) (normal range 3–7.4 nmol/mmol creatinine).

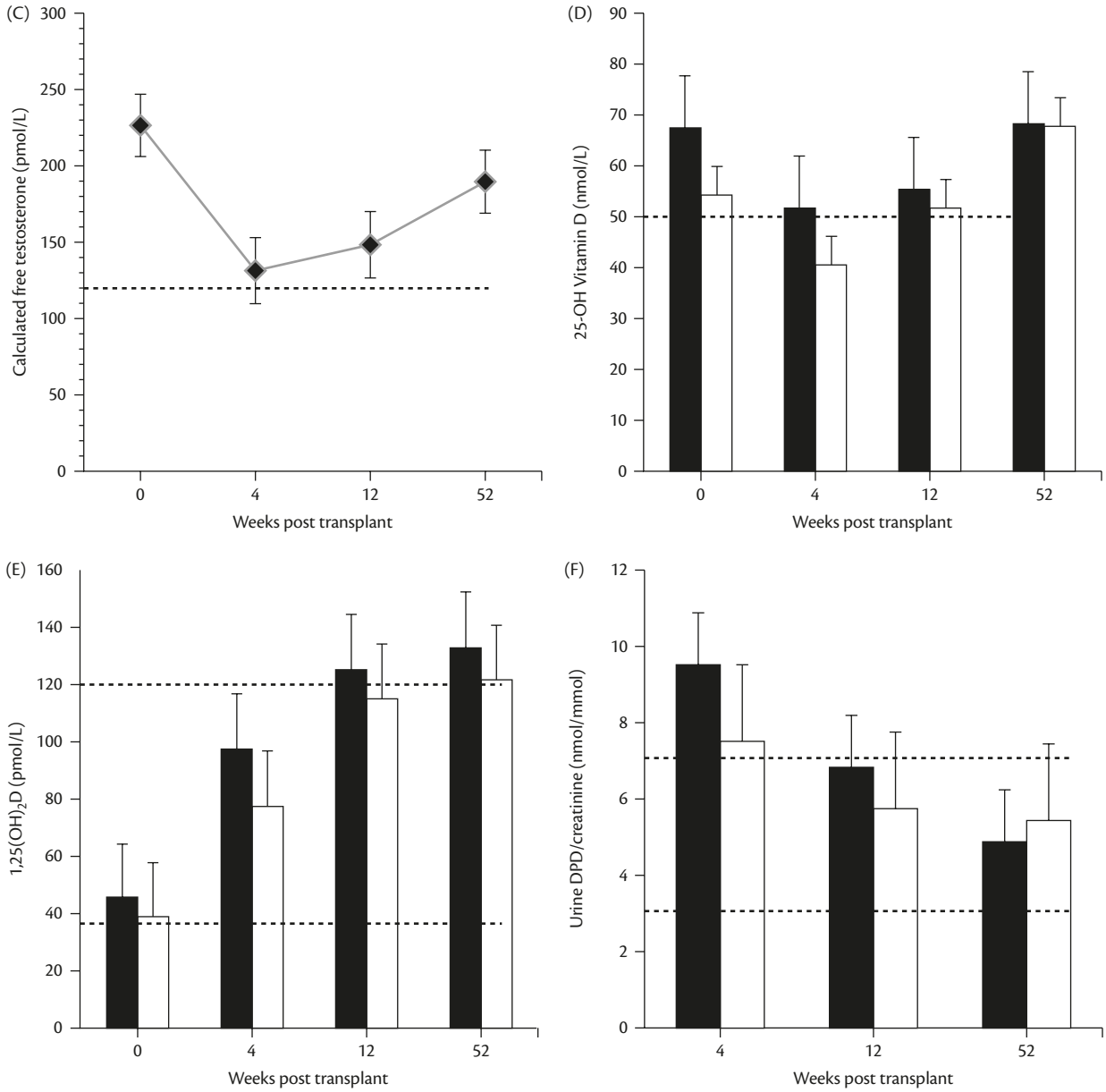

Fig. 288.2 (Continued)

hyperparathyroidism and longer dialysis vintage. A study from the United States reported that 24% of kidney recipients (estimated glomerular filtration rate (eGFR) 40–60 mL/min/1.73 m^2) had iPTH values more than twice the normal upper range of the assay (Egbuna et al., 2007) and even 7 years post transplant, only 27% of a Swiss cohort of 823 patients had normal PTH values (Yakupoglu et al., 2007). Persisting hyperparathyroidism influences vitamin D metabolism directly by inducing 1-alpha hydroxylase activity and the conversion of 25(OH)D to $1,25(OH)_2D$. Hyperparathyroidism influences serum calcium by increasing tubular calcium reabsorption and osteoclastic resorption of bone, and serum phosphate by directly reducing tubular phosphate reabsorption, and indirectly by stimulating FGF23 production. Of course, post-transplant hyperparathyroidism can also be 'appropriate', to maintain normal values of serum phosphate and calcium, particularly when there is continued end organ resistance due to post-transplant CKD.

FGF23

After transplantation, FGF23 levels fall to around 46% of pre-transplant levels during the first week (Bhan et al., 2006) and to 5% by 3 months (Evenepoel et al., 2007). Nevertheless, 3-month levels remain above the normal range in around 60% of patients. Patients with higher FGF23 levels before transplantation are those most likely to have sustained post-transplant elevations; a condition that has been termed tertiary hyperphosphatoninism (Bhan et al., 2006). This is possibly caused by osteocytes and osteoblasts developing resistance to suppression of FGF23 synthesis during prolonged exposure to elevated values of phosphate and PTH,

analogous to the calcium set-point shift that occurs with the development of parathyroid hyperplasia.

Calcium

Hypercalcaemia, measured as total or corrected serum calcium, is present in 8% of our local patients awaiting transplantation with little change over the first transplant year. While similar percentages were reported in a US study (Egbuna et al., 2007), corrected serum calcium was elevated in 40% of recently transplanted recipients and 25% of patients more than a year after transplantation in a study from the United Kingdom (Stavroulopoulos et al., 2007). Total serum calcium values may underestimate hypercalcaemia defined by ionized calcium, because of a high prevalence of metabolic acidosis; in fact, hypercalcaemia, defined by ionized calcium >1.29 mmol/L, has been reported in 59% of patients at 3 months and 44.8% at 12 months, despite elevated total serum calcium values being observed in only 13.1% of these patients (Evenepoel et al., 2010). The most common cause of post-transplant hypercalcaemia is persisting hyperparathyroidism, which increases bone turnover, tubular reabsorption of calcium, and calcitriol production. In this setting, bisphosphonate therapy may prove ineffective in reducing calcium levels, because any reduction in serum calcium caused by suppression of bone resorption may increase PTH levels, and, as a consequence, renal and gastrointestinal calcium absorption. In this situation, 'off-label' treatment with cinacalcet will often return serum calcium values towards the normal range. Interestingly, bone biopsy studies do not show consistent increases in bone turnover when hypercalcaemic hyperparathyroidism is present, supporting the important role of these other mechanisms. However, when ALP and osteocalcin levels are elevated (indicating increased osteoblast activity), high bone turnover is generally present (Borchhardt et al., 2007).

Phosphate

Hypophosphataemia occurs in up to 93% of patients after kidney transplantation (Ambuhl et al., 1999; Levi, 2001), reaching a nadir around 4 weeks and generally returning to the normal range by 12 months (Fig 288.2B). Nevertheless, a urinary phosphate leak may persist, causing ongoing hypophosphataemia in up to 22% of patients 7 years post transplant (Felsenfeld et al., 1986; Ghanekar et al., 2006; Yakupoglu et al., 2007). Calcineurin inhibitors and glucocorticoids have been associated with urinary phosphate wasting (Graf et al., 1979; Loffing et al., 1998; Falkiewicz et al., 2003) but this is not a feature of other solid organ transplants, suggesting facilitation by tubular mechanisms. While pre-transplant PTH levels correlate to post transplant hypophosphataemia and phosphate excretion (Evenepoel, 2007; Trombetti et al., 2011), hypophosphataemia also occurs when PTH levels are low or normal (Graf et al., 1979; Rosenbaum et al., 1981; Parfitt et al., 1986; Green et al., 2001). In such cases, tertiary hyperphosphatoninism is likely to be the principal cause, with levels of FGF23 inversely related to the phosphate levels. In fact, even when PTH levels are normal or elevated, FGF23 may accentuate hypophosphataemia or be its principal cause (Bhan et al., 2006; Evenepoel, 2007; Trombetti et al., 2011). Hypophosphataemia can be treated cautiously with phosphate supplementation, but high doses of phosphate or calcitriol may induce FGF23 secretion, reducing the effectiveness of phosphate therapy (Ito et al., 2005).

25-Hydroxyvitamin D

Many patients undergoing transplantation have low levels of 25(OH)D. While there is no consensus on definitions of vitamin D deficiency and insufficiency, values below 25 nmol/L or 50 nmol/L are generally considered deficient, and from 25–50 nmol/L or 50 – 75 nmol/L insufficient. For patients on dialysis, values of 25(OH)D are lower for women, patients with diabetes or on continuous ambulatory peritoneal dialysis (Elder and Mackun, 2006), and are lower skin pigmentation is darker. After transplantation, further reductions of 25(OH)D values are often seen by 4–12 weeks (Fig 288.2D), as persisting hyperparathyroidism facilitates the tubular conversion of 25(OH)D to calcitriol and elevated levels of FGF23 facilitate its catabolism (Wesseling-Perry et al., 2013). Avoidance of sun exposure contributes to suboptimal levels of 25(OH)D in patients who remain unsupplemented, and in one study, up to half of renal transplant recipients over 1-year duration were reported to have levels of 25(OH)D below 40 nmol/L, while 5% had levels in the severely deficient range below 12 nmol/L (Stavroulopoulos et al., 2007).

1,25-Dihydroxyvitamin D

At the time of transplantation, most patients have low $1,25(OH)_2D$ levels, but these improve rapidly after transplantation (Bhan et al., 2006; Evenepoel, 2007), with only 13% of local patients recording subnormal values at 4 weeks, 5% at 12 weeks, and 1% at 12 months (Fig. 288.2E). Despite this, some studies have reported $1,25(OH)_2D$ values to be lower than predicted from ambient low phosphate and elevated PTH values, which should stimulate $1,25(OH)_2D$ synthesis providing allograft function is normal (Riancho et al., 1988; Steiner et al., 1993; Claesson et al., 1998). Tertiary hyperphosphatoninism may contribute to lower than predicted values, because in studies evaluating levels of FGF23, an inverse relationship has been reported to $1,25(OH)_2D$ levels after adjusting for PTH and 25(OH)D (Bhan et al., 2006). At 3 months post transplant, FGF23 and creatinine (inverse) and PTH (positive), are reported to account for 50% of the variation in levels of $1,25(OH)_2D$ (Evenepoel, 2007).

Sex hormone levels

Sexual dysfunction is common in patients on dialysis (Toorians et al., 1997), with abnormalities at all levels of the hypothalamic–pituitary–gonadal axis (Handelsman, 1985). From 22% to 66% of men with CKD are estimated to have testosterone deficiency (Iglesias et al., 2012) and elevated prolactin levels are common (Gomez et al., 1980). However, uraemic hypogonadism is reversible in most individuals after successful kidney transplantation (Palmer, 1999). Elevated prolactin levels are reported to decline towards the high normal range by the fourth post-transplant week (Shamsa et al., 2005) and testosterone levels improve after a fall over the first 4 weeks that coincides with higher glucocorticoid doses (Fig. 288.2C). Around 70% of pre-menopausal women report the resumption of a regular menstrual cycle following transplantation, and around 45% of these cycles are ovulatory, which is comparable to healthy women (Pietrzak et al., 2006). Oestradiol levels rise progressively from the time of transplantation to 12 months. In fact, compared to healthy women, increased levels of oestrogen have been reported in kidney transplant recipients, despite similar levels of serum follicle stimulating hormone, luteinizing hormone, and prolactin (Pietrzak et al., 2006).

Biochemical bone turnover markers

Measurement of turnover markers is often incorporated into evaluations of fracture risk in the general population, and may be useful in determining and monitoring treatment after successful transplantation. Levels show wide diurnal and dietary fluctuations (Hannon et al., 2004) and accuracy is improved by collecting a fasting, morning blood sample, or a second morning urine sample. Markers reflecting osteoblast activity and/or bone formation generally decline with high-dose glucocorticoids that induce osteoblast and osteocyte apoptosis, or after antiresorptive therapies. However, post-transplant levels may also rise, which may reflect increased skeletal responsiveness to PTH or, for patients with osteitis fibrosa, the maturation of inchoate pre-osteoblasts to osteoblasts due to reduced levels of PTH. Bone resorption markers are often elevated after transplantation and generally decline when PTH levels normalize, glucocorticoid doses are reduced, or following antiresorptive therapy (Fig. 288.2F).

Bone and fracture risk

Bone histomorphometry

The gold standard for evaluating bone is double tetracycline-labelled bone histomorphometry, but bone biopsy data following transplantation remains scarce. In general, bone densitometry and biochemical markers of bone turnover are used as poor surrogates. From available post-transplant bone biopsy studies, around 5–16% of patients are reported to have normal bone histomorphometry, with normal bone volume and turnover reported in 28%, mixed renal osteodystrophy reported most commonly, and adynamic bone or osteomalacia reported in 20–37% (Cueto-Manzano et al., 1999; Monier-Faugere et al., 2000; Rojas et al., 2003). A negative association has been reported between cumulative prednisone dosage and bone volume and turnover. A recent bone biopsy study of patients 2–5 years post transplant, reported normal bone histomorphometry in only 19% of patients (Neves et al., 2013). Bone turnover was normal in 48%, high in 26%, low in 26%. Mineralization was delayed in 48%, and bone volume was low in 37%. The complex nature of these bone changes is illustrated by typical changes of osteomalacia being found on trabeculae adjacent to others showing marked osteoclastic resorption and marrow 'fibrosis'.

Bone mineral density

Bone mineral density (BMD) is generally measured by dual-energy X-ray absorptiometry (DXA), and values predict fracture risk in the general population and following other solid organ transplants. BMD measurement is less informative in patients undergoing kidney transplantation, for whom the term 'low BMD' is often more appropriate than 'osteoporosis'. Early studies demonstrated rapid BMD loss over the initial 6–12 months after kidney transplant (Julian et al., 1991), but lower dose glucocorticoid regimens mitigate such rapid declines (Wissing et al., 2005; Nikkel et al., 2012). In fact, over 30% of kidney transplant recipients in the United States are discharged on no glucocorticoid (Luan et al., 2009). BMD loss after transplantation has been associated with both high and low levels of PTH, hypophosphataemia, and elevated FGF23 levels (Kanaan et al., 2010), and as in the general community, BMD decreases more rapidly with postmenopausal or hypogonadal status and with reduced physical activity. Calcineurin inhibitors may influence BMD indirectly, by causing impairment of renal function, a reduction in calcitriol levels, and increase in PTH, but their direct influence is contested (McIntyre et al., 1995; Josephson et al., 2004). It will be interesting to see whether use of the trabecular bone score, which is derived from DXA data, but reflects changes to bone microarchitecture or its deterioration not directly related to BMD, will provide additional insights into bone fragility after kidney transplantation.

Fracture risk

Fracture risk after kidney transplantation exceeds that of patients on dialysis (Ball et al., 2002) and fractures are associated with increased mortality (Abbott et al., 2001). Within 3 years of transplantation, the adjusted fracture incidence is 4.6 times that of the general population and the cumulative fracture incidence 15 years post transplant is 60% compared to an expected rate of 20% (Abbott et al., 2001; Vautour et al., 2004). Common fracture sites are the femur, ankle, and spine, and risk factors for post-transplant fracture include Caucasian race, female sex, CKD due to diabetes, low body weight, longer dialysis vintage, with a 4% increased fracture risk after transplantation for each year of dialysis before transplantation (Nikkel et al., 2012), and prevalent fracture (Abbott et al., 2001). Persisting hyperparathyroidism, with iPTH values > 14.3 pmol/L (130 ng/L) at 3 months, is also associated with increased fracture rates in the 5 years after kidney transplantation (Perrin et al., 2013). Lower limb fractures are more common in patients who are elderly or have diabetes, and vertebral fractures are predicted by a history of osteoporosis, but not cumulative corticosteroid dose (Vautour et al., 2004; Nikkel et al., 2012). Despite much concentration on therapies to maintain BMD, there are scant data assessing relationships of BMD to post-transplant fracture. One study that evaluated repeated DXA measures in 238 kidney recipients with transplant function ranging from CKD-T stages 1–5, demonstrated an association of lower BMD at the hip and increased fracture risk (assessed by questionnaire) (Akaberi et al., 2008). The Kidney Disease Improving Global Outcomes workgroup suggested that BMD should not be measured routinely in patients with CKD-T stages 4–5, because fracture risk is predominantly due to compromised bone quality (Kidney Disease Improving Global Outcomes (KDIGO) CKD-MBD Work Group, 2009). These guidelines are currently under review.

Prevalent vascular calcification

Vascular calcification (VC) develops over the course of CKD, and on lateral abdominal X-ray, abdominal aortic calcification, assessed using a validated scoring system (Kauppila et al., 1997), is detected in 48% of our kidney recipients and 46% of SPK recipients at the time of transplantation (Chau et al., 2014). When assessed at the time of transplantation, both the abdominal aortic VC score (using the same scoring system) and pulse wave velocity are reported to strongly predict cardiovascular events up to 36 months (Claes et al., 2013). Coronary artery calcification is also reported to predict cardiovascular risk over the first 2–3 post-transplant years (Nguyen et al., 2010). One preliminary study suggests the progression of coronary artery calcification may be slowed or arrested after renal transplantation (Moe et al., 2004), although another reported progression of coronary but not aortic calcification up to 4 years post transplant (Mesquita et al., 2010).

Laboratory values, graft loss, and mortality

A few studies have described relationships of pre-transplant CKD-MBD to post-transplant outcomes. In one study that assessed PTH levels from 1 year before to 2 months after transplantation, patients with PTH values of 90 pmol/L had double the graft failure of patients with a PTH of 7 pmol/L and a higher risk of death (Roodnat et al., 2006). Higher calcium and phosphate levels have also been associated with increased graft loss (Sampaio et al., 2011). However, another study of 773 patients found no relationships between serum calcium, phosphorus, or PTH and mortality (Schaeffner et al., 2007).

Assessing patients for treatment

Patients who fracture after transplantation often have risk factors predictive of osteoporosis in the general population, plus factors that are specific for CKD. The patient history should include general risk factors, including older age, female sex and post-menopausal or male hypogonadal status, prior fracture, a low BMI, postural instability, a history of falls, and a family history of osteoporosis. Risk factors more common in the transplant setting include glucocorticoid exposure, diabetes, hyperparathyroidism, and time on dialysis (Nikkel et al., 2012). Although pre-transplant dialysis and discharge on corticosteroids increase risk by 56% and 45% respectively, general population factors have a greater impact. Fracture risk calculators, such as FRAX® (<http://www.shef.ac.uk/FRAX/>) and the Garvan Institute calculator (<http://garvan.org.au/promotions/bone-fracture-risk/calculator/>) that incorporate demographic details and risk factors in addition to BMD may be helpful in identifying high-risk patients. Recently FRAX®, with or without BMD input, has been reported to provide modest fracture prediction after kidney transplantation (Naylor et al., 2014).

Functional tests of muscle strength, postural stability, and assessment for sarcopenia, (all of which worsen with any prolonged hospital stay) may also prove useful, but have not yet been investigated.

Bone densitometry and lateral spine X-ray

Within the first 3 months after kidney transplantation, it has been suggested that patients receiving corticosteroids or considered at high risk should undergo DXA examination if they have a well-functioning allograft (CKD-T stages 1–3) (Kidney Disease Improving Global Outcomes (KDIGO) CKD-MBD Work Group, 2009). A lateral spine X-ray is an additional useful test. It can identify vertebral fractures that would otherwise go unrecognized and that increase the risk of future fracture, and can be used to semiquantitatively assess vascular calcification of the abdominal aorta (Kauppila et al., 1997).

Laboratory assessments

When serum calcium and phosphate values are abnormal they should be monitored regularly, and 25(OH)D and PTH values should be checked when renal function has stabilized. Locally available bone formation and resorption markers may help guide therapy; ALP levels correlate closely to b-ALP levels if liver function tests are normal. Subsequent measures should be determined by the level of CKD-T.

Bone biopsy

In special circumstances, bone biopsy with tetracycline labelling has been suggested after transplantation. These circumstances may include determining that hyperparathyroidism is the predominant pathology when parathyroidectomy is being considered, when patients suffer recurrent fragility fractures, or when antiresorptive therapy with bisphosphonates or denosumab is being considered for a patient with suppressed bone turnover markers.

Management

The severity of pre-transplant CKD-MBD predicts post-transplant bone and mineral abnormalities and appears to impact both graft and patient survival. Optimizing the management of pre transplant CKD-MBD, and maintaining normal levels of calcium, phosphate, PTH, vitamin D, and bone turnover after successful renal transplantation, has potential to improve post-transplant patient-level outcomes.

Bone mineral density

Much of the focus of management has been on preserving BMD, although there are no adequately powered trials in this population, to provide fracture data for agents such as bisphosphonates, denosumab, or calcitriol and its analogues. Nevertheless, meta-analysis of available data has demonstrated that patients treated with placebo have higher fracture rates than patients receiving active interventions (Palmer et al., 2007). Available treatments can be considered as general or targeted. General treatment includes supplementation with cholecalciferol for vitamin D insufficiency or deficiency, which is common after transplantation, particularly as patients are counselled to avoid sun exposure and to use effective ultraviolet skin protection (Reichrath et al., 2008). Values of 25(OH)D recommended for the general population are generally used as target levels (Kidney Disease Improving Global Outcomes (KDIGO) CKD-MBD Work Group, 2009). Calcium supplementation has been questioned in the general community (Bolland et al., 2010), but may be used cautiously if dietary calcium intakes are low (Elder, 2011). Correction of metabolic acidosis and magnesium depletion is warranted but unproven, and when low, phosphate may be replenished, although the efficacy of replacement therapy will be limited by reciprocal increases in PTH and FGF23. Where possible, glucocorticoid reduction is another general bone-protective measure, because glucocorticoids have a major effect on bone volume and turnover (Monier-Faugere et al., 2000). Weight-bearing exercise should also be encouraged.

Calcitriol

Specific drug therapy includes the use of calcitriol or its analogues. Calcitriol appears to provide moderate protection against BMD loss, and generally has no deleterious effects (De Sevaux et al., 2002; Josephson et al., 2004; Torres et al., 2004; Palmer et al., 2005; Mainra and Elder, 2010). Providing hypercalcaemia is avoided, calcitriol may be useful when PTH levels remain elevated or phosphate levels are reduced. In addition, alfacalcidol or calcitriol and its analogues may exert immunomodulatory effects that positively influence long-term graft survival (Ozdemir et al., 2011). Although 1 year of calcitriol treatment appears to improve BMD, a bone biopsy study has suggested that beneficial effects may not continue long term, so ongoing therapy should be individualized (Cueto-Manzano et al., 2000). For prevention of BMD loss, bisphosphonates are more effective than calcitriol (Palmer et al., 2007), and when $1,25(OH)_2D$ values are high, the usefulness of calcitriol therapy is questionable because calcitriol may increase osteoclastic resorption.

Bisphosphonates

Compared to controls, bisphosphonates given soon after transplantation preserve BMD (Grotz et al., 2001; Coco et al., 2003; Palmer et al., 2005; Torregrosa et al., 2010, 2011; Abediazar and Nakhjavani, 2011) but they also reduce osteoblast activity, with the potential to cause adynamic bone. This was demonstrated by a small bone biopsy study performed before and after five doses of pamidronate therapy over 6 months (Coco et al., 2003). Similar features of low bone turnover have even been noticed when teriparatide (a potent anabolic agent) has been used (Cejka et al. 2008). For preservation of BMD, lower-dose bisphosphonate therapy may be both efficacious and prudent. Oral risedronate or low-dose (30 mg) alendronate given once a week are reported to preserve BMD in the first year after transplantation (Torregrosa et al., 2010; Abediazar and Nakhjavani, 2011) and lower-dose pamidronate (30 mg at baseline and 3 months) reduced spinal BMD loss accompanied by a return of turnover markers to the normal range (Torregrosa et al., 2011). Although one study evaluating ibandronate showed preservation of BMD with fewer vertebral deformities than controls (Grotz et al., 2001), these studies do not provide adequate fracture data; so the verdict remains unclear on the benefits or harms of reduced bone turnover, prolonged crystal development, and maintenance of BMD, that may even increase fracture risk over time. As a general rule, bisphosphonates are unlikely to impact fracture risk in patients who have T-scores above −2 even in the general community. Luckily, adverse renal events are uncommon with oral or intravenous bisphosphonates when used appropriately, but gastrointestinal side effects can cause volume depletion. Acute influenza-like symptoms are generally minor and osteonecrosis of the jaw and atypical fragility fractures, including subtrochanteric femoral fracture, are rare. However, these risks increase over time, and even rare events should be considered when the fracture efficacy of these drugs after kidney transplantation is unproven.

Denosumab

The potent antiresorptive agent denosumab has been used following kidney transplantation, but its long-term effects are unknown. Certainly it increases BMD in this setting, and reduces fracture risk for women with post-menopausal osteoporosis, including those with CKD stages 1–3 (Jamal et al., 2011). While caveats regarding low bone turnover also apply to denosumab, it has the advantage of a 6-month window of activity, and unlike bisphosphonates is not retained in bone. However, denosumab has been associated with severe hypocalcaemia in some patients with CKD, who develop a 'hungry bone' syndrome (Block et al., 2012). Because of this, additional treatment with calcium and vitamin D should be considered, and denosumab should be avoided in patients who have severe CKD-T.

Strontium ranelate

This drug increases osteoblastic activity and reduces bone resorption (Yamaguchi and Weitzmann, 2012). In postmenopausal women it is reported to increase BMD up to 10 years from the commencement of treatment while reducing fracture risk (Reginster et al., 2012). Reports of renal impairment following its use are rare (Iyer et al., 2009) and it has a low serious side effect profile, although recent concerns have been raised regarding cardiovascular risk. It has not been proven in the kidney transplant setting.

Teriparatide

Teriparatide has been used infrequently for patients after kidney transplantation, with no proven benefit to BMD or bone histopathology (Cejka et al., 2008). Case reports suggest that in parathyroidectomized patients, severe post-transplant hypocalcaemia can be successfully treated with this drug (Nogueira et al., 2011).

Hormone replacement therapies

Use of low-dose oral contraceptive agents has been associated with improved quality of life after transplantation (Pietrzak et al., 2006) and should be considered for maintenance of BMD in younger women who have undergone premature menopause or have amenorrhoea, once other secondary causes have been excluded. For postmenopausal women, tibolone and the selective oestrogen receptor modulator raloxifene can also be considered. Apart from a potential interaction of tibolone to increase tacrolimus levels (Clark et al., 2010), no data are available for these drugs following transplantation. For males there are few data available for the influence of testosterone therapy on BMD or fracture after transplantation (Palmer et al., 2007), although in long-term kidney transplant patients, levels of sex hormones and BMD do not correlate significantly (Brandenburg et al., 2005). Nevertheless, providing there are no contraindications, it is reasonable to consider a trial of testosterone therapy using gels or sustained-release intramuscular preparations in men with symptomatic hypogonadism, or when BMD does not respond to other therapies and testosterone levels remain low.

Cinacalcet

Patients treated with cinacalcet before transplantation are at increased risk of nephrocalcinosis and parathyroidectomy after transplantation, due to a rebound in levels of PTH (Evenepoel et al., 2012). On the other hand, when cinacalcet is introduced after transplantation for hyperparathyroidism and hypercalcaemia, calcium levels fall, phosphate levels increase, and there is a lowering of PTH levels (Schwarz et al., 2011). Of some concern, increased creatinine levels are reported to correlate to the decline in PTH, analogous to the effects observed after parathyroidectomy. Although some small studies have not observed changes in renal function (Copley et al., 2010; Guerra et al., 2011; Pinho et al., 2011), a meta-analysis of eight observational studies (N = 115) reported reductions in renal function that correlated to changes in levels of serum calcium (Henschkowski et al., 2011). A trend to increased BMD has been reported with post-transplant cinacalcet therapy (Cho et al., 2010), but no fracture data are available.

Parathyroidectomy

After transplantation, parathyroidectomy is an alternative therapy for persisting hyperparathyroidism and hypercalcaemia, but some data suggest that renal function may decline following this procedure. In fact, retrospective studies report that reductions in postoperative GFR correlate to the extent of parathyroid gland resection, but there is no increased risk to graft function providing one gland remains *in situ* (Jager et al., 2011; Park et al., 2011). Changes in renal function that do occur may not persist in the long term (Ferreira et al., 2011). Overall, surgical intervention, with potential for postoperative hypo- and hypercalcaemia and an increased pill burden, may be best avoided during the first post-transplant year when renal function is less stable. For further reading, Alshayeb et al. (2013) provides a useful summary of available treatments.

Algorithm to maintain BMD by assessing common risk factors while maintaining bone turnover markers in the normal range.

Step 1 BMD by DXA and lateral thoracic and lumbar spine radiograph.

Step 2 Laboratory investigations: PTH, 25(OH)D and bone formation and resorption markers such as bone-specific ALP and deoxypyridinoline: creatinine ratio or TRAcP-5b. Checking an oestradiol for younger women with amenorrhoea and a serum testosterone for men may be useful.

Note: if the eGFR is reduced, markers affected by renal function, such as P1NP, osteocalcin and β-CTX, are difficult to interpret. Check fasting, morning bloods, and urinary markers on a second morning sample.

Step 3 Score factors that increase fracture risk:	
Age > 50 years	1
Hypogonadal male or female	1
Prior fragility fracture including vertebral fracture	1
Prolonged oral glucocorticoid use before transplant	1
Low body mass index	1
First-degree relative with osteoporosis	1
Postural instability, peripheral neuropathy, reduced visual acuity, falls in past 6 months	1
Pre-transplant iPTH > 50 pmol/L	1
Type 1 diabetes	2

Step 4 Commence cholecalciferol if 25(OH)D is <65 nmol/L.

Consider hormone replacement for younger hypogonadal women.

Step 5 Commence calcitriol or an analogue if T-score is ≥ 0.

Commence calcitriol or an analogue if the bone formation marker is low.

Step 6 For other patients, use the following table reading from left to right.

Antiresorptive agents will generally be a bisphosphonate or denosumab.

Calcitriol or analogues are contraindicated if serum calcium is elevated.

BMD T-score	Fragility fracture	Resorption marker	Score	Treatment
< −2.5				Antiresorptive
−2.5 to −1	Yes	High	≥ 3	Antiresorptive
	No	Normal	0–2	Antiresorptive
	No	Normal		Antiresorptive
	No			Calcitriol or analogue
> −1.0 to 0	Yes	High	≥ 3	Antiresorptive
	Yes	Normal		Calcitriol or analogue
	No	Normal		Calcitriol or analogue

Adapted from Mainra, R, and Elder, G. J. (2010). Individualized therapy to prevent bone mineral density loss after kidney and kidney-pancreas transplantation. *Clin J Am Soc Nephrol*, 5(1), 117–24 with permission.

Fig. 288.3 This algorithm includes an assessment of common risk factors and aims to improve the targeting of therapy while maintaining normal bone turnover.

Management of CKD-T stages 3–5

If patients progress to CKD-T stages 3–5 (see Fig. 288.1), management principles are those that apply to other patients at similar stages of CKD (Kidney Disease Improving Global Outcomes (KDIGO) CKD-MBD Work Group, 2009).

Fig. 288.3 illustrates a schema that assists the assessment of risk factors and allocates therapy with calcitriol or bisphosphonate while avoiding the suppression of bone turnover.

Clearly we need more information on the optimal management of post-transplant mineral metabolism and bone disease, and in a number of areas, randomized controlled trials are necessary to assess which therapies will improve patient-level outcomes. In circumstances when best practice is unclear, discussion with an endocrinologist, rheumatologist, or renal physician with a special interest in mineral and bone disorders may prove valuable in helping to decide the most appropriate management.

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CHAPTER 289

Recurrent renal disease: prophylaxis, diagnosis, and management

Philip Clayton and Steven Chadban

Epidemiology: general concepts

The exact incidence of recurrence of renal disease is difficult to determine and will vary according to whether it is diagnosed by screening tests or protocol biopsy (subclinical recurrence), by clinical manifestations which lead to biopsy (clinical recurrence), or by graft loss due to recurrence. Most evidence comes either from case series or registry studies. Most case series are small, retrospective, and come from centres with an interest in recurrent disease. They capture clinical recurrences and underestimate the prevalence of subclinical recurrence. Registry studies provide higher patient numbers and less selection bias, but are susceptible to errors in classification (e.g. a slowly progressive case of recurrent immunoglobulin A nephropathy (IgAN) might be misclassified as chronic allograft nephropathy) and may lack detailed clinical and histological information. Registries typically provide reliable estimates of graft loss from recurrence, representing the more severe end of the spectrum of recurrent disease (Fig. 289.1).

Those whose disorders recur are at increased risk of premature graft loss (Hariharan et al., 1998). Although some conditions, such as focal and segmental glomerulosclerosis (FSGS) and haemolytic uraemic syndrome (HUS), may recur within days after transplantation, the incidence of clinical recurrence and graft loss due to recurrence increases over time post transplant. In one large registry study of patients transplanted because of glomerulonephritis (GN), recurrence was the cause of graft failure in < 1% at 1 year post transplant, increasing to 8% by 10 years, at which time recurrence was the third most common cause of graft failure after chronic rejection and death with a functioning graft (Briganti et al., 2002). The rate and impact of recurrence varies by disease. For several forms of GN, recurrence is common and also a common cause of graft failure. In contrast, systemic diseases such as systemic lupus erythematosus (SLE) and vasculitis may recur in the allograft but are uncommon causes of graft loss. Predicting the risk of recurrence in an individual relies on the rate of decline to renal failure and genetic predisposition. For most forms of GN the risk of recurrence is not a contraindication to transplantation; however, patients who have lost a graft from recurrent disease are at the greatest risk of recurrence in a subsequent graft and this should be taken into account when considering re-transplantation.

Graft loss from recurrent disease appears to be diminishing in the modern era. In one report, the risk of graft loss from recurrent GN was reduced by 61% in patients transplanted in 2001–2003 compared with those transplanted in 1990–1994 (Mulay et al., 2009). The reason for this is unclear. Possible explanations include changing patient demographics, better management of recurrent disease, better supportive care, or changes in immunosuppression. Two more recent studies suggested that steroid use might be associated with lower rates of graft loss from recurrence of IgAN (Clayton et al., 2011; Kukla et al., 2011). T-cell depleting induction immunosuppression has been associated with lower rates of GN recurrence in one small study. With these exceptions, however, current data are insufficient to conclude one immunosuppressive strategy is superior to any other in terms of preventing recurrence.

Pre-transplant assessment

It is important to consider the risk of recurrence prior to transplantation (Fig. 289.2). Precise diagnosis of the patient's primary disease is invaluable. In most cases this will require a kidney biopsy, and in selected cases, testing for specific genetic mutations. Accurate diagnostic information can inform prognosis, donor selection, perioperative management, and in some cases alter therapy in the event of recurrence. Patients should be counselled as to the risk and likely outcome of disease recurrence, especially if living donor kidney transplant is planned.

Perioperative management

In most cases perioperative management is the same as for patients without diseases liable to recur. However, in some diseases such as FSGS and HUS, recurrence can occur almost immediately and specific perioperative strategies may be helpful. For patients with proteinuric kidney disease, measurement of pre-transplant proteinuria is a useful baseline. Native kidney proteinuria typically resolves within 4 weeks after transplant in patients receiving calcineurin inhibitors. Protein excretion can then be measured regularly post transplant and a significant increase over baseline early after transplant, or any significant proteinuria beyond week 4, should prompt consideration of a kidney biopsy to look for recurrence. For those with HUS, regular screening for thrombotic microangiopathy post transplant may enable early diagnosis of recurrence and timely institution of treatment.

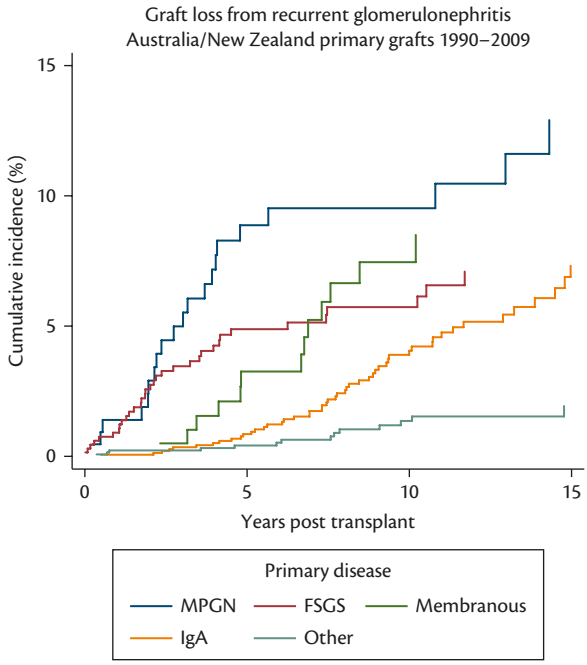

Fig. 289.1 Cumulative incidence of graft loss from recurrence of glomerulonephritis in transplant recipients with glomerulonephritis as the cause of end-stage kidney failure.

Renal allograft dysfunction

In patients with unexplained graft dysfunction, the possibility of recurrence should be entertained early, especially if the patient's primary disease was not clearly defined. A full investigation, including an immunology screen, urine microscopy, proteinuria measurement, and renal biopsy are indicated. Biopsy examination should routinely include immunofluorescence and electron microscopy as these can be critical in differentiating disease recurrence from other causes of kidney injury.

Treatment of recurrent disease has not been studied systematically, and treatment strategies are therefore empirically based on the treatment of the primary disease. The exception to this rule is FSGS, in which early and aggressive plasmapheresis is effective in inducing disease remission in a majority of cases. The absence of specific therapies should not diminish enthusiasm for obtaining a biopsy diagnosis which provides prognostic, in addition to diagnostic, information. In all cases of GN recurrence, conservative care should be provided including blood pressure control, renin–angiotensin blockade if proteinuria is present, avoidance of nephrotoxins, smoking cessation, and management of cardiovascular risk factors. In general, mammalian target of rapamycin inhibitors (mTORi), such as sirolimus, should be avoided in patients at high risk of, or with proven recurrence of GN, due to the propensity of mTORi to promote proteinuria.

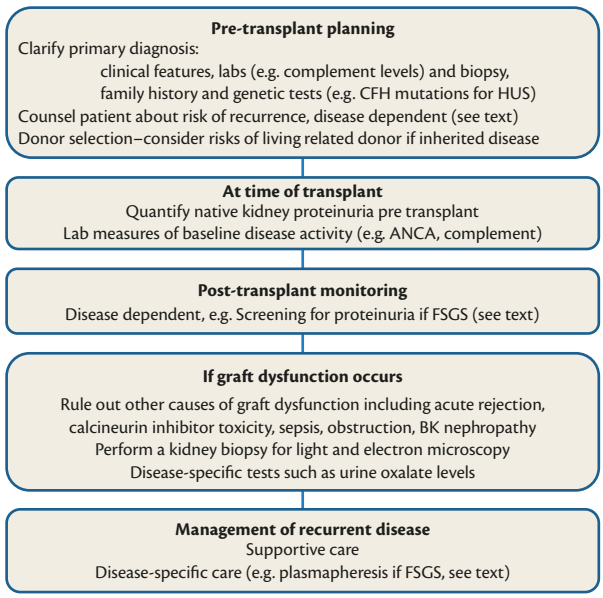

Fig. 289.2 Flow chart of an approach to the patient with possible recurrent glomerulonephritis.

Disease-specific information

Focal and segmental glomerulosclerosis

Primary FSGS often recurs, usually within days of transplantation. In one recent series, 36% of patients developed recurrence after a median of 13 days and the majority of recurrences were within 6 months. In contrast, recurrence risk is minimal in patients with secondary FSGS, for example, caused by reflux nephropathy, ischaemic nephropathy, or familial FSGS. Mutations in podocyte genes are of course cured by transplantation. In one series with a mean follow-up of over 12 years, 11 patients with homozygous or compound heterozygous *NPHS2* mutations were all free of recurrence, whereas 45% of patients without identified genetic mutations developed recurrence (Jungraithmayr et al., 2011). Recurrent disease is commonly, but not always, the same histologic pattern as was seen in the native kidney (IJpelaar et al., 2008; Canaud et al., 2010; Schachter et al., 2010). In patients who have lost a previous graft due to recurrence, recurrence is usually repeated with any subsequent grafts (Artero et al., 1992).

Recurrence frequently causes graft loss. In a large registry study of primary grafts for FSGS, 12.7% of patients had lost their graft from recurrence by 10 years (Briganti et al., 2002). Predictors of graft loss from recurrence include white recipients, younger recipient age, and treatment for rejection. Grafts from living donors show superior overall graft survival (Abbott et al., 2001).

A circulating 'permeability factor' with molecular mass of about 50 kDa has been reported to be present in many patients with recurrent disease (Savin et al., 1996). In these patients, *in vitro* glomerular permeability was increased. Plasmapheresis, administered to remove the circulating permeability factor, was subsequently reported to reverse recurrent FSGS in a high proportion of cases (Ohta et al., 2001; Gohh et al., 2005; Garcia et al., 2006; Canaud et al., 2009; Fuentes et al., 2010; Schachter et al., 2010) and for this reason plasmapheresis is the mainstay of treatment. The scientific rationale of plasmapheresis has been better defined by elegant studies in humans and mice published recently by Wei et al, who have identified suPAR as one such soluble permeability factor responsible for FSGS recurrence in a majority of their patients with recurrence (Wei et al., 2011). Early treatment is associated with improved outcomes, and for this reason it is essential to monitor patients post transplant for the development of proteinuria. Pre-emptive plasma

exchange has also been used with a reduction in recurrence rates, though only in comparison with historical controls (Ohta et al., 2001; Gohh et al., 2005). We recommend measurement of urinary protein excretion by protein:creatinine ratio (PCR) in a spot urine sample pre transplant and weekly post transplant to detect recurrence. Significant proteinuria (PCR > 100mg/mmol) should prompt consideration of a kidney allograft biopsy and plasmapheresis, with treatments given three times per week for a total of nine cycles in biopsy confirmed cases. Substantial proteinuria, however, often precedes light microscopic histological changes in cases of recurrent FSGS and thus treatment may be needed prior to confirmation by biopsy. In many cases, once remission has been achieved, the frequency of plasma exchange can be safely weaned. However, some patients remain dependent on plasma exchange long term. Other strategies reported to be effective include immunoadsorption (Dantal et al., 1998), high-dose ciclosporin (Raafat et al., 2004; Canaud et al., 2009), and rituximab (Dello Strologo et al., 2009). Whether measurement of suPAR or a related 'permeability factor' in blood or urine will become useful in the diagnosis and management of recurrence remains to be determined.

Membranous nephropathy

Recurrence of membranous nephropathy (MN) is common, with clinical recurrence reported in approximately one-third with primary MN who receive a transplant (El-Zoghby et al., 2009; Moroni et al., 2010). This causes graft loss in 12.5% at 10 years (Briganti et al., 2002). *De novo* MN is less common (Schwarz et al., 1994); 60% of patients with *de novo* disease lose their graft from the disease. PLA2R1 antibodies, recently found to be associated with the majority of cases of idiopathic MN, are predictive of recurrent but not *de novo* disease (Debiec et al., 2011). There is no clear best treatment but spontaneous remission is less common than in native disease. In small case series, rituximab has been reported effective (El-Zoghby et al., 2009, Sprangers et al., 2010). In the authors' opinion a trial is justified in cases which progress despite supportive care. Re-transplantation following graft loss due to recurrence is associated with a very high risk of repeat recurrence and cannot be recommended. This advice can be modified if the graft loss took >5 years.

Membranoproliferative glomerulonephritis

Recurrence of idiopathic type I MPGN occurs in about a quarter of primary grafts (Andresdottir et al., 1997; Lorenz et al., 2010; Moroni et al., 2011). This increases to 80% if a previous graft was lost from recurrent disease (Andresdottir et al., 1997). The incidence of graft loss from recurrence increases steadily over time, reaching 14% at 10 years in one large registry study (Briganti et al., 2002). In the second series, recurrence was the cause of 14.5% of graft losses. Risk factors for recurrence include younger age at diagnosis (Moroni et al., 2011) and crescents on the initial native kidney biopsy (Little et al., 2006). Recurrence is associated with low C3, heavy proteinuria (Moroni et al., 2011), and serum monoclonal proteins (Lorenz et al., 2010). Recurrence may be subclinical. In one small case series, only one-third of cases of recurrence presented clinically with haematuria, proteinuria, and declining glomerular filtration rate (Lorenz et al., 2010). Diagnosis requires kidney biopsy and examination by immunostaining and electron microscopy. The appearance must be distinguished from transplant glomerulopathy. Features favouring MPGN include subendothelial electron-dense deposits and C3 deposition (Andresdottir et al., 1998). Treatment is supportive, but also requires exclusion of causes of secondary MPGN, such as hepatitis C infection.

Type II MPGN (dense deposit disease) is more likely to recur than type I and for these patients, recurrence is the most common cause of graft loss, accounting for 30% of graft failures. In general, however, disease progression is relatively slow and grafts may last well over 10 years despite recurrence. Recurrent disease is associated with proteinuria, and glomerular crescents are associated with impaired graft function (Braun et al., 2005). In the absence of any proven therapies for this disease, recurrence is best managed with supportive care with ongoing standard immunosuppression and renin–angiotensin blockade.

Type III MPGN is rare and although recurrence has been reported (Morales et al., 1997), the incidence is not known.

Immunoglobulin A nephropathy

Recurrence of IgAN is common and increases with duration of follow-up. Protocol biopsy data has demonstrated that histological recurrence exceeds 50% and is more frequent than clinical recurrence (Odum et al., 1994). Clinical recurrence has been estimated at 31–44% at 10 years (Kim et al., 2001; Han et al., 2010a), but in most of these cases it does not lead to graft loss. In a recent large registry study, graft loss from recurrent IgAN was estimated at 4.3% at 10 years (Clayton et al., 2011).

Risk factors for IgAN recurrence include steroid-free maintenance therapy (Clayton et al., 2011; Kukla et al., 2011), receiving a zero human leucocyte antigen mismatched kidney (McDonald and Russ, 2006), and possibly a shorter pre-transplantation dialysis time (Freese et al., 1999). Induction therapy with antithymocyte globulin was also protective in one small study (Berthoux et al., 2008). There are no trials to guide therapy in recurrent IgAN. Tonsillectomy has been reported to reduce proteinuria (Kennoki et al., 2009) but this observational finding requires confirmation. We recommend standard conservative treatment including blood pressure control and renin–angiotensin system blockade.

Henoch–Schönlein purpura

Patients with Henoch–Schönlein purpura have equivalent graft survival to those with IgAN (Han et al., 2010b; Samuel et al., 2011). In a large registry study, 13.6% of graft losses were attributed to recurrent disease. Patients with necrotizing and crescentic lesions in the native kidney biopsy are more likely to suffer recurrence, and half of recurrent cases lead to graft loss (Moroni et al., 2008). Treatment is as for IgAN.

Lupus nephritis

Histological recurrence of lupus nephritis is common, with around half of patients showing biopsy evidence of lupus nephritis (Goral et al., 2003; Norby et al., 2010). However, lesions are usually milder than in the native kidney, generally showing only mesangial changes. Given the efficacy of mycophenolate as an induction and maintenance agent in lupus nephritis, it is not surprising that the disease is attenuated post transplant under standard immunosuppression. Recurrence is not always accompanied by systemic features of lupus (Stone et al., 1998), but is associated with proteinuria. Despite frequent histologic recurrence, graft loss from recurrent disease is rare. Registry studies have reported graft loss due to recurrence in only 0–2.4% (Contreras et al., 2010). Risk factors for

recurrence include young age, African American race, and female sex. In the authors' opinion, treatment of recurrence should include an increase in baseline immunosuppression; maintenance steroids; and maximal-dose mycophenolate. Pulse steroids may also be used in cases of diffuse proliferative lupus and switch from mycophenolate to cyclophosphamide should be considered in refractory cases. Patients should be screened for the presence of a lupus anticoagulant pre transplant, with consideration given to either perioperative anticoagulation or antiplatelet agents if this test is positive.

Haemolytic uraemic syndrome

The incidence of recurrent HUS depends on the type of HUS. It is very rare for diarrhoea-associated HUS to recur (Ferraris et al., 2002), but much more common for atypical HUS. HUS associated with complement factor H (CFH) or factor I mutations is the most likely to recur, with an incidence of approximately 80% (Bresin et al., 2006; Noris and Remuzzi, 2010), whereas membrane cofactor protein (MCP) mutations lead to recurrence in around 20% of cases. Recurrence typically occurs within days to months and generally leads to graft loss. Complement mutations can also lead to *de novo* thrombotic microangiopathy (Le Quintrec et al., 2008); some of these cases probably represent previously undiagnosed HUS. Calcineurin inhibitors, mTORi, and T-cell depleting antibodies can cause thrombotic microangiopathy and whilst their use is avoided in an attempt to prevent recurrence, calcineurin inhibitor-free immunosuppression does not prevent recurrence (Quan et al., 2001).

When considering transplanting a patient with atypical HUS, a full diagnostic workup should ideally be performed including the measurement of complement components, anti-CFH antibodies, and complement genotyping (Loirat and Frémeaux-Bacchi, 2008). Living related donors are at risk of developing HUS after donation and so should be screened for shared genetic mutations and only be accepted as donors if they do not share the mutations possessed by the recipient.

Treatment of recurrent HUS requires cessation of any potential inciting agents (such as calcineurin inhibitors) and plasma exchange with fresh frozen plasma replacement. Eculizumab has been reported to be effective in case reports and could be trialled as a second-line agent (Hadaya et al., 2011). In patients with a genetic cause of HUS, liver-kidney transplant should be considered as a definitive treatment (Saland et al., 2009).

Greater understanding of the mechanisms of atypical HUS, and treatment options after transplantation, have been facilitated by the International Registry Of Recurrent And Familial Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP) (<http://negribergamo.marionegri.it/content/view/170>). Clinicians are encouraged to enrol all consenting patients with HUS in this registry.

Goodpasture disease

Recurrence of Goodpasture disease is rare if the antiglomerular basement membrane (anti-GBM) antibodies have been undetectable for at least 6 months. In a large registry study, the practice of delaying transplantation prevented any cases of graft loss from recurrence for up to 10 years (Briganti et al., 2002). Should the disease recur, it should be treated in the same manner as for disease in the native kidney.

Patients with Alport disease can develop *de novo* anti-GBM antibodies due to neo-antigen exposure (the alpha chain of type IV collagen) from the transplanted kidney. This syndrome is rare, is typically less severe than Goodpasture disease, and responds well to plasmapheresis and immunosuppression (Göbel et al., 1992).

ANCA-associated vasculitis

ANCA-associated vasculitis recurs in 15–20% of patients, with around half of recurrences being systemic rather than renal (Westman et al., 1998). Graft losses from recurrent disease may occur in up to 10% of patients (Briganti et al., 2002). There are no definite predictors of recurrent disease, but we recommend deferring transplantation until the ANCA has been negative for 6 months. We recommend using maintenance steroids. Management includes pulse steroids and switching patients from mycophenolate to oral cyclophosphamide until remission is achieved.

Scleroderma

Recurrent kidney disease has been reported among patients transplanted for end-stage renal disease caused by scleroderma; however, in such cases the kidney is frequently only one site of progressive disease (Chang and Spiera, 1999). Each case requires therapy tailored to the individual set of manifestations. Tight blood pressure control with an angiotensin-converting enzyme inhibitor may be important for renoprotection.

Diabetes

The finding of pathologic features of diabetic nephropathy is common in the grafts of diabetics who have been transplanted, but this is a rare cause of graft loss (Najarian et al., 1989). In addition to recurrent disease, new-onset diabetes after transplantation (NODAT) can cause diabetic nephropathy and can, rarely, lead to graft loss (Salifu et al., 2004). However, the primary impact of NODAT is diminished patient rather than graft survival (Cole et al., 2008). Graft diabetic nephropathy is characterized by proteinuria and extensive vascular changes on biopsy; but typical Kimmelstiel–Wilson nodules are uncommon (Hariharan et al., 1996). There is no proven treatment and we recommend the usual supportive treatments including tight blood pressure control, renin–angiotensin system blockade, and glycaemic control. mTORi are best avoided because of their tendency to promote proteinuria.

Oxalosis

For patients with oxalosis, recurrence is the most common cause of kidney graft loss but still only accounts for a minority of graft failures in this group (Bergstralh et al., 2010). Severe graft dysfunction may occur early, particularly following episodes of graft inflammation caused by acute rejection or urinary sepsis, precipitated by crystal deposition within tubules (Fig. 289.3). Indeed, such graft dysfunction occurring in a graft recipient with unknown primary disease should prompt a diagnostic evaluation for oxalosis including kidney histology, urinary oxalate concentration, and genetic studies. Graft outcomes are generally worse than those of patients with GN as their underlying diagnosis (Cibrik et al., 2002), but may be improving (Bergstralh et al., 2010). Total body oxalate load is the key determinant of risk of recurrence and pre-emptive transplantation may lead to improved outcomes, perhaps by preventing

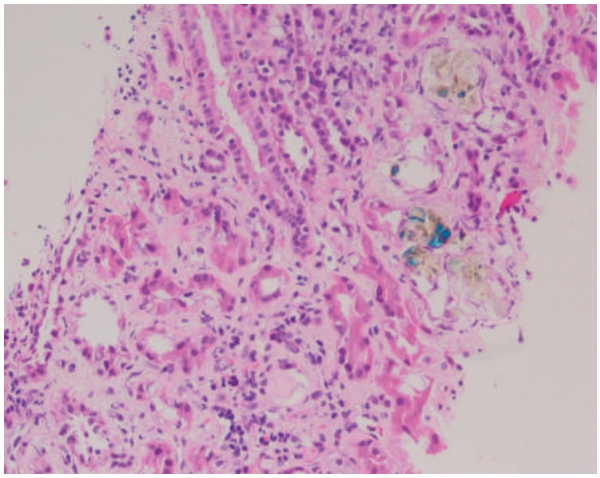

Fig. 289.3 Histological appearances of recurrent oxalosis.

systemic build-up of oxalate (Watts et al., 1988). The ideal approach in cases of primary oxalosis is combined kidney-liver transplantation which restores both kidney function and capacity to metabolize oxalate. This strategy is not useful for patients with secondary oxalosis. Diagnosis of recurrent disease requires biopsy (Fig. 289.1). Empiric therapy to prevent and treat recurrent oxalosis includes avoiding high-oxalate foods, reducing oxalate levels with dialysis and possibly pyridoxine, avoiding dehydration, and urinary tract infection prophylaxis.

Amyloidosis

Recurrent AA amyloidosis occurs in 10–14% of grafts and is associated with an increased risk of death (Hartmann et al., 1992; Kofman et al., 2011). Recurrence occurs late with one series reporting a mean time to recurrence of 118 months (range 99–233) (Kofman et al., 2011). Recurrent disease is usually associated with proteinuria and reduced renal function. Treatment is supportive. Experience with transplantation in AL amyloidosis is more limited. In one series of 25 patients, recurrence occurred in seven (28%) after a median of 5.9 years. It is recommended that patients be in haematological remission prior to transplantation (Herrmann et al., 2011; Pinney et al., 2013).

Light chain nephropathy

There are limited data on recurrence of light chain nephropathy. In one series of seven patients, recurrence occurred in five patients after a median of 33.3 months (range 2–45) (Leung et al., 2004). Recurrence of disease was followed by a rapid progression to end-stage kidney disease. Patients with light chain nephropathy should ideally be in remission before being transplanted.

Fibrillary glomerulonephritis

In a series of four patients receiving five grafts, recurrence occurred in three. In each case, the rate of deterioration of kidney function was slower than it had been in the native kidneys (Pronovost et al., 1996). In another series of five patients with fibrillary GN, there was no recurrence but five of seven patients diagnosed with monoclonal gammopathy and fibrillary deposits developed recurrence after 3–87 months (Czarnecki et al., 2009).

BK nephropathy

In one series of 10 patients with graft loss due to BK nephropathy, one patient developed recurrent BK nephropathy 8 months after re-transplantation but did not lose the graft (Ramos et al., 2004). In all 10 cases the urine was negative for decoy cells prior to transplantation, and in seven of the patients nephroureterectomy of the first graft was performed. It may be advisable to ensure the absence of viraemia prior to re-transplantation in such patients. As BK virus is usually of donor origin, graft nephrectomy prior to re-transplantation appears unlikely to be helpful in diminishing recurrence risk.

Sickle cell disease

As with other systemic diseases, sickle cell disease may recur in the transplant kidney. However, graft survival in patients with sickle cell disease is comparable to that of patients with alternative renal diagnoses (Huang et al., 2013).

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CHAPTER 290

Paediatric renal transplantation

Minnie M. Sarwal and Ron Shapiro

Introduction

Kidney transplantation is the best renal replacement treatment for children with end-stage renal disease (ESRD) (Fine, 1985). Five-year survival rates in paediatric renal transplant recipients exceed those of patients on dialysis.

The special issues in children and adolescents with ESRD include achieving normal growth and cognitive development. Successful transplantation improves linear growth and allows a nearly normal lifestyle, including fewer dietary restrictions and better school attendance. Newer immunosuppressive regimens which include steroid minimization or avoidance have been associated with a significant improvement in growth (Sarwal et al., 2003; Li et al., 2009). Better surgical techniques, with fewer early complications, especially graft thrombosis, and more effective prevention and treatment of rejection and infectious complications have led to young children having the best long-term outcomes (Sarwal et al., 2000; US Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients, 2007). Nonetheless, success in paediatric kidney transplantation is still challenging in children and adolescents.

Incidence and aetiology of end-stage renal disease in children

The incidence of ESRD is substantially lower in children, 14.4 per million, than in adults, where the incidence is as high as 1505 per million population in the 70–79-year age group.

The aetiology of ESRD in paediatric patients is quite different from that in adults. It varies by age. Congenital, hereditary, and cystic diseases are responsible for over half of the cases of ESRD in children 0–4 years of age, while glomerulonephritis and focal segmental glomerulosclerosis (FSGS) account for 38% in patients 10–19 years of age. The most common diagnosis is structural disease (posterior urethral valves being the major cause in this group, 49%), followed by glomerulonephritis (14%) and FSGS (12%).

Indications for transplantation

In contrast to adults, almost all children with ESRD are potential transplant candidates because there are few absolute contraindications in children. Relative contraindications include metastatic malignancy or HIV. Children in remission from their cancer for at least 2 years and HIV-positive patients with undetectable viral loads and CD4 counts > 200 may be considered for transplantation. Patients with autoimmune disease are candidates for transplantation after a year of immunological remission of the primary disease. Severe neurologic dysfunction is a relative contraindication, but these patients should be considered on an individual basis, as the degree of neurologic recovery is unpredictable, and the decision is really whether to initiate dialysis. If a decision is made to offer dialysis, transplantation should be considered.

Dialysis may be necessary before transplantation to optimize the child's nutritional and metabolic state, achieve a sufficient size, or maintain stability until a suitable donor is found. Infants need to weigh at least 8–10 kg, both to minimize the risk of vascular thrombosis and to accommodate an adult-sized kidney. They may, therefore, require dialysis until they are at least 12–18 months of age. Specialist paediatric transplant centres do transplant an adult-sized kidney in children < 10 kg or < 6 months of age.

Pre-emptive transplantation (i.e. transplantation performed before the need for dialysis) is achieved in 25% of paediatric renal transplants. There are advantages related to less infection and cardiovascular morbidity. There is also the advantage of avoiding dialysis and its attendant morbidity. Pre-emptive renal transplantation should be considered when the glomerular filtration rate (GFR) is <10–15 mL/min/1.73 m^2, in symptomatic patients, or when the projected need for dialysis is within 6–12 months (Lerner et al., 1999). The rates of pre-emptive transplantation vary slightly among different age groups, and are 20%, 24%, 28%, and 22% in recipients aged 0–2, 2–5, 6–12, and 13–17 years, respectively.

Characteristics of donors and recipients

Over the past 10 years in the USA, the greatest increase in new paediatric patients listed for transplantation has been in the 11–17-year age group. About two-thirds of paediatric transplants are performed in children 11–17 years of age, 17% in patients 6–12 years of age, and 17% in patients 1–5 years of age.

About half of paediatric kidney transplants are from living donors. Between 1998 and 2003, 58% were from living donors. The rates for both living and deceased donor renal transplantation are higher in children than in adults, and are 29 live donor and 27 deceased donor transplants per 100 dialysis patient-years. These are over twice the rate in patients 20–44 years of age. The highest rates are in the 5–9-year age group, with 40 live donor and 46 deceased donor transplants performed per 100 dialysis patient-years.

The US Organ Procurement and Transplantation Network (OPTN) instituted an allocation priority for children waiting for a deceased donor transplant in 2005, giving priority for kidneys from deceased donors < 35 years of age. These kidneys were assigned to recipients < 18 years, after zero mismatch transplants, recipients with a panel reactive antibody (PRA) > 80, or candidates receiving a kidney with a non-renal organ. This new policy shortened

the waiting time for children, but had the unintended consequence of increasing the percentage of deceased donor versus live donor kidneys, without increasing the overall number of kidneys transplanted into children.

In 2004, there were an equal number of living and deceased donor kidney transplants in children, but in 2005, the recipients of living donor kidney transplant rate dropped to 47% and declined further in 2006 to 35%.

Living donor kidney transplantation graft survival is excellent and has not changed much over the past 13 years. From 2003 to 2007, the 1-year graft survival was 96.1%, and from 1999 to 2002, it was 95.9%. Graft survival rates for deceased donors improved modestly and were 94.4% from 2003 to 2007 compared to 92.7% between 1992 and 2002.

In paediatric patients awaiting deceased donor transplantation, the goal is to minimize waiting time, and to transplant children aged 1–6 years within 6 months, aged 7–12 within a year, and aged 13–18 within 18 months (US Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients, 2007).

Transplant evaluation/preparing for transplantation

The transplant evaluation team includes the surgeon, nephrologist, nutritionist, social worker, psychologist, financial counsellor, pre-transplant nurse, pharmacist, and dialysis nurse. The patient and family are told what to expect before, during, and after transplantation, with an emphasis on the importance of perfect adherence.

Primary renal disease recurrence can occur in a few specific diseases, but is not a contraindication to transplantation (see Chapter 289). Recurrent disease accounts for graft loss in about 7% of first and 10% of re-transplantations compared to 2% in adults.

FSGS and primary oxalosis are two diseases which can recur and irreversibly damage the transplanted kidney (see also Chapter 289). Twenty to 30% of FSGS patients lose their kidneys to recurrence, within a mean of 17 months Oxalosis recurs with such regularity that kidney transplantation alone is not indicated. Combined or staged liver/kidney transplantation is necessary. Patients with Alport syndrome can rarely develop antiglomerular basement membrane (anti-GBM) glomerulonephritis with an incidence of 3–4%, with resultant graft loss. Histological recurrence of MPGN type I varies from 20% to 70%, with graft loss occurring in up to 30% of cases. Histopathological recurrence of MPGN type II occurs in most patients, with graft loss rates as high as 50%. Histological recurrence of immunoglobulin A nephropathy is common and occurs in about half of the patients, but is associated with graft loss in only 5%. Henoch–Schönlein purpura recurs in approximately 30% of patients. Congenital nephrotic syndrome rarely recurs after transplantation, though patients can develop antinephrin antibodies and become nephrotic. Approximately 25% of the nephrotic syndrome which develops after transplantation is probably *de novo* rather than recurrent disease. Membranous nephropathy occurs very rarely in children. The turnover recurrence rate in children with tumour is about 13%, and patients with Denys–Drash syndrome should thus undergo bilateral nephrectomy prior to transplantation.

The indications for *bilateral native nephrectomies* include hyposthenuria with polyuria, significant proteinuria, resistant hypertension, and persistent infection. Nephrectomies are also indicated in patients with polycystic kidney disease to make space for the transplanted kidney.

Urologic problems, including vesico-ureteral reflux, posterior urethral valves, abnormal urinary bladders, and/or neurogenic bladders should be dealt with prior to transplant. Children with a urologic condition may require multiple operations to improve urinary tract anatomy and function. This can include ureteral re-implantation, bladder augmentation, creation of a vesico-cutaneous fistula by using the appendix to allow for easy intermittent catheterization (Mitrofanoff procedure), and excision of duplicated systems that could cause recurrent infections. In children with posterior urethral valve resection and bladder rehabilitation without augmentation, regimented double voiding improves (Bartsch et al., 2002).

A *nutritional assessment* should be performed to ensure optimal nutritional status before transplantation. Many children with ESRD, especially those on dialysis, require nutritional supplements. Infants and young children on dialysis may require nasogastric or gastrostomy tube feedings to compensate for decreased oral intake as a result of nausea and anorexia related to uraemia (Sarwal et al., 2000; Warady et al., 2004).

Most children with renal failure have poor linear growth, and administration of growth hormone may be appropriate. Growth tends to improve after transplantation, particularly in children <12 years of age. Eighty-one per cent of transplanted children have improved growth as a function of no longer being in renal failure (Warady et al., 2004). Growth hormone can be resumed after the first post-transplant year if necessary but there remains a concern of being associated with rejection if it is given early after the transplant. Steroid-free immunosuppressive regimens are associated with improved linear growth after transplantation (Sarwal et al., 2003; Li et al., 2009). The diagnosis and treatment of bone disease starts early. Secondary hyperparathyroidism starts early in chronic kidney disease and needs to be treated well before the need for transplantation to avoid post-transplant urinary phosphate wasting and hypercalcaemia. A high calcium phosphorus product prior to transplantation can lead to vascular stiffness and calcification and increases the risk of cardiovascular disease.

Cardiovascular disease is the leading cause of death in paediatric ESRD patients. Over 25% of the mortality in children on dialysis is related to cardiovascular disease. Cardiac death is also the leading cause of death in children after transplantation. Thus, cardiac evaluation with at least an echocardiogram and electrocardiogram is needed. Hypertension is common and must be controlled. If medical management is inadequate, bilateral nephrectomy may be needed.

Anaemia needs to be managed early. Patients usually require an erythropoiesis stimulating agent (ESA), folic acid, and iron to ensure haemoglobin levels are maintained between 11 and 12 g/dL. Blood transfusions should be avoided to avoid sensitization. If blood transfusion is necessary, it should be with filtered red blood cells to reduce the risk of cytomegalovirus (CMV) and sensitization.

Excluding *hypercoagulability* is a necessary step before transplantation. The third (10%) leading cause of graft failure is vascular thrombosis. The risk factors include technical error, reperfusion injury, young donor age (<2 years), young recipient (<5 years), cold ischaemia time >24 hours, arterial hypotension, history of peritoneal dialysis, and/or hypoperfusion of an adult kidney. It is also essential to evaluate patency of the iliac veins

and vena cava if the patient has had previous surgery or central line placement. Femoral lines can increase the risk of inferior vena cava thrombosis. Children with large protein losses from nephrotic syndrome and/or peritoneal dialysis are also at increased risk for thrombosis because of protein loss of protein S, C, and antithrombin III. Doppler ultrasound, computed tomography angiography, and magnetic resonance angiography (MRA) have all been used to evaluate the vessels. MRA is used less because of the concern of exposure to gadolinium causing nephrogenic systemic fibrosis (Chrysochou et al., 2009). In patients with renal dysfunction who receive contrast media, intravenous fluids to maintain pre and post hydration are needed for patients with residual renal function, correction of acidosis should be carried out before giving contrast, and *N*-acetylcysteine should be administered before and after any dye study to minimize the risk of contrast nephropathy.

Any *persistent infection* needs to be eradicated before transplantation. Screening includes a history of treatment for active or latent tuberculosis, a vaccine history for varicella and pertussis, a travel history within the past 2 years, a history of BCG, animal and/or insect exposure, sexual activity, and consumption of high-risk foods such as unpasteurized products. Testing includes purified protein derivative, CMV IgG, Epstein–Barr virus (EBV) antibody panel, varicella titre, measles antibody, hepatitis B serologies, hepatitis C antibody, and HIV antibody. Additional testing for patients who lived in or visited the Central Valleys of California, Utah, Nevada, Arizona, and/or New Mexico includes *Coccidioides* immunodiffusion. Patients from the Ohio River valley should also be checked for *Histoplasma* antibody. Patients from Mexico should have *Coccidioides* immunodiffusion, *Histoplasma* antibody, and ova and parasite screening to exclude *Strongyloides*. Those from South American should have *Coccidioides* immunodiffusion, *Histoplasma* antibody, and toxoplasma antibody. Sexually active patients should also be screened for syphilis, gonorrhoea, and chlamydia.

Immunizations need to be current before transplantation. All live virus vaccines must be given before transplantation, as they cannot be given to immunosuppressed patients. Therefore, MMR and varicella should be administered before transplantation, and antibody titres checked to assess the response. MMR may be given as early as 6 months of age. Inhaled influenza vaccine (a live virus vaccine) should not be given to transplant patients, family members, or healthcare providers (Anonymous, 2004).

Psychological and social service evaluation before transplantation is an essential part of the pre-transplant assessment. Screening for the ability to care for the child after transplantation, depression, substance abuse, and/or non-adherence is important.

ABO blood group, human leucocyte antigen (HLA) typing, and a PRA are documented early in the assessment. Patients can become sensitized from prior transplants, blood transfusions, and/or pregnancy.

Immunosuppression

Most paediatric renal transplant patients receive a combination of immunosuppressive agents consisting of a calcineurin inhibitor (CNI) and steroids with or without an antiproliferative agent. The North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) reported that in 2003, approximately 80% of transplanted patients were receiving three agents by 6 months after transplantation. The goal is to provide adequate immunosuppression but to minimize side effects.

Antibody induction therapy is routinely administered to paediatric recipients to minimize the risk of acute rejection. NAPRTC data in 1996 showed that 50% of patients were receiving induction, and by 2006, 69% were receiving induction treatment. Antilymphocyte globulin has been reported to be associated with improved graft survival. In paediatric deceased donor transplantation, there is close to a 10% advantage in the 5-year graft survival rate in patients receiving antibody induction. The incidence of acute rejection is about 30% lower and occurs later. Antibody induction can allow for corticosteroid avoidance and a reduced need for immunosuppressive medications. Induction can include T-cell antibodies, interleukin (IL)-2 receptor antibodies, and/or anti-B-cell antibodies.

T-cell antibodies: antithymocyte globulin (thymoglobulin) is a lymphocyte depleting polyclonal antibody preparation that is administered intravenously through a central line. It is used to prevent rejection and leads to a rapid depletion of T lymphocytes (Moudgil and Puliyanda, 2007). The dose is 1–1.5 mg/kg/day with daily monitoring of CD3+ subsets; the dose is not given if the CD3+ count is < 20 cells/mm^3.

IL-2 receptor antibodies: these are chimeric (basiliximab) or humanized (daclizumab—no longer available) monoclonal anti-CD25 receptor antibodies. They prevent T-cell proliferation, but are not lymphocyte depleting.

Basiliximab is given on day 0 and postoperative day 4 at a dose of 12 mg/m^2/dose in children (Moudgil and Puliyanda, 2007). Daclizumab was given on day 0, then every 2 weeks for a total of five doses at 1 mg/kg/dose in patients on steroid-based immunosuppression. Patients who were on a steroid avoidance regimen received a 2 mg/kg dose on day 0 then the 1 mg/kg dose at weeks 2, 4, 6, 8, 11, 15, 19, and 23. This provided the patients with extra immunosuppression for 3 months if they were receiving steroids and 6 months for those patients who were not. IL-2 receptor antagonists are well tolerated (Li et al., 2003), and the double dose of daclizumab given in steroid-free induction appears to be a critical dose to support the very low rates of acute rejection seen despite complete steroid avoidance (Li et al., 2003).

Alemtuzumab (Campath-1H®): is a depleting humanized monoclonal antibody against the CD52 antigen (present on T and B cells, monocytes, and NK cells). The paediatric data regarding alemtuzumab are encouraging, but more studies are needed.

Additional potential agents for highly sensitized patients include rituximab, a monoclonal anti-CD20 antibody (Zarkhin et al., 2008), the proteasome inhibitor bortezomib (Perry et al., 2009), plasmapheresis with intravenous immunoglobulin (IVIG) for the removal and suppression of donor-specific antibodies (Vo et al., 2008), and eculizumab.

Maintenance immunosuppression

For maintenance immunosuppression a combination of CNIs, mycophenolate, corticosteroids, azathioprine, and/or a mammalian target of rapamycin inhibitor (mTORi) may be used. Most paediatric renal transplant patients are prescribed three immunosuppressive agents: a CNI (ciclosporin or tacrolimus) in combination with corticosteroids and an adjunctive antiproliferative agent (azathioprine, mTORi, or mycophenolate). Mycophenolate is used in over two-thirds of paediatric kidney patients. Sirolimus

is used in 10–15%, while azathioprine is used in only about 2%. Corticosteroids continue to be employed in approximately 80–85% of transplant recipients. However, steroid minimization or steroid avoidance protocols are now preferred.

Calcineurin inhibitors

Ciclosporin

Ciclosporin was the first CNI to be used clinically. Children usually require higher doses of ciclosporin than adults on a milligram per kilogram basis. This is related both to a higher rate of metabolism by the hepatic cytochrome P450 (CYP)-3A4 and decreased gastrointestinal absorption of the drug in children. The side effect profile of ciclosporin in children is similar to that seen in adults, but the impact on children is more profound. Hypertrichosis, gingival hyperplasia, and coarsening facial features are particularly problematic. Hispanic and African American children appear to be at higher risk of significant hypertrichosis. In the adolescent population, especially girls, these side effects may be associated with severe emotional distress, and may lead to non-adherence. Seizures, although uncommon, are observed more commonly in children than in adults. Children, like adults, are prone to develop hypercholesterolaemia and hypertriglyceridemia. Hyperglycaemia is less common in children than in adults, occurring in < 5% of children on ciclosporin.

Tacrolimus

The second CNI to be used in clinical practice was tacrolimus. The hyperlipidaemia associated with ciclosporin is not seen with tacrolimus. Post-transplantation glucose intolerance, tremor, alopecia, and mild sleep disturbances are, however, more common with tacrolimus. The lack of cosmetic side effects makes tacrolimus an attractive immunosuppressive agent for children. This is especially true for adolescents and females. Direct comparative data in paediatrics between ciclosporin and tacrolimus are limited. The only randomized, controlled, multicentre clinical trial in paediatric renal transplantation comparing these two agents was performed in Europe (Filler et al., 2005). It showed that overall acute rejection rates at 6 months were 59.1% versus 36.9% (P = 0.003) for ciclosporin and tacrolimus, respectively. In the tacrolimus group, graft function was better at 1-year post transplantation, with a clearance of 62 mL/min/1.73 m^2 versus 56 mL/min/1.73 m^2 in the ciclosporin group. In addition, 4-year graft survival was superior in the tacrolimus group. The mean total corticosteroid dose at 6 months post transplantation was lower in the tacrolimus group (112 vs 141 mg/kg; P = 0.009). The safety profiles of the two agents were equivalent, with essentially no difference in the incidence of post-transplant lymphoproliferative disorder (PTLD) or in diabetes requiring insulin treatment. Tacrolimus has replaced ciclosporin as the most commonly used CNI in transplantation.

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is the morpholinoethyl ester pro-drug of mycophenolic acid (MPA), an inhibitor of *de novo* purine synthesis, and has largely replaced azathioprine because it is associated with a lower incidence of acute rejection (Ettenger and Sarwal, 2005). It is not nephrotoxic.

MMF has allowed lower doses of other immunosuppressive agents, including corticosteroids and CNIs. Its principal side effects are gastrointestinal (diarrhoea and gastritis) and haematological (neutropenia and thrombocytopenia). These toxicities respond to dosage reduction. An alternative formulation, mycophenolate sodium, is similar to MMF.

Sirolimus

Sirolimus, an inhibitor of the mammalian target of rapamycin (mTOR), is used primarily as an adjunctive immunosuppressive agent in combination with a CNI in children. It is used in approximately 10–15% of paediatric renal transplant recipients. Its toxicities include hyperlipidaemia, thrombocytopenia, impaired wound healing, joint pain, and diabetogenicity and synergistic toxicity with CNIs.

Corticosteroids

Corticosteroids are used in most transplant patients in spite of their side effects, that is, growth retardation, infection risk, hypertension, obesity, diabetes mellitus, hyperlipidaemia, osteopenia and aseptic necrosis of bone (particularly the femoral heads), Cushingoid changes, and acne. Unfortunately, only a minority of transplant programmes attempt to stop or avoid corticosteroids.

Steroid withdrawal in patients on ciclosporin has been associated with high rates of acute rejection (Roberti et al., 1994) but in those receiving tacrolimus it is less of a problem. A steroid withdrawal trial conducted by the NAPRTCS using sirolimus had low rates of acute rejection, but revealed a high incidence of PTLD which resulted in early discontinuation of the study.

Corticosteroid avoidance has been pioneered in several programmes, including those in Stanford and Pittsburgh, United States. Studies from Stanford have demonstrated that complete steroid avoidance can successfully be achieved with excellent long-term outcomes at 8 years, in children on tacrolimus in combination with MMF, and an extended 6-month course of daclizumab (Li et al., 2009; Sutherland et al., 2009) (Fig. 290.1 outlines a proposed maintenance immunosuppression protocol with and without maintenance steroid usage). A similar experience has been seen at other centres with complete steroid avoidance, using a similar protocol with tacrolimus and MMF, and induction with either extended daclizumab or thymoglobulin (Smith et al., 2003; Silverstein et al., 2005). The Stanford steroid avoidance protocol has been studied as a randomized, multicentre US trial (NIH/NIAID/CCTPT UO1 AI-55795; 'New NAPRTCS Trials in Steroid-Free Immunosuppression'), with similar good outcomes (Sarwal et al., 2012). This trial also provided the largest prospective histological analysis of protocol biopsies in paediatric renal allograft recipients (Naesens et al., 2012), and contrary to earlier concerns, clearly demonstrated that there was no adverse effect of steroid avoidance on fibrosis or chronic injury (Naesens et al., 2012). Experience at the University of Pittsburgh using alemtuzumab induction and tacrolimus monotherapy looks promising.

Fluid management in infants and small children

It is important to maintain a good blood flow for an adult-sized kidney transplanted into an infant or small child (Sarwal et al., 2000). Recipient aortic blood flow after transplantation of an adult-sized kidney more than doubles compared to pre-transplant aortic blood flow (Salvatierra et al., 1998). The maximum flow obtainable in an adult-sized kidney transplanted into a small child is about

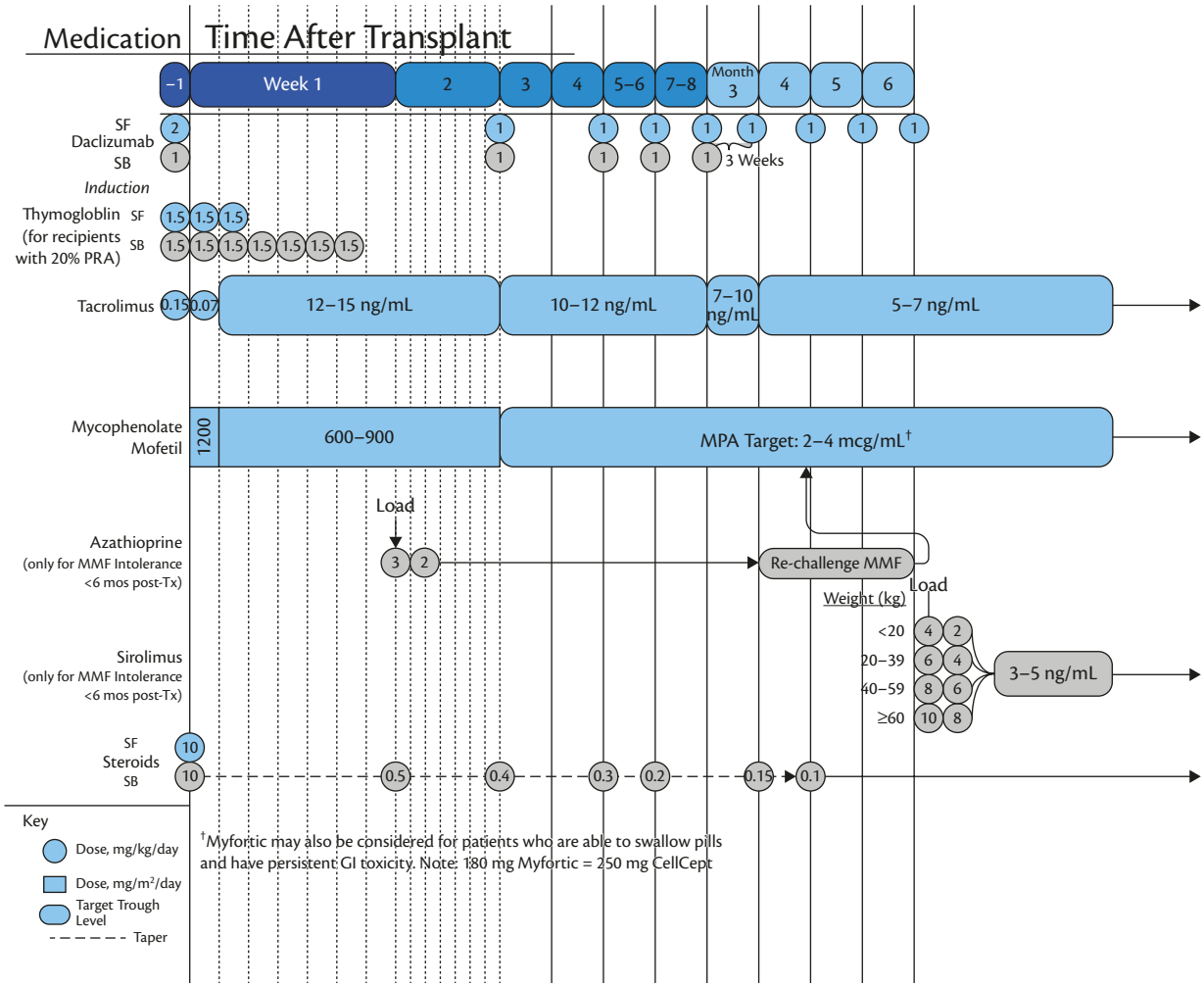

Fig. 290.1 Maintenance immunosuppression protocol grid for paediatric renal transplantation with options for steroid-free or steroid-based regimens with proposed target trough levels.

two-thirds of the flow in the donor. The goal is to achieve a systolic blood pressure of 120 mmHg with crystalloid, colloid, and blood as needed. Low-dose dopamine can also be given.

Lasix 1 mg/kg and mannitol 1 g/kg are administered as the kidney is being implanted. It is essential to *over*-hydrate the small recipient to prevent hypovolaemia and hypotension, which can lead to acute tubular necrosis or graft thrombosis. Over-hydration has to be continued in the postoperative period.

Infants will require aggressive fluid administration via nasogastric or gastrostomy tube. At least 2500 mL/m^2/day will be required for at least 6 months after transplantation if the child is unable to take in a sufficient volume. This fluid management strategy is associated with a 30mL/min increase in GFR in infants receiving adult-sized kidneys, compared to that in infants not as aggressively hydrated (Salvatierra and Sarwal, 2000).

Renal biopsy

Acute allograft dysfunction in children usually requires an allograft renal biopsy. The role of protocol biopsies is still not established, and in the absence of sensitive and specific non-invasive biomarkers of different phenotypes of graft injury, the protocol biopsy has emerged as a surveillance tool for subclinical acute rejection and CNI nephrotoxicity in some paediatric programmes. There have been a few studies demonstrating the utility of protocol biopsies in paediatric patients, but the largest study of serial protocol biopsies in paediatric renal transplant patients (a National Institutes of Health randomized trial (Vidhun et al., 2003; Naesens et al., 2012)) conclusively showed that chronic tubulointerstitial fibrosis is progressive over time, irrespective of incidents of clinical acute rejection; is not influenced by steroid usage or avoidance; and is significantly greater in infant recipients of adult-sized kidneys, a consequence of the discrepancy between recipient vasculature and donor blood flow demand.

Rejection

Hyperacute rejection occurs immediately after a kidney is transplanted in the presence of preformed antibodies against the donor HLA, ABO, or other antigens. Fortunately, it is rare, as the kidney has to be removed.

The diagnosis of acute rejection in the very young transplant recipient can be difficult. A proposed diagnostic workup, stratified by recipient age is shown in Fig. 290.2. Because many small

children are transplanted with adult-sized kidneys, a rise in serum creatinine may be a late sign of rejection. This explained by the large renal reserve compared with the body mass. Thus, substantial allograft dysfunction may be seen with minimal change in the serum creatinine level. An early and sensitive sign of rejection is the development of hypertension and low-grade fever. In children, any rise in the serum creatinine, especially if it is accompanied by hypertension, should be attributed to acute rejection until proven otherwise (Fig. 290.2). A percutaneous biopsy in an infant recipient of an adult-sized kidney may be difficult because of the intraperitoneal location of this kidney and its proximity to the bowel. Guidelines on a safe approach for the closed biopsy in this clinical setting have been outlined by Vidhun et al. (2003). A late diagnosis of rejection is often confounded by the association of

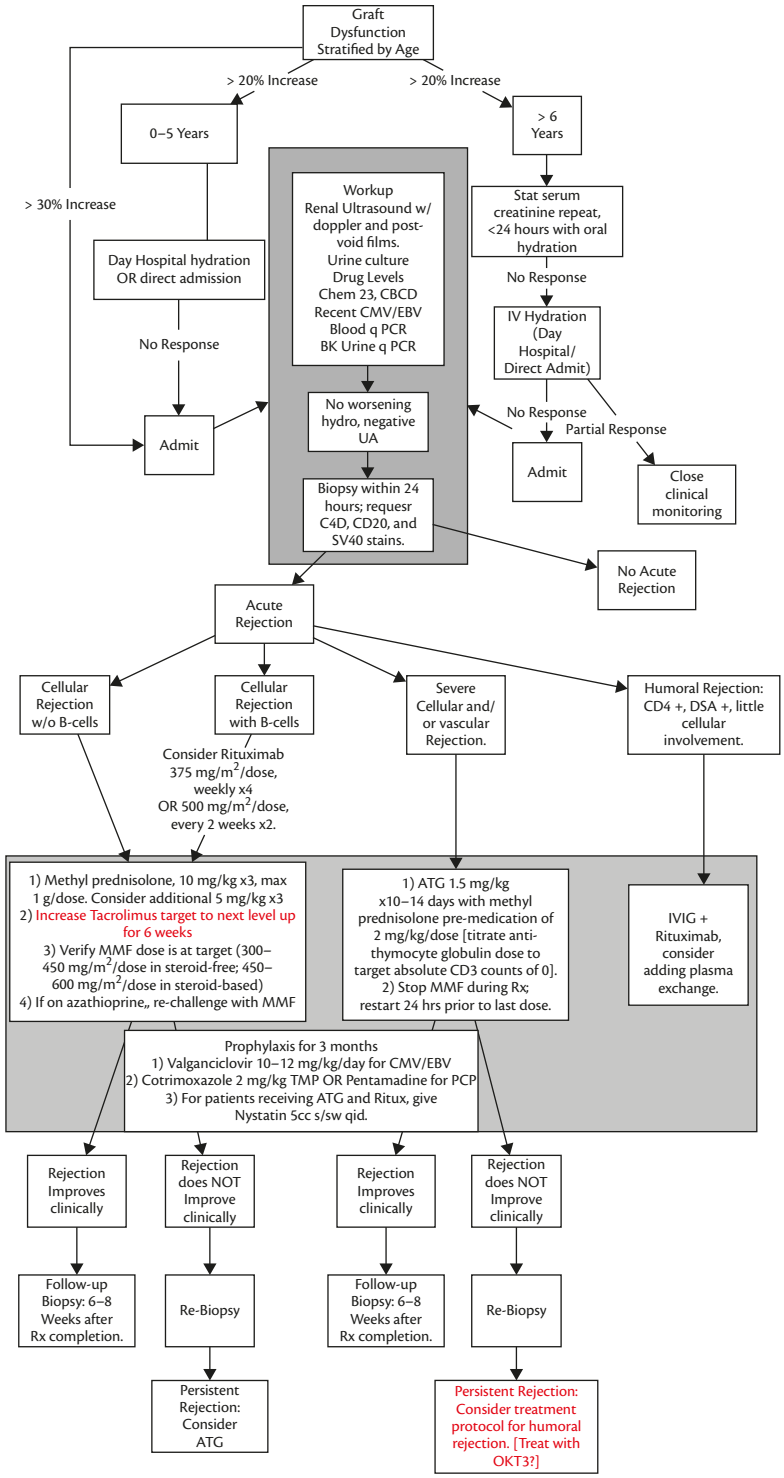

Fig. 290.2 Proposed protocol for management of graft dysfunction, stratified by recipient age. ATG = Anti-thymocyte globulin (Thymoglobulin); MMF = Mycophenolate Mofetil; MPA = Mycophenolic Acid; TMP = Trimethoprim.

chronic injury because of either immune or non-immune causes. Standardized scoring criteria have been developed for differentiating chronic calcineurin toxicity from chronic rejection in paediatric renal transplant recipients (Kambham et al., 2007). Late acute rejection is more likely to be refractory and lead to graft loss (Racusen et al., 2004). The inclusion of a large genomic biomarker study in the randomized, multicentre trial of steroid-avoidance versus steroid-based immunosuppression in 130 low-risk paediatric transplant recipients (SNSO1) (Sarwal et al., 2012), identified blood-based gene markers for the non-invasive diagnosis and prediction of biopsy confirmed acute renal allograft rejection (Li et al., 2012). The availability of this assay has the potential to change the number of steps used in the current clinical management algorithm shown in Fig. 290.2, and thereby reduce graft injury by early detection of rejection, early treatment, and early reversal of tissue injury.

Chronic rejection (known officially as interstitial fibrosis/tubular atrophy (IF/TA)) is the leading cause of graft loss, and occurs because of immune and non-immune injuries, including hypertension, diabetes, and hyperlipidaemia. Children may have a gradual deterioration of their renal function with proteinuria and hypertension. Although there was interest in the ability of MMF and sirolimus to reduce the incidence of chronic graft injury, this has not proved effective (see Chapter 281).

Graft survival

Five-year graft survival is better in living donor recipients than in deceased donor recipients (Tables 290.1 and 290.2). Children under 10 years of age have the best long-term graft and patient survival rates (Magee et al., 2004). Graft survival in adolescent patients is the worst, probably because of non-adherence to immunosuppressive drugs. Other risk factors for graft failure are race, previous transplant history, history of multiple blood transfusions, HLA-B matches, gender, and transplant year.

About 25% of paediatric renal transplants are pre-emptive. Graft survival for both living and deceased donor kidneys is significantly better in the pre-emptive group than in patients on dialysis. The three most common causes of graft failure include IF/TA (34.9%), acute rejection (13%), and thrombosis (10.1%); 6.4% had graft failure related to recurrence of primary disease (NAPRTCS Annual Report, 2007). The NAPRTCS 2003–2007 data showed that the incidence of acute rejection was 8.7% for living donor and 17.7% for deceased donor recipients.

Graft survival is significantly worse in the presence of delayed graft function (DGF). DGF is defined as the need for dialysis within the first week after transplant. The incidence of DGF is 5.1% in living donor renal transplants and 16.4% in deceased donor transplants.

Table 290.1 Graft survival. OPTN/SRTR 5-year graft survival 2000–2005

	1–5 years	6–10 years	11–17 years
Living donors	88.5%	84.6%	74.4%
Deceased donors	74.4%	72.1%	63%

Table 290.2 NAPRTCS 2003–2006 1-year graft survival

Living donor	95.7%
Deceased donors	95%

Infectious complications

While the incidence of acute rejection has declined over the past 15 years, the incidence of infections has increased. In 1987, there were more hospitalizations for rejection than for infection, while in 2000, there were over twice as many hospitalizations for infection as for rejection.

Pneumonia and urinary tract infections (UTIs) are the most common bacterial infections (see Chapter 284). UTI can progress rapidly to pyelonephritis. Trimethoprim/sulfamethoxazole is prescribed for UTI prophylaxis as well as for *Pneumocystis* pneumonia prophylaxis for at least the first 6 months after transplant. Opportunistic infections associated with unusual organisms usually occur after the first month following transplantation. The herpes viruses, CMV, varicella zoster, and EBV, are a particular problem in children as they have generally not been exposed to those viruses. The incidence of CMV seropositivity is about 30% in children > 5 years of age and rises to about 60% in teenagers. Younger patients are, therefore, at higher risk for viral infection when a CMV-positive donor kidney is transplanted. About 90% of children are seronegative for EBV, and infection will occur in about 75% of these patients. Most EBV infections are clinically silent, but PTLD is the possible serious consequence. The incidence of PTLD is higher in children who receive antibody induction and after treatment of acute rejection, and prophylactic therapy is recommended. Recently, it has also been shown in children that subclinical replication of these viruses is much higher in children on steroids than in those not on steroids, and that even subclinical viral replication may have a detrimental effect on the incidence of acute rejection and graft function (Dharnidharka et al., 2004; Li et al., 2007). Antiviral prophylaxis with ganciclovir and valganciclovir for 3–12 months after transplantation, especially in the higher-risk groups (recipient seronegative, donor seropositive), has been effective in reducing the incidence of clinical CMV and EBV disease. Serial surveillance for these viruses by quantitative polymerase chain reaction (PCR) has also allowed for minimization of immunosuppression to reduce the risk of infection (Fig. 290.3).

Polyomavirus nephropathy (PVN) is now an important cause of allograft dysfunction; almost a third of children will have BK viruria, although allograft dysfunction is observed in perhaps 5%. The increased incidence of PVN is the result of more potent immunosuppression. A renal biopsy identifying the virus by immunoperoxidase staining may be required to make the diagnosis with certainty. Reducing immunosuppression is the first line of therapy, and very low-dose cidofovir, leflunomide, and ciprofloxacin are used as adjunctive therapies.

The risk of *PTLD* is highest in the EBV+ donor/EBV– recipient population. A plan to monitor for EBV, CMV, and PTLD is provided in Fig. 290.3.

Hypertension, hyperlipidaemia, and *post-transplant diabetes mellitus* are the important potential complications of immunosuppressive drugs. *Non-adherence* is one of the most important and difficult problems in paediatric transplantation. By one estimate,

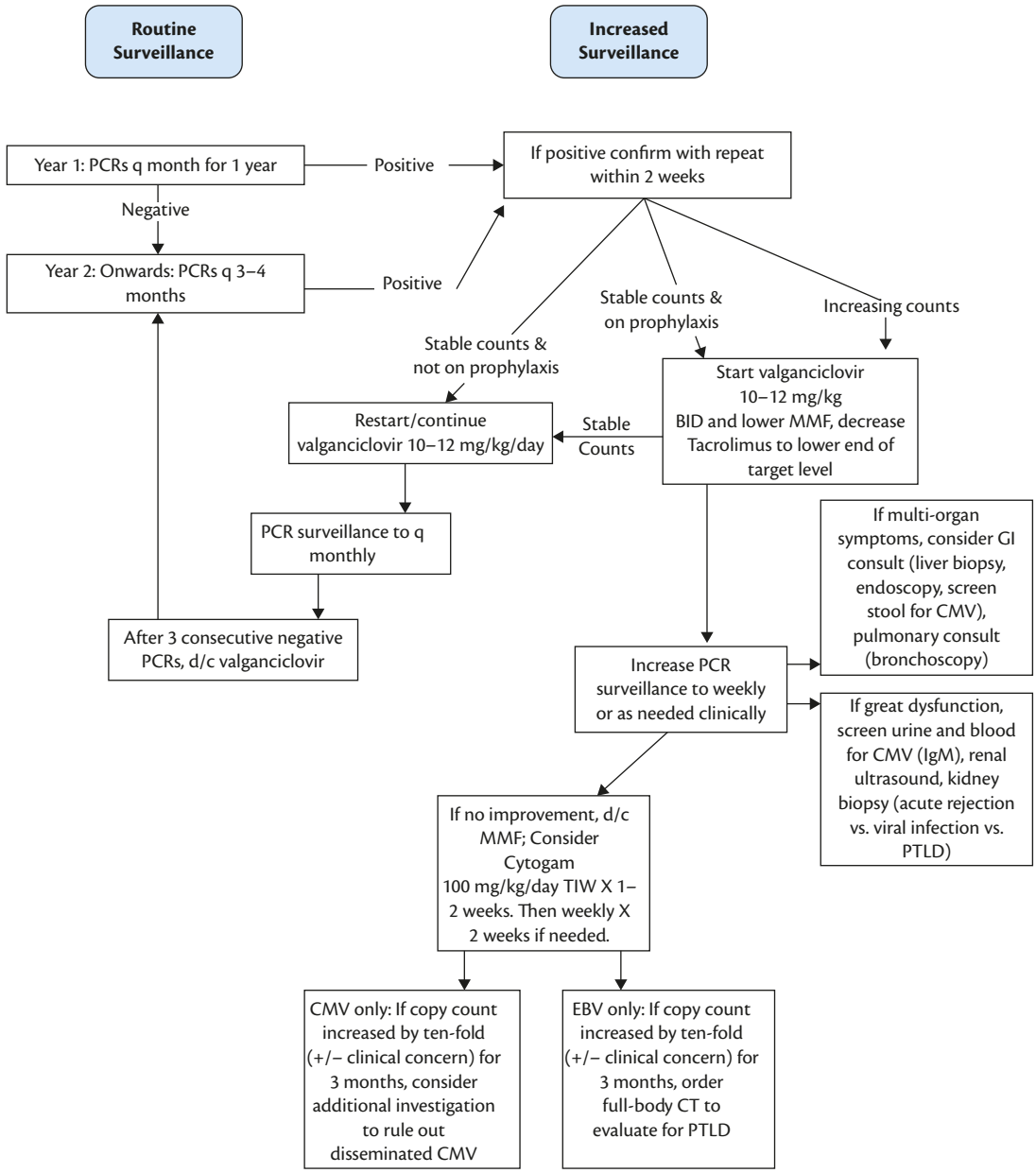

Fig. 290.3 Surveillance for CMV and EBV viral infections after paediatric kidney transplantation.

at least half of the paediatric deceased donor transplant recipients demonstrated significant medication non-adherence in the post-transplantation period. This figure exceeded 60% in adolescents (Shaw et al., 2003), and the true incidence may be higher. Non-adherence is the main cause of graft loss in 10–15% of paediatric kidney transplant recipients; in re-transplant patients, this figure may exceed 25%. Risk factors for non-adherence include female sex, adolescent age, family instability, insufficient emotional support, lower socioeconomic class, and maladaptive behaviour.

Though growth improves after transplantation, chronic corticosteroids will inhibit growth. The mechanism is unknown. Corticosteroids may reduce the release of growth hormone, reduce insulin growth factor (IGF), impair cartilage growth, decrease calcium absorption, and increase phosphate wasting. The administration of recombinant growth hormone in paediatric renal transplant recipients can improve growth. A GFR of < 60 mL/min/l.73 m^2 is associated with poor growth and low IGF levels; optimal growth occurs when the GFR is > 90 mL/min/1.73 m^2. Impaired graft function is the second most important cause, after corticosteroids, of post-transplantation growth retardation. Steroid minimization and withdrawal protocols are associated with improved growth, and steroid avoidance in children allows for significant catch-up growth at 5 years post transplantation. Optimal growth is achieved in children with a well-functioning kidney who are not on corticosteroids.

Long-term outcome

With better immunosuppression and close attention to psychosocial, educational, vocational, and developmental issues, the social and emotional health of the child returns to pre-transplant levels

within a year after successful transplantation. Intellectual development improves after renal transplantation too (Fennell et al., 1986; Mendley and Zelko, 1999). Most patients can resume school and social activities 4–6 weeks after surgery. Three years after transplantation, nearly 90% of children are in their appropriate level of school. Ten-year survivors report that their health is good, and they are able to engage in appropriate social, educational, and sexual activities and experience a very good to excellent quality of life.

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