

Continuing Medical Education

KIDNEY TRANSPLANTATION: CURRENT ISSUES AND FUTURE PROSPECTS

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INTRODUCTION

Renal transplantation has evolved from an experimental treatment for end-stage renal disease (ESRD) into routine clinical therapy in less than five decades. The success story of kidney transplantation is closely linked to important synchronous breakthroughs in the understanding of fundamental immunology, the identification of the Human Leukocyte Antigens (HLA) and the development of powerful immunosuppressive drugs. Today, the geographical penetration of renal transplantation as renal replacement therapy is still characterized by a very large variation and is virtually absent in developing countries. Reported prevalent rates of (functioning) renal grafts range from as low as 23 per million population (Russia) over 398 (Belgium) to as high as 584 per million in some Spanish regions, while incident transplant rates follow a similar pattern (from 2 and 29.3 to 64.4 per million population) (1). World-wide initiatives for the promotion of organ transplantation have led, at least in some regions, to a sharp increase in transplantation rates but unfortunately do not provide any counterbalance against the enormous increase in

patients with ESRD (1). While short-term transplantation results are excellent nowadays with first year graft survival rates of +95% and even higher patient survival rates, no dramatic improvements in terms of long-term outcome have been established in the last decades (2). This discrepancy is reflected by the increasing rates of secondary transplantations and the growing waiting lists. Many initiatives have been launched in the last decade in order to fight this looming threat of an ever-increasing organ shortage and also characterize the way basic and clinical transplantation research is currently directed.

Programmes to expand and extend the different types of donor pools have been successful in recent years and have, at least temporary, caused some improvement in donor rates but do not provide a sole solution for achieving a steady-state equilibrium between demand and supply (3). Attempts to improve long-term outcome by optimizing the use of our current immunosuppressive arsenal with the goal of prolonging graft half-lives and patient survival have yielded disappointingly limited results (4). And while xenotransplantation still tries to cope with overcoming fundamental barriers, attention has currently turned into the exploration of immunological tolerance with the hope of providing eternal life for a transplanted kidney. In this exiting era of great endeavours it is not only a privilege to work in the field of transplantation but also a mission to spread the awareness that perhaps, one day in the future, we all may need a transplant organ.

DONATION

The obvious way of improving transplantation rates is increasing donation rate. Many regional and national initiatives have been launched in order to sensitize in-

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tensive care and emergency department personnel for the growing demand of organs and the need for early potential donor identification and improved management (5). The majority of these "donor action" programmes have had a measurable success but all seem to require regular reinforcement in terms of new public campaigns and promotions in order to sustain their beneficial effects.

An alternative method to increase effective donation rate is to extend the criteria for donor acceptance which very often signifies an important lowering of donor quality. The use of extended criteria donors (ECD, Table 1) has resulted in increased transplantation rates in many countries (1,6) while the use of non-heart beating donors (NHBD) has received less widespread acceptance but is particularly successful in a limited number of countries like Spain and the Netherlands (7,8). Initial reports suggest that overall graft survival and patient survival with ECD and NHBD donors are not very different from so-called "normal deceased donor" pro-

grammes provided that some adaptations in clinical management are taken into account (9). Recent studies have started to assess if the former donor categories also require different methods of preservation and immunosuppressive therapeutic protocols in order to compensate for the initial suboptimal graft condition at the time of implantation (10).

While living donor transplantation programmes have always been characterized by a very heterogeneous geographical distribution, a lot of new ideas have been developed in recent years in order to increase the use of this vast organ pool. At the same time ethical considerations of living donation have been brought to the public view, exposing the dreadful practices of organ trade and the use of donors in violation with human rights legislation (11-13). While living-related (LRD) and unrelated (LURD) donation have been practiced for decennia, living donor exchange programmes (LDEP) have only emerged recently in some countries like the Netherlands (14). These new initiatives seem to provide a solution for over more than half of ABO-incompatible and positive cross-match donor-recipient pairs (15). The principle of donating a kidney into the deceased donor pool in return for a prioritized deceased donor kidney has been intensively debated but not yet applied in clinical practice. This kind of practices, when performed in vast numbers, could potentially offset balances in deceased donor pools with the risk of extending the time on the waiting list for certain blood group and tissue types. More simulations are needed to clear this difficult question. Finally, the emergence of more and more altruistic donations is another way of increasing transplantation rates but the former complicate the ethical debate even further and intensify the need for careful legislation (16). Innovations in the surgical aspects of living donation techniques like laparoscopic donor nephrectomy have contributed to the growing confidence in these procedures (17). However, better guidelines for long-term medical follow-up of living donors are mandatory as an essential part of a truly complete transplantation programme (18-20).

Table 1: Types of donors currently employed in renal transplantation.

Type of donor	Characteristics
Deceased donor	Deceased unrelated anonymous donor
Extended criteria donor (ECD)	Age \geq 60 years OR Age 50-59 years and either: - Cerebrovascular accident (CVA) + arterial hypertension (AHT) + serumcreatinine > 1.5 mg/dL OR - CVA + AHT OR - CVA + serumcreatinine > 1.5 mg/dL OR - AHT + serumcreatinine > 1.5 mg/dL OR Cold ischaemia time \geq 24 hours
Non-heart beating donor (NHBD)	
Maastricht category I	Donor is dead on arrival at the hospital
Maastricht category II	Donor has undergone unsuccessful resuscitation
Maastricht category III	Donor in whom cardiac arrest is awaited
Maastricht category IV	Donor with cardiac arrest while brain dead
Living related donor	Parents – children Siblings
Living unrelated donor	Spouses – life partners
Living exchange donor	Parents – children - siblings - spouses – life partners
(Living donor deceased donor pool exchange)	(Parents – children -siblings - spouses – life partners)
Altruistic donor	Living unrelated unknown donor

ALLOCATION

The allocation of kidney allografts is primarily based on the point score system which takes into account HLA-A, -B, -DR-matching, percentage panel-reactive T-cell antibodies (PRA), ABO blood group rules, waiting time, donor region and medical urgency to determine the rank order on the waiting list. A couple of years ago

an initiative was started by Eurotransplant to assess whether shortening of the cold ischaemia time of kidneys of older deceased donors (≥ 65 years) for 65+ recipients improved outcome. In this "Eurotransplant Senior Programme" (ESP) allocation was done without taking into account HLA-matching (21-22). Within the framework of Eurotransplant's aim to keep or even increase the number of renal transplants, the ESP has proven to be quite successful without compromising the outcome for the individual end-stage renal disease patient (23).

IMMUNOSUPPRESSION

Standard immunosuppressive therapy.

Calcineurin-inhibitors (CNI). Cyclosporine A (Neoral™, Novartis) and tacrolimus (Prograf™, Astellas) inhibit lymphocyte proliferation by preventing the translocation of the transcription factor NF-(kappa)B into the nucleus and blocking transcription of the IL-2 gene. Both compounds are characterized by a highly variable oral bioavailability and a narrow therapeutic window, reinforcing the need for close therapeutic drug monitoring, usually based on pre-dose trough blood concentrations (24). Calcineurin-inhibitors form the basis of most currently used "standard triple immunosuppressive drug regimens" in renal transplantation. Usually they are combined with corticosteroids and an anti-metabolite drug (25,26, see below). The introduction of CNI has led to a significant improvement of short-term efficacy (avoidance of acute allograft rejection)

but has not altered long-term graft survival (27,28). The long-term disadvantages of CNI are the unfavourable profile of cardiovascular side-effects like arterial hypertension, hyperlipidaemia, diabetes mellitus (see Table 2) and their intrinsic nephrotoxic properties, leading to chronic graft damage (29). While attempts to transplant without the use of CNI have been occasionally successful (30,31), it is still not common practice because equally frequent failures have been reported (32,33). A more promising approach seems to be the substantial reduction of CNI target blood concentrations while at the same time compensating for the overall degree of immunosuppression by using non-nephrotoxic and less cardiovascular toxic alternatives (34). Because CNI are substrate of the p-glycoprotein (P-gp, ABCB1) transporter and metabolized mainly by cytochrome P450 3A (CYP3A) enzymes, they are subject to important drug-drug interactions with inducers or inhibitors of the former. A few examples of clinically relevant drug-drug interactions which lead to significant changes in CNI exposure are summarized in Table 3.

Anti-metabolites. The introduction of mycophenolate mofetil (MMF, Cellcept™, Roche) in the mid-nineties further reduced acute rejection rates and resulted in an almost complete discontinuation of azathioprine (Imuran™, Glaxo-Smith-Kline) as primary concomitant drug for CNI together with corticosteroids (35,36). Mycophenolate mofetil is a selective blocker of the *de novo* purine salvage pathway in lymphocytes through binding with the inosine-monophosphate-dehydrogenase enzyme (IMDPH2 iso-enzyme) (37). MMF is rapidly and almost completely absorbed from the gastrointestinal

Table 2: Clinically relevant side-effects of current immunosuppressive drugs.

Adverse effects	Cyclosporine	Tacrolimus	MMF / EC-MPS	Corticosteroids	Sirolimus / Everolimus	IL-2R mAb
Arterial hypertension	++	+		+		
Hyperlipidaemia	++	+		++	+++	
Nephrotoxicity	++	++			+*	
Bone marrow toxicity			++		+	
NODAT	++	+++		+++		
Gastrointestinal side-effects	+	+	+++ [§]		+	
Neurotoxicity	++	++				
Esthetical side-effects	++	+		+++	+	

MMF/EC-MPS: mycophenolate mofetil / enteric-coated mycophenolate sodium; IL-2-R mAb: IL-2 receptor blocking monoclonal antibodies (daclizumab, basiliximab); NODAT: new-onset diabetes mellitus after transplantation. *sirolimus / everolimus without calcineurin-inhibitor. §gastrointestinal disorders (diarrhea, abdominal pain, nausea & vomiting) exclusive hepatotoxicity, probably less upper gastrointestinal side-effects with EC-MPS.

Table 3: Examples of clinically relevant drug-drug interactions affecting immunosuppressive drug exposure.

Immunosuppressive drug	Effect on exposure	Concomitant drug
Calcineurin-inhibitors	Increased exposure	Macrolide antibiotics Azole antifungals Calcium channel blockers Grapefruit, Seville oranges (naringenin)
	Decreased exposure	Phenytoin Rifampin St. John's wort (<i>Hypericum perforatum</i>)
Mycophenolate mofetil / EC-MPS	Increased exposure	Probenicid Ganciclovir Acyclovir
	Decreased exposure	Cyclosporine Iron (oral) supplement Rifampin Cholestyramine Magnesiumoxide, Aluminiumhydroxide Antibiotics
Sirolimus / everolimus	Increased exposure	Cyclosporine Macrolide antibiotics Azole antifungals Calcium channel blockers
	Decreased exposure	Phenytoin Rifampin

tract and hydrolyzed into its active form mycophenolic acid (MPA) (37). Similar to its previous non-specific predecessor (azathioprine), MMF is a bone marrow suppressive agent, requiring dose adjustments in case of severe leukopenia or anaemia. Clinically, the most important side-effect of the drug is gastrointestinal intolerance with upper GI-tract symptoms and/or diarrhoea (37). Recently, a bioequivalent modified release formulation of MPA has been developed, mycophenolate disodium (Myfortic™, Novartis) which seems to cause less upper GI-tract symptoms although trials directly comparing both formulations are currently still lacking (38). Mycophenolic acid is devoid of any cardiovascular side-effects, making it the drug of choice to compensate for reduced concentrations of CNI in immunosuppressive protocols (39). At the moment the therapeutic window of MMF is not delineated as well as for CNI. Especially the upper target ranges, indicating toxicity, are still not clearly defined while pre-dose trough plasma concentrations of MPA do not seem to reliably

mark the lower target range for maintaining efficacy. Currently, large international multi-centre trials (e.g. FDCC-trial) are examining the need and the optimal way of measuring MPA exposure, in order to achieve superior results in terms of efficacy and toxicity. MPA undergoes extensive enterohepatic recirculation (EHC) and while cyclosporine inhibits the EHC of MMF, tacrolimus does not (41). This results in a higher dose-corrected MPA exposure with tacrolimus and hence lower dose requirements. Currently, a daily MMF dose of one or two grams is advocated in combination with tacrolimus while recent data indicate that in combination with cyclosporine two and even better three grams are necessary, especially in the early post-transplantation period when the risk of acute rejection is higher (32). Drug interaction with MMF are less frequent than for example with CNI; attention is warranted when using concomitant cholestyramine, magnesium oxide, sodium bicarbonate, oral iron supplements, rifampicin and certain antibiotics. As a selective anti-metabolite, MMF is also more and more successfully used outside the transplantation setting, as treatment for different types of glomerulonephritis and systemic vasculitis (42).

Corticosteroids. At present, glucocorticosteroids still form an undeniable part of maintenance immunosuppressive therapy but are used in dramatically reduced doses. Few studies have actually addressed the possibility of transplanting without corticosteroids but have met with variable success (43,44). Early discontinuation of corticosteroids is probably a more realistic procedure and currently steroids can be stopped safely from triple regimens including a calcineurin-inhibitor and MMF, as early as 3 months after grafting (45) or even earlier (46). While discontinuation of steroids has favourable effects on bone mineral density and hyperlipidaemia (46,47), it is not yet established whether this can also lead to a state of long-term under-immunosuppression and hence more chronic kidney damage.

m-TOR inhibitors. The m-TOR (mammalian target of rapamycin) inhibitors are potent blockers of cell cycle progression and have anti-proliferative effects on lymphocytes and non-immune cells like vascular smooth muscle cells and fibroblasts (48). Sirolimus (Rapamune™, Wyeth) and everolimus (Certican™, Novartis) are, similar to calcineurin-inhibitors, characterized by a narrow therapeutic index and require therapeutic drug monitoring because of important variability in oral availability (49). Because in the pivotal clinical studies the combination of m-TOR inhibitors with CNI was

found to increase CNI-related nephrotoxicity as a result of pharmacokinetic and pharmacodynamic drug-drug interactions, subsequent studies mainly explored the use of m-TOR inhibitors without concomitant CNI (50). Two different approaches seem to provide the best use of these compounds: either m-TOR inhibitors are only briefly combined with CNI after transplantation (first 3 months) and then maintained in combination with MMF and corticosteroids (51) or m-TOR inhibitors are combined with MMF and steroids from the start in combination with induction therapy (31) (see below). In these latter instances m-TOR inhibitors seem to provide better long-term allograft function, both functionally and histologically, at least when used in immunological low to moderate risk recipients. m-TOR inhibitors have a specific side-effect profile (see Table 2) that requires specific attention in the immediate postoperative period (impaired wound healing), in the long-term (hyperlipidaemia) and when used in the setting of switching from CNI-based therapy to m-TOR inhibitors because of chronic graft dysfunction (proteinuria, anaemia) (50). Because m-TOR inhibitors have anti-proliferative effects on vascular smooth muscle cells, they have been successfully incorporated in drug-eluting coronary stents (52). Currently, the final balance between the favourable vascular effects of m-TOR inhibitors and their negative effect on lipids, has not yet been studied in controlled long-term studies. The lack of effect on arterial hypertension and diabetes mellitus makes m-TOR inhibitors an interesting alternative to be considered in case of severe CNI toxicity. m-TOR inhibitors are metabolized through cytochrome P450 enzymes and are transported by p-glycoprotein, making them susceptible to very similar types of drug-drug interactions as calcineurin-inhibitors (see Table 3).

Induction therapy.

Anti-lymphocyte antibodies. Thymoglobulin™ (Imtix Sangstat) is a rabbit-derived polyclonal antibody specific for T-cell epitopes including CD2, CD3, CD4, CD8+, CD25, and is used in the immediate posttransplantation period (induction phase) in patients at increased risk for acute rejection. The drug induces a rapid dose-dependent decline in blood lymphocytes and T-cells in spleen and lymph nodes which lasts up to six months (52). Because of its animal origin, anti-lymphocyte antibodies can cause a serious "cytokine release" syndrome characterized by fever, chills, generalized illness, arthralgia and headache (52). In order to reduce these symptoms prophylactic corticosteroids and H1- and

H2-blockers are co-administered, especially during the initial doses. On rare occasions, anti-lymphocyte antibodies can produce a serum sickness type of reaction (52). Apart from their role as induction agent, anti-lymphocyte globulines are also used in case corticosteroids fail to control an acute rejection episode -a so-called steroid-resistant acute rejection- or if the use of high doses of corticosteroids is contraindicated (53).

Interleukin-2-receptor blocking monoclonal antibodies (IL-2R-mAb). These chimeric and humanized anti-IL-2-receptor monoclonal antibodies produce a durable receptor blockade, lasting for up to 3 months (54,55). This makes them ideal induction agents for recipients at increased immunological risk treated with triple immunosuppression. In contrast to anti-lymphocyte globulines, these agents are devoid of serious cytokine release effects, except for the occasional mild infusion reaction. Basiliximab (Simulect™, Novartis) and daclizumab (Zenapax™, Roche) are both IL-2R-mAb of which only the former is currently available in Belgium. IL-2R-mAb cannot be used in the treatment of acute rejection. Maintenance therapy with these compounds is going to be explored in future clinical trials.

COMPLICATIONS

Patient survival.

The most frequent cause of renal allograft failure is still death of the patient with a functioning graft (DWFG) which accounts for over 50% of all graft losses (56). In the past decade improvements in patient survival (DWFG occurred in 38% of patients transplanted between 1988 and 1997) (57) have been difficult to discern due to the negative consequences of increasing donor and recipient age and the important morbidity associated with the latter. Mortality after transplantation remains associated with infectious complications, cardiovascular events and malignancies.

Infectious complications. The immune-compromised transplant recipient is susceptible for a variety of opportunistic infectious agents including bacteria, viruses, fungi, protozoa and parasites. In the past, infections affected up to 80% of patients and were responsible for 40% of the first year mortality (58). Bacterial infections including wound infections, pneumonia, urinary

tract infections and sepsis, often of unknown origin, were frequently encountered after transplantation (59). Risk factors for this type of bacterial infection were (and still are) diabetes mellitus, smoking, obesity, urinary tract abnormalities, the presence of foreign materials and indwelling catheters (59). Subsequent improvements in surgical techniques, the availability of more accurate diagnostic tools and more effective therapies have contributed to a gradual decline in number and severity of infections (60). While the use of effective antibiotics has dramatically improved outcome of bacterial infections, an increasing number of multi-resistant bacterial strains are posing an enormous future threat. The use of prophylactic antimicrobial drugs in the initial postoperative period for prevention of opportunistic infections like cytomegalovirus, *pneumocystis jirovecii* and *candida albicans* have shown their usefulness while for chronic pre-existing infections like hepatitis B and C, treatment is currently scheduled prior to transplantation (61). Finally, effective vaccination schemes comprising hepatitis A and B, tetanus, pneumococcal disease and influenza, have helped to further reduce posttransplantation infectious complications. Despite all these measures, infectious complications still account for 9 to 25% of all deaths occurring after transplantation in all age groups (62). These relatively high mortality numbers indicate that clinical vigilance in transplant recipients is still warranted. Given the recent availability of new types of powerful anti-infective drugs (for example caspofungin and voriconazole), prompt diagnostic work-up and treatment are fundamental steps towards a better control of infectious complications after transplantation.

Cardiovascular complications. Cardiovascular disease (CVD) is the leading cause of death after transplantation with a reported prevalence ranging between 20 and 63% (56). The annual CVD death rate among renal recipients is significantly higher than in the general population, even after correction for age, gender and race. Similar to patients in renal replacement therapy, graft recipients are exposed to a higher cardiovascular risk compared to non-renal patients. Apart from specific transplantation-related risk factors, classical cardiovascular risk factors can be identified: male gender, arterial hypertension, hyperlipidaemia, smoking, obesity, diabetes mellitus or impaired glucose metabolism. Left ventricular function, pre-existing CVD, anaemia, hyperuricaemia, hyperhomocysteinaemia, corticosteroid (acute rejection treatment) and CNI use, chronic renal allograft failure, chronic graft rejection and pro-

teinuria -to name only a few- are more typically related risk factors a transplant recipient is exposed to (56,63). Addressing these risk factors in terms of optimal prevention and treatment is probably one of the biggest challenges in current transplantation medicine. While life style modification and medications used for cardiovascular prevention and therapy are similar to those advocated for non-transplanted patients, the obligatory use of cardiovascular toxic immunosuppressive medication (see Table 2) often hampers achievement of optimal results (64). Tailoring immunosuppression to the individual needs defined by the specific cardiovascular patient profile is the aim of many recent clinical studies examining the feasibility of transplanting without or with minimal doses of corticosteroids, using reduced CNI protocols or CNI avoidance regimens or combining exclusively non-cardiovascular toxic drugs (45-47,65). Whether these initiatives will produce detectable improvements, taking into account the overall challenging risk profile of today's average renal transplant candidate, remains to be determined. Encouraging results of recent large intervention trials in transplant populations, like for example the ALERT trial, demonstrate that the use of classical preventive drugs like statins can achieve comparable improvements in cardiovascular risk factor as in the non-transplanted population, albeit not to the same extent (66,67).

As pre-existing CVD is the strongest predictor of posttransplant CV mortality, strong emphasis should also be placed on the use of stringent screening strategies for potential transplant candidates. It is clear that the cumulative CV damage, acquired during years of renal insufficiency and/or dialysis therapy, is worsened by additional CV stress after transplantation. Guidelines have been proposed on how to effectively identify transplant candidates at risk for CVD but studies comparing different screening strategies in terms of long-term posttransplantation outcome have not yet been performed. With increasing recipient age, validated CVD screening algorithms are necessary to enable pre-emptive counterbalanced actions to remediate the negative effects of a gradually aging transplant population on CVD incidence and mortality.

Malignancies. Malignancies account for 9 to 12% of deaths after renal transplantation (57,62). Skin cancer (non-melanoma) is the most common malignancy but is usually not lethal. The overall incidence of invasive (non-skin) malignancies is significantly higher in the transplant population compared with age- and gender-matched controls in the general population (68,69). The annual

incidence in the former group ranges between 0.8 and 2.2% per year with post-transplantation lymphoproliferative disease (PTLD) and Kaposi's sarcoma being the most common tumours (70). Risk factors for development of malignancies after transplantation include age, smoking, viral infections (hepatitis B and C, EBV and HHV8) and the use of powerful immunosuppressive drug combinations (69,71). Curative treatment of invasive malignancies after transplantation is often hampered by reduced (allo)graft function, important drug-drug interactions, maintained immunosuppression and important pre-existing comorbidity. This generally results in suboptimal treatment results compared to the non-transplanted population with reduced survival rates and increased recurrence rates. Fortunately, for some types of malignancies, specific therapeutic agents with low toxicity have been developed. For example, PTLD is increasingly and effectively treated with monoclonal antibodies directed against the CD20-molecule expressed on B-lymphocytes (rituximab), thereby reducing the need for more toxic chemotherapeutic protocols (72). It is strongly advocated to provide regular cancer screening for transplant recipients, comparable with their age-matched controls in the general populations (prostate, breast, colon cancer, ...) while at the same time educate and teach the patient basic clinical self-examination techniques like skin inspection, breast palpation etc... .

Graft survival.

After death with a functioning graft, chronic allograft nephropathy (CAN) is the leading cause of graft loss despite improvements in immunosuppressive therapy (73,74). CAN is a non-specific histological mixture of cumulative acquired damage to the tubulointerstitial, glomerular and microvascular compartment of the allograft, ultimately resulting in progressive loss of function. An adequate clinical management of CAN is hampered by its insidious onset and hence late diagnosis, its intrinsic phenotypic heterogeneity caused by a variety of variables including donor and receptor characteristics, immunological and non-immunological factors and other clinical variables and finally the lack of pathophysiological insights in the aetiological processes (73,74). Fortunately, efforts are made to dissect the multiple overlaying causal pathologies resulting in the final common pathway CAN, leading to a better understanding of this complex problem. Tubular atrophy and interstitial fibrosis (IF/TA) are considered to be the principal histological changes in progressively failing

grafts (previously described as CAN) but are almost invariably accompanied by either (micro)vascular changes (denoting true chronic rejection) or signs of calcineurin-inhibitor-mediated nephrotoxicity (CNIT), acute and chronic glomerular lesions (transplant glomerulopathy), humoral changes, donor pathology, recurrence of original diseases and recently BK-(polyoma)virus nephropathy (BKVN; 73-75). BKVN is a tubulointerstitial type of viral nephritis associated with rapid graft failure in over 50% of infected cases. This opportunistic infection has emerged only in recent years and currently no effective treatment is available except for drastic reduction of immunosuppression (75). The unravelling of all these concordant processes occurring in the majority of initially well-functioning renal grafts will hopefully lead to the development of clinical tools that are necessary to modify the natural course of CAN and hence prolong graft survival.

FUTURE CHALLENGES

Pharmacogenetics. Calcineurin-inhibitors are metabolized by cytochrome P450 enzymes (CYP) 3A4 and 3A5 and are also substrate for P-glycoprotein (P-gp), an energy-dependent transmembrane efflux pump [ATP-binding cassette B1 (ABCB1)] encoded by the multidrug resistance 1 (*MDR1*) gene (25). The different levels of intestinal and hepatic expression and bioactivity of P-gp and the cytochrome P450 enzymes regulate both the absorption and the clearance of calcineurin-inhibitors, determining their first pass and oral bioavailability (76,77). Significant inter-individual variation in the expression and function of CYP3A5, CYP3A4 and *MDR1* is caused by single nucleotide polymorphisms of genes encoding these proteins (25). Several studies have shown that *CYP3A* genotypes are associated with significant differences in CNI dose requirements between individuals. These findings have caused speculations concerning the individualization of CNI therapy based on the genetically determined expression of *CYP3A*, with the goal of improving efficacy (avoidance of acute rejection) by achieving earlier adequate drug exposure after grafting. For example, Anglicheau et al. reported 40% higher tacrolimus dose requirements in patients homozygous for the exon 21 SNP of the *MDR1* gene compared to wild-type individuals (78).

In analogy, for mycophenolate mofetil, different effects of SNPs of genes encoding for enzymes involved in mycophenolic acid metabolism (*UGT1A9*) and transport (*MRP2*) have been identified (79,80). These poly-

morphisms significantly affect dose-corrected MPA exposure in renal recipients and potentially determine the individual susceptibility for drug-related side-effects. Further exploration of this challenging area of research could potentially lead to methods for pre-transplantation pharmacogenetic fingerprinting in order to pre-emptively individualize and optimize immunosuppressive therapy, both in terms of early efficacy and long-term toxicity.

Graft monitoring. In the last 10 years an increasing number of studies have demonstrated that well-functioning stable kidney grafts can contain tubulointerstitial inflammatory infiltrates, histological similar to clinical acute cellular infiltrates seen in biopsies of rejecting grafts (81). This has motivated some investigators to systematically search for these subclinical inflammatory changes using surveillance or protocol biopsies; i.e. routine biopsies performed at regular time intervals without changes in graft function. The previously unnoticed acute inflammatory infiltrates could be one of the reasons why, despite the use of efficient immunosuppressive drug regimens resulting in strongly reduced clinical acute rejection rates, not much improvement has been observed in long-term graft survival (82,83). Subsequently the term subclinical acute rejection (SCAR) was proposed to describe these inflammatory changes. The recognition of the importance of this cumulative chronic damage to the graft (84) has further helped to develop more refined sensitive and specific non-invasive methods to monitor the transplanted kidney. Current innovations in genomics, proteomics, metabolomics and gene chip techniques (85,86) have been successfully applied to blood, urine and tissue samples to obtain more information about the subclinical graft status. These non-invasive techniques could potentially replace tissue biopsies in the future as a safe, fast and repetitive method of graft monitoring. Exploring the subclinical natural history of the kidney graft with the goal of optimizing immunosuppressive therapy could ultimately lead to prolonged graft survival and improved overall clinical care.

CONCLUSION

In the last decades renal transplantation has become a successful routine treatment for patients with end-stage renal disease. The price for this success are huge waiting lists populated by elderly patients with increased and complex comorbidity. It is a challenge for

transplantation practitioners to find ways of improving long-term graft survival as part of a solution for the growing demand for organs. Simultaneously, joined efforts are needed to improve the overall clinical care of the vulnerable transplant recipient in an attempt to further reduce morbidity and mortality.

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