Chronic rejection in renal transplantation

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Cover: Chronic transplant glomerulopathy

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Chronic rejection in renal transplantation

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Voor de transplantatiepatiënten
Voor Marie-Christine en de jongens

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Chronic rejection in renal transplantation

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Case history

A woman, born in 1972, had a history of proteinuria secondary to focal segmental glomerulosclerosis diagnosed in 1982. Renal failure developed gradually and she started peritoneal dialysis in 1991. In 1992 she received a cadaveric renal transplant from a 19-years old male donor who had died from a trauma. At time of transplantation she had 4% panel-reactive antibodies and denied cigarette smoking. There was a 1-2-0 human leukocyte antigen (HLA)-A,B,DR mismatch with three class I cross-reactive group (CREG) mismatches and one shared CREG. She was treated with prednisone and cyclosporine Sandimmune. Her initial post-transplant course was uncomplicated without delayed graft function or early acute rejection episodes (ARE). At six months the serum creatinine level was 111 µmol/l, which corresponded with an endogenous creatinine clearance of 85 ml/min and there was no proteinuria. In 1993, one and a half years after transplantation, she participated in a steroid withdrawal trial and was assigned to prednisone withdrawal. After several weeks her creatinine level rose to 165 µmol/l and a biopsy showed an interstitial infiltrate and tubulitis (figure 2A). This ARE responded favourably to the administration of a steroid pulse. However, after reaching a nadir of 125 µmol/l her creatinine level gradually increased again and she developed 3.7 g/day of

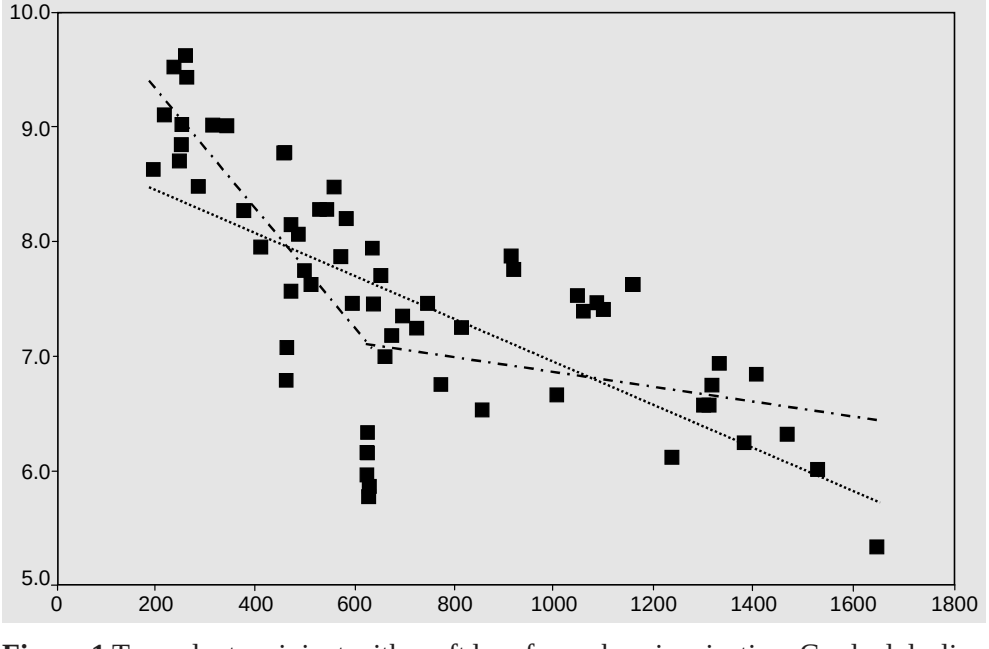

Figure 1 Transplant recipient with graft loss from chronic rejection. Gradual decline in renal function, expressed as a negative slope of the reciprocal creatinines (1000/serum creatinine) over time (days post-transplantation) curve.

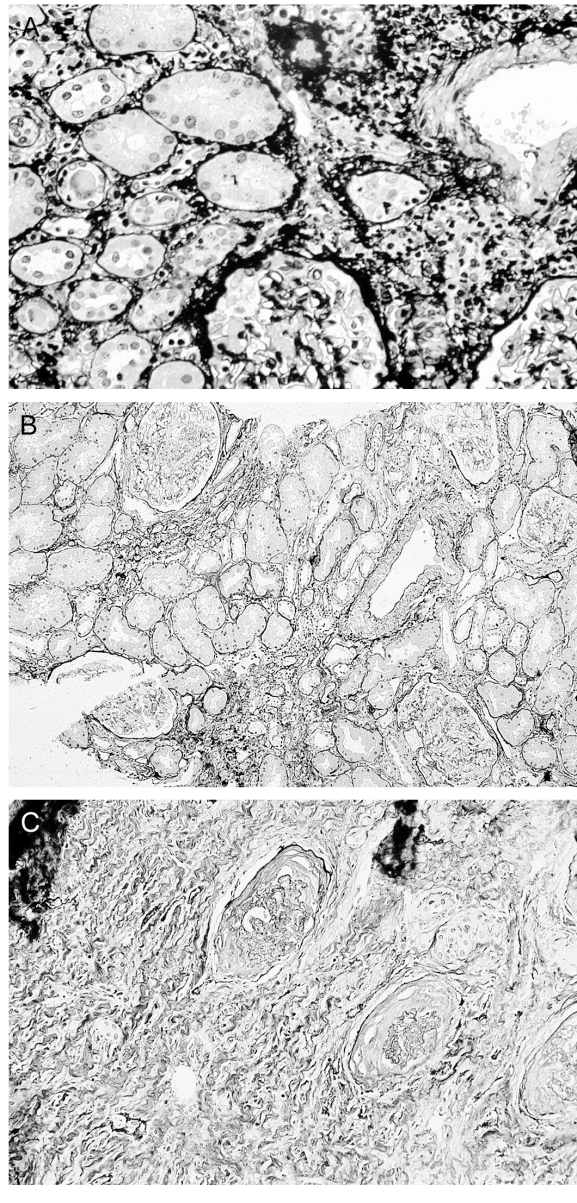

Figure 2 Three consecutive biopsies of a transplant recipient with graft loss from chronic interstitial rejection. (A) Acute rejection episode, one and a half year after transplantation, following prednisone withdrawal. The histology is characterized by an interstitial infiltrate and tubulitis. The artery did not show endothelialitis. (B) Chronic allograft nephropathy, three years after transplantation, consisting of mild interstitial fibrosis and tubular atrophy in the absence of fibrous intimal thickening of arteries and transplant glomerulopathy. (C) Chronic allograft nephropathy, five years after transplantation, showing severe interstitial fibrosis and glomerulosclerosis.

proteinuria (figure 1). A biopsy obtained in 1995 because of declining function showed evidence of chronic allograft nephropathy (CAN). Specifically, the interstitium was expanded by connective tissue and focal mononuclear infiltrates and moderate tubular atrophy was present (figure 2B). Some glomeruli were globally sclerosed but the arteries were normal. Retrospective C4d staining was negative. The patient was treated with enalapril that resulted in a decline of proteinuria to 0.7 g/day. However, proteinuria resumed and renal transplant dysfunction progressed. Another biopsy, taken in 1997 showed extensive glomerulosclerosis and severe interstitial fibrosis (figure 2C). Reinstitution of haemodialysis therapy was necessary in 1998. In 2001 she received a renal transplant from her sister, which is functioning well.

Definitions

This patient developed chronic transplant dysfunction (CTD) after a late acute rejection episode (ARE) following prednisone withdrawal in a randomised trial (1). Histology revealed chronic allograft nephropathy (CAN) without signs of chronic cyclosporine toxicity or de novo or recurrent glomerular disease. Clinically, a putative diagnosis of chronic rejection (CR) was made. Subsequently, she developed premature graft failure.

ARE may occur early, i.e. in the first three months or late, i.e. beyond three months post-transplantation. ARE is clinically characterised by a rapid rise in serum creatinine level in absence of other causes of renal dysfunction. In the European best practice guidelines for renal transplantation change of function has been defined as an increase of > 10-25% compared to baseline within 1-2 days (2). The presence of a mononuclear cell infiltrate in the renal biopsy at time of acute transplant dysfunction confirms the diagnosis. Infiltrating cells can be observed in one or more compartments of the kidney. The presence of an interstitial infiltrate with tubulitis, glomerulitis or intimal arteritis is known as acute interstitial rejection, acute transplant glomerulopathy or glomerulitis and acute vascular rejection, respectively (3). A histological picture characterized by neutrophils and C4d deposition in peritubular capillaries has been assigned as acute humoral rejection in the presence of de novo donor specific antibodies (4-6).

CTD is a clinical syndrome occurring beyond three months post-transplantation and is characterized by a slowly rising plasma creatinine concentration, and is associated with an increase in proteinuria and blood pressure. CTD is often the functional consequence of chronic allograft nephropathy (CAN) which is a descriptive term for histological changes consisting of arteriosclerosis, glomerulosclerosis, interstitial fibrosis and tubular atrophy (7,8). Hence, both CTD and CAN are not specific diagnoses but clinical and histopathologic

syndromes with a differential diagnosis including different recurrent or de novo diseases.

Chronic rejection (CR) is an alloantigen-dependent immune process ultimately leading to CAN and CTD (7). Clinically, the presence of immunological risk factors and the absence of other causes of CTD support the diagnosis. De novo fibrointimal thickening of arteries, also called transplant vasculopathy, or the presence of glomerular basement membrane reduplication characteristic for chronic transplant glomerulopathy in absence of other causes of CAN favour the diagnosis CR. Ongoing humoral mechanisms can be detected by circulating donor specific antibodies and peritubular capillary (PTC) staining for C4d, a breakdown product of complement that binds tightly to tissue (9).

The term "chronic" has been debated because it has to do with time (CTD), histopathology (CAN) and mechanism of injury (CR) (8). However, in most patients with CR all three meanings are applicable. In the patient presented, we diagnosed CR as cause of CTD and CAN despite the lack of vasculopathy, glomerulopathy and C4d deposits in consecutive biopsies. Absence of other causes of CTD and an immunological risk profile, including histoincompatibility and a late ARE, support the presence of chronic interstitial rejection. This process could be explained by a fibrotic response to injury from an increased cellular immune response induced by prednisone withdrawal.

Incidence

The incidence of CR is unknown, since there are no universally accepted diagnostic criteria. In general, the incidence increases with time; at five years, CR affects 30-40% of renal transplants. In single centre studies the cumulative incidence of CR depends on the follow-up period and ranges from 11 to 34% in transplants that had a minimum follow-up time of 3 months to 2.5 years. The incidence has decreased significantly since the early 1990s (10-12).

Clinical features

CR presents clinically as CTD, a syndrome characterized by a decline in renal function after the initial post-transplant months, often in combination with proteinuria and hypertension.

Changes in function may occur at any time beyond 3 months posttransplantation (13). To evaluate the timing of CTD, plots of the GFR or reciprocal of the serum creatinine concentration over time have been used. In a cohort of 200 patients transplanted from 1978 to 1982 who survived more than one year, monthly estimates of glomerular filtration rate (GFR) were made. Of these, 50 patients (25%) had a gradual decline of GFR. In most cases, the onset was early but in 28% CTD began 2.2 ± 1.2 years after transplantation (14). In 1663

patients transplanted between 1983 and 2000, the date of the first persistent decline in renal function was assessed using inverse serum creatinine over time slopes. It was found that a thirty percent chronic decline in inverse creatinine first occurred in 792 transplants (48%) at a median of 1.0 years posttransplantation and 3.0 years before graft failure (13).
Proteinuria is variably present in CR; 20 to 28% of patients excrete more than 0.5 g/day compared with 6 to 8% of patients free from CR (15). Proteinuria is usually within the range of 1-2 g/day but nephrotic-range proteinuria and hypoalbuminemia, occurring in 15 to 25% of a group of patients with CR, has also been reported (15).
Hypertension is a common finding in renal transplant recipients, and its prevalence has increased to 80% in current era (16,17). Pre-transplant hypertension of the recipient, the presence of native kidneys, hypertension of the donor, immunosuppressive drugs such as cyclosporine, tacrolimus and steroids and ARE correlate with post-transplant hypertension. Furthermore, every cause of CTD is associated with hypertension. Therefore, the significance of hypertension to diagnose CR is limited.
Graft loss is the ultimate result of CR and is preceded by all manifestations of chronic renal failure, such as anemia, secondary hyperparathyreoidism and acidosis. Renal allograft failure is associated with significant mortality (13,18).

Histopathological features

CR causes CAN, which is characterized by arteriosclerosis, glomerulosclerosis, interstitial fibrosis and tubular atrophy (7,8,19). Since 1991, there has been an ongoing effort to standardize renal transplant pathology interpretation which lead to the Banff working scheme and subsequent adjustments in an attempt to promote uniform allograft biopsy grading for drug trials and routine diagnostic use (3,20-22). This 'Banff' classification includes grading of ARE and CAN (tabel 1, http://tpis.upmc.edu/). Recognizing that the tubulointerstitial changes are most accurately sampled and correlate well with progressive loss of graft function (19), CAN has been graded by the severity of interstitial fibrosis and tubular atrophy, changes that are often accompanied by patchy interstitial infiltrates and tubulitis (23). In the scheme it has been denoted that specific vascular or glomerular changes are needed to diagnose CR on a tissue section. Transplant vasculopathy or chronic vascular rejection consists of de novo, generalised and concentric fibrointimal thickening of arteries. This is caused by smooth muscle cell proliferation in the intima and mononuclear cell infiltration in the vessel wall. Chronic transplant glomerulopathy is considered the most specific lesion of CR, but emerges in only 5-15% (3,24). It is characterized by splitting of the glomerular basement membrane leading to

Tabel 1. Banff '97 classification of chronic allograft nephropathy

Chronic Allograft Nephropathy (CAN) §	
Grade	**Histopathological Findings**
Grade I (mild)	Mild interstitial fibrosis and tubular atrophy without (a) or with (b) specific vascular changes suggesting chronic rejection
Grade II (moderate)	Moderate interstitial fibrosis and tubular atrophy without (a) or with (b) specific vascular changes suggesting chronic rejection
Grade III (severe)	Severe interstitial fibrosis and tubular atrophy without (a) or with (b) specific vascular changes suggesting chronic rejection
§ Glomerular and vascular lesions help define type of chronic nephropathy; chronic/recurrent rejection can be diagnosed if typical vascular lesions are seen	
Quantitative Criteria for Fibrous Intimal Thickening ("cv")	
cv0	No chronic vascular changes
cv1	Vascular narrowing of up to 25% lumenal area by fibrointimal thickening of arteries ± breach of internal elastic lamina or presence of foam cells or occasional mononuclear cells*
cv2	Increased severity of changes described above with 26 to 50% narrowing of vascular lumenal area*
cv3	Severe vascular changes with >50% narrowing of vascular lumenal area*
Quantitative Criteria for Allograft Glomerulopathy ("cg")	
cg0	No glomerulopathy, double contours in <10% of peripheral capillary loops in most severely affected glomerulus
cg1	Double contours affecting up to 25% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
cg2	Double contours affecting 26 to 50% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
cg3	Double contours affecting more than 50% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
Quantitative Criteria for Interstitial Fibrosis ("ci")	
ci0	Interstitial fibrosis tissue in up to 5% of cortical area
ci1	Mild- Interstitial fibrosis tissue in 6 to 25% of cortical area
ci2	Moderate- interstitial fibrosis of 26 to 50% of cortical area
ci3	Severe interstitial fibrosis of >50% of cortical area
Quantitative Criteria for Tubular Atrophy ("ct")	
ct0	No tubular atrophy
ct1	Tubular atrophy in up to 25% of the area of cortical tubules
ct2	Tubular atrophy involving 26 to 50% of the area of cortical tubules
ct3	Tubular atrophy of >50% of the area of cortical tubules

double contours accompanied by variable degrees of infiltration with mononuclear cells and mesangial matrix expansion (25). On immunofluorescence, patients with transplant glomerulopathy show a nonspecific pattern of IgM deposits in the glomeruli (26). Electronmicroscopy shows an electron-lucent zone of fine floccular material in the subendothelial zone (26). Splitting and multilayering of peritubular capillary basement membranes is strongly associated with transplant glomerulopathy, suggesting immunological endothelial injury (27,28). Only extensive multilayering is specific for CR because mild lesions are also observed in native kidney diseases (28,29).

Immunohistochemical analysis of transplant biopsies is not routinely used but provides valuable insights into the immunological events that occur in the transplanted kidney. Both cellular and humoral responses have been demonstrated in CR. In a miniature swine model persistent T cell infiltration accompanied PTC capillaritis and tubulitis during the development of CR following ARE (30). Signalling via costimulatory molecules is important for the interaction of T cells and parenchyma cells in the development of CR. For example, CD40 and CD40 ligand immunoreactivity has been demonstrated in renal biopsies undergoing CR (31). CD40 expression is not only present on most graft infiltrating cells but also on resident tubular epithelial cells stimulated by CD40 ligand on T cells (32). Delayed type hypersensitivity involving macrophages plays also a role in CR. In a CR group of 17 patients there were significantly more CD68 positive macrophages in the tubulointerstitium than in those with temporary dysfunction (33). C4d is reported as useful marker for in situ humoral CR (6). C4d is a fragment of the classical complement pathway component C4, which is activated by antigen-antibody complexes and in contrast to immunoglobulins, binds covalently to tissue by its reactive thiol group (6). C4d deposits in PTC were detected in 61% of biopsies that had been diagnosed as CR in contrast to 2% in controls. Most of the C4d positive cases had anti-donor HLA antibodies (9). The histology of C4d-positive CR is similar to C4d-negative CR but the presence of C4d correlates with multilayering in the PTC on electron microscopy (9,34). Endothelial C4d deposition in PTC is associated with actual or forthcoming transplant glomerulopathy and associated with inferior graft outcome (35).

Both cellular and humoral responses may result in a fibrotic response to injury. Myofibroblasts play an important role in this process as documented with an increased staining of smooth muscle actin (SMA) over time in conjunction with worsening fibrosis (36). Using a laminin and cytokerarin stain of respectively tubular basement membrane and distal tubular cells it has been observed that tubular cells may herniate into the interstitium (37). These

separated cells may transdifferentiate in myofibroblasts and thereby link ARE related tubulitis and CAN (38,39). Using SMA staining and in situ hybridisation to identify Y-chromosome DNA, mesenchymal cells of host origin have also been found in the vascular and interstitial compartments of grafts undergoing CR but also in controls with normal function (40). Finally, CR may be associated with unique changes of interstitial extracellular matrix composition consisting of new expression of collagen IVα3 and laminin β2 in the proximal TBM (41). Hence, in the process of CR, a role of cellular and humoral immunity, on one hand, and myofibroblasts, on the other hand, could be made plausible.

Risk factors

Acute rejection episodes

The most important risk factor of CR are previous ARE (42). The estimated half-life for cadaveric transplants is shorter in patients who had ARE than those who did not, 6.6 years versus 12.5 years (43). Not all recipients with ARE develop CR; type, pathogenesis, severity, number and timing determine outcome. Acute vascular rejection is an adverse prognostic feature compared with tubulointerstitial rejection (44,45). Independent of the histological type, peritubular C4d deposition in acute humoral rejection is a significant predictor of worse graft survival rates (46). ARE followed by partial loss of graft function exert a more detrimental effect on long-term outcome than episodes with complete functional recovery (47,48). Recipients with repeated rejection episodes have lower graft survival rates than those with no or only one episode (49). Finally, timing of the first rejection episode has an impact on the long-term outcome. ARE within the first three months may have no effect on CR whereas ARE occurring after two to six months confer the greatest risk (15,50-52). Apart from clinical ARE, patients may have subclinical rejection that causes ongoing immunologic injury leading to CR (53).

Recipient age

Young age is associated with a relatively high state of immune responsiveness to alloantigens, as documented by a more frequent production of lymphocytotoxic antibodies in response to blood transfusions (54). Young individuals are also more likely to forget to take immunosuppressive medication (55). In single center studies, young recipient age appears to be predictive of CR and graft loss censored for patient death with a functioning graft (56,57).

Race

Graft survival in blacks is poor as illustrated by a current projected half-life of 7.2 years compared with 13.3 years in whites (11). ARE occurs more common in blacks than in white recipients, a finding that is mainly caused due to

differences in immunological responsiveness (58). In several single center studies black race is a risk factor of CR (56,59,60).

Sensitization

Antibodies against HLA antigens elicited by pregnancies, blood transfusions or failed transplants are determined by testing the serum against a panel of HLA-typed leucocytes. Due to a reduction in transfusion since the introduction of erythropoietin, there is a substantial decrease in mean value of panel reactive antibodies (PRA) (11). Despite a negative crossmatch at time of transplantation, sensitized recipients have an increased risk of CR (10). Especially, sensitization against both HLA class I and class II results result in an increased rejection of HLA mismatched grafts (61,62). De novo anti-HLA antibodies post-transplantation has also been correlated with CR (61,63). More specifically, post-transplant antibodies could be detected in 24 to 56% of the patients and predate renal dysfunction and graft loss from CR (64). Therefore, the presence of anti-HLA antibodies, both before and after transplantation are associated with CR.

HLA matching

Major histocompatibility complex (MHC) molecules of the graft are the principle targets of the immune response post-transplantation. Class I MHC, consisting of HLA-A, B and C antigens is expressed on all nucleated cells while class II MHC, including HLA-DR antigens, is more restricted to cells of the immune system. The clinical benefits of HLA matching on graft survival, as appreciated in large registries, persists in the recent era despite new immunosuppressive drugs (65-68). HLA-matched grafts have an estimated half-life of 12.4 years, as compared with 8.6 years for HLA-mismatched grafts (69). However, in single center studies focusing on CR or death censored graft loss, the effect of HLA matching is small (70,71). MHC class I antigens share immunogenic epitopes, which have been assigned to one or more crossreactive groups (CREG). In the UNOS database the risk of CR is 62% higher in CREG-mismatched patients compared with those receiving a HLA and CREG-matched kidney (10). HLA-B or CREG matching is associated with a reduced frequency of late ARE and improved graft function at 2 years (72).

Peritransplantation injury

Donor factors such as old age, shock, brain death, and long cold ischemia time (CIT) are the most important events pre-transplantation that may culminate clinically in delayed graft function (DGF) (73). Brain death and ischemia / reperfusion injury trigger an inflammatory cascade with upregulation of cytokines, adhesion and HLA-DR molecules (74). This ‘injury’ response increases the graft immunogenicity leading to more early ARE (74,75). Delayed graft function, mostly defined as requirement of dialysis during the first week

after transplantation, remains at 20% of all cadaveric transplants (73). DGF is associated with a small increased risk of CR in the UNOS database of almost 89000 cadaveric donor transplant recipients (10). In single center studies the risk of DGF on long-term outcome depends on the presence of ARE and the requirement of a follow-up time of at least 6 to 12 months (76-78). Fully recovered DGF without ARE may not necessarily be detrimental for long-term graft survival (79-81).

Inadequate immunosuppression

Low dose, low serum levels of the drug, and variable oral bioavailability of cyclosporine in the early posttransplant period have been reported to correlate with higher rates of CR (42,82,83). Non-compliance with immunosuppressive treatment occurs in about a quarter of recipients, as assessed by interview, and is associated with lower graft survival at 5 years after transplantation (84).

Progression factors

Renal function

Beyond certain time points progression of chronic transplant dysfunction is largely dependent on non-immune factors. CR is characterized by systemic hypertension, vasculopathy, glomerulopathy and chronic tubulointerstitial nephritis, features that may lead to glomerulosclerosis. Loss of renal mass with subsequent intraglomerular hypertension and proteinuria and further loss of nephrons play a role as a progression factor that controls the rate of decline to end-stage renal failure (85). The importance of this type of injury is illustrated by a lower graft survival rate of transplants that come from female, black, very young, or very old donors compared with transplants from donors supposed to be endowed with a larger nephron mass (86-88). However, other studies could not confirm an effect on graft outcome of donor kidney size or the ratio of donor versus recipient body surface area as surrogate marker of renal mass (89,90).

The relation between renal dysfunction and subsequent CR or graft failure has been reported in several ways. Most investigators analyzed renal function, measured at an arbitrarily chosen time point after transplantation as time-fixed covariate of the dependent variable and found that an elevated serum creatinine value at 6 months or one year predicts subsequent outcome in patients who have already survived to that time with a functioning graft (60,91,91,92). However, the relation between renal dysfunction at this time point and late failure might be confounded by other risk factors like donor age and early ARE (93). Analysis of the course of renal function is another way to assess the relationship between renal dysfunction and graft failure. A negative slope of glomerular filtration rate between 6 and 12 months is significantly associated

with the occurrence of CR after 12 months (94). Chronic declines in renal function modeled by one or two least-squares-fitted regression lines of inverse creatinines may begin at variable times after transplantation and precede graft failure for several years (14). The vast majority of patients with CR progress linearly, although a change in the rate of decline revealed by a breakpoint test occurs frequently (95). Recently, Kasiske et al. systematically investigated what changes in chronic allograft function best predict subsequent graft failure. They examined the independent effects of relative declines in function, creatinine clearance and inverse creatinine over time slopes separately as time-dependent covariates. The best predictor of failure, a thirty percent decline in inverse creatinine, was superior to baseline function and independent of other risk factors of CR (96). In a subsequent report they validated this factor as the best forecaster of outcome (13).

Donor age

Increasing donor age is associated with arteriosclerosis, glomerulosclerosis, tubular atrophy and interstitial fibrosis and is associated with decreased long-term graft function (97,98). In single center studies, old donor age is an independent risk factor of CR (60,99). Kidneys from donors older than 55 years have an increased risk of CR in the UNOS database, but also of non-rejection failure (10). These findings are ascribed to the reduced renal mass, leading to glomerular hypertension, or more recently to accelerated senescence (86,100). Furthermore, it has been suggested that the higher rate of ARE in kidneys from older donors reflects increased immunogenicity (101). With the reduction of ARE and progression of transplant care, the impact of donor age on outcome has been attenuated (102,103).

Donor source

The higher graft survival of living donor kidneys compared with cadaveric kidneys is often used to illustrate the importance of early injury. Recipients of unrelated living donors have better long-term survival than recipients from cadaveric donors with better degrees of HLA matching (104). However, differences in graft survival are evident only in recipients undergoing ARE (105,106). In a group of 588 recipients (326 cadaveric, 260 living) treated for ARE, a 10 year censored graft survival of 45% was recorded compared to 91% in recipients without rejection. Graft loss from CR occurred in 30% of cadaveric and 16% of living donors (105). These data indicate that the benefit of living related transplantation results from the fact that a living related graft progresses from acute to chronic rejection at a slower rate than a cadaveric graft and that the higher rate of survival is attributed to the fact that kidneys from living donors are uniformly healthy (104,106).

Hypertension

Graft survival is inferior in hypertensive patients but the relation has been shown to be confounded by renal function (16,107). Both high systolic and diastolic blood pressures at one year post-transplant are significant predictors of long-term graft survival (108). The rate of deterioration of graft function is associated with diastolic blood pressure (95). Hypertension is associated with graft dysfunction both in cyclosporine- and azathioprine-treated patients (17). Blood pressure after an ARE correlates with graft survival, in contrast to patients without rejection (109). Hypertension may promote arteriosclerosis within renal blood vessels and glomerular hypertension, which can increase glomerular permeability and consequently enhance protein trafficking (110).

Proteinuria

Proteinuria at one year post-transplantation is an important risk factor for CR (57,111,112). Transplant patients with persistent proteinuria of more than 2 grams per day have a high risk of subsequent deterioration of renal function (113,114). Patients on cyclosporine and persistent proteinuria of greater than 1 g/day as a result of CR have a compromised five-year graft survival (115). Resorption of excessive amounts of protein by proximal tubular epithelial cells leads to release of inflammatory mediators from tubular cells and subsequent interstitial injury (116).

Hyperlipidemia

Hyperlipidemia is a common problem as elevated cholesterol levels are present in 70 to 80% and hypertrygliceridemia in 30 to 40% of transplant patients (117). Hypertrygliceridemia is correlated with graft dysfunction in some studies (117-120). Hypercholesterolemia at 6 months, 1 and 2 years is also associated with graft dysfunction or death-censored graft loss (118,121,122). Hypercholesterolemia is an independent risk factor for kidney graft loss from CR in male patients with previous ARE (123). Outcome may be adversely affected through the accumulation of oxidized low-density lipoprotein (LDL) in the renal interstitium and the development of fibrosis (124).

Smoking

Smoking is a risk factor for renal outcome as documented in several studies (125). A recent report revealed that 24% of transplant recipients smokes cigarettes at time of transplantation, of which 90% continues this habit after transplantation. Smokers had a relative risk on death-censored graft loss of 2.3, which was independent of ARE (126). Chronic cigarette smoking reduces renal plasma flow, probably by increasing the synthesis of the vasoconstrictor endothelin and by reducing the generation of the vasodilatory endothelial nitric oxide (127).

Genetic polymorphisms

Genetic factors may also play a role in the pace of graft failure. For instance, it has been shown that the DD genotype of the ACE insertion/deletion (I/D) polymorphism is associated with a shorter graft survival (128,129)

Differential diagnosis

CR should be differentiated from other causes of CTD, such as ARE, calcineurin inhibitor (CNI) nephropathy, recurrent or de novo glomerulonephritis, nephrosclerosis, transplant renal artery stenosis or BK virus nephropathy (figure 3).

Acute rejection episodes

ARE may occur late after transplantation, especially in the setting of withdrawal studies or patient incompliance with immunosuppressive drugs (1). The renal biopsy shows an interstitial infiltrate and tubulitis and renal function usually restores with anti-rejection treatment. However, late ARE may lead to CR or may develop on top of CR making a clear clinical distinction between ARE and CR difficult.

Drug toxicity

CNI nephrotoxicity is a significant and dose-limiting side effect of both cyclosporine and tacrolimus that may occur even in those patients with trough-levels maintained at the currently acceptable levels (130). Other adverse effects such as gum hyperplasia, hypertrichosis, gout and tremor may also be present. Histopathology reveals arteriolar hyalinosis, (focal) glomerulosclerosis and

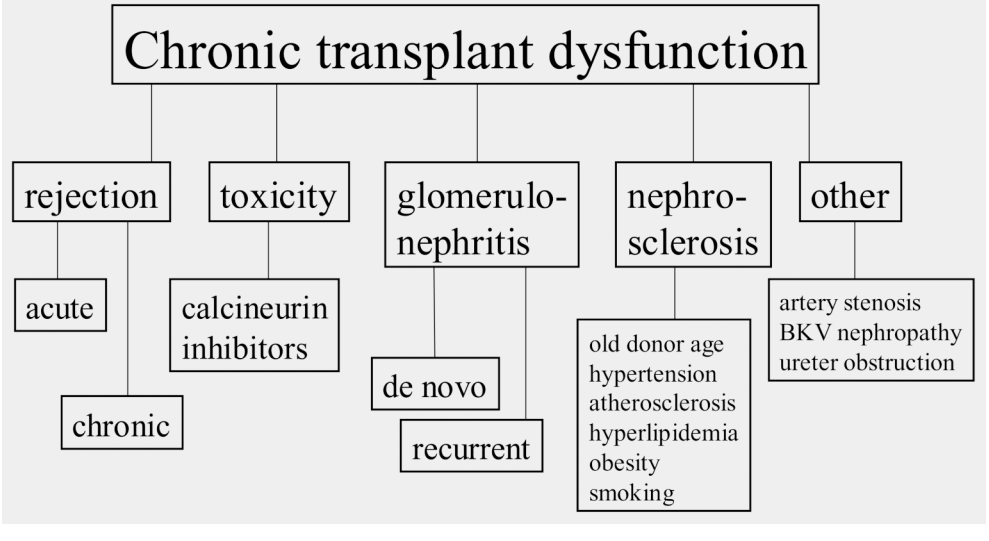

Figure 3 Differential diagnosis of chronic transplant dysfunction

sometimes striped interstitial fibrosis, albeit non of these lesions should be considered as specific (131-133). Reduction of the drug dose may improve or stabilize renal function in patients with CAN and deteriorating function (134). However, those with cyclosporine associated focal glomerular sclerosis and increasing proteinuria exceeding 2 g per day lost graft function even after reducing cyclosporine administration (133). Area under the curve (AUC)-based monitoring of cyclosporine should help to optimise therapeutic drug monitoring (135)

De novo and recurrent glomerulonephritis

Late recurrences of renal diseases may be seen in IgA nephropathy, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis and diabetic nephropathy (136,137). The diagnosis is based on the original renal disease, donor source, presence of erythrocyturia and renal biopsies, including immunofluorescence and electron microscopy (138,139). Membranous nephropathy is considered the most common de novo renal disease usually presenting with nephrotic proteinuria (140). De novo FSGS is mostly regarded as a secondary phenomenon of CR, CNI nephrotoxicity, nephrosclerosis or obesity and is more often diagnosed in young or black recipients with elevated cholesterol levels or proteinuria (141).

Nephrosclerosis

In recipients with a long interval between transplantation and CTD in conjunction with older donor age, hypertension, smoking, hyperlipidemia and peripheral artery diseases, a putative diagnosis of nephrosclerosis could be made. Nephrosclerosis is characterized histologically by arterial intimal thickening, arteriolar hyalinosis, glomerulosclerosis and interstitial disease, changes difficult to distinguish from CR and CNI nephrotoxicity (142).

Transplant renal artery stenosis

Renal artery stenosis is a well-known cause of post-transplant hypertension that also may result in graft dysfunction. The reported incidence ranges from 1 to 12% depending on indications for radiological investigation and on the degree of stenosis (143-145). Hypertension of recent onset or refractory hypertension at any time after transplantation is the most common presentation. Collor Doppler ultrasonography and magnetic resonance imaging are increasingly used to screen for renovascular disease. Arteriography is done to confirm significant stenosis and to apply treatment with angioplasty, which improves graft function and blood pressure control in most patients (143,144). CTD can also result from stenosis of the external iliac artery proximal to the graft anastomosis site which could be managed by placement of an intravascular stent (146,147).

BK virus nephropathy

BK virus (BKV), a polyoma virus, may reactivate from latency under immunosuppression and cause nephropathy and hemorraghic cystitis. BKV nephropathy occurs on average 6 to 18 months posttransplantation in relation to intense immunosuppression, particularly anti-rejection treatment with corticosteroids (148,149). Histologically, it is characterized by inclusion bodies and patchy tubulointerstitial inflammation that may culminate in CAN (148). The presence of decoy cells in the urine and BKV DNA in plasma are useful tools for early detection of BKV disease (148,150). Early reduction in immunosuppression and treatment with cidofovir may prevent graft loss.

Pathogenesis

Alloimmune response

T-cell recognition of donor HLA antigens encoded within the major histocompatibility complex (MHC) is the central event of the rejection process. There are two distinct, although not mutually exclusive, pathways of allorecognition. The direct response is characterized by T-cell recognition of MHC molecules on the surface of donor antigen presenting, so-called passenger cells. It has been suggested that direct allorecognition is confined to the early post-transplantation phase, because later on, the donor passenger cells are depleted from the graft and donor specific T helper cell hyporesponsiveness occurs with time (151-153). Direct allorecognition therefore appears unlikely to be responsible for CR, implicating indirect allorecognition as the predominant immunological driving force. In this pathway, donor antigens are shed from the graft and taken up by the recipients' immune system. T cells recognize these antigens after they are processed and presented as peptides by recipient antigen presenting cells (154). This response is donor-specific and directed to different MHC antigens over time, a phenomenon termed epitope shifting (155). A second costimulatory signal provided by the engagement of CD28 or CD40 ligand on T-cell receptors with their respectively ligands B7 and CD40 on antigen presenting cells is required for full T cell activation. Once activated, T cells undergo clonal expansion, mainly under influence of interleukin 2 and differentiate into CD8 cytotoxic and CD4 helper T-cells. In the effector phase, CD8 T cells induce donor cell death and CD4 T cells help B cells to produce antibodies and help macrophages to induce delayed type hypersensitivity.

The extent of the alloresponse is a balance between the immunogenicity of the transplanted kidney, recipient responsiveness and the level of immunosuppression. Immunogenicity depends mainly on the degree of histoincompatibility between donor and recipient as HLA-mismatched transplants fare worse than HLA-matched transplants (69). Furthermore, it

has been shown that some mismatched donor antigens are differentially recognised depending on the HLA phenotype of the recipient and as taboo combinations confer lower graft survival (156). An increased incidence of ARE in kidneys from older donors may suggest enhanced immunogenicity in ageing (101). Histoincompatibility differences between donor and recipient may stimulate the production of anti-donor HLA antibodies. These antibodies are associated with C4d deposition in patients with steroid-insensitive ARE and CR (4,5,9). In addition, a tissue specific response might also be involved. In the Fisher to Lewis rat model of chronic transplant glomerulopathy, IgG antibodies were found against the glomerular basement membrane (GBM) with perlecan as one of the antigens recognized (157). Recipients of younger age, with sensitization and after a previous failed transplant show increased humoral alloreactivity (5,54). The physiological indirect route of allorecognition is characterized by much lower frequencies of allopeptide-specific T cells compared to the direct route explaining the more indolent course of CR (158). Immunosuppression may decrease the intensity of this type of alloresponse but will not abolish it as illustrated by a normal clearance of viral infections in most transplant recipients (154). In conclusion, grafts develop CR from a persistent or intermittent alloantigen driven immune response.

Response to injury

Injury from clinical or subclinical ARE results in an inflammatory cascade consisting of myofibroblast proliferation, deposition of extracellular matrix proteins, scar formation and ultimately tissue restoration (159). Whereas ischemic damage and most ARE resolve more or less completely, irreversible chronic changes may ensue in more severe or longer lasting ARE. Acute vascular rejection, glomerulitis and acute interstitial rejection are linked to the subsequent development of vasculopathy, glomerulopathy and interstitial fibrosis / tubular atrophy, respectively (160-162). Mononuclear cells causing endothelialitis produce cytokines and growth factors which stimulate smooth muscle cell proliferation and synthesis of extracellular matrix proteins culminating in concentric intima fibrosis. Independent of arterial lesions, tubulitis may cause tubular basement membrane defects allowing herniation of tubular cells which subsequently can transdifferentiate into myofibroblasts (37). This process is stimulated by immunoregulatory T cells that may persist long-term within tubules after ARE (39). The response to injury promotes more immune recognition of upregulated major and minor histocompatibility antigens, which results in a self-propagating feature leading to CR (163).

Treatment

Immunosuppressive treatment

There is no established treatment for CR, mainly because of the presence of irreversible damage at time of diagnosis. However, in early phases of the disease or in those patients in whom inadequate immunosuppression is the precipitating cause, a change in the immunosuppressive regimen may stabilize or even reverse part of the renal dysfunction. However, randomised trials regarding the treatment of CR have not been reported. If there is evidence of coexisting ARE, a beneficial response of a trial with methylprednisolone has been observed (23). In patients with CR who are not treated with a CNI, institution of one of these agents might be effective (164). On the other hand, addition of azathioprine may improve renal function in cyclosporine-treated patients with allograft dysfunction (165). In some recipients on cyclosporine Neoral conversion to tacrolimus resulted in sustained improvement of renal function (166). Adding mycophenolate mofetil to maintenance immunosuppression provided no clear benefit in a small retrospective study (167). On the other hand, reduction and possible withdrawal of CNI with either the addition or continuation of mycophenolate mofetil slowed the rate of loss of renal function in patients with CAN (134). Reduction of antidonor antibody synthesis by the combination of mycophenolate and tacrolimus is a novel promising approach for the treatment of humoral CR (168).

Nonimmune interventions

Nonimmunological measures to halt or retard progression of CTD focus on aggressive control of blood pressure, proteinuria and hyperlipidemia. Treatment of hypertension reduces progression to renal failure in native kidney diseases but this effect has not yet been proven in renal transplantation. In patients on CNI dose reduction or withdrawal may improve blood pressure (134). Calcium entry blockers, beta blockers and ACE inhibitors have similar antihypertensive efficacy after renal transplantation and are often used in combination to achieve adequate control (169,170). Significant reduction of proteinuria has been reported as a beneficial effect of ACE inhibitors and angiotensin II receptor antagonists in clinical transplantation (171,172). These drugs have the potential to prevent the progression of chronic failure (173). In a small group of transplant recipients the slope of the curve of inverse serum creatinine and time decreased when they were subjected to a low-protein diet of 0.6 g/kg (174). It is not yet clear whether treatment of hyperlipidemia slows the progression of CTD, but in the presence of concomitant risk factors of cardiovascular disease an increasing number of patients are being treated with statins (175,176).

Prevention

Because of the lack of effective treatment, efforts should be made to prevent CR. Measures are directed to the risk factors of CR including sensitisation, histoincompatibility, ARE, and insufficient immunosuppression. Allocation strategies should primarily aim for HLA matched transplants that have an established superior long-term outcome compared to HLA-mismatched grafts (65,69). In the case of mismatches, functional matching should aim for the selection of donors with HLA molecules non-stimulatory to both the cellular and humoral immune system of the recipient (177,178). In this way, sensitisation due to a transplant could be prevented which may also facilitate future transplants in the case of graft loss.

The introduction of cyclosporine Neoral, tacrolimus and mycophenolate mofetil in the 1990s has been associated with a reduction in the incidence of ARE during the first year after transplantation (179-181). Initially, longer follow-up of these agents did not reveal much effect on graft survival or the prevalence of CR (181,182). However, a tendency towards improved graft survival by the prevention of late ARE has been observed in patients who stay on mycophenolate for a prolonged period of time (183,184). Rapamycine (Sirolimus) may also have the ability to reduce the rates of CR by further reduction of the incidence of ARE and inhibition of smooth muscle cell proliferation (185).

Protocol biopsies and immune monitoring of both the cellular and humoral response are potential tools to detect subclinical rejection activity beyond the early phase after transplantation. Protocol biopsies and treatment of subclinical rejection with corticosteroids may prevent CR (53). The enzyme-linked immunosorbent spot assay (ELISPOT) of peripheral blood lymphocyte reactivity to HLA peptides or donor stimulator cells might be a useful method of measuring indirect alloreactivity (186,187). Early detection of in-situ C4d deposition and circulating donor specific antibodies may lead to specific strategies for humoral rejection (6).

Besides optimal immunosuppression, prevention of premature graft failure requires a multifactorial approach aiming at early and tight control of blood pressure, proteinuria, lipids, glucose and weight (188,189).

Summary

CR is an antigen driven immune process ultimately leading graft loss. Clinically, CR is characterized by CTD consisting of a gradual increase in serum creatinine, increasing proteinuria and worsening hypertension, features that present at various intervals after transplantation. Histologically, CR results in CAN including interstitial fibrosis and tubular atrophy with or without transplant

vasculopathy, glomerulopathy, interstitial infiltrate and tubulitis. Extensive multilayering of PTC, visible at electron microscopy, is considered a specific feature of CR. Young recipient age, black race, pre-sensitization, histoincompatibility and acute, especially vascular and late ARE are dominant risk factors, compatible with immunological mechanisms. CR should be differentiated from chronic toxicity of CNI, de novo or recurrent glomerulonephritis, nephrosclerosis, transplant renal artery stenosis and polyoma (BK) virus nephropathy. Cellular and humoral responses resulting from indirect recognition of alloantigens with subsequent fibrotic sequellae play a central role in the pathogenesis. Detection of donor and possibly tissue specific antibodies and C4d deposits in PTC support humoral mediated CR. The prognosis depends on alloreactivity and the presence of progression factors such as increased donor age, donor source, renal failure, hypertension, proteinuria, hyperlipidemia and smoking. Therapeutic strategies in established CR consist of a putative change of immunosuppressive drugs and non-immune interventions to manage blood pressure, proteinuria, lipids and smoking behaviour. Prevention of CR by a multifactorial approach directed to its risk factors is the hallmark in further improvement of long-term outcome after renal transplantation.

Scope of this thesis

The goal of this thesis is to study the clinical, epidemiological and histopathological features of CR to elucidate its pathogenesis. This first chapter offers an extensive review of the literature. All studies are done in the Leiden cohort of renal transplant recipients. Recipient, donor, transplant, follow-up and outcome variables of transplants performed since 1983 and functioning for more than 6 months were collected in a database. Biopsies obtained beyond 6 months were evaluated according the Banff ’97 classification, blinded for clinical information. In chapter 2 the risk factors of graft loss from CR are identified using uni- and multivariate Cox regression analysis with special emphasis on the impact of HLA matching and ARE. Late ARE, defined as the last ARE occurring beyond 3 months, appears to be the strongest risk factor for CR. Therefore, the prognosis and the risk factors of early versus late ARE are determined in chapter 3. To identify progression factors, i.e. parameters related to a decline in renal function, the predictive factors of a low intercept, defined as a low creatinine clearance at 6 months are compared with those of a negative slope of reciprocal creatinines from 6 months onwards in chapter 4. Next (chapter 5), the multivariate model with time fixed covariates, known at six months post-transplantation is extended with time dependent renal function covariates beyond 6 months to allow an accurate prediction of graft loss at any

time point. In the Leiden cohort, chronic cyclosporine toxicity was mainly observed after the conversion of Sandimmune to Neoral. Chapter 6 shows the clinical features and risk factors of cyclosporine nephrotoxicity to allow better differentiation with CR. In several patients we observed CAN without transplant vasculopathy or cyclosporine toxicity. Therefore, we assess prognosis and risk factors of CAN with and without vasculopathy to answer the question in chapter 7 whether CR could occur without obliterative intima fibrosis. Chapter 8 focuses on the question whether transplant glomerulopathy is a manifestation of CR. It reports the incidence, risk factors, clinical and histological characteristics, and prognosis of late transplant glomerulopathy in comparison with CR without glomerular lesions. Because of the presence of immunological risk factors, such as pre-transplant sensitization, C4d staining was used to detect in situ evidence of humoral rejection. The studies are summarized and discussed in chapter 9. Finally, a summary in Dutch is given in chapter 10.

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2

Sharing cross-reactive groups of MHC class I improves long-term graft survival

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Abstract

Background Renal transplant loss from chronic rejection remains substantial. To increase our understanding of this syndrome we identified risk factors predicting late graft loss with special emphasis on the impact of HLA matching.
Methods We studied all 654 cadaveric kidney transplants performed in our center between 1983 and 1996 that had survived for more than six months. Eighty-two transplants, lost because of chronic rejection, were used as the outcome variable. The influence of HLA mismatches and shares on long-term graft survival was evaluated at the level of private antigens and cross-reactive groups (CREG) of MHC class I. HLA and other recipient, donor and transplant parameters were studied using univariate and multivariate Cox regression analysis.
Results The cohort had a mean number of 1.9 HLA mismatches. Because of homozygosity of HLA antigens, HLA mismatches were not reciprocal to shares. CREG and HLA-A-B mismatches had a relative risk for graft loss of 1.19 (95% confidence interval 0.97 to 1.45) and 1.05 (0.84 to1.32) per mismatch. In contrast, the relative risk per shared CREG and broad HLA-A-B antigen was 0.76 (0.63 to 0.92) and 0.79 (0.61 to 1.03). Multivariate analysis revealed that individuals sharing less than four CREG had a relative risk of 2.13 (1.29 to 3.75) for late graft loss. Other independent predictors were recipient age < 50 years, relative risk 1.95 (1.02 to 3.71); donor age > 50 years, relative risk 1.68 (1.01 to 2.80); acute rejection (vascular vs. no rejection), relative risk 3.52 (1.72 to 7.18); proteinuria (dipstick >1+ vs. negative), relative risk 2.86 (1.29 to 6.35) and serum creatinine concentration > 150 μmol/l at six months, relative risk 3.41 (1.96 to 5.94).
Conclusion We identified several coexisting recipient, donor and transplant related risk factors for graft loss from chronic rejection. In this well matched group of renal transplants HLA mismatches and shares had a non-reciprocal relationship. Sharing of HLA antigens, especially CREG of MHC class I, was associated with improved long-term survival.

Introduction

Graft loss from chronic rejection is the major obstacle to successful long-term outcome following renal transplantation and results in an increasing number of patients on dialysis who are awaiting a repeat transplant. Both alloantigen-dependent and -independent factors have been identified that contribute interactively to the deterioration of graft function and structure that ultimately results in graft loss (1, 2). A high donor age is the best recognized antigen-independent factor that affects graft survival adversely (3, 4) whereas the acute rejection history is the most important immunological predictor for late graft loss (5, 6). Specifically, the number of acute rejection episodes, their timing, their severity and their histological type seem to be important (7-13). The influence of HLA matching on long-term survival is less clear. The detrimental effect of HLA-disparity between donor and recipient as observed in large registries (4, 14-17) has not been found in most single center studies (18, 19). Matching for split HLA antigens has been reported to result in a better transplant outcome than matching for broad HLA antigens (20), but this does not seem practical to implement because of the enormous polymorphism of the HLA system. Recently, it has been suggested that matching for the public HLA epitopes of MHC class I, also called crossreactive groups (CREG), increases the likelihood of recipients to obtain histocompatible kidneys (21, 22). However, the effect of CREG matching is still controversial and has not been fully addressed (23, 24).

The aim of the present study was to identify recipient, donor and transplant related factors predicting late graft loss with special emphasis on the impact of HLA matching.

Patients and methods

Patients and immunosuppression

654 cadaveric renal transplants done in the Leiden University Medical Center between January 1983 and July 1996 that had survived at least 6 months were included in the study. There were 106 repeat transplants. Patients were followed until death, return to dialysis, or until July 1, 1998. The median follow-up was 74 months (range, 6 to 186 months). Kidneys were allocated according to the matching algorithm used by Eurotransplant. In our center, we aimed for no more than 2 HLA mismatches and, preferably, no DR mismatches. The standard immunosuppressive regimen consisted of prednisone and cyclosporine (Sandimmune). Fifty-four patients (8.3%) were initially treated with prednisone and azathioprine. Sixty patients (9.2%) in the study were converted to azathioprine at 3 months posttransplant as part of a prospective

randomized trial (25); another 51 patients (7.8%) were converted in the first six months posttransplant from cyclosporine to azathioprine for clinical reasons, mostly because of suspected cyclosporine toxicity. Beyond three months posttransplant, the once-daily cyclosporine dose was adjusted to a desired 24-hour trough concentration between 250 and 500 µg/l (polyclonal radioimmunoassay, Sandoz) and from 1989 onwards to a range of 50-150 µg/l (monoclonal radioimmunoassay, Incstar). During the first six months acute rejection episodes occurred in 56% of the cases and these were confirmed by biopsies in 88%. Interstitial rejection was diagnosed when tubulitis with a widespread interstitial infiltrate was present; a diagnosis of vascular rejection was made when arteritis was present. Rejection episodes were treated according to a standard protocol consisting of 3 one-gram intravenous doses of methylprednisolone, a 10-day course of anti- thymocyte globulin at a dose of 5 mg/kg guided by absolute lymphocyte counts, or a course of methylprednisolone for the first, second or third rejection episode, respectively.

Study design

Graft loss from chronic rejection was defined as graft failure beyond six months and exclusion of other obvious causes of graft loss. Therefore, analysis was censored for graft loss from recurrent or de novo glomerulonephritis, transplant renal artery stenosis or thrombosis, acute rejection as result of discontinuation of immunosuppressive drugs or patient death with a functioning graft (26). Furthermore, we reviewed all biopsies and nephrectomy specimens obtained beyond the first six months from patients with late graft loss. Graft histology was evaluated according the recently published Banff '97 classification (27)

A number of recipient, donor and transplant related characteristics were evaluated as risk factors of late graft loss. The following recipient variables were tested: age, original disease, cigarette smoking, pretransplant blood pressure, peak and current panel reactive antibodies defined by % PRA. The original diseases were grouped according to the UNOS report in inherited (polycystic disease, Alport's disease, dysplasia), glomerular (primary glomerulonephritis) and systemic (nephrosclerosis, diabetes mellitus, vasculitis, systemic lupus) diseases (4). Donor variables studied were age and cause of death. Transplant parameters studied included: year of transplant, repeat transplant, gender match, cold ischemic time, delayed graft function defined as the need of dialysis in the first week, and the baseline immunosuppressive drug regimen. All kidney donors and recipients were typed using the standardized Eurotransplant serum set for HLA -A,-B and –DR antigens. All donors were retyped at the Eurotransplant Reference Laboratory (28). MHC class I antigens were assigned to one or more CREG (table 1) based on the amino acid residue system proposed for UNOS allocation (21). We studied the impact of

Table 1. Crossreactive-groups (CREG) used in the present study

CREG	Antigens included
A01C	A1, 3, 11, 29, 30, 31, 36
A02C	A2, 23, 24, 28, B 57, 58
A10C	A25, 26, 32, 33, 34, 66
B05C	B18, 35, 51, 52, 53
B07C	B7, 8, 13, 27, 41, 47, 55, 56, 60, 61
B08C	B8, 14, 18, 38, 39
B12C	B13, 37, 41, 44, 45, 47, 49, 50, 60, 61
B21C	B35, 49, 50, 51, 52, 53, 57, 58, 62, 63, 70
BW4	A23, 24, 25, 32, B13, 27, 37, 38, 44, 47, 49, 51, 52, 53, 57, 58, 63
BW6	B7, 8, 14, 18, 35, 39, 41, 45, 50, 55, 56, 60, 61, 62, 70

HLA matching of broad antigens as well as antigenic splits and crossreactive antigens (CREG). Not only was the degree of mismatching between donor and recipient studied but also the effect of sharing HLA antigens. The term 'shares' was used for the number of corresponding HLA antigens between donor and recipient. We made crosstabulations of HLA mismatches and shares to investigate their relationship. Furthermore, we compared the percentage of homozygosity between donors and recipients. We also evaluated risk factors observed up to 6 months posttransplant. Clinical and histological acute rejection parameters were studied in detail: number of acute rejection treatments in the first six months, time to the first and last acute rejection episode and histological type of rejection. The occurrence of an acute rejection episode beyond six months posttransplant was studied as a time dependent variable. CMV serology was routinely performed pretransplant and on clinical indication after transplantation. The occurrence of CMV seroconversion in the first six months was analyzed as a risk factor. As a result of the randomized and clinical conversion from cyclosporine to azathioprine in many patients, maintenance drug regimen at six months posttransplant was studied as a separate variable. The effect of cyclosporine trough levels could not be evaluated because of the mentioned change in assays in 1989. Therefore, we used the drug dose (mg/kg) at six months as a measure in patients on cyclosporine. Blood pressure, number of antihypertensive drugs, dipstick proteinuria, serum creatinine and endogenous creatinine clearance at six months were evaluated as clinical parameters.

Statistical analysis

A crosstabs procedure and a chi-square test tested the relationship between HLA mismatches and shares. Late graft loss from chronic rejection was used as the outcome

Table 2. Demographic, clinical and laboratory characteristics of study subjects

Characteristic	Value
Recipient factors	
Age (years)	45 ±12
Gender (% female)	37
Original disease: inherited / glomerular / systemic (%)	19 / 40 /17
Cigarette smoking (%)	43
Systolic blood pressure (mm Hg)	149 ± 26
Diastolic blood pressure (mm Hg)	89 ± 13
Peak panel reactive antibodies (%)	31 ± 32
Current panel reactive antibodies (%)	11 ± 22
Donor factors	
Age (years)	37 ± 15
Gender (% female)	40
Cause of death: trauma / cardiovascular (%)	45 / 50
Transplant factors	
Year of transplant: 1983-86 / 1987-91 / 1992-96 (%)	30 / 37 / 33
Repeat transplant (%)	16
Gender: match / female to male / male to female (%)	54 / 24 /22
Cold ischemic time (hours)	29 ± 7
Delayed graft function (%)	24
Immunosuppression: cyclosporine / azathioprine (%)	92 / 8
HLA CREG mismatches	1.1 ± 1.0
HLA A, B and DR broad mismatches	1.9 ± 1.1
HLA A, B and DR split mismatches	2.2 ± 1.2
HLA CREG shares	4.5 ± 1.2
HLA A, B and DR broad shares	3.6 ± 1.0
HLA A, B and DR split shares:	3.4 ± 1.1
Acute rejection factors	
Histological type: interstitial / vascular (%)	34 / 15
Number of rejection episodes: one / two / three or more (%)	23 / 22 / 11
First episode: within one month / between month one and six (%)	45 / 11
Last episode: within two months / between month three and six (%)	40 /10
Rejection episode(s) after six months (%)	8
Factors at six months posttransplant	
Cytomegalovirus: sero-negative / -positive / -conversion (%)	25 / 57 / 18
Systolic blood pressure (mm Hg)	144 ± 20
Diastolic blood pressure (mm Hg)	86 ± 10
Number of antihypertensive drugs	1.4 ± 0.9
Immunosuppression: cyclosporine / azathioprine (%)	77 / 23
Cyclosporine dose (mg/kg)	4.6 ± 1.8
Dipstick proteinuria: trace, + / > 1+ (%)	42 / 6
Serum creatinine (µmol/l)	152 ± 66
Creatinine clearance (ml/min)	62 ± 23

Data are expressed as mean ± SD unless otherwise stated.

variable. We used the Cox proportional hazard model to assess the predictive value of the studied risk factors. The effect of acute rejection episodes occurring after six months was tested as a time dependent variable. Significant predictors ($P<0.05$) of late graft loss in univariate analysis were fitted into a multivariate model. Other potential confounders such as prior graft loss, panel reactive antibodies delayed graft function and HLA mismatches were also added to the analysis. Forward selection techniques were used to choose significant risk factors. The histological type of acute rejection was used as rejection factor and serum creatinine >150 μmol/l as renal function parameter. Multivariate analysis was performed with and without inclusion of the serum creatinine concentration at six months into the model. We used the SPSS software package (8.0) for all our analyses.

Results

The demographic and clinical characteristics for the entire study population are presented in table 2. A total of 224 transplants were lost; 14 (6%) due to de novo or recurrent glomerulonephritis, 9 (4%) due to thrombosis or stenosis of the transplant renal artery, 3 (1%) due to discontinuation of immunosuppressive drugs, and 116 (52%) due to patient death with a functioning graft. After censoring for these causes late graft loss occurred in 82 (37%) transplants; in 62 cases graft histology was available from biopsies or nephrectomy specimens, which showed chronic allograft nephropathy in all cases. Concomitant borderline or acute rejection was present in 18 and 25 cases, respectively. The median follow up in the 82 lost transplants was 34 months (range, 6 to 132 months).

Our cohort of patients studied was well matched for HLA antigens. The mean number of CREG and HLA-A,-B,-DR mismatches was 1.1 and 1.9, respectively. Crosstabulation of mismatches and shares revealed that mismatches and shares had a non-reciprocal relationship (table 3). The mean number of CREG and HLA-A,-B,-DR shares was 4.5 and 3.6. The percentage of homozygosity was higher in the donors compared with the recipients; A locus: 21 versus 17%, B locus: 12 versus 9% and DR locus: 20 versus 14%. The quantitative impact of HLA matching on graft loss is shown in Table 4. Although a higher number of HLA mismatches did not predict graft loss, sharing broad HLA-A,-B,-DR antigens had a beneficial effect on long-term outcome. Matching at the level of split antigens showed similar results. Sharing of HLA-DR antigens did not impact on outcome whereas the effect of sharing of HLA-A,-B antigens was almost significant. However, sharing CREG of MHC class I improved outcome significantly (figure 1).

The univariate effects of the various other risk factors are shown in table 5. The relative risk of graft loss was increased in recipients younger than 50 years.

Table 3a. Cross tabulation of HLA-A,-B,-DR mismatches and shares (Pearson r = 0.76)

		HLA mismatches							
		0	1	2	3	4	5	6	Total
HLA shares	0				1			2	3
	1				1	3	2		6
	2		1	5	18	34			58
	3	15	24	78	139				256
	4	20	59	143					222
	5	20	57						77
	6	30							30
	Total	85	141	226	159	37	2	2	652

Table 3b. Cross tabulation of CREG mismatches and shares (Pearson r = 0.45)

		CREG mismatches						
		0	1	2	3	4	5	Total
CREG shares	1				4	1	1	6
	2		1	6	1	1		9
	3	27	25	32	21	4	2	111
	4	46	69	59	24	1		199
	5	74	72	38	7			191
	6	45	36	5	2			88
	7	26	7					33
	8	5						5
	Total	223	210	140	59	7	3	642

Pretransplant blood pressure was not significantly associated with graft failure. Patients with inherited renal diseases did better than patients with glomerular or systemic diseases. Cigarette smoking had a detrimental effect on graft outcome. Grafts from older donors (over age 50) experienced decreased graft survival. There was no difference between donors who died from cardiovascular causes or as a result of a trauma. The gender mismatch factor was not associated with an effect on graft loss. Patients who were on an initial immunosuppressive drug regimen not containing cyclosporine did worse compared with patients treated with cyclosporine. No significant long-term effect was found of the degree of sensitization, duration of cold ischemic time or delayed graft function. Also, first and repeat transplants had an equivalent outcome. Transplants performed in the first five years of the study period were more at risk for graft loss compared with the transplants done more recently. We found a strong influence of acute rejection on long-term graft survival. The occurrence of interstitial and especially vascular rejection within six months was associated with poor outcome (figure 2). An increasing number and a later occurrence of rejection episodes were also associated with graft loss. Acute rejection beyond six months, tested as a time dependent variable, had a strong adverse effect on graft

Table 4. HLA matching and relative risk of late graft loss using univariate Cox analysis

Characteristic	RR	95% CI	*P*
Mismatches			
HLA CREG	1.19	0.97 – 1.45	0.10
HLA-A-B broad	1.05	0.84 – 1.32	0.65
HLA-A-B split	1.05	0.84 – 1.30	0.67
HLA-DR broad	1.02	0.68 – 1.53	0.92
HLA-DR split	1.08	0.75 – 1.55	0.69
HLA-A-B-DR broad	1.04	0.86 – 1.26	0.67
HLA-A-B-DR split	1.06	0.89 – 1.27	0.51
Shares			
HLA CREG	0.76	0.63 – 0.92	0.005
HLA-A-B broad	0.79	0.61 – 1.03	0.08
HLA-A-B split	0.78	0.61 – 1.00	0.05
HLA-DR broad	0.81	0.56 – 1.19	0.29
HLA-DR split	0.78	0.54 – 1.12	0.17
HLA-A-B-DR broad	0.79	0.63 – 0.99	0.04
HLA-A-B-DR split	0.77	0.62 – 0.95	0.01

RR, Relative risk and 95% CI, confidence interval, calculated per mismatch or share.

survival. Patients showing CMV seroconversion, presumably due to a CMV positive donor, had comparable results as patients with persistent negative CMV serology. The need of antihypertensive drugs, dipstick proteinuria and an elevated serum creatinine concentration (>150 µmol/l) at six months post-transplant were also associated with a higher risk of graft loss.

The result of the multivariate analysis is given in Table 6. It shows that recipients younger than 50 years were at risk for late graft loss. Recipients with an inherited original disease had a favorable outcome. The detrimental effect of grafts from donors older than 50 years was only present after discarding renal function at six months from the model. Sharing less than four CREG and a baseline immunosuppressive regimen consisting of prednisone and azathioprine predicted late graft loss independent from acute rejection. The negative effect of acute rejection on outcome was especially present in case of vascular rejection. Proteinuria and renal dysfunction at 6 months were independent clinical factors.

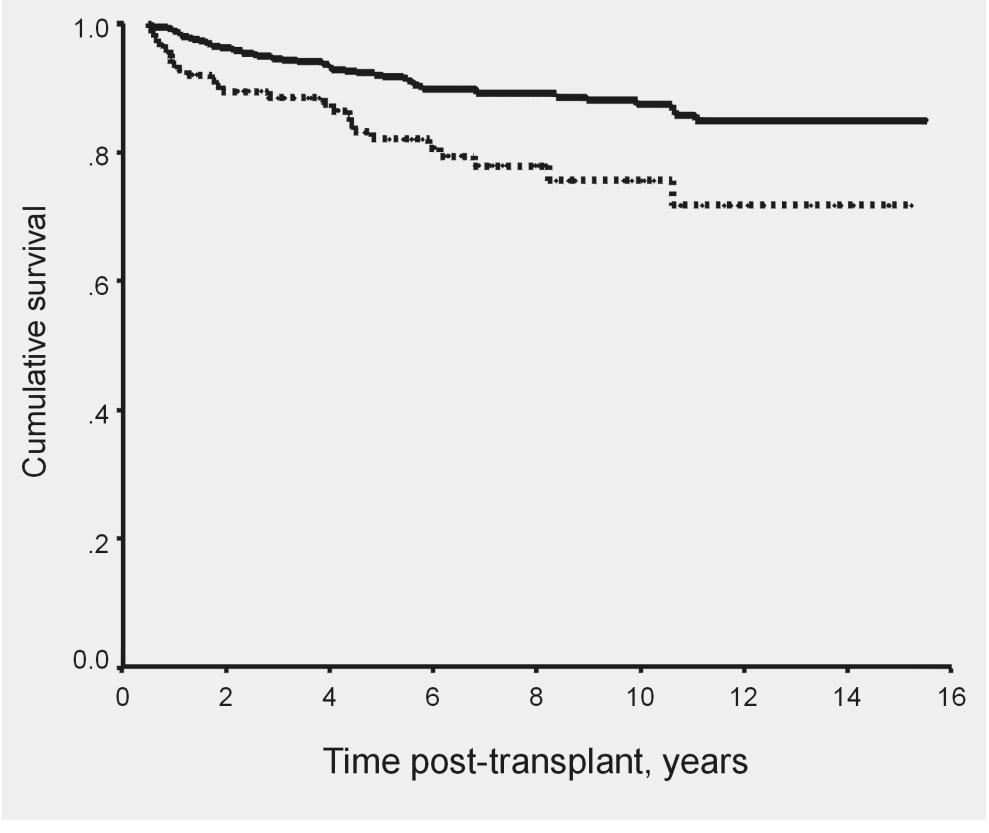

Figure 1 Graft survival and CREG sharing. Kaplan Meier estimates for transplants sharing 4 - 8 CREG (——; *N* = 516) and sharing 1 - 3 CREG (- - - -; *N* = 126). Log-rank test, *P* = 0.0011.

Discussion

The aim of this study was to identify risk factors predicting late graft loss in renal transplant patients. We found that a higher number of HLA mismatches did not predict graft loss whereas sharing of HLA antigens, especially MHC class I CREG had a beneficial effect on long-term outcome. In contrast to North-American databases, our cohort of patients was very well matched. Therefore, it is not surprising that the mismatch level did not further affect long-term survival. Even when HLA antigenic splits were considered in the evaluation, outcome did not correlate with the degree of mismatching. However, we clearly demonstrated that HLA mismatches and shares had a non-reciprocal relationship. This phenomenon is explained by homozygosity of HLA specificities and the allocation algorithm aiming at a low number of mismatches and was confirmed by a higher percentage of homozygosity of the different HLA

Table 5. Risk factors of late graft loss using univariate Cox regression analysis

Characteristic	RR	95% CI	*P*
Recipient factors			
Recipient age (< 50 years)	2.72	1.53– 4.84	< 0.001
Original disease: glomerular versus inherited	2.25	1.09 – 4.67	0.03
Original disease: systemic versus inherited	3.62	1.65 – 7.92	0.001
Systolic blood pressure (per 10 mm Hg increase)	1.02	0.93 – 1.10	0.61
Diastolic blood pressure (per 10 mm Hg increase)	1.17	0.98 – 1.39	0.08
Cigarette smoking	1.55	1.00 – 2.39	0.05
Peak panel reactive antibodies (> 50%)	1.21	0.75 – 1.95	0.37
Current panel reactive antibodies (> 50%)	1.69	0.87 – 3.28	0.14
Donor factors			
Donor age (> 50 years)	1.88	1.19 – 2.96	0.007
Cause of donor death: cardiovascular versus trauma	1.24	0.79 – 1.95	0.42
Transplant factors			
Year of transplant: 1983-86 versus 1992-96	2.67	1.31 – 5.43	0.007
Year of transplant: 1987-91 versus 1992-96	1.88	0.91 – 3.87	0.09
Repeat transplant	1.24	0.72 – 2.14	0.43
Gender match: female to male versus match	1.53	0.93 – 2.50	0.09
Gender match: male to female versus match	0.97	0.54 – 1.73	0.93
Cold ischemic time (> 36 hours)	1.43	0.84 – 2.44	0.19
Delayed graft function	0.90	0.52 – 1.54	0.69
Immunosuppression: azathioprine versus cyclosporine	1.90	1.06 – 3.40	0.03
Acute rejection factors			
Acute rejection type: interstitial	2.36	1.33 – 4.20	0.003
Acute rejection type: vascular	5.10	2.81 – 9.28	< 0.001
Number of rejection episodes: one	1.87	0.94 – 3.36	0.08
Number of rejection episodes: two	3.03	1.68 – 5.47	< 0.001
Number of rejection episodes: three or more	5.58	2.86 – 10.1	< 0.001
First rejection episode: within one month	2.22	1.33 – 3.71	0.02
First rejection episode: between month one and six	4.17	2.18 – 7.98	< 0.001
Last rejection episode: within two months	0.81	0.48 – 1.38	0.44
Last rejection episode: between month two and six	5.01	2.97 – 8.47	< 0.001
Acute rejection episode(s) after six months	5.71	3.46 – 9.42	< 0.001
Clinical factors at six months posttransplant			
Cytomegalovirus: seroconversion versus seronegative	0.78	0.40 – 1.50	0.45
Systolic blood pressure (per 10 mm Hg increase)	1.04	0.93 – 1.17	0.46
Diastolic blood pressure (per 10 mm Hg increase)	1.28	0.99 – 1.58	0.06
Number of antihypertensive drugs	1.27	1.00 – 1.62	0.05
Immunosuppression: azathioprine versus cyclosporine	1.12	0.70 – 1.81	0.63
Cyclosporine dose (< 3 mg/kg)	1.55	0.78 – 3.08	0.21
Dipstick proteinuria: trace, + versus negative	2.46	1.48 – 4.09	< 0.001
Dipstick proteinuria: >1+ versus negative	6.26	3.12 – 12.54	< 0.001
Serum creatinine (> 150 µmol/l)	4.37	2.72 – 7.00	< 0.001
Creatinine clearance (< 50 ml/min)	4.18	2.69 – 6.51	< 0.001

loci in the donors. This finding is important because a significant long-term beneficial effect of histocompatibility was only apparent in the analyses of shared HLA antigens. The beneficial effect of sharing HLA antigens has been reported (29, 30) and is illustrated by the excellent survival of six-antigen shared grafts (31). However, the difference between shares and mismatches with respect to their effect on graft survival has not been demonstrated previously in the same study population. HLA-A and -B matching seems to have a more profound effect on long-term survival compared to HLA-DR matching (32, 33). Private class I antigens share antigenic determinants which can be assigned in public groups, also called CREG (21, 22, 29, 34, 35). We found a beneficial effect of sharing CREG on long-term graft survival that was independent from acute rejection in the multivariate analysis. A minimum of

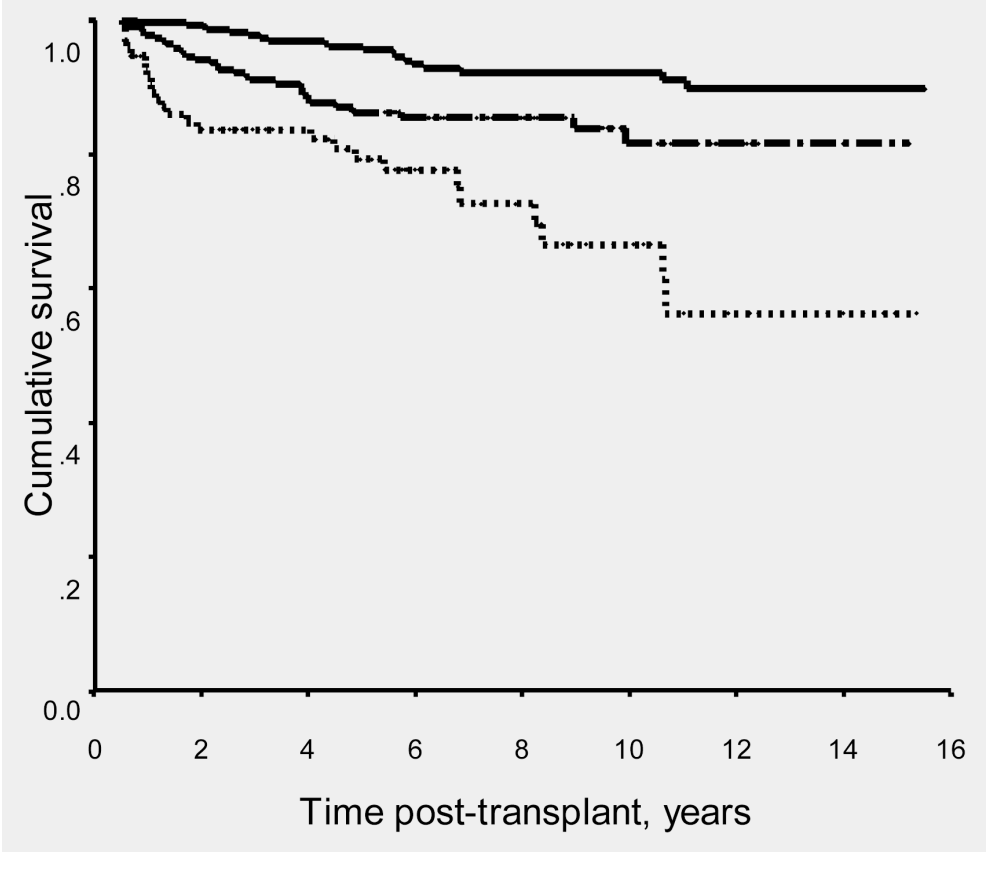

Figure 2 Graft survival and acute rejection type. Kaplan Meier estimates for transplants without rejection (——; *N* = 292), interstitial rejection (— - — -; *N* = 222) and vascular rejection **(**- - - - -; *N* = 95) within six months post-transplant. Log-rank test *P* = 0.0026, interstitial vs. no; *P* < 0.001 vascular vs. no; *P* = 0.0038, vascular vs. interstitial rejection.

Table 6. Independent risk factors of late graft loss using multivariate Cox regression analysis

Characteristic	Relative risk	95% Confidence interval	*P*
Recipient age < 50 years	1.95	1.02 – 3.71	0.04
Original disease: glomerular versus inherited	2.59	1.05 – 6.38	0.04
Original disease: systemic versus inherited	4.99	1.91- 13.1	0.001
Donor age > 50 years[a]	1.68	1.01 – 2.80	0.04
Sharing HLA CREG (1-3 versus 4-8)	2.20	1.29 – 3.75	0.004
Immunosuppression: azathioprine versus cyclosporine	2.13	1.06 – 4.28	0.03
Acute rejection: interstitial versus no rejection	2.04	1.04 – 3.97	0.04
Acute rejection: vascular versus no rejection	3.52	1.72 – 7.18	0.001
Proteinuria at six months (>1+ dipstick versus negative)	2.86	1.29 – 6.35	0.01
Serum creatinine at six months (> 150 μmol/l)	3.41	1.96 – 5.94	< 0.001

[a]Donor age is an independent risk factor after removal of serum creatinine from the model.

four shared CREG seems to be required for optimal graft survival. This result extends a recent report that showed that CREG matching was associated with a reduced frequency of late rejection episodes and improved 2-year graft function (23). It has been suggested that self-restricted recognition of donor derived HLA peptides contributes to the pathogenesis of chronic rejection (36, 37). Both mismatches and shares might play a role in this indirect allorecognition pathway. A low number of mismatches reduces the number of foreign peptides presented. Shared HLA antigens might result in downregulation or clonal deletion of T cells directed to these peptides. Prospective studies should reveal whether allocation algorithms based on CREG matching would reduce the number of failed grafts.

Recipient, donor and transplant related factors all influenced outcome. After censoring for death with a functioning graft, recipients younger than 50 years were more likely to have late graft loss compared with older recipients (26). This observation is consistent with other studies that have shown that young age is associated with a state of heightened immune responsiveness to alloantigens (38). Moreover, young patients tend to have a lower compliance with prescribed drugs (39). Another important factor in late graft loss is the age of the donor. The use of kidneys from older donors is correlated with an increased risk of late graft loss. Donor age influences the initial renal function and possibly also the quality of the tissue (40). The detrimental effect of a higher donor age appears to be mediated by a decreased renal mass, as suggested by our observation that this risk was independent only after discarding renal function at six months from the multivariate model. Contrary to some earlier reports that showed no impact of smoking (41), in the current study smoking significantly increased the risk of graft loss. Interestingly, smoking is also an emerging risk factor in other, non-transplant renal diseases (42). Patients with inherited renal diseases such as polycystic disease did better than patients with systemic or

glomerular disease, which is in agreement with large registry data (4). From our study it appears that the occurrence of late graft loss decreases over the long course of the study period. Indeed, patients receiving azathioprine as the baseline immunosuppressive regimen did worse than patients on a cyclosporine-based regimen did. These findings might be partially explained by the higher incidence of vascular rejection in the azathioprine treated patients (9). On the other hand, conversion from cyclosporine to azathioprine in the first six months is safe (25). We could not demonstrate an effect on late graft failure of pretransplant sensitization to lymphocyte antigens, ischemic damage and repeat transplantation. These variables are probably more related to early graft loss, with little effect on long-term survival (15, 43).

At six months posttransplant pending graft loss could be independently predicted by the histological pattern of previous acute rejection episodes, confirming previous data from our center and others that vascular rejection is a strong risk factor for late graft loss (9, 44). The incidence of vascular rejection should be a valuable short-term endpoint in clinical trials comparing different immunosuppressive regimens. Patients with multiple and late acute rejection episodes also showed an increased risk for late graft loss, as shown previously (7, 8, 10, 11, 13). Proteinuria is not only an important prognostic factor of outcome (45), it may also play a role in the pathogenesis of renal injury (46). Maneuvers to reduce proteinuria may stabilize deteriorating graft function and may ultimately affect prognosis (47). The serum creatinine concentration at 6 months was strongly correlated with subsequent graft loss.

Although there is no direct clinical test to diagnose chronic rejection, the gradual deterioration in graft structure and function as a result of antigen-dependent mechanisms, it seems to constitute the predominant cause of late graft loss in our study population. We studied only patients with graft survival beyond six months, a point in time at which most graft failures from acute rejection have occurred. Chronic rejection was diagnosed by exclusion of graft failure as result of recurrent glomerulonephritis and patient death with a functioning graft albeit that graft loss due to cyclosporine toxicity is not completely excluded. However, most patients were treated with once daily Sandimmune, aimed at 24-hour trough levels between 50 and 150 μg/l (25). Furthermore, cyclosporine nephrotoxicity has not been reported as major cause of graft loss (48). Moreover, biopsies and nephrectomy specimens obtained beyond six months posttransplant confirmed the presence of chronic allograft nephropathy. The concomitant histological presence of borderline or acute rejection supports ongoing rejection activity for the most part of the patients. Finally, antigen-dependent risk factors seem to play a dominant role in our study and resemble the established correlates of biopsy confirmed chronic rejection (40, 49-51).

We identified several coexisting recipient, donor and transplant related risk factors

for graft loss from chronic rejection after six months. In this well matched group of renal transplants HLA mismatches and shares had a non-reciprocal relationship. Sharing of HLA antigens, especially CREG of MHC class I, was associated with improved long-term survival

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3

Early versus late acute rejection episodes in renal transplantation

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Abstract

Background Acute rejection is a major complication after renal transplantation and the most important risk factor for chronic rejection. We investigated whether the timing of the last treated acute rejection episode (ARE) influences long-term outcome and compared the risk profiles of early versus late ARE.

Methods A cohort of 654 cadaveric renal transplants (1983-1997) that functioned for more than six months was studied. In 384/654 transplants, one or more treated ARE were documented; the last ARE occurred in 297/384 within 3 months and in 87/384 after 3 months. Applying multivariate logistic regression analysis, we compared the predictor variables of the two groups with transplants without ARE.

Results Ten-years graft survival rates censored for causes of graft loss other than chronic rejection were 94, 86 and 45% for patients without, with early and with late ARE, respectively. Delayed graft function, odds ratio (OR) 2.37 (1.55-3.62) and major histocompatibility complex (MHC) class II incompatibility, OR 2.28 (1.62-3.20) per HLA-DR mismatch, were independent risk factors for early ARE. In contrast, recipient age, OR 0.75 (0.61-0.93) per 10 years increase, donor age, OR 1.28 (1.07-1.53) per 10 years increase, female donor gender, OR 1.74 (1.03-2.94) and MHC class I incompatibility, OR 1.35 (1.07-1.72) per mismatch of cross reactive groups (CREG) were associated with late ARE.

Conclusions Late ARE have a detrimental impact on long-term graft survival and are associated with MHC class I incompatibility whereas transplants with early ARE are correlated with HLA-DR mismatches and have a better prognosis. These data are consistent with the role of direct and indirect allorecognition in the pathophysiology of early and late ARE, respectively.

Introduction

Acute rejection is still a major complication after renal transplantation. The overall incidence of acute rejection varies between 10 and 50% within the first six months, depending on the degree of HLA matching and treatment used for immunosuppression (1-3). Although novel regimes have reduced its incidence, the improvement of long-term graft survival is relatively small (2). Chronic rejection is the most prevalent cause of late renal transplant failure with acute rejection being one of its most important risk factors (4).

Not all acute rejection episodes (ARE) result in an adverse outcome. Therefore, it is important to define what kind of ARE is associated with late graft loss. Acute vascular rejection is an adverse prognostic feature compared with tubulointerstitial rejection (5,6). The occurrence of both interstitial and vascular rejection is associated with HLA-DR mismatches and delayed graft function but the risk of developing vascular rejection is decreased in patients using cyclosporine as compared with azathioprine (5). Severe ARE exert a more detrimental effect on long-term outcome than ARE with complete functional recovery. No single factor could differentiate between the two entities but the risk of severe ARE was decreased with the use of cyclosporine as primary immunosuppression (7). Recipients with repeated ARE have lower graft survival rates than those with no or only one ARE and were predicted by delayed graft function and earlier severe ARE (8). Finally, timing of ARE has an impact on long-term outcome. ARE within the first three months may have no effect on chronic rejection (9) whereas ARE occurring after three or six months confer the greatest risk (10,11). HLA mismatches were more frequent in early versus no or late ARE (11). In most studies early and late ARE are divided by the onset of the first ARE. However, we previously observed more contrast in prognosis when the onset of the last treated ARE was used as time factor (12). Assessing the risk profile of late ARE is important to earmark transplants with an adverse prognosis and to get insight in the pathophysiology of late rejection activity. MHC antigens play a key role in allorecognition. Class II antigens are the counterparts of helper T cells while class I antigens are recognized by cytotoxic T cells. In the first period after transplantation, activation of helper T cells is achieved predominantly in the direct pathway via allo-class II antigens expressed on antigen presenting cells of the donor. Once these passenger cells are disappeared, donor HLA antigens can only be recognized after internalization, processing and presentiation via MHC antigens of dendritic cells of the recipient, the indirect pathway (13,14). Therefore, we hypothesize that early ARE as result of the direct alloresponse are related with HLA-DR mismatches and that late ARE from the indirect response are associated with class I histoincompatibility.

In the present study, we compared prognosis and risk factors of early versus late ARE, defined by the timing of the last treated episode before and after three months, respectively.

Patients and methods

Study population

We studied all cadaveric kidney transplants performed in our center between 1983 and 1997, i.e. before major changes in immunosuppressive treatment took place. Kidneys were allocated according to the matching algorithm used by Eurotransplant. Transplants that functioned for less than six months were excluded as this study was designed to evaluate the effect of ARE on late survival. The standard immunosuppressive regimen consisted of prednisone and cyclosporine or azathioprine. Patients on cyclosporine were generally treated with once-daily Sandimmune and had a dose adjustment 3 months after transplantation, dependent on a desired 24-hour trough concentration of 50-150 μg/l. Patients were followed until death, return to dialysis, or until July 1, 1998. The clinical characteristics of the cohort have been described in detail in a previous report (12).

Acute rejection episodes

ARE were defined clinically by an acute deterioration of allograft function without an obvious other cause and confirmed histologically in most cases. Interstitial rejection was diagnosed when tubulitis with an infiltrate was present (Banff grade borderline or I); the diagnosis of acute vascular rejection was made when arteritis was present (Banff grade II or III). If a biopsy also had features of chronic allograft nephropathy the diagnosis of acute rejection was based on the clinical decision to administer Solumedrol or ATG. The term clinical rejection was used for a treated ARE not confirmed by biopsy. ARE were treated with Solumedrol, antithymocyte globuline (ATG) or Solumedrol for the first, second, or third rejection episode, respectively.

We used the time between transplantation and the last biopsy displaying acute rejection followed by anti-rejection treatment to define early and late ARE. In clinical rejection, the period until the first day of the last anti-rejection treatment was used. Early ARE were defined as ARE within three months; late ARE if the last ARE occurred after three months independent of previous early ARE. The 3 months cut-off was taken because of the routinely reduction in cycloporine dose afterwards and the hypothesis that the direct pathway of allorecognition is confined to the first months post transplantation and therefore related with early ARE. Ten-year graft survival rates of transplants without ARE and cases

with early or late ARE were estimated using Kaplan-Meier methods, including a log-rank test and assigning graft loss from chronic rejection as outcome variable. In addition, we compared graft survival rates of transplants with late ARE with or without previous early ARE. The clinical characteristics of the groups without ARE, with early ARE, and with late ARE were compared using the Chi-square or independent samples T test.

Logistic regression analysis

Using univariate logistic regression analysis we assessed the risk profiles of transplants with early or late ARE. Recipient age and percentage of panel reactive antibodies at time of transplantation were tested as well as donor age and gender. Transplant parameters included year of transplantation, repeat transplant, cold ischemic time, delayed graft function defined as the need for dialysis in the first week, and the baseline immunosuppressive drug regimen. The influence of HLA mismatches on ARE was evaluated at the level of private antigens and crossreactive groups (CREG) of MHC class I, as described previously (12). Odds ratios and 95% confidence intervals were estimated for each variable in the model. Significant predictors (P value < 0.05) were fitted in a multivariate model. Forward selection techniques were used to assess independent risk factors of transplants with early or late ARE.

Results

Between 1983 and 1997, 762 cadaveric transplants were performed. In 108 cases, graft loss occurred in the first six months due to acute rejection (n=61), patient death with functioning graft (n=14), primary non-function (n=10), technical failure (n=8), recurrent disease (n=3) and other causes (n=12). Therefore, a total of 654 transplants functioned for more than six months. The mean number of CREG, HLA A and B and HLA-DR mismatches was 1.9, 1.5 and 0.4, respectively. Late graft loss censored for causes other than chronic rejection occurred in 82 cases.

Early versus late acute rejection episodes

One or more treated ARE were documented in 384/654 (59%) transplants. ARE were confirmed histologically in 94% of the cases. ARE were present within 3 months in 297/384 cases (77%), whereas in 87/384 (23%), the last treated ARE occurred after 3 months. The median time to the last ARE was 23 days (range 2-88) in the early ARE group and 244 days (range 92-2411) in the late ARE group. In the latter group 50/87 transplants (58%) had also previous ARE during the first three months. Ten-year graft survival censored for causes

of graft loss other than chronic rejection was 94, 86 and 45% for patients without, with early, and with late ARE, respectively (Figure 1). These differences were significant (P<0.01). In the 87 patients with late ARE ten year graft survival was similar (45%) between the 51 patients with and the 36 patients without ARE within 3 months. Table 1 compares the clinical characteristics of the groups with early or late ARE with the 270 transplants without ARE. There was no difference in year of transplantation, but the group with early ARE had more repeat transplants. Recipient age was lower in the late ARE group. Sensitization at time of transplantation was not significantly different with or without ARE. Transplants with late ARE had mostly older donors, and were more frequently of female gender. The early ARE group showed a higher number of HLA-DR mismatches whereas the late ARE group had a higher number of mismatches on the HLA-A locus and on the level of CREG. Cold

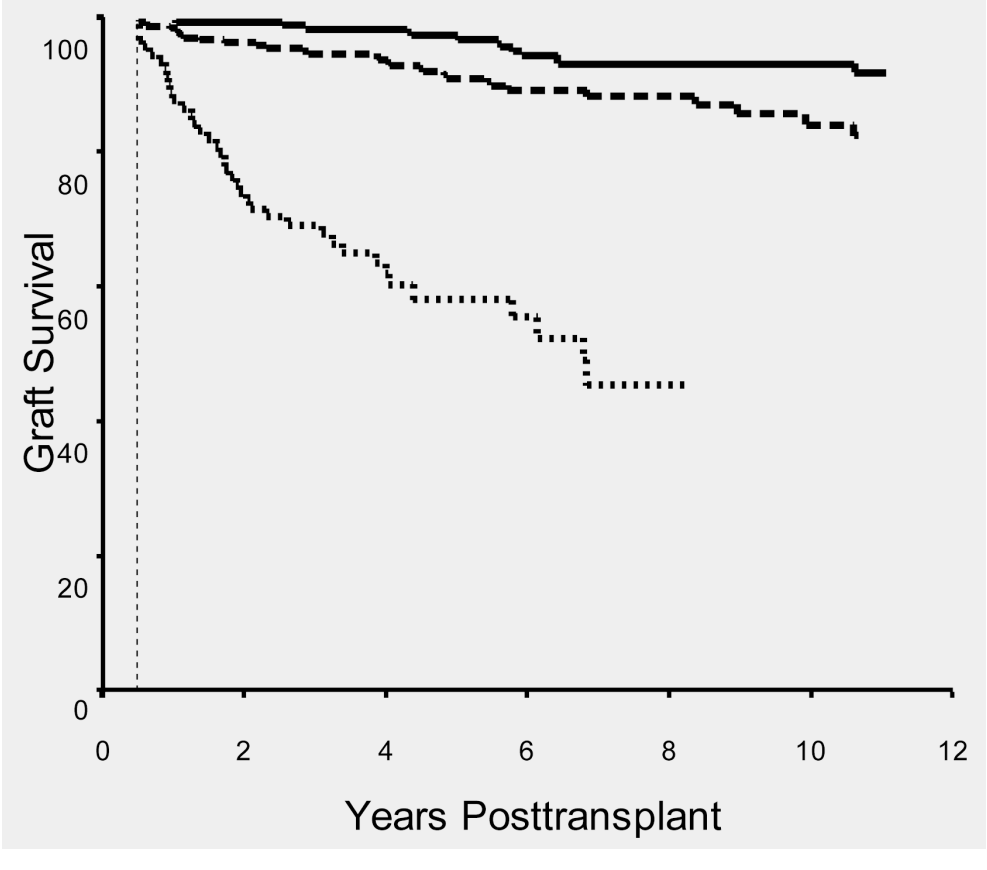

Figure 1 Kaplan-Meier graft survival, censored for other causes than from chronic rejection, for transplants without ARE (solid line), with ARE within 3 months (dashed line) and with ARE after three months (dots).

ischemic time was similar in the three groups, but delayed graft function was more prevalent in the ARE groups. Patients with late ARE were significantly more often treated with an azathioprine-based regimen. The total number of ARE was higher in the late ARE group. Looking at the histological type of the ARE, vascular rejection occurred in 26% of the transplants with early ARE and in 28% of the cases with late ARE. Taking into account only the biopsies obtained after three months vascular rejection was present in 16%. At 6 months posttransplantation the late ARE group showed decreased renal function and more proteinuria.

Risk profiles of early versus late acute rejection episodes

Table 2 and 3 show the independent risk factors of early and late ARE, respectively. Delayed graft function, OR 2.37 (1.55-3.62) and MHC class II histoincompatibility, OR 2.28 (1.62-3.20) per HLA-DR mismatch, were risk factors for early ARE while recipient age, OR 0.75 (0.61-0.93) per 10 years increase, donor age, OR 1.28 (1.07-1.53) per 10 years increase, female donor gender, OR 1.74 (1.03-2.94) and class I histoincompatibility, OR 1.33 (1.05-1.68) per mismatch of CREG were associated with late ARE.

Table 1. Clinical characteristics of transplants without, with early, and with late ARE

	no ARE (n=270)	early ARE (n=297)	late ARE (n=87)
Year of transplantation (% 1980s)	54	48	54
Repeat transplant (%)	13	20[a]	12
Recipient age (years)	46 ± 12	46 ± 13	42 ± 13[ab]
Panel reactive antibodies (%)	9 ± 12	12 ± 19	11 ± 22
Donor age (years)	36 ± 14	37 ± 15	41 ± 16[ab]
Donor gender (% female)	34	42	49[a]
HLA-A mismatches	0.6 ± 0.6	0.7 ± 0.7	0.8 ± 0.6[a]
HLA-B mismatches	0.8 ± 0.6	0.8 ± 0.7	0.8 ± 0.6
CREG mismatches	1.0 ± 1.1	1.1 ± 1.0	1.3 ± 1.0[a]
HLA-DR mismatches	0.3 ± 0.5	0.5 ± 0.6[a]	0.3 ± 0.5[b]
Cold ischemic time (hours)	29 ± 7	29 ± 7	29 ± 7
Delayed graft function (%)	16	30[a]	27 [a]
Azathioprine-based regimen (%)	7	8	15[ab]
Number ARE 1 / 2 / > 2 (%)		41 / 38 / 21	21 / 46 / 33[b]
Interstitial / vascular / clinical ARE (%)		60 / 26 / 14	69 / 28 / 3
Interstitial / vascular / clinical ARE (%)[c]			78 / 16 / 6
Dipstick proteinuria >1+ 6 m. (%)	4 ± 19	6 ± 24	11 ± 32[a]
Creatinine clearance 6 m. (ml/min)	69 ± 21	60 ± 25[a]	51 ± 21[ab]

Data are expressed as mean ± SD unless otherwise stated. [a]P<0.05, transplants with ARE compared with cases without ARE. [b]P<0.05, transplants with late ARE compared with cases with early ARE. [c]Biopsies obtained after three months.

Table 2. Independent risk factors for transplants with early ARE

	Odds ratio	95% confidence interval
HLA-DR mismatches (per antigen)	2.28	1.62-3.20
Delayed graft function	2.37	1.55-3.62

Discussion

These studies shows that transplants with ARE after three months had a worse survival and a different risk profile compared to transplants with ARE confined to the first three months. The strong correlation of late ARE with chronic rejection and late graft loss has been reported consistently in the literature (10,15-17). In these studies late ARE is usually defined by the timing of acute rejection onset. We used the timing of the last treated ARE for several reasons. First, we have followed the generally accepted policy to decrease the cyclosporine dose at three months, which may result in rejection activity afterwards. Second, we previously reported that in contrast to early ARE, a later occurrence of the last ARE had a strong adverse effect on long-term graft survival (12). Third, we hypothesized that the temporal direct anti-donor response is related with early ARE and that the continual indirect alloresponse is responsible for late rejection activity (13,14).

We found that early ARE were associated with HLA-DR mismatches and delayed graft function. HLA-DR mismatches increase the occurrence of early ARE (5,18) and the risk of early graft failure (19) which is consistent with the initial recognition of HLA-DR molecules on donor dendritic cells by T helper cells of the recipient. This direct pathway and therefore the HLA-DR effect diminishes over time once the passenger cells disappear and donor-specific T helper cell hyporesponsiveness possibly has been induced (20). Delayed graft function also increases the risk of ARE without an independent effect on graft survival (21). We confirmed previous studies that graft survival after early ARE is not severely compromised (9), a feature which preferentially applies for interstitial rejection (5,6).

Table 3. Independent risk factors for transplants with late ARE

	Odds ratio	95% confidence interval
Recipient age (per 10 years increase)	0.75	0.61-0.93
Donor age (per 10 years increase)	1.28	1.07-1.53
Donor gender (female)	1.74	1.03-2.94
CREG mismatches (per CREG)	1.33	1.05-1.68

By contrast, late ARE appear to be correlated with young recipient age, old donor age, female donor gender and class I CREG mismatches. Recently we described that kidneys from older donors are prone to undergo acute interstitial rejection and have an adverse outcome possibly due to an impaired ability to restore tissue (22). Others found similar results for kidneys from female donors (23). Young recipient age is associated with a state of increased immune responsiveness, but also with non-compliance (24). The incidence of ARE has been reported earlier to be increased by CREG mismatches (25). In our study, late ARE were also associated with HLA-A mismatches and CREG shares, factors both reported to influence long-term graft survival (12,26). These findings support the evidence that recipient T cells recognizing donor class I molecules, presented by recipient antigen presenting cells are important mediators of late ARE and chronic rejection (13,14). Interaction of helper T cells with B cells might be relevant for the induction of anti-donor antibodies possibly leading to subsequent chronic humoral rejection (27). Late rejection activity via the indirect pathway and development of chronic allograft nephropathy might happen even under modern immunosuppressive treatment (28). This explains the little change in long-term outcome despite significant reduction in the incidence of ARE and the increased impact of ARE on chronic allograft failure in recent era (2,29). The persistent association of the number of HLA mismatches and graft outcome (30) warrants further improvement in matching algorithms to prevent chronic rejection (31).

Patients with late ARE had a higher number of ARE, but a similar vascular rejection rate compared to early ARE. Within the late ARE group graft survival was similar between the patients with and the patients without previous early ARE confirming the importance of rejection activity over time. Damage to tubular basement membranes as a consequence of persistent interstitial inflammation and tubulitis in late ARE have been reported to correlate with later development of chronic interstitial rejection (32). The finding that acute interstitial rejection can lead to interstitial fibrosis without transplant vasculopathy or glomerulopathy has earlier been observed (33). We recently categorized chronic allograft nephropathy in a group with and without vasculopathy and found an immunological risk profile in the latter group consistent with chronic interstitial rejection (34).

This study shows that late ARE have a detrimental impact on long-term graft survival compared to early ARE. In this well-matched cohort of renal transplants, HLA class II mismatches predict early ARE, whereas HLA class I mismatches are correlated with late ARE. These data are consistent with the role of direct and indirect allorecognition pathways in the pathophysiology of early and late rejection activity, respectively.

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4

Intercept and slope analysis of risk factors in chronic renal allograft nephropathy

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Abstract

In the Leiden cohort of cadaveric renal transplants we compared the risk factors of a low intercept, defined as a creatinine clearance lower than 50 ml/min at 6 months, and of a negative slope of the reciprocal creatinine concentrations after 6 months. Two hundred of 654 grafts (31%) failed to reach optimal function because of old donor age, female gender of the donor, histoincompatibility, delayed graft function or acute rejection episodes in the first 6 months. Forty-four percent of all grafts displayed progressive deterioration of function over time. The association with younger recipient age, sensitization, class I histoincompatibility, baseline immunosuppression and late acute rejection episodes suggest an underlying immunological process, very likely activated through the indirect antigen-presenting route. The negative impact of proteinuria and diastolic hypertension at six months on the slope is compatible with their role as progression factors. Although associations are not necessary causally related and interventions do not necessary result in an improvement in outcome, it is conceivable that better matching, optimal immunosuppression and a more aggressive antihypertensive and antiproteinuric treatment results in improvement of long-term graft survival.

Key terms

Intercept: the point at which the graph that describes the level of function over time intercepts with the ordinate.

Slope: the point at which the graph that describes the level of function over time intercepts with the abscissa.

Introduction

Chronic rejection is the most prominent cause of chronic allograft nephropathy and graft loss beyond the initial six post-transplant months. Clinically it results in chronic graft dysfunction which is characterized by a decline in glomerular filtration rate over months or years, often in combination with proteinuria and hypertension (1). The histopathology of chronic rejection shows arterial intimal thickening, transplant glomerulopathy, glomerulosclerosis, interstitial fibrosis and tubular atrophy (2). Several risk factors have been identified but it is not clear how they operate in the process of graft attrition. In the present study we defined transplants with a low intercept or a negative slope and compared their risk profiles.

Patients and methods

We analyzed the course of the renal function in 654 patients transplanted between 1983 and 1979 who survived at least 6 months (3). The endogenous creatinine clearance at 6 months was used to categorize the intercept of the graph that describes graft function over time as lower or higher than 50 ml/min. Chronic decline in function was modeled by one or two least-squares-fitted regression lines (4), which determines the slope of the graft function over time graph. A negative slope significantly different from zero categorized transplants as having a decline in function beyond 6 months. Using logistic regression techniques, we assessed the risk profiles of a low intercept and a negative slope, respectively.

Results

Four patterns of “evolution of graft function over time” were created (figure 1). 41% of grafts resumed and maintained optimal function post-implantation (creatinine clearance > 50 ml/min) whereas another 28% achieved a similar function but experienced functional deterioration afterwards. The remaining 31% had at 6 months a creatinine clearance < 50 ml/min; about half of these maintained this function whereas the remaining grafts displayed progressive loss of function. We found a significant correlation between a low intercept and a negative slope in a chi-square test ($p=0.025$). Furthermore, the slope expressed as decline of the reciprocal creatinine concentration per month was significantly steeper in the group with a low intercept (-1.09) compared to the group with a high intercept (-0.44). Figure 2 shows the graft survival censored for patient death in the groups with a negative slope stratified by the intercept. Univariate analysis showed that old donor age, female gender of the donor,

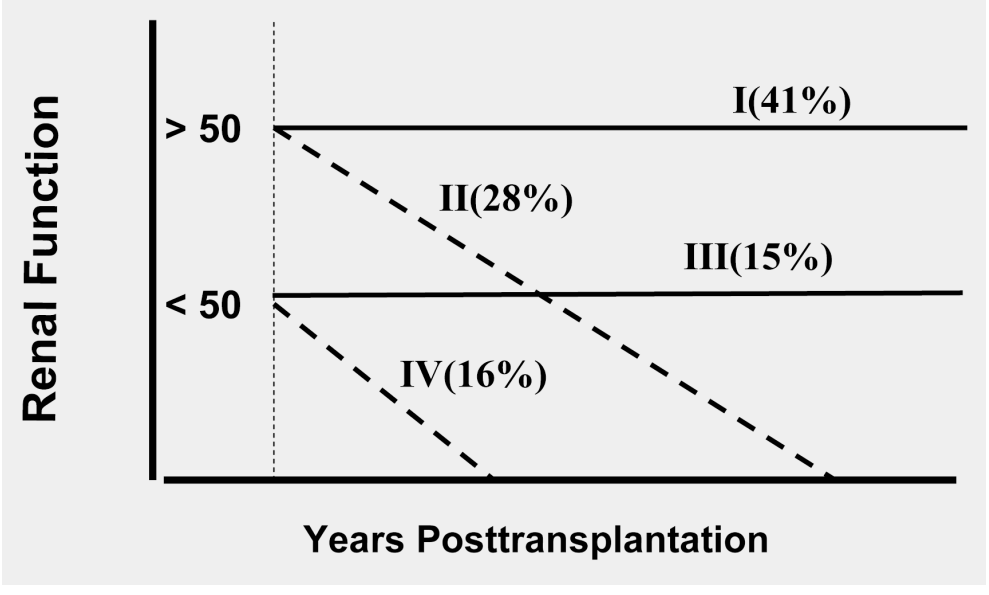

Figure 1 604 transplants categorised according to intercept (endogenous creatinine clearance at 6 months) and slope of reciprocal creatinine concentrations after six months post-transplant: I > 50 ml/min, stable function (n=246), II > 50 ml/min, decline in function (n=171), III < 50 ml/min, stable function (n=92), IV < 50 ml/min, decline in function (n=95).

histoincompatibility of MHC class I antigens, delayed graft function or acute rejection episodes were associated with a low intercept (table 1). Donor age, odds ratio (OR) 1.45 (1.27-1.65) per 10 years increase, CREG matching, OR 0.77 (0.66-0.91) per shared CREG and acute rejection, OR 3.09 (2.02-4.79) for episodes within he first two months and OR 7.73 (4.26-14.04) for episodes between month 2 and 6 posttransplantation were independent factors in multivariate analysis. Younger recipient age, sensitization, year of transplantation, repeat transplantation, histoincompatibility, baseline immunosuppression, late acute rejection episodes, diastolic blood pressure and proteinuria at 6 months were associated with progressive loss of renal function. In multivariate analysis, transplantation in the eighties, OR 2.77 (1.95-3.93), HLA matching, OR 0.81 (0.68-0.96) per shared antigen and acute rejection episodes between month 2 and 6 posttransplantation, OR 1.91 (1.09-3.33) were independently predictive of a negative slope.

Discussion

Although the zenith of renal function post-transplantation may be after the first year (5), this intercept and slope analysis allows an interesting insight into

the evolution of graft function over time. In the present study, old donor age, female gender of the donor and delayed graft function were the discriminating factors correlated with a low intercept, defined as a creatinine clearance of less than 50 ml/min at 6 months posttransplantation. Kidneys from old and female donors may have a lower renal mass. Moreover, old donor kidneys are more likely to develop delayed graft function and to undergo acute rejection episodes (6,7). We have previously shown that delayed graft function is associated with an impaired long-term outcome, dependent on the level of renal function (8), and is therefore an intercept problem. Grafts with delayed function have also a higher likelihood of acute rejection episodes (8). Acute rejection episodes had a strong impact on the intercept. Interstitial and vascular rejection had a similar effect. Previous studies have shown that acute vascular rejections are of prognostic significance but graft loss in this group occurs early after

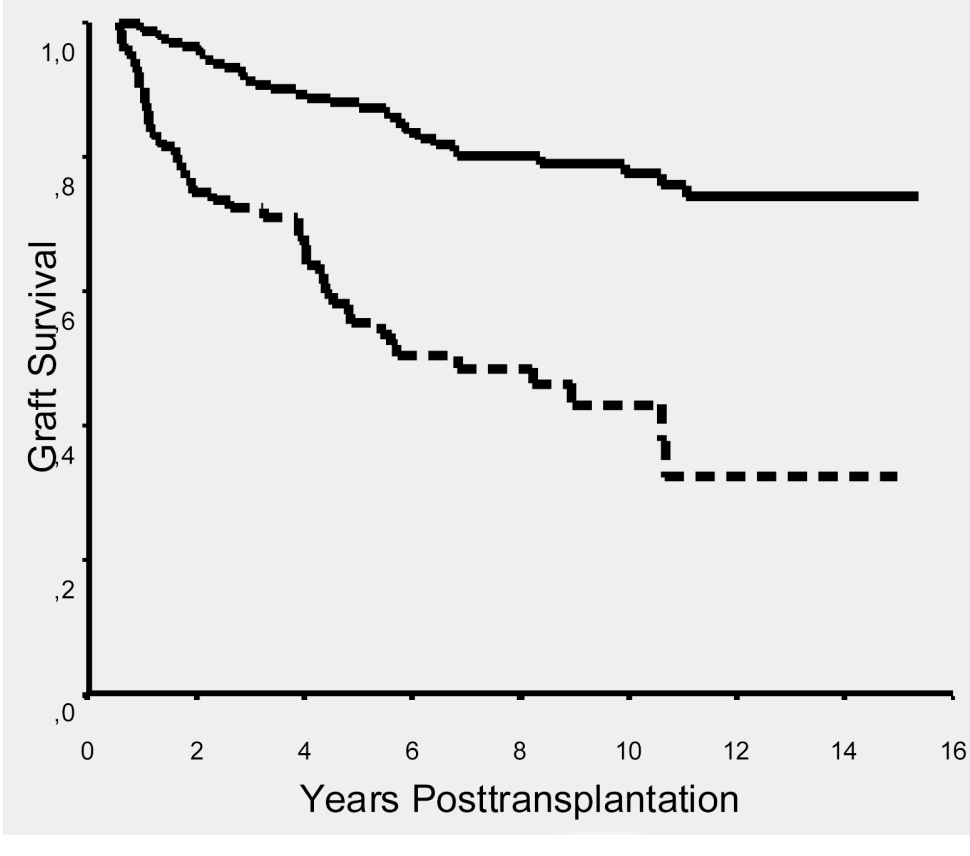

Figure 2 Graft survival censored for patient death of transplants with a creatinine clearance at 6 months > 50 ml/min and a decline in function after 6 months (solid line) and transplants with a creatinine clearance at 6 months < 50 ml/min and a decline in function after 6 months (dashed line). Log rank test: $P < 0.001$.

Tabel 1. Univariate analysis of risk factors for a low intercept and for a negative slope

	low intercept	negative slope
Recipient age (per 10 years increase)	1.10 (0.96-1.26)	0.84 (0.74-0.96)*
Panel reactive antibodies (per 10% incr)	1.04 (0.96-1.12)	1.09 (1.01-1.18)*
Donor age (per 10 years increase)	1.49 (1.32-1.68)*	1.03 (0.92-1.15)
Donor gender (female)	1.88 (1.34-2.64)*	0.78 (0.56-1.08)
Year of transplantation (83-89 vs. 90-97)	0.95 (0.68-1.33)	2.69 (1.29-3.76)*
Repeat transplant	1.40 (0.91-2.16)	1.70 (1.10-2.61)*
HLA-A,B,DR mismatches (per antigen)	1.05 (0.91-1.22)	0.99 (0.86-1.15)
HLA-A,B,DR shares (per antigen)	0.90 (0.76-1.06)	0.82 (0.70-0.96)*
CREG mismatches (per group)	1.27 (1.09-1.49)*	1.02 (0.88-1.19)
CREG shares (per group)	0.80 (0.69-0.92)*	0.84 (0.73-0.96)*
Azathioprine versus cyclosporine	0.95 (0.52-1.75)	2.25 (1.26-4.02)*
Delayed graft function	1.90 (1.30-2.78)*	0.81 (0.56-1.19)
Acute interstitial rejection episodes < 6 months	3.67 (2.45-5.51)*	0.90 (0.62-1.30)
Acute vascular rejection episodes < 6 months	4.20 (2.54-6.96)*	1.31 (0.81-2.12)
Acute rejection episodes 0 - 2 months	2.68 (1.81-3.95)*	0.93 (0.66-1.31)
Acute rejection episodes 3 - 6 months	8.04 (4.62-14.0)*	1.74 (1.03-2.95)*
Systolic RR 6 months (per 10 mmHg incr.)		1.06 (0.98-1.15)
Diastolic RR 6 months (per 10 mmHg incr.)		1.26 (1.07-1.51)*
Dipstick proteinuria >1+		2.72 (1.33-5.55)*
Creatinine 6 months (per 10 µmol/l increase)		1.01 (0.99-1.04)

transplantation (9). Other investigators have also noted the lack of correlation of acute rejection pathology and survival beyond six months post-transplantation (10). Multivariate analysis showed that acute rejection episodes occurring later in the initial 6 months had a stronger impact on the intercept than the histopathological type of acute rejection. Moreover, in contrast to early acute rejection episodes late acute rejection activity appeared to be the link between a low intercept and a negative slope. We recently confirmed previous data that patients undergoing acute rejection episodes > 60 days have an increased incidence of chronic rejection (3,11). In our well matched cohort of renal transplants sharing cross reactive groups of MHC class improved long-term graft survival (3) and influenced both the intercept and the slope in this study. This observation is consistent with the hypothesis that an immunological mechanism is involved in chronic rejection as is the association with late acute rejection episodes. While freedom from chronic rejection is associated with lack of donor-specific immune reactivity, patients with chronic rejection often have circulating T lymphocytes that recognize incompatible donor MHC peptides presented on recipient antigen presenting cells (12) or anti-HLA antibodies or anti-kidney antibodies (13,14). We hypothesized that sharing HLA antigens down-regulates the conventional MHC directed response that is also attenuated by immunosuppressive treatment (3). However, the correlation between the degree of MHC matching and graft loss from chronic rejection is variable (15-17), suggesting that the specificity of the immune response might

also be directed to non-MHC determinants. It is therefore also conceivable that chronic rejection results from an immune reaction against damaged tissues and that such responses result in perpetuation of chronic inflammation and impairment of the tissue repair process, resulting in excessive fibrosis and tissue remodeling (18).

The discriminating impact of recipient age, sensitization, and repeat transplant on the slope confirms the importance of an antigen-driven process in the attrition of graft function. Young age is associated with noncompliance (19) but also with a state of heightened immune response to alloantigens (20). Losing a kidney increases the risk of broad sensitization with a subsequent risk of acute and chronic rejection (21). Patients using baseline immunosuppression with azathioprine and prednisone were transplanted in the early eighties and had therefore a longer follow-up to develop a negative slope whereas patients randomized to conversion from cyclosporine to azathioprine at 6 months did not have an inferior graft survival (22).

The level of renal function, proteinuria, and hypertension are non-immunological risk factors that have also been identified as risk factors in the progression of native kidney diseases. In experimental animals, reduction in renal mass results in an increase in the glomerular capillary hydrostatic pressure and glomerular filtration rate in the remaining nephrons as a result of afferent arteriolar dilatation (23) and it has been proposed that increased hydrostatic pressure results in glomerulosclerosis and progressive renal damage. We demonstrated in a rat model that following reduction in renal mass or transplantation the glomerular hydrostatic pressure increases in some rat strains but not others (24). We have previously shown that the rate of human long-term graft loss depends on the level of function as assessed by the creatinine clearance (8). The present analysis confirms that if functional deterioration occurs, the rate of decline is different for grafts with a creatinine clearance of < 50 ml/min compared with grafts with a clearance of > 50 ml/min at 6 months posttransplantation.

Hypertension is an established progression factor in renal transplantation as it is in native kidney diseases (25). The association between the diastolic blood pressure and the slope of reciprocal creatinines has been reported previously (26). Experimental data in a rat renal transplant model of chronic kidney graft rejection are consistent with the hypotheses that this effect is mediated by glomerular hypertension (24,27). Lowering of the blood pressure has a beneficial effect on the rate of progression of chronic rejection in animals (28) although there are very few clinical data available.

The amount of proteinuria has traditionally been considered a marker of the severity of a renal disease. Recent studies indicate, however, that proteins filtered through the glomerular capillaries may have intrinsic renal toxicity which play a role in the progression of renal damage (29) and that the amount

of urinary proteins correlates with the tendency of a given disease to progress (30). Dietary protein restriction, ACE inhibitors, and other anti-hypertensive drugs are capable of limiting the progressive decline in glomerular filtration rate to the extent that they effectively lower the urinary protein excretion rate in native kidney disease (31) and in chronic rejection (32).
The combination of a decline in renal function, hypertension and proteinuria, characterize the syndrome of chronic transplant dysfunction, which might begin weeks to months after transplantation. Chronic rejection is the most important cause of chronic transplant dysfunction which is supported by the impact of histoincompatibility of class I antigens and late acute rejection episodes on both the intercept and the slope in this study. Graft survival is further modified by pure intercept factors such as donor age, female gender of the donor and delayed graft function and the presence of hypertension and proteinuria as progression factors. Better matching, optimal immunosuppression and a more aggressive antihypertenisve and antiproteinuric treatment should improve long term graft survival.

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5

Predicting kidney graft failure using time-dependent renal function covariates

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Abstract

Chronic rejection and recurrent disease are the major causes of late graft failure in renal transplantation. To assess outcome, most researchers use Cox proportional hazard analysis with time-fixed covariates. We developed a model adding time-dependent renal function covariates to improve the prediction of late graft failure. We studied 692 kidney transplants at the Leiden University Medical Center that had functioned for at least six months. Graft failure from chronic rejection or recurrent disease occurred in 106 patients. The reciprocal of last recorded serum creatinine (RC), the ratio of RC and RC at 6 months (RC_6) and the time elapsed since last observation (TEL) were used as time-dependent covariates. Cadaveric donor transplantation, a lower RC and a lower ratio of RC/ RC_6 were independently associated with graft failure. The impact of the last recorded RC was dependent on its value, TEL and the time since transplantation. Validation of the model confirmed much higher failure predictions in those with subsequent graft failure compared to non-failures. In conclusion, this study illustrates that the prediction of late graft failure could be improved significantly by using time-dependent renal function covariates.

Introduction

Long-term success of renal transplantation is hampered by the steady loss of transplants mainly because of chronic rejection and recurrent disease (1,2). Graft failure is usually preceded by chronic transplant dysfunction, which is routinely assessed by measurements of serum creatinine concentrations. Plotting the reciprocal serum creatinine versus time is useful in monitoring renal disease progression and predicting the start of dialysis (3,4). However, in renal transplant patients the nature of the chronic decline is quite variable (5). Another way to predict graft failure is the assessment of risk profiles using Cox proportional hazard analysis (6). In our center, younger recipient age, older donor age, histoincompatibility and acute vascular rejection independently predicted late graft failure from chronic rejection (7). Increased serum creatinine level at 6 months post-transplantation also increases the risk of a worse outcome (7-9). In addition of the absolute level of the serum creatinine, studies have reported on the relationship between the course of renal function over time and subsequent graft failure (5,9-11).

Renal function covariates obtained at follow-up visits might be useful to update the prognosis during the course of the disease. Several models have been developed for survival studies in which a covariate is measured repeatedly across time (12-14). Christensen et al performed a Cox proportional hazards model with time-dependent covariates on data of patients with cirrhosis that could be used to update prognosis whenever changes occur in the clinical status (15). However, this approach is less accurate when the repeated follow-up measurements are collected irregularly as usually occurs in clinical practice or when the frequency of data collection is associated with the end-point under study (16). Therefore, we previously extended the Cox model with an additional term to correct for irregularly collected observations. This term is the time elapsed since last observation (TEL) and defined as the actual time minus the time of the last recording of the time-dependent covariate. It proved to be an important prognostic covariate in patients with chronic myeloid leukemia using time-dependent white blood cell counts (17).

In the present study, we applied the multivariate Cox proportional hazards model with time-dependent renal function covariates to the Leiden cohort of renal transplant patients with the aim of improving the prediction of late graft failure.

Patients and methods

Patients and graft failure

All 692 patients who underwent their first kidney transplantation in the Leiden University Medical Center between 1 January 1983 and 1 July 1996 and who had a functioning graft for at least six months were included in the study. Ninety-seven patients had been transplanted with a kidney from a living donor. Late graft failure, defined as return to dialysis, from chronic rejection or de novo or recurrent disease was used as outcome variable. Patient death with a functioning graft (n=108) was counted as a non-failure. Maintenance immunosuppressive regimen consisted of prednisone and either cyclosporine (Sandimmune) or azathioporine.

Time-fixed covariates

Several recipient, donor and transplant characteristics, available at 6 months posttransplantation, were evaluated as risk factors of late graft failure. The following recipient covariates were tested: age, gender, original disease, smoking, and panel reactive antibodies. Donor covariates included age, cause of death and donor source. Transplant covariates studied included year of transplant, repeat transplant, gender match, cold ischemia time, delayed graft function and the baseline immunosuppressive drug regimen. We studied the impact of the number of HLA mismatches and shares between donor and recipient, both at the level of private antigens and at the level of cross-reactive groups (CREG) of major histocompatibility complex (MHC) class I molecules (7). As rejection factor we used the number of treated acute rejection episodes. Finally, dipstick proteinuria and the reciprocal of the serum creatinine concentration at 6 months (RC_6) were tested as time-fixed covariates.

Time-dependent covariates

In contrast to time-fixed covariates, time-dependent covariates are measured repeatedly over time, where the number of observations and the time between the observations may vary between patients. We collected all serum creatinine values measured at unspecified time points beyond 6 months after transplantation and used all the available information in the analysis. The mean number of recordings was 55 with a range between 2 and 468 values. Because of the reciprocal relationship between the creatinine clearance and serum creatinine level, we used 1000 times the reciprocal of the serum creatinine concentration (RC) for all our analyses. RC was centered on the overall mean of 7.5, corresponding to a serum creatinine concentration of 133 µmol/L. Figure 1 illustrates four individual plots of RC versus time courses after transplantation. In our model, we used the last recorded RC as a covariate of patients' most up-

to-date renal function. We chose to use the last recorded value only, instead of all preceding ones, because our goal was to develop a prognostic model using the Cox model. To assess the impact of the rate of decline of renal function we used the ratio of the last measured RC and the RC at 6 months, further denoted as RC/ RC_6. To account for irregular observations, we included the time elapsed since the last recorded creatinine value (TEL) in the model.

Statistical analysis

The Kaplan-Meier procedure was used to estimate graft failure counting patient death with a functioning graft as non-failures. We applied the Cox proportional hazards model to study the effects of the time-fixed covariates on graft failure. The assumed proportionality of these time-fixed covariates was checked by examining the Schoenfeld residuals. The covariates were selected using stepwise selection with $P<0.05$ as level of significance.

Next, we fitted a multivariate Cox proportional hazards model including the time-dependent renal function covariates. To apply such a Cox proportional

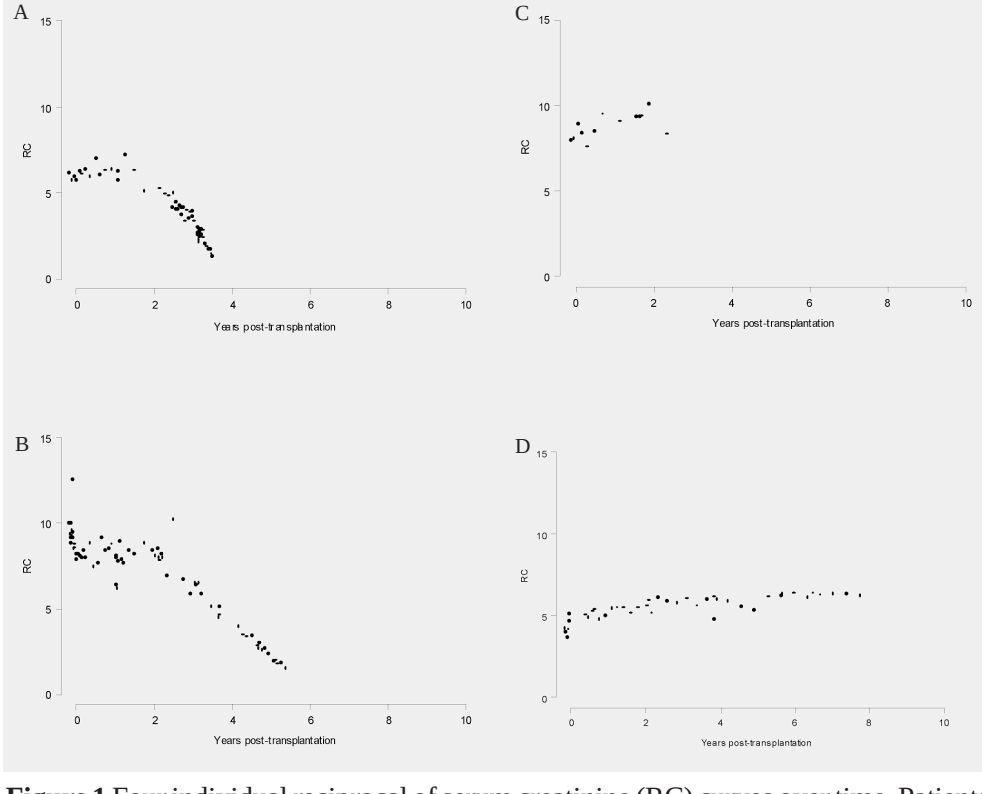

Figure 1 Four individual reciprocal of serum creatinine (RC) curves over time. Patients A and B had graft failure after 4 and 6 years, respectively. Patients C and D ended follow-up after 3 and 8 years, respectively.

hazards model with time-dependent covariates in SPSS, data were reorganized in risk sets. At each event time, the patients at risk and the recent values of the time-dependent covariates were determined. These risk sets were stacked into one large data set and then analyzed using Cox regression with stratification on the risk sets. The hazard $\lambda(t|Z,X(t),TEL(t))$ at time t in the multivariate Cox proportional hazards model with time-dependent covariates was defined as:

$$\ln(\lambda(t|Z,X(t),TEL(t)) = \ln(\lambda_0(t)) + Z\beta + \eta\, X(t)\gamma(TEL(t)) + \omega\, \delta(TEL(t))$$

with $\lambda_0(t)$ an unspecified baseline hazard function at time t, X(t) the vector of the last recorded value of the reciprocal of serum creatinine at time t, its ratio to the baseline value at 6 months and their interactions with TEL and follow-up time, the vector Z = (z1, z2, …,zp) of the p fixed covariates available at 6 months after transplantation. The regression coefficient β represents the effect of the time-fixed covariate Z on the logarithm of the hazard, and the regression coefficient η represents the effect of the time-varying covariates X(t). All significant factors from the multivariate analysis with time-fixed covariates were included in Z. The shape of the $\gamma(.)$-and $\delta(.)$-functions of TEL(t) were allowed to be different and estimated by trial-and-error. The interaction term $X(t)\gamma(TEL(t))$ with regression parameter η models the decaying (none)-linear effect of older recordings of X on the hazard, i.e. the term illustrates that the effect of X(t) on the hazard is not constant over time. The term $\delta(TEL(t))$ with regression parameter ω models the possibility that patients without complications are monitored less often than patients with complications. We checked the assumption of a constant effect of the prognostic covariates on the hazard by adding the interaction of the time-dependent covariates with time since transplantation t to the model. The underlying cumulative baseline hazard function was estimated using the Breslow estimator. The prognostic value of the Cox model with time-dependent covariates was compared to the prognostic value of the Cox model with only time-fixed covariates using the likelihood ratio test. Degrees of freedom were the number of time-dependent covariates.The probability of graft failure was calculated using the following model for the hazard prediction at some time $t_0+\Delta$:

$$\ln(\lambda(t_0+\Delta|Z,X(t_0),TEL(t_0+\Delta))) = \ln(\lambda_0(t_0+\Delta)) + Z\beta + \eta\, X(t_0)\gamma(TEL(t_0+\Delta)) + \omega\delta(TEL(t_0+\Delta)) + \kappa X(t_0)(t_0+\Delta)$$

with κ the regression coefficient of the interaction between X(t) and follow-up time, and t_0 the moment of starting prediction and Δ the time since t_0.
Finally, we determined the accuracy of the failure predictions. Both the first

year and the period beyond ten years post-transplantation were left out of validation because there were hardly any RC recordings and failures, respectively. So, prognosis was validated for the time period of 1 to 10 years post-transplant with intervals of one year each. For each interval, the patients at risk at the start of an interval and the most recent values of the covariates were determined. Hence, the probability of graft failure was calculated for each patient at risk at the start of that interval using our prognostic model. Next, the accuracy of failure predictions was assessed for each interval by comparing the failure predictions between failures and non-failures.

Results

Patients

The demographic and clinical characteristics of the study population are presented in table 1. A total of 106 graft failures occurred, 95 from chronic rejection and 11 from recurrent disease or de novo glomerulonephritis. The median follow-up time was 7.5 years (range 0.5 to 15.5). The Kaplan-Meier curve illustrates the overall time to graft failure (figure 2).

Table 1. Characteristics of 692 renal transplant recipients included in the analysis

Characteristic	Value
Recipient age (year)	45 ± 13
Donor age (year)	37 ± 14
Males (%)	63
Living related transplantation (%)	14
Repeat transplant (%)	12
Peak panel reactive antibodies	28 ± 30
Current panel reactive antibodies	9 ± 19
Number of HLA CREG mismatches	1.1 ± 1.0
Number of HLA-A, -B and -DR mismatches	1.9 ± 1.1
Number of HLA CREG shares	4.6 ± 1.2
Number of HLA-A, -B and -DR shares	3.6 ± 1.0
Delayed graft function (%)	24
Type of rejection (%)	
Interstitial / vascular / none	40 / 14 / 46
Number of acute rejections episodes (%)	
0 / 1 / 2 / 3 or more	43 / 22 / 22 / 13
Dipstick proteinuria at 6 months (%)	
Negative / trace,1+ / >1+	60 / 34 / 6
RC_6	7.6 ± 2.4

HLA: human leucocyte antigen, CREG: crossreactive groups of MHC class I, RC_6: reciprocal of serum creatinine at 6 months. Data are expressed as mean ± SD unless otherwise stated.

Table 2. Multivariate Cox model for graft failure with time-fixed covariates

Covariate	β	SE(β)	*P*
Recipient age	-0.038	0.009	<0.001
Current panel reactive antibodies	0.016	0.005	0.001
CREG, per share	-0.184	0.091	0.044
Number of acute rejections	0.362	0.107	<0.001
RC_6	-0.256	0.059	<0.001
Dipstick proteinuria			
Trace, 1+ versus negative	0.160	0.125	0.201
>1+ versus negative	0.511	0.093	<0.001

SE, standard error; CREG, crossreactive groups of MHC class I; RC_6, reciprocal of serum creatinine at 6 months.

Time-fixed covariates

The multivariate analysis of time-fixed risk covariates is given in table 2. The risk of late graft failure was increased in younger recipients and in patients sensitized at the time of transplantation. Furthermore, donor-recipient combinations sharing less CREG and transplanted patients with a high number

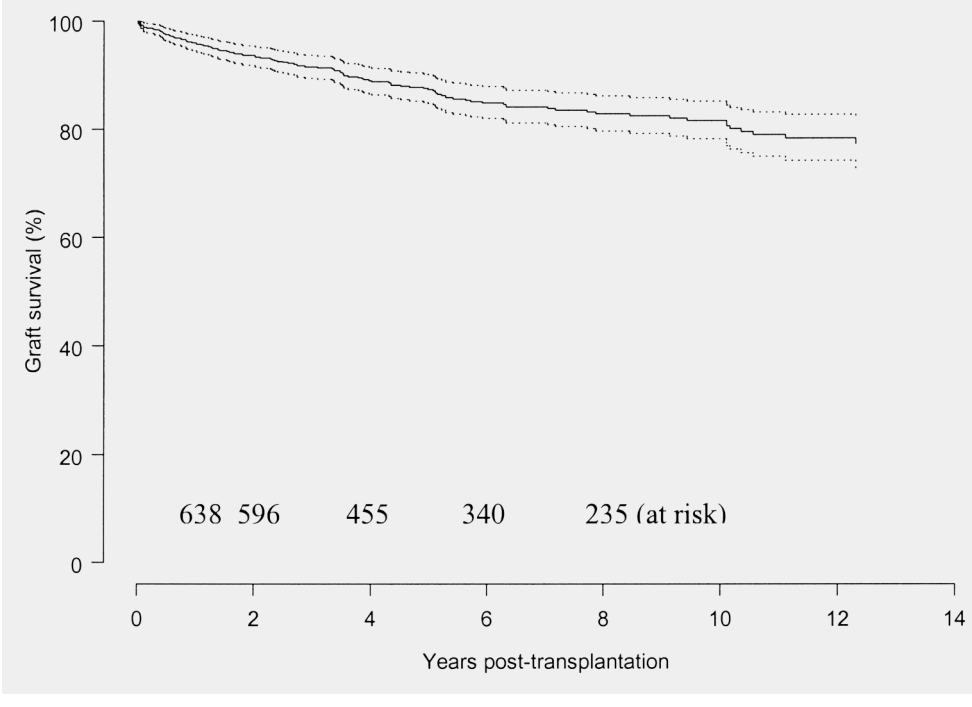

Figure 2 Kaplan-Meier estimation of time to graft failure with pointwise 95% confidence intervals (dotted lines). Patient death with a functioning graft was counted as non-failure.

Table 3. Multivariate Cox proportional hazard model for graft failure including time-fixed and time-dependent covariates

Covariate	In the model as	β	SE(β)	*P*
Living related transplant		-0.750	0.375	0.046
Serum creatinine	RC – 7.5	-1.383	0.352	<0.001
Decline of serum creatinine	RC / RC_6	-5.057	0.820	0.001
Time elapsed since last observation (TEL)	1 - exp(-10xTEL(t))	8.847	2.695	<0.001
Serum creatinine x TEL	(RC – 7.5) x exp(-10xTEL(t))	-1.353	0.475	0.004
Serum creatinine x time post-transplant	(RC – 7.5) x t	0.100	0.038	0.009

The mean ± SD of RC-7.5 was 0.0 ± 3.1, the mean of RC/RC_6 was 0.18 ± 0.56, the mean of TEL was 92 ± 65 days.

of rejections had also a high risk of late graft failure. Finally, dipstick proteinuria and a lower reciprocal of serum creatinine at 6 months post-transplantation were associated with a worse outcome. The Schoenfeld residuals showed that the proportional hazard assumption of above time-fixed covariates was not violated.

Time-dependent covariates

Table 3 shows the results of the multivariate Cox analysis after inclusion of the time-dependent renal function covariates. The likelihood ratio test comparing this model with the model including only time-fixed covariates showed a p-value of <0.001. The beneficial effect of a living donor was the only significant time-fixed covariate in the final model. The results show that a higher serum creatinine (lower RC) and a steeper decline in renal function (lower RC/RC_6) were independently associated with graft failure. The interaction between RC and TEL was highly significant, and the shape of the gamma-function was estimated as exp(-10 x TEL). This means that the prognostic value of the RC-values decreased sharply towards zero with increasing TEL. Therefore, RC values recorded at the same day as prognosis was calculated had more predictive value than RC values recorded weeks or months before. In addition, we found that TEL itself was significant and the shape of the delta-function was estimated as 1 - exp(-10 x TEL), pointing to the fact that the graft-failure hazard decreased with increasing TEL. This means that patients of whom RC values were less often determined had better prognosis. However, this effect was only significant when the interaction between RC and TEL was in the model. The impact of RC on outcome was also dependent on the time since transplantation as illustrated by the significant effect of the interaction term (RC-7.5) x t, and this pointed to the fact that RC values had more prognostic value early than late in the follow-up. Figure 3 gives illustrates the findings of the Cox model with time-dependent covariates. At one year post-transplantation the log relative risks of several RC values

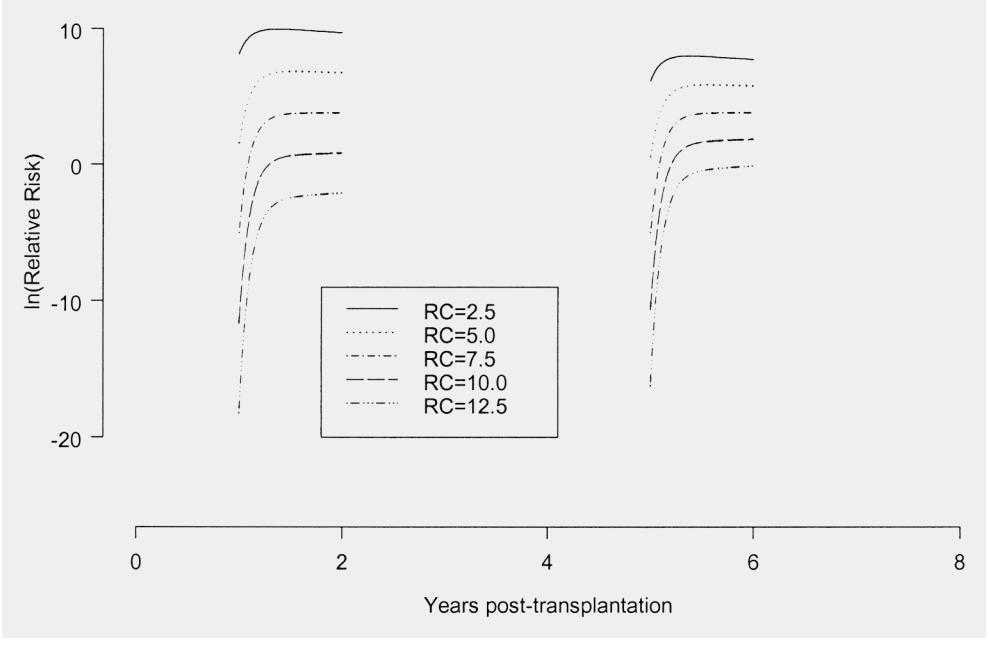

Figure 3 Effect of centered reciprocal of serum creatinine values (RC = 2.5, 5.0, 7.5, 10.0, 12.5) on the relative risk of renal failure as a function of the time elapsed since last observation TEL(t) by making failure prognosis at 1 and 5 years post-transplantation. In both situations TEL(t) runs from 0 to 1 years and $RC/RC_6 = 1$ in all cases.

rapidly converge towards each other with increasing TEL(t), indicating that older observations of RC are less prognostic. For instance, the relative risk of graft failure of a patient with RC value of 5.0 compared to a patient with RC value 7.5 is about e^3 when TEL is zero, but it is about e^1 when TEL is 180 days. This phenomenon of decreasing prognostic value of RC values was also observed at five years post-transplantation but the differences in log relative risk of the several RC values were smaller compared to 1 year post-transplantation. This indicates that the prognostic effect of RC on graft failure becomes smaller when time post-transplantation increases. The log-relative risks in this figure were very extreme for very low and very high RC values; this represents the fact that almost all events occurred in patients with decreasing RC-values, and therefore the baseline hazards involved in the log relative risks were very small. Also, the overall majority of RC values were close to 7.5, meaning that the extreme log relative risks were based on few patients only. Resuming, the prognostic value of a RC value becomes lower when it ages and when time after transplantation elapses.

Failure prediction

The prediction model with parameter estimates given in table 3 could be used to predict graft failure for each patient at any moment during the follow-up. First, failure prediction is illustrated in a single patient. This specific patient had a kidney graft from a living donor and a RC course as given in figure 4A. Failure prediction was done at t_0 = (1, 4, 7) years post-transplantation. At these three time points, TEL(t_0) values were 0.23, 0.44 and 0.11 years, respectively. The cumulative baseline hazard is given in figure 4B. The baseline hazard was relatively constant during follow-up. Beyond approximately 10 years, the cumulative baseline hazard quickly increased due to decline in patient number and graft failure rate. Figure 4C illustrates failure prognosis at t_0 = (1, 4, 7) years. At t_0 =1 year, RC was more or less constant at a high level. As a consequence the predicted survival curve at this time point remained at 100% approximately. At t_0 = 4 years, the RC deteriorated and consequently the survival curve decreased considerably. At t_0 = 7 years the RC values had deteriorated so much that the survival curve showed a very sharp decline.

Similar calculations were performed in all other patients. Table 4 shows that patients with graft failure had a higher failure probability than non-failures. The failure probability of the former was diverse ranging between 0 and 1 (i.e. 100%), whereas the failure probability of the latter was near 0 in all patients without failures. The average failure probability decreased from 0.52 at year 1 to 0.22 at year 9, confirming that the prognostic power of the model decayed over time since transplantation.

Discussion

In this study we improved the prediction of late kidney graft failure by incorporating time dependent renal function covariates in a Cox proportional hazard model. For our analysis we used the reciprocal of all serum creatinine (RC) values that were routinely measured beyond 6 months after transplantation. After fitting the time- dependent renal function covariates, we found that the last recorded RC and a low ratio of RC and RC at 6 months (RC_6) appeared to be the strongest predictors of late graft failure. Furthermore, the impact of RC was not only depended on its last value, but also on the time elapsed since last recording and on the time since transplantation.

Chronic transplant dysfunction (CTD) usually precedes graft failure from chronic rejection or recurrent disease. This relation between renal dysfunction and subsequent chronic rejection or graft failure has been reported in several ways. Most investigators analyze renal function, measured at an arbitrarily chosen time point after transplantation as time-fixed covariate of the dependent

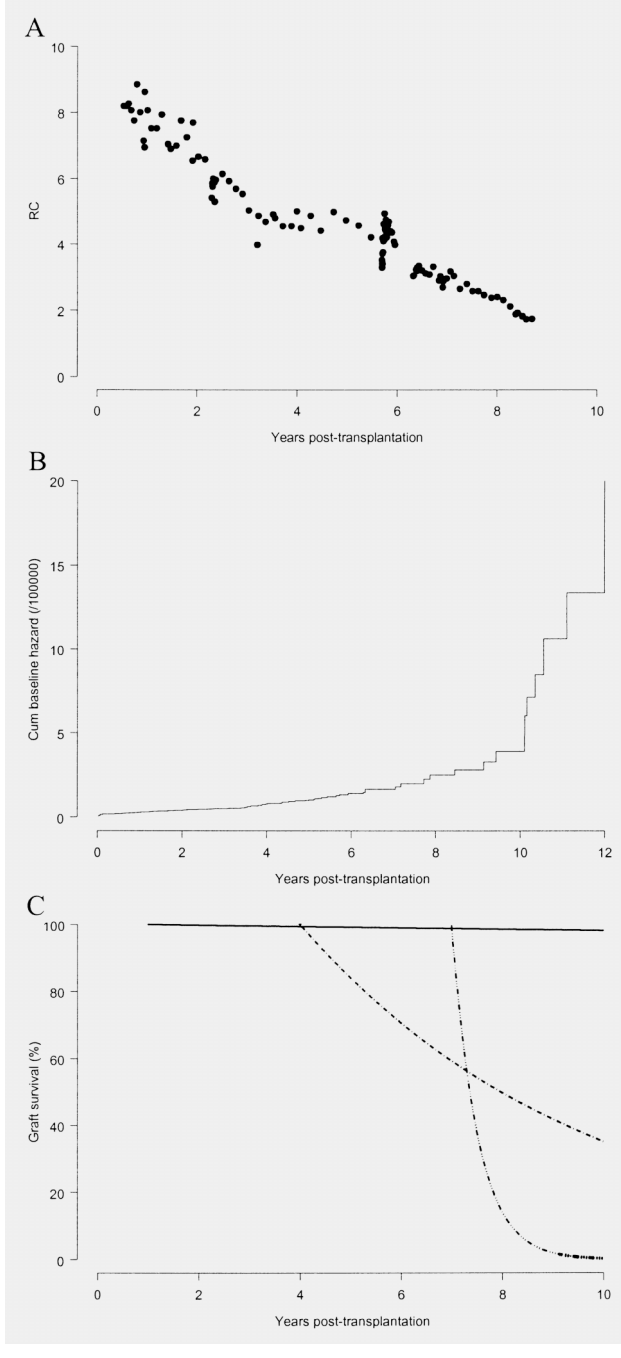

Figure 4 Prediction of graft failure in an individual patient. A: Reciprocal of serum creatinine (RC) over time. B: Cumulative baseline hazard using the Kaplan-Meier estimate with $RC/RC_6 = 1$, RC = 7.5 and no living transplant. C: Failure prognosis at 1, 4 and 7 years post-transplantation.

variable. We and others have previously found that an elevated serum creatinine value at 6 months predicts subsequent outcome in patients who have already survived to that time with a functioning graft (7,9,18,19). However, the relation between renal dysfunction at this time point and late failure might be confounded by other risk factors like donor age and early acute rejection episodes (7,8). On the other hand, the process of chronic rejection or recurrent disease may already be present at that time after transplantation (20). Finally, injury from glomerular hypertension in the setting of a reduced renal mass has been used as an explanation for the development of chronic allograft nephropathy (21). Analysis of the course of renal function is another way to assess the relationship between renal dysfunction and graft failure. In transplants functioning for at least 10 years the natural history of renal function, estimated by creatinine clearances, is to increase for several years and then to decline linearly (22). In contrast, a negative slope of glomerular filtration rate between 6 and 12 months is significantly associated with the occurrence of chronic rejection after 12 months (9,23). Chronic declines in renal function modeled by one or two least-squares-fitted regression lines of RC may begin at variable times after transplantation and precede graft failure for several years (5). The vast majority of patients with chronic rejection progress linearly although a change in the rate of decline revealed by a breakpoint test occurs frequently (10). Recently, Kasiske et al. systematically investigated what changes in chronic allograft function best predict subsequent graft failure. They examined the independent effects of declines in RC, creatinine clearance and slope of RC separately as time-dependent covariates. The best predictor of failure, a thirty percent decline of RC, was superior to baseline function and independent of other risk factors of chronic rejection (11).

Our study confirmed that the last recorded RC and the slope of RC, measured

Table 4. The accuracy of model prognoses for patients with and without graft failure

Interval	At risk at the start of the interval	Observed failures	Predicted failure in non-failures	Predicted failure in failures
1 – 2	638	16	0.01 (0.08)	0.52 (0.49)
2 – 3	596	13	0.01 (0.08)	0.56 (0.44)
3 – 4	509	14	0.01 (0.04)	0.54 (0.39)
4 – 5	455	8	0.01 (0.04)	0.48 (0.34)
5 – 6	408	11	0.01 (0.05)	0.38 (0.32)
6 – 7	340	3	0.01 (0.05)	0.33 (0.31)
7 – 8	279	4	0.01 (0.05)	0.32 (0.32)
8 – 9	235	2	0.01 (0.05)	0.31 (0.38)
9 – 10	189	2	0.01 (0.04)	0.22 (0.13)

Mean prognoses are given as probabilities with standard deviation in parentheses

as the RC/ RC_6 ratio, were superior predictors compared to several time-fixed covariates including baseline function RC6. The present model allows a clinician to re-calculate the prognosis of an individual patient each time he or she wishes to do that. Take for example two forty year old patients both who received cadaver transplants with 10% panel reactive antibodies, 5 shared CREG, 1 acute rejection episode and no proteinuria. Suppose one patient has a serum creatinine concentration of 150 µmol/l at 6 months post-transplantation and the other 100 µmol/l. Using the Cox model with only time-fixed covariates (table 2) it can be calculated that the relative risk of graft rejection is 2.3 for the former against the latter patient. Suppose that after two years follow-up serum creatinine has increased to 200 µmol/l in the first patient, and that creatinine remained stable at 100 µmol/l in the other patient. Then, the Cox model with time-dependent covariates (table 3) results in an update of the original prognosis to a relative risk of over 100.

In clinical practice, creatinine values are sampled at intervals of varying lengths. To allow predictions at any time we fitted the time elapsed since the last observation (TEL) to the model to account for irregular sampling. TEL and its interaction with longitudinal white blood cell counts as time-dependent covariate appeared to be strong predictors of mortality in patients with chronic myeloid leukemia (17). In the present study, the independent effect of the interaction term RC x TEL(t) revealed that older RC recordings are less prognostic for graft failure. The prognostic value of the last recorded RC also declines with time after transplantation, as demonstrated by the independent effect of the interaction term RC x t. This finding is explained by the decline of the graft failure rate over time in the cohort under study.

The obtained multivariate prognostic model with these time-dependent covariates allows updates of prognosis at any time after transplantation, independent of the presence of an actual serum creatinine sampling. We validated the accuracy of our model by comparing prognosis between failures and non-failures at different intervals after transplantation. We made survival predictions for one year ahead and found that patients with graft failure in the subsequent year had indeed a high failure probability. The accuracy of the model was illustrated by the decay of prognostic power over time after transplantation. Patients without failures had a failure probability of almost zero. Therefore, predictions that substantially differ from 0 are an indication of transplant dysfunction and may warrant more frequent renal function measurements and therapeutic interventions.

In brief, our results suggest that late kidney graft failure could be predicted for each patient at any time during the follow-up using a Cox proportional hazards model including time-dependent renal function covariates. The effect of the

last recorded serum creatinine concentration on outcome depended on its value, the time since the last observation and the time since transplantation.

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Risk factors of of cyclosporine nephrotoxicity after conversion from sandimmune to neoral

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Abstract

Background In 1995-1996, we switched from a once-daily Sandimmune dose to a twice-daily dose regimen of Neoral. Concurrent with the switch we changed our target trough level from 100 µg/l at 24 hours to the generally accepted 12 hours level of 150 µg/l. We performed a retrospective cohort study to assess cyclosporine toxicity following this switch and to identify risk factors for nephrotoxicity.

Patients and methods Of 212 patients with a stable graft function pre-conversion clinical parameters at 1 and 12 months post-conversion were compared with those at time of conversion. Cyclosporine nephrotoxicity was defined as a significant decline of the reciprocal of the serum creatinine concentration over time post-conversion in the absence of other obvious causes for declining graft function. Risk factors of cyclosporine nephrotoxicity were assessed using logistic regression analysis.

Results The mean cyclosporine trough level rose from 87 µg/l at the time of conversion to 139 µg/l at 12 months post-conversion whereas the daily drug dose increased over the same period from 233 mg to 252 mg. Mean serum creatinine increased by 10% from 135 to 148 µmol/l ($P<0.001$). Cyclosporine nephrotoxicity was present in 42 patients (20%). Cyclosporine dose and trough level did not predict nephrotoxicity but beta blockers (OR 0.35, 95% CI 0.17 - 0.72) and calcium channel blockers (OR 0.35, 95% CI 0.19 - 0.82) reduced the risk of nephrotoxicity, independent from an effect on blood pressure.

Conclusions Twenty percent of stable renal transplant patients experienced chronic cyclosporine nephrotoxicity after conversion from a once-daily Sandimmune regimen to a twice-daily Neoral regimen with dose adjustments to a trough level of 150 µg/l. Beta blockers and calcium channel blockers reduced the risk of nephrotoxicity.

Introduction

Maintenance immunosuppression with cyclosporine after kidney transplantation has substantially improved the one-year graft survival rate compared with regimens not containing cyclosporine albeit that cyclosporine nephrotoxicity has remained a concern. It causes a reversible, dose-related decrease in glomerular filtration rate but its role in the pathogenesis of permanent structural lesions is less well understood. The assumption that it causes progressive nephropathy has been questioned because many patients receiving long-term therapy have stable graft function (1). In fact, it has been suggested that higher cyclosporine blood concentrations are associated with better long-term graft function (2). On the other hand, several studies have shown that discontinuation of cyclosporine does not adversely affect graft outcome (3,4).

Neoral, the micro-emulsion form of cyclosporine with a better and more consistent bioavailability has replaced Sandimmune in most centres. Neoral administration results in higher peak levels and a 30% increase in the dose-normalised area under the curve (5). In most conversion studies Neoral replaced Sandimmune in a one-to-one dosing ratio but the doses were subsequently reduced by 4 to 23% to maintain the same trough level range (6-10). Transient or persistent declines in renal function after conversion have also been reported (9,10).

Cyclosporine-based regimens remain largely empirical. Dosing regimens and target drug levels vary according to local practice. In our centre, Sandimmune used to be administered once-daily with dose adjustments to aim for a target 24 hours trough level of 100 µg/l (3). When we switched from Sandimmune to Neoral, we followed the generally used guideline to dose the drug twice-daily and to aim at a 12 hours trough level of 150 µg/l. The present paper describes a retrospective cohort study to assess cyclosporine toxicity following this switch and to identify risk factors for nephrotoxicity.

Patients and methods

Patients and immunosuppressive treatment

The renal transplant patients studied received Sandimmune and prednisone since transplantation. In our centre, Sandimmune was administered once-daily and we aimed at 24 hours trough levels of 100 µg/l (range 50-150), as determined by the Incstar RIA kit using a specific monoclonal antibody. A total of 302 patients who were more than six months after transplantation were switched from Sandimmune to Neoral between April 1995 and November 1996 using an initial one-to-one dosing conversion ratio. Neoral was administered

twice-daily and dose adjustments were made to aim at a 12 hours trough level of 150 µg/l. To monitor the conversion clinical and biochemical evaluations were done before conversion, at one and at 12 months post-conversion. To identify patients with a stable graft function before conversion, regression lines were retrospectively constructed of the reciprocal of the serum creatinine levels from 6 months after transplantation onwards to the date of conversion. In 288 patients sufficient data points were available. Seventy-six patients had a negative slope of 1/Cr, which was significantly different from zero, and were considered to have a declining renal function pre-conversion and were therefore excluded from further evaluation. Two hundred and twelve patients with a stable graft function before conversion and a follow-up after conversion of at least 12 months were included in the study.

Cyclosporine toxicity

Cyclosporine toxicity in patients with a stable graft function pre-conversion was assessed by comparing clinical and biochemical data obtained 1 and 12 months post-conversion with those at the time of conversion. The following parameters were evaluated: cyclosporine dose and trough level, serum creatinine concentration, endogenous creatinine clearance, proteinuria, serum cholesterol and uric acid concentration, the use of HMG CoA-reductase inhibitors, the blood pressure and the number and the kind of antihypertensive drugs.

Cyclosporine nephrotoxicity

Cyclosporine nephrotoxicity was defined as a statistically significant ($P<0.05$) decline of the reciprocal of the serum creatinine concentration over time post-conversion in the absence of other obvious causes for declining graft function. Regression lines of 1/Cr were constructed from the date of conversion to the end of follow-up, or until January 1, 1998. Figure 1 illustrates the pre- and post-conversion regression lines of an individual patient. Chart and graft histology data of patients with a decline in renal function post-conversion were reviewed to exclude other causes of graft dysfunction. In the patients with cyclosporine nephrotoxicity we extended the follow-up of graft function and immunosuppressive treatment until January 1, 1999.

We studied the following risk factors for cyclosporine nephrotoxicity: donor source, donor and recipient age, delayed graft function, presence of previous acute rejection episodes, Sandimmune dose (> 300 mg/day) and trough level (< 100 µg/l), serum creatinine, mean arterial pressure and the number and the kind of antihypertensive drugs. Patients with a stable graft function post-conversion were used as control group.

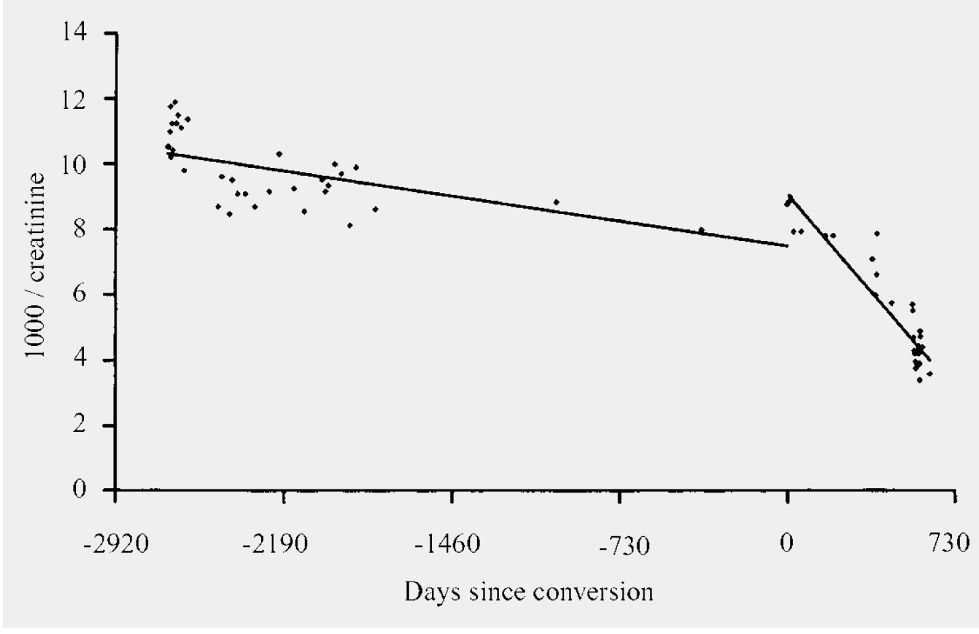

Figure 1 Regression lines of the reciprocal creatinines in a patient with a stable renal function before conversion and a significant decline in renal function after the switch from Sandimmune to Neoral

Statistical analysis

Clinical and biochemical parameters at 1 and 12 months post-conversion were compared with the parameters at time of conversion using a paired-samples *t* test. This test compares the mean of the differences with zero. We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for risk factors of cyclosporine nephrotoxicity. Data are expressed as mean ± SD or number (%). A P value < 0.05 was considered significant.

Results

Patients

Two hundred and twelve patients with a stable graft function pre-conversion had a mean serum creatinine concentration of 135 ± 46 µmol/l at time of conversion; these patients were 4.6 ± 2.8 years after surgery; their mean age was 50 ± 13 years, 80 (38%) were women and 22 (10%) patients received their graft from a living donor.

Table 1. Clinical and biochemical parameters before and after conversion from Sandimmune to Neoral in 212 kidney transplant patients with a stable graft function pre-conversion

	Conversion	1 month	12 months
Cyclosporine dose (mg)	233 ± 75	251 ± 69[c]	252 ± 69[c]
Cyclosporine dose/kg (mg/kg)	3.2 ± 1.1	3.5 ± 1.1[c]	3.5 ± 1.1[c]
Cyclosporine trough level (µg/l)	87 ± 27	124 ± 35[c]	139 ± 36[c]
Serum creatinine (µmol/l)	135 ± 46	138 ± 49[b]	148 ± 60[c]
Creatinine clearance (ml/min)	70 ± 27	69 ± 25	62 ± 25[c]
Proteinuria (g/24h)	0.3 ± 0.7	0.4 ± 0.8[a]	0.6 ± 1.2[b]
Cholesterol (mmol/l)	6.1 ± 1.0	6.0 ± 1.0	6.2 ± 1.2
HMG-CoAreductase inhibitors – nr. (%)	87 (41)	98 (41)	111 (52)
Uric acid (mmol/l)	0.44 ± 0.11	0.46 ± 0.12[c]	0.48 ± 0.12[c]
Systolic blood pressure (mmHg)	139 ± 17	140 ± 19	143 ± 18[b]
Diastolic blood pressure (mmHg)	82 ± 10	83 ± 9	83 ± 9
Mean Arterial Pressure (mmHg)	101 ± 11	102 ± 11	103 ± 10[a]
Antihypertensive medication – nr	1.6 ± 1.0	1.7 ± 1.0[a]	1.9 ± 1.1[c]

Results are reported as mean ± SD unless otherwise stated. [a]P<0.05, [b]P<0.01, [c]P<0.001: 1 and 12 months post-conversion versus time of conversion tested by a paired-samples *t* test.

Cyclosporine toxicity

Table 1 shows the clinical parameters of the 212 patients with a stable graft function pre-conversion. To reach the target cyclosporine trough level of 150 µg/l after conversion the mean cyclosporine dose had been increased from 233 to 252 mg at 12 months (P<0.001). Mean serum creatinine concentration increased by 10% from 135 to 148 µmol/l (P<0.001) and the mean endogenous 24 hours creatinine clearance decreased from 70 to 62 ml/min (P<0.001). Proteinuria, serum uric acid concentration, systolic blood pressure and the need of antihypertensive drugs increased significantly at 12 months post-conversion. We did not observe any acute rejection episodes after the conversion.

Cyclosporine nephrotoxicity

Of the 212 patients with a stable graft function pre-conversion 70 (33%) had a significant decline of 1/Cr post-conversion. In 28 patients obvious causes for the decline in function were evident and included chronic rejection (9), recurrent original disease (6), infection (6) and other causes (7). By exclusion, cyclosporine nephrotoxicity was the most likely diagnosis in 42 patients (20%). Tabel 2 compares the baseline characteristics of these 42 patients with 142 patients without cyclosporine toxicity. Donor source, recipient or donor age, delayed graft function and presence of previous acute rejection episodes were not associated with nephrotoxicity. In the patients with nephrotoxicity the mean cyclosporine dose and trough level rose from 231 to 256 mg and from 85 (24 hours target) to 141 µg/l (12 hours target) respectively at 12 months post-conversion. The mean slope of 1/Cr pre- and post-conversion of the 42 patients with nephrotoxicity

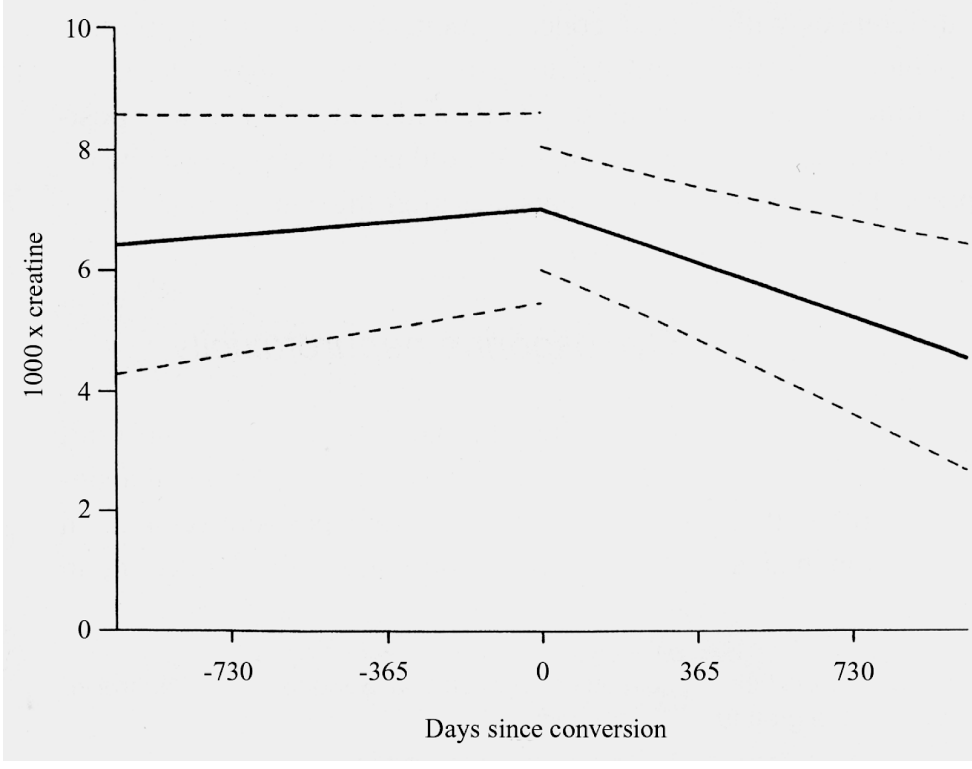

Figure 2 Mean regression line (95% confidence interval) of the reciprocal creatinines pre- and post-conversion in 42 patients with cyclosporine nephrotoxicity after the switch from Sandimmune to Neoral

is shown in Figure 2. In patients without nephrotoxicity the mean serum creatinine increased from 135 to 139 µmol/l at 12 months post-conversion whereas in patients with nephrotoxicity it rose from 131 to 161 µmol/l. In 10/42 patients, a graft biopsy was done on clinical indication post-conversion and the presence of significant arteriolar hyalinosis confirmed the diagnosis of cyclosporine nephrotoxicity in all cases. Patients who were biopsied had a higher mean serum creatinine level at time of conversion (145 µmol/l) and at 12 months post-conversion (195 µmol/l) compared with the 32/42 patients who were not biopsied (125 and 151 µmol/l, respectively). At the end of the extended follow-up, 33 ± 4 months post-conversion, dose reduction or conversion to mycophenolate was done in 23/ 42 and 6/42 patients, respectively. The decline in renal function was not progressive in the remainder 13/42 patients and no dose-reduction was instituted; in this group the mean serum creatinine stabilised at 163 µmol/l.

Table 3 shows the risk factors of cyclosporine nephrotoxicity. A high Sandimune dose (>300 mg) or a low trough level (<100 µg/l) before conversion was not associated with toxicity. Neither renal dysfunction (creatinine > 150 µmol/l)

Table 2. Baseline characteristics of kidney transplant patients with or without cyclosporine nephrotoxicity after switch from Sandimmune to Neoral

	Stable function (142)	Cyclosporine toxicity (42)
Transplantation to switch (years)	4.8 ± 3.0	4.2 ± 2.5
Recipient age (years)	46 ± 13	45 ± 13
Donor age (years)	37 ± 14	40 ± 13
Delayed graft function - no (%)	35 (25)	9 (20)
Acute rejection - no (%)	82 (58)	22 (48)
Cyclosporine dose (mg)	234 ± 74	231 ± 75
Cyclosporine level (µg/l)	88 ± 26	85 ± 30
Serum creatinine (µmol/l)	135 ± 47	131 ± 45
Creatinine clearance (ml/min)	71 ± 28	69 ± 23
Proteinuria (g/24hr)	0.2 ± 0.4	0.3 ± 0.5
Mean arterial pressure (mmHg)	100 ± 10	100 ± 12
# antihypertensive drugs	1.7 ± 1.0	1.3 ± 1.0 [a]
Diuretics - no (%)	28 (20)	4 (9)
Beta blockers - no (%)	87 (62)	17 (38) [a]
Calcium channel blockers - no (%)	78 (55)	16 (36) [a]
Converting enzyme inhibitors - no (%)	42 (30)	17 (38)

Results are reported as mean (SD) unless otherwise stated. [a]$P < 0.05$

nor hypertension (mean arterial pressure > 100 mmHg) at time of conversion predicted nephrotoxicity. However, patients treated with antihypertensive drugs (OR per drug 0.59, 95% CI 0.41 – 0.86), especially beta blockers (OR 0.35, 95% CI 0.17 – 0.72) or calcium channel blockers (OR 0.35, 95% CI 0.19 – 0.82), had a lower risk of nephrotoxicity.

Discussion

We observed cyclosporine nephrotoxicity after conversion from Sandimmune to Neoral in 20% of stable renal transplant patients. Our original cyclosporine protocol differed from that in most other centres in that we used to administer Sandimmune once-daily, aiming at a 24 hours trough levels of 100 µg/l (range 50-150 µg/l) (3). After conversion to twice-daily Neoral a target 12 hours trough level of 150 µg/l was adopted as per a nationwide guideline. To reach this level the mean cyclosporine dose was increased from 3.2 to 3.5 mg/kg. Nephrotoxicity was not observed in the first month post-conversion. Deteriorating graft function, proteinuria, hyperuricemia and hypertension, all known manifestations of cyclosporine toxicity (11) became evident only late after conversion.

Other groups studying toxicity after the conversion from Sandimmune to Neoral in stable renal transplant patients reported variable results. A single centre study from Berlin reported that a one-to-one conversion was efficacious and safe (6) but at 12 months the trough levels were lower (102 µg/l) compared with baseline (114 µg/l). The International Sandimmune Neoral study group

has confirmed safety and tolerability of Neoral and found no significant difference in renal function when switched from Sandimmune to Neoral (7). In another conversion study, nephrotoxicity was prevented using an individualised approach of rapid dose reduction in response to increased cyclosporine levels (8). The randomised, multicentre study of the Canadian Neoral Renal Study Group reported a transient increase in serum creatinine concentration after the switch to Neoral whereas the mean cyclosporine trough levels were similar in both groups (9). In another study, serum creatinine concentration remained elevated more than 30% above baseline in 16% of patients converted from Sandimmune to Neoral and followed for up to 11 months despite substantial dose reductions and a decrease in mean cyclosporine level from 156 to 130 µg/l (10). A recent study of cardiac transplant patients who were switched from Sandimmune to Neoral reported that an increase in cyclosporine trough level from 119 to 175 µg/l in parallel with a rise in serum creatinine concentration from 136 to 162 µmol/l 12 months post-conversion despite of a decrease in dose (12).

In our cohort of patients, it is likely that the changes in cyclosporine administration resulted in increased drug exposure and concomitant nephrotoxicity post-conversion. The 12 hours target trough level of 150 µg/l for Neoral appeared to be too high. We had favourable results with our once-daily Sandimmune regimen aiming at 24 hours trough levels of 50-150 µg/l as the 8-year graft survival censored for death with functioning graft was 80% (3). Trough levels, however, are poor indicators for total drug exposure. Sparse-sampling algorhytms are currently being developed to estimate area under the curve (AUC) values (13). Correlations between predicted and actual AUC are stronger for Neoral compared to the convential formulation (14). Long-term prospective studies are needed to define the optimal AUC target range for maintenance therapy. Awaiting these data, we reduced the target level to 100 - 125 µg/l for all patients.

Table 3. Risk factors for cyclosporine nephrotoxicity

	OR	95% CI
Serum creatinine (> 150 µmol/l)	0.96	0.42 – 2.14
Cyclosporine dose (> 300 mg)	0.94	0.41 – 2.16
Cyclosporine trough level (< 100 µg/l)	1.75	0.77 – 3.98
Mean arterial pressure (>100 mmHg)	1.66	0.33 – 1.32
Number of antihypertensive drugs	0.59	0.41 – 0.86[a]
Diuretics	0.43	0.19 – 1.30
Beta blockers	0.35	0.17 – 0.72[a]
Calcium channel blockers	0.35	0.19 – 0.82[a]
Converting enzyme inhibitors	1.22	0.62 – 2.64

OR: Odds Ratio, CI: Confidence interval. [a] P<0.05

We attempted to identify risk factors for cyclosporine nephrotoxicity by stringently defining patients with nephrotoxicity and to compare them with patients with a stable graft function post-conversion. The diagnosis of cyclosporine nephrotoxicity was based on a statistically significant deterioration of graft function in temporal relation to the switch of cyclosporine administration after exclusion of other obvious causes of graft dysfunction. Patients with nephrotoxicity had a stable graft function for a mean period of 4.2 years pre-conversion. Graft histology in ten patients, showing severe arteriolar hyalinosis (15) confirmed cyclosporine nephrotoxicity. Furthermore, cyclosporine dose reduction (16) or conversion from cyclosporine to mycophenolate mofetil (17) resulted in stabilisation of renal function, another feature consistent with cyclosporine nephrotoxicity.

Reports assessing risk factors of cyclosporine nephrotoxicity are scarce. We found that factors associated with pre-existent graft damage such as older donor age, delayed graft function or previous acute rejection episodes did not account for the nephrotoxicity. A Sandimmune dose of at least 300 mg/d, suggestive of poor absorption and a pre-conversion trough level lower than 100 µg/l were also not predictive for nephrotoxicity. Drug exposure, defined by AUC, could have been different between patients with and without nephrotoxicity, but this was not measured. In the Sandimmune era, cyclosporine induced episodes of acute deterioration in renal function and trough levels correlated best with the development chronic cyclosporine nephrotoxicity (18). In patients with autoimmune diseases who develop cyclosporine induced nephropathy initial higher doses of cyclosporine were given and patients with toxicity were older compared with patients in whom nephropathy did not develop (19).

We found that patients treated with calcium channel blockers or beta blockers were less likely to develop nephrotoxicity. This effect seems independent from their effect on the blood pressure. The renoprotective effect of calcium channel blockers in cyclosporine treated transplant patients has recently been reported by other investigators (20,21). Our observation that beta blockers have a similar effect has not been reported before. If there is reinnervation of the graft following transplantation (22), such a beneficial effect could be explained on the basis of cyclosporine's ability to increase sympathetic nerve activation (23), which in turn could result in renal vasoconstriction and increased renin production. Angiotensin-converting enzyme inhibitors did not affect the risk of nephrotoxicity. In a retrospective analysis, these agents were safe and well tolerated in a cohort of renal transplant recipients using cyclosporine in 86% (24).

In summary, we report considerable nephrotoxicity of cyclosporine late after conversion from Sandimmune to Neoral and twice-daily dosing aiming at a 12 hours target trough level of 150 µg/l, the standard practise in many centres. Beta blockers

and calcium channel blockers reduced the risk of cyclosporine nephrotoxicity, independent from their effect on blood pressure.

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Chronic rejection with or without transplant vasculopathy

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Abstract

Background Chronic allograft nephropathy (CAN) is defined and graded in the Banff '97 scheme by the severity of interstitial fibrosis and tubular atrophy. It has been denoted that chronic rejection can be diagnosed if the typical vascular lesions are seen, consisting of fibrointimal thickening. We observed several patients who developed CAN without vascular changes or signs of cyclosporine toxicity. Therefore, we assessed the risk factor profiles of CAN with and without transplant vasculopathy.

Methods A cohort of 654 cadaveric renal transplants performed between 1983 and 1997 that functioned for more than six months was studied. Fifty-four transplants had CAN defined by a significant decline in renal function together with interstitial fibrosis and tubular atrophy without signs of cyclosporine nephrotoxicity or recurrent disease. Using the Banff CV score, 23 of 54 cases (43%) had a chronic vasculopathy score of 0 or 1 whereas 31 cases (57%) had a CV score of 2 or 3. Applying multivariate logistic regression, predictor variables of the two groups were compared with 231 transplants with a stable function for at least five years.

Results Graft histology was obtained at a mean of 2.4 and 2.9 years after transplantation in the group with or without vasculopathy, respectively. Acute rejection episodes (ARE) after three months post-transplantation were the strongest risk factor for both forms of CAN, odds ratio (OR) 14.7 (6.0-36.0). CAN with vasculopathy was also associated with transplants performed in the 1980s, OR 4.95 (1.65-14.9) and with creatinine clearance at 6 months, OR 0.58 (0.44-0.75) per 10 ml/min increase. In contrast, young recipient age, OR 0.69 (0.47-0.99) per 10 years increase, and the presence of panel reactive antibodies at the time of transplantation, OR 1.26 (1.08-1.47) per 10% increase, were independent risk factors for CAN without vasculopathy.

Conclusions After exclusion of cyclosporine toxicity or recurrent disease CAN occurred without moderate or severe transplant vasculopathy in 43% of the cases. The correlation with young recipient age, sensitization and late ARE suggest an immune pathogenesis, consistent with chronic rejection.

Introduction

Chronic allograft nephropathy (CAN) is the term used to describe the fibrosclerotic changes in long-surviving renal transplants. The lesions may involve all anatomic compartments of the kidney and give rise to fibrous intimal thickening of the arteries, transplant glomerulopathy and glomerulosclerosis, interstitial fibrosis and tubular atrophy (1). CAN is considered to result from both immune and non-immune injury to the graft (2). Associated risk factors such as a young recipient age, high panel reactive antibodies (PRA), histoincompatibility and acute rejection episodes (ARE) suggest an immune pathogenesis, consistent with 'rejection' (3). On the other hand, non-immune damage due to old donor age, hyperlipidemia, arterial or glomerular hypertension, smoking or cyclosporine exposure may also contribute to the development of CAN (4).

In the Banff '97 classification, CAN has been graded by the severity of interstitial fibrosis and tubular atrophy, recognizing that these changes are present in almost all biopsies and have the strongest correlation with outcome (5). However, given the lack of specificity of these lesions, it has been suggested that chronic rejection can be diagnosed if typical vascular lesions are observed. This transplant vasculopathy consists of fibrointimal thickening of arteries, breaks in the elastic layer and vessel wall infiltration with inflammatory cells. The vascular narrowing with downstream ischemia is held responsible for the chronic glomerular and tubulointerstitial lesions. However, we observed several patients who developed CAN without obvious vasculopathy or signs of cyclosporine toxicity suggesting that chronic rejection may emerge independent of vascular obliteration.

In the present study CAN was defined in grafts that show a significant decline in renal function together with tubulointerstitial damage without signs of late cyclosporine nephrotoxicity or recurrent disease. To determine the risk profiles of the various forms of CAN, we categorized CAN on the basis of whether significant vasculopathy was present and compared these two groups with a group of patients with long-term stable graft function.

Patients and methods

We studied 654 cadaveric kidney transplants performed in our center between 1983 and 1997 that functioned for more than six months. The standard immunosuppressive regimen consisted of prednisone and once-daily cyclosporine (Sandimmune) or azathioprine. Patients were followed until death, return to dialysis, or until July 1, 1998. CAN, our study end-point, was defined

Table 1. Clinical characteristics of cases with CAN or stable graft function

	CAN (n=54)	Stable function (n=231)	*P*
Year of transplantation (% 1980s)	67	54	0.08
Repeat transplants (%)	20	14	0.27
Recipient age (years)	41 ± 13	45 ± 13	0.03[a]
Cigarettes smoking (%)	59	40	0.01[a]
Current panel reactive antibodies (%)	19 ± 31	9 ± 19	0.01[a]
Donor age (years)	38 ± 16	35 ± 14	0.25
HLA-A,B,DR mismatches (#)	1.9 ± 1.0	1.9 ± 1.1	0.75
HLA-A,B,DR shares (#)	3.4 ± 0.9	3.7 ± 1.0	0.09
CREG mismatches (#)	1.3 ± 0.9	1.1 ± 0.9	0.12
CREG shares (#)	4.2 ± 1.2	4.6 ± 1.1	0.02[a]
Cold ischemia time (hours)	31 ± 7	29 ± 7	0.20
Delayed graft function (%)	23	30	0.36
Interstitial / vascular / clinical ARE (%)	48 / 26 / 6	35 / 13 / 8	<0.01[a]
Last ARE < 3 months / > 3 months (%)	35 / 44	50 / 6	<0.01[a]
Azathioprine / cyclosporine @ 6 months (%)	30 / 70	28 / 72	0.87
Dipstick proteinuria >1+ @ 6 months. (%)	14	3	<0.01[a]
Serum creatinine @ 6 months (µmol/l)	178 ± 77	149 ± 62	<0.01[a]
ECC @ 6 months (ml/min)	50 ± 25	64 ± 23	<0.01[a]
Slope 1000/creatinine > 6 months (l/µmol/yr)	-16.1 ± 9.4	0.4 ± 1.1	<0.01[a]

Data are expressed as mean ± standard deviation. [a]P<0.05. CAN: chronic allograft nephropathy, CREG: crossreactive groups of MHC class I, ARE: acute rejection episode, ECC: endogenous creatinine clearance.

functionally and histologically according to the diagnostic criteria from the fourth Alexis Carrel conference (6). To assess whether there was significant deterioration of renal function, regression lines were constructed of the reciprocal of the serum creatinine levels from 6 months after transplantation until the end of follow-up. To improve the estimate of the regression lines, especially in patients with a variable decline in renal function, a breakpoint analysis was included (7). The curves of the reciprocal creatinine-versus-time were also used to define stable graft function. We examined all biopsies and nephrectomies from beyond 6 months performed in patients with a significant decline in renal function. Blinded for clinical information, graft histology was read according to the Banff ’97 classification (YS, FK, JAB). Biopsies or nephrectomies with unsatisfactory specimen adequacy (less than 7 glomeruli or no arteries) were discarded from evaluation whereas all marginal (8-10 glomeruli and 1 artery) and adequate (>10 glomeruli and more than one artery) biopsies were included in the study (5). CAN was defined as the presence of interstitial fibrosis and/or tubular atrophy with or without histopathologic features of obliterative vascular disease or glomerulopathy. Patients with clinical or histological signs of de novo or recurrent glomerulonephritis or predominant cyclosporine toxicity, i.e. the presence of arteriolar hyalinosis (8), were excluded from analysis. Most biopsies were taken before the conversion from Sandimmune to Neoral, a switch that increased the problem of cyclosporine

nephrotoxicity in our population (9). Nephrectomies were done in a minority of the patients on the clinical indication of pain, fever or hematuria.
We used the Banff chronic vascular (CV) score to categorize CAN in a form without or with minimal vasculopathy (CV 0-1) and in a form with moderate or severe vasculopathy (CV 2-3) (5). Using uni- and multivariate logistic regression analysis, predictor variables of CAN were compared with those in 231 cases with a stable graft function for at least 5 years post-transplantation. Panel reactive antibodies, age and cigarette smoking at time of transplantation were the recipient variables investigated. Donor age was used as a donor variable. Transplant parameters included: year of transplantation, repeat transplant, cold ischemia time and delayed graft function defined as the need for dialysis in the first week. The influence of HLA mismatches and shares on the occurrence of CAN was evaluated at the level of private antigens and crossreactive groups (CREG), as described previously (10). An acute rejection episode was defined by a rapid decline in renal function; in 94% of the cases this diagnosis was confirmed by histopathology. Interstitial rejection was diagnosed when an interstitial infiltrate and tubulitis were present (Banff grade borderline or I); the diagnosis of acute vascular rejection was made whenever an arteritis was present (Banff grade II or III). We used the time interval between transplantation and the last treated ARE (within or after three months) and the histological type of acute rejection as rejection factors. Dipstick proteinuria, serum creatinine concentration and endogenous creatinine clearance at six months were evaluated as clinical parameters. As a result of the randomized and clinical early conversion from cyclosporine to azathioprine in many patients, maintenance drug regimen at six months posttransplant was studied as variable. Since the reciprocal of the serum creatinine concentration decreases linearly with time in the majority of patients with chronic renal disease, we used the slope of this relationship as a measure of the rate of decline in renal function. Deterioration of renal function may start at any time point after transplantation. To identify a change in the slope of the regression line we included a breakpoint analysis (11). We used the broken line in case of a significantly better fit of the residual sum of squares than the straight line. Patients with a negative slope of the straight or broken line, significantly different from zero, were considered to have a decline in renal function. Stable graft function was defined as having not a significant decline of the regression line for at least five years post-transplantation.
The two samples t test for continuous and dichotomous variables was used to determine the significance of differences of baseline characteristics between the CAN group and the patients with stable function, and of biopsy data of the two forms of CAN. Graft survival of the two groups was compared using

Kaplan-Meier curves, including a log-rank test.
Logistic regression models were used to predict the presence of CAN based on the set of recipient, donor and transplant variables. CAN and its forms with and without vasculopathy were compared with 231 stable transplants. Odds ratios (OR) and 95% confidence intervals were estimated for each of the variables in the model. Significant predictors (P value < 0.05) were fitted in a multivariate model. Forward selection techniques were used to choose independent risk factors.

Results

In 604 transplants (92%) sufficient data points were available to construct the regression line of 1/Cr. The mean number of creatinine readings per transplant was 53, range 7-468. Breakpoint analysis revealed a better fit of the broken line in 129/604 cases (21%). In 266 cases (41%) there was a negative slope of the straight (n=170) or broken (n=96) line of 1/Cr, which was significantly different from zero. Graft loss censored for patient death with a functioning transplant occurred in 99/266 cases (37%). Graft histology was available in 111/266 (42%) cases. After exclusion of recurrent/de novo glomerulonephritis (28), acute rejection (9), cyclosporine toxicity (6) or inadequate specimens (12), CAN was diagnosed in 54 cases; in 45 patients based on biopsies and in 9 cases on nephrectomy specimens. Specimen adequacy was scored marginal

Table 2. Clinical characteristics of CAN with or without vasculopathy

	No vasculopathy (n=23)	Vasculopathy (n=31)	*P*
Year of transplantation (% 1980s)	57	74	0.18
Repeat transplants (%)	22	19	0.83
Recipient age (years)	40 ± 13	42 ± 14	0.55
Cigarettes smoking (%)	61	58	0.84
Current panel reactive antibodies (%)	22 ± 34	16 ± 29	0.42
Donor age (years)	31 ± 14	43 ± 16	<0.01[a]
HLA-A,B,DR mismatches (#)	2.0 ± 1.0	1.9 ± 1.0	0.76
HLA-A,B,DR shares (#)	3.5 ± 1.1	3.4 ± 0.7	0.56
CREG mismatches (#)	1.4 ± 1.0	1.3 ± 0.9	0.62
CREG shares (#)	3.9 ± 1.4	4.4 ± 1.1	0.20
Cold ischemia time (hours)	29 ± 7	32 ± 7	0.08
Delayed graft function (%)	16	29	0.35
Interstitial / vascular ARE (%)	52 / 17	45 /32	0.56
Last ARE < 3 months / > 3 months (%)	26 / 48	42 / 42	0.57
Azathioprine / cyclosporine @ 6 months (%)	22 / 78	35 / 65	0.37
Dipstick proteinuria >1+ @ 6 months (%)	10	17	0.52
Serum creatinine @ 6 months (μmol/l)	148 ± 45	201 ± 87	0.01[a]
ECC @ 6 months (ml/min)	60 ± 30	42 ± 17	0.01[a]
Slope 1000/creatinine > 6 months (l/μmol/yr)	-14.9 ± 9.3	-13.7 ± 7.7	0.60

Data are expressed as mean ± standard deviation. [a]P<0.05. Abbreviations as in table 1.

Table 3. Univariate analysis of risk factors of CAN and its forms with or without vasculopathy

	CAN (n=54)	No vasculopathy (n=23)	Vasculopathy (n=31)
Year of transplantation (83-89 vs. 90-97)	1.73 (0.93-3.21)	1.12 (0.47-2.66)	2.48 (1.07-5.77)[a]
Recipient age (10 years increase)	0.78 (0.62-0.98)[a]	0.72 (0.52-1.00)[a]	0.83 (0.61-1.10)
Smoking cigarettes	2.14 (1.17-3.92)[a]	2.29 (0.95-5.51)	2.04 (0.95-4.36)
Panel reactive antibodies (10% increase)	1.16 (1.04-1.31)[a]	1.22 (1.05-1.41)[a]	1.12 (0.97-1.31)
Donor age (10 years increase)	1.13 (0.92-1.37)	0.81 (0.32-2.08)	1.41 (1.08-1.82)[a]
HLA-A,B,DR mismatches (antigen)	1.04 (0.80-1.37)	1.07 (0.73-1.57)	1.02 (0.73-1.44)
HLA-A,B,DR shares (antigen)	0.77 (0.55-1.08)	0.84 (0.54-1.30)	0.71 (0.48-1.07)
CREG mismatches (group)	1.22 (0.92-1.63)	1.29 (0.87-1.91)	1.16 (0.81-1.67)
CREG shares (group)	0.73 (0.56-0.98)[a]	0.59 (0.40-0.88)[a]	0.85 (0.60-1.20)
Cold ischemia time (hour)	1.03 (0.99-1.08)	0.99 (0.92-1.05)	1.06 (1.01-1.13)[a]
Delayed graft function	0.71 (0.34-1.47)	0.45 (0.13-1.58)	0.95 (0.38-2.15)
Interstitial vs. no ARE	2.98 (1.31-6.40)[a]	2.25 (0.91-7.02)	3.54 (1.22-10.2)[a]
Vascular vs. no ARE	4.29 (1.76-10.4)[a]	2.24 (0.59-8.48)	6.73 (2.14-21.2)[a]
Last ARE < 3 months vs. no ARE	1.52 (0.69-3.34)	0.88 (0.27-2.81)	2.28 (0.79-6.23)
Last ARE > 3 months vs. no ARE	14.7 (6.00-36.0)[a]	12.3 (3.98-38.3)[a]	17.5 (5.46-56.1)[a]
Azathioprine vs. cyclosporine @ 6 months	1.08 (0.56-2.06)	0.71 (0.25-1.99)	1.41 (0.64-3.09)
Dipstick proteinuria >1+ @ 6 months (%)	5.21 (1.74-15.6)[a]	3.55 (0.69-18.4)	6.40 (1.89-21.7)[a]
Serum creatinine @ 6 months (10 µmol/l increase)	1.06 (1.02-1.20)[a]	1.00 (0.93-1.07)	1.08 (1.03-1.14)[a]
ECC @ 6 months (10 ml/min increase)	0.73 (0.62-0.85)[a]	0.92 (0.76-1.02)	0.56 (0.44-0.71)[a]

Data are expressed as odds ratio (95% confidence interval). [a]$p<0.05$. Abbreviations as in table 1.

in 12 and adequate in 42 transplants. Table 1 compares the characteristics of these 54 patients with 231 cases with a stable transplant function for at least five years. Recipient age was lower in the CAN group, pre-transplant sensitization and percentage of smokers were higher. While the number of HLA mismatches was not different, CAN patients had a significantly lower number of shared CREG. CAN patients had more ARE, especially those of a vascular type and those that occurred after three months. Furthermore, they had more proteinuria and a lower renal function at 6 months. Maintenance drug regimen was not different between those with CAN or those with stable function.

The 54 cases with CAN were analyzed according the Banff CV score. Twenty-three grafts (43%) had a CV score 0 (n=9) or 1 (n=14), while 31 (57%) had a CV score of 2 (n=11) or 3 (n=20) and were categorized as CAN without or with vasculopathy, respectively. Mean time between transplantation and the diagnosis of CAN with or without vasculopathy was 2.4 and 2.9 years, respectively. Subsequent graft loss, censored for other causes than chronic rejection, occurred in 77 and 61% respectively, almost significantly different (figure 1). In the 45 biopsy-confirmed cases there were no significant differences in mean number of arteries (2.0 versus 2.2) and number of glomeruli (15 versus 13). Immunosuppressive agents, renal function, blood pressure and proteinuria at time of the biopsy were not different between the two groups. Transplant

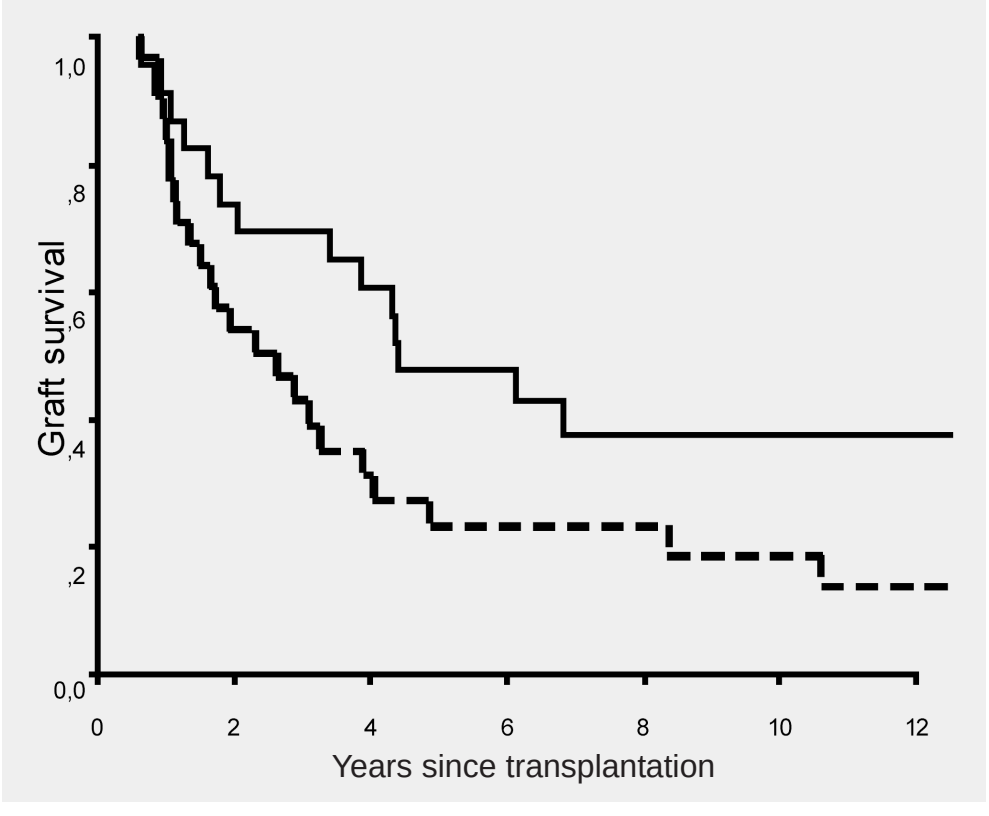

Figure 1 Kaplan-Meier graft survival for CAN without (solid line) or with (dashed line) vasculopathy. Log-rank test: P = 0.06

glomerulopathy (CG score 1-3) was present in 8/23 cases without vasculopathy and in 3/31 transplants with vasculopathy. The extent of tubulointerstitial changes was comparable. Of note, all nephrectomies showed diffuse and concentric thickening of the arterial intima (CV score of 3) and signs of ischemia such as bleeding and edema. Table 2 compares the clinical characteristics of both forms of CAN. Mean donor age was 12 years higher and renal function was worse at six months in the group with vasculopathy compared to the group without vasculopathy.

Tables 3 and 4 compares the risk profiles of the two forms of CAN after uni- and multivariate analysis, respectively. CAN with vasculopathy was associated with transplantation in the 1980s, older donor age, cold ischemia time, vascular and late ARE and graft dysfunction at 6 months. Stepwise selection of these significant factors revealed the following risk factors in a multivariate model: year of transplantation, 1983-1989 versus 1990-1997, OR 4.95 (1.65-14.9), ARE after 3 months, OR 15.0 (4.08-50.0) and creatinine clearance at 6 months,

OR 0.58 (0.44-0.75) per 10 ml/min increase. CAN without vasculopathy was predicted by younger recipient age, sensitization at time of transplantation, sharing less CREG and late ARE in univariate analysis. Independent risk factors were recipient age, OR 0.69 (0.49-0.99) per 10 years increase, panel reactive antibodies, OR 1.26 (1.08-1.47) per 10% increase and also ARE after 3 months, OR 10.4 (3.13-34.5).

Discussion

This study shows that chronic renal graft rejection can occur in the absence of apparent transplant vasculopathy. In our cohort of renal transplants we defined CAN by a significant decline in renal function, interstitial fibrosis and tubular atrophy without signs of cyclosporine nephrotoxicity or recurrent disease (6). Based on the semiquantitative CV score of the Banff schema, we divided CAN in a form with moderate or severe vasculopathy and a form with no or minimal vasculopathy. It was found that 43% of the cases did not have significant fibrointimal thickening of arteries and therefore had parenchymal deterioration from a mechanism other than ischemia. Although the biopsy specimens fulfilled the adequacy criteria of the Banff classification (5) and comparable numbers of arteries were assigned in the two groups, we cannot exclude that sampling variation has led to an underestimation of the CV score. However, we found remarkable differences in the risk profiles of the two forms of CAN; in the absence of moderate or severe thickening of arteries, CAN was independently associated with young recipient age, sensitization at the time of transplantation and late ARE, three markers of presumed immunological reactivity. Young age has been associated with a state of relatively high immune responsiveness to alloantigens (12) as well as medication non-compliance (13). Pretransplant sensitization increases the risk of chronic rejection (14) and late graft loss (15). This study confirms our previous report showing a beneficial effect of sharing CREG on long-term graft survival (10). From our multivariate analysis we conclude that histoincompatibility of MHC class I is associated with late ARE, the major risk factor of CAN. In accordance with a recently published study, we found that transplant glomerulopathy occurred preferentially in the group without vasculopathy (16). As transplant glomerulopathy is considered to result from rejection, this is another argument that chronic rejection may emerge without vasculopathy. Despite the lack of obliterative vasculopathy, sixty percent of these transplants were lost during the observation period, which is compatible with the poor prognosis of chronic tubulointerstitial changes (1,17). Thus, chronic tubulointerstitial lesions in graft biopsies may develop independent of chronic vascular obliteration in association with a risk factor

profile consistent with an immunological pathogenesis.
CAN with vasculopathy was found mainly in transplants done in the 1980s. The strong association with acute vascular rejection suggests a transition from acute to chronic vascular rejection, a correlation reported in subsequent protocol biopsies at 3 and 12 months (18). The detrimental effect of acute vascular rejection on later outcome and its relation with HLA-DR mismatches, cold ischemia time and immunosuppression has been observed previously (19). The Eurotransplant matching policy, aiming for no more than 2 HLA mismatches and, preferably, no DR mismatches may have resulted in a relatively low frequency of severe vascular rejection in our cohort. However, the association of the vascular lesions with older donor age questions the specificity of the extensive vascular lesions for the diagnosis of chronic rejection. Differentiation between donor-derived atherosclerosis and non-immune driven vascular damage by recipient factors such as hypertension, hyperlipidemia, smoking, and chronic rejection associated vasculopathy is difficult, especially when these entities coexist. Donor-derived fibrointimal thickening might already be present at implantation of kidneys of older donors (20) and constitutes the main determinant of outcome at 1-2 years (21). Furthermore, arteriosclerotic wall thickening in baseline biopsies correlates with its presence in late biopsies (17). Others demonstrated that the presence of vascular lesions in protocol biopsies at 3 months post-transplantation has strong prognostic implications, as cases with CAN and transplant vasculopathy had a 10 year graft survival of 41% compared to 82% among those with CAN but without vasculopathy (22).

Table 4. Multivariate analysis of risk factors of CAN and its forms with or without vasculopathy

	OR (95% CI)	*P*
CAN (n=54)		
Recipient age (10 years increase)	0.71 (0.53-0.96)	0.025
Smoking cigarettes	2.14 (1.17-3.92)	0.006
Panel reactive antibodies (10% increase)	1.28 (1.09-1.48)	0.002
Last ARE > 3 months vs no ARE	12.6 (4.36.-36.7)	<0.001
Dipstick proteinuria >1+ @ 6 months (%)	4.77 (1.12-20.3)	0.034
ECC @ 6 months (10 ml/min increase)	0.78 (0.64-0.93)	0.006
CAN without vasculopathy (n=23)		
Recipient age (10 years increase)	0.69 (0.47-0.99)	0.044
Panel reactive antibodies (10% increase)	1.26 (1.08-1.47)	0.006
Last ARE > 3 months vs no ARE	10.4 (3.13-34.5)	<0.001
CAN with vasculopathy (n=31)		
Year of transplantation (83-89 vs. 90-97)	4.95 (1.65-14.9)	0.004
Last ARE > 3 months vs no ARE	15.0 (4.08-50.0)	<0.001
ECC @ 6 months (10 ml/min increase)	0.58 (0.44-0.75)	<0.001

Abbreviations as in table 1.

In another study graft loss was independently predicted by either fibrointimal thickening or interstitial fibrosis at three months (18). In contrast to tubulointerstitial damage, arterial wall thickening at time of late dysfunction lacks prognostic impact (17,23). In transplant nephrectomies, performed because of pain or hematuria in a selected group of patients, we observed the most severe form of vasculopathy consisting of concentric thickening of the arterial intima with infiltration of mononuclear cells, features that are considered more specific of true chronic rejection (5). The larger number of arteries in the nephrectomies allowed us to appreciate the diffuse nature of chronic vascular rejection. Old donor age and acute vascular rejection episodes obviously contributed to graft dysfunction at 6 months and subsequent lower graft survival in this group.

In our multivariate analysis however, the timing of ARE was more predictive of CAN than the histological type. ARE after three months were the strongest risk factor for both forms of CAN whereas acute rejection within three months did not have a long-term adverse effect. This strong association of late ARE and subsequent CAN has consistently been reported in the literature (24-26). The notion that acute interstitial cellular rejection can lead to interstitial fibrosis without chronic vasculopathy or glomerulopathy has earlier been reported (27). Novel immunosuppressive regimens might be less capable to prevent late rejection activity compared to early ARE which explains the relatively lack of improvement of long-term graft survival (28) and the increased impact of acute rejection on chronic transplant failure in recent era (29).

The data presented in this paper suggests the existence of a pure interstitial and a more generalized interstitial and vascular type of chronic rejection. Such a distinction is well accepted for acute rejection because it is easily recognized and has prognostic implications (5,19). Since the introduction of cyclosporine in the 1980s the incidence of vascular rejection has diminished which suggests that vascular rejection results from the most vigorous immune response (19). Rather than intensity of the response, the specificity of graft infiltrating T cells is also important (30). An immune response against endothelial antigens would result in vascular rejection whereas interstitial rejection would emerge from a reaction against antigens on tubular epithelium. Induced expression of class II MHC antigens and interactions between adhesion molecules on T lymphocytes and endothelial cells has been shown to play a role in graft vasculitis (31). Although little information is available regarding the antigenic profile of tubular epithelial cells, specific cytotoxic T cells have been isolated from rejecting kidneys that lyse tubular cells (30,32). The capacity of T cells, expressing upregulated integrin (CD103), to bind E-cadherin on tubular epithelial cells is an additional factor in the pathogenesis of specific tissue damage in allograft

rejection (33). Disruption of the tubular basement membrane and proliferation of myofibroblasts as consequence of tubulitis has recently been shown to correlate with CAN in the absence of arterial injury (34).
In our well-match cohort of renal transplants, chronic allograft nephropathy occurred without moderate or severe transplant vasculopathy in 43% of the cases. The association with young recipient age, sensitization and late ARE suggests immune-mediated graft destruction. Fibrous intimal thickening of arteries, related with donor age, is not a condition sine qua non for chronic rejection in clinical transplantation.

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8

Immunological risk factors and glomerular C4d deposits in chronic transplant glomerulopathy

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Abstract

Background Chronic transplant glomerulopathy (CTG) is an uncommon cause of chronic transplant dysfunction of unknown pathogenesis. We evaluated the epidemiological, clinical and histological features of CTG. To determine the possible contribution of humoral immune responses we assessed glomerular deposition of C4d.

Methods From a cohort of 1111 kidney transplants (1983-2001) with at least 6 months of graft function we identified 18 cases with CTG (1.6%) showing double contours of the GBM on light microscopy. To assess the risk factors, this group was compared with 739 patients with stable function using multivariate Cox regression analysis. Paraffin sections of 11/18 biopsies were stained with polyclonal C4d antibodies. Sera of 13/18 patients could be tested for anti-HLA antibodies by ELISA. Patients with chronic rejection without CTG were used as controls.

Results CTG was diagnosed at 7.5 $\pm$ 3.2 years post transplantation. Panel reactive antibodies at time of transplantation, RR 1.23 (1.05-1.45) per 10% increase, and late acute rejection episodes, RR 7.6 (1.8-31.7) were independently associated with CTG. We found glomerular C4d deposits in 10/11 biopsies showing CTG and in only 2/13 controls. Peritubular capillary C4d deposits and donor-specific anti-HLA antibodies were demonstrated in respectively 4 and 3 of the 10 patients with glomerular C4d deposits.

Conclusions Pre-sensitization and late acute rejection episodes were the risk factors identified. Glomerular C4d deposits suggest that CTG emerges from in situ humoral rejection. Not all glomerular C4d positive cases had peritubular capillary C4d deposits or anti-donor HLA antibodies suggesting that other (tissue specific) antibodies might be involved. CTG should be considered as a manifestation of chronic rejection.

Introduction

Chronic transplant glomerulopathy (CTG) emerges in approximately 5-15% of transplants with chronic rejection (1-3), and is characterized by reduplication of the glomerular basement membrane (GBM) in the absence of de novo or recurrent glomerulonephritis (2). The extent of membrane reduplication is used to grade the severity of this entity in the Banff 97 classification (4). CTG usually occurs in the background of chronic allograft nephropathy (CAN), i.e. interstitial fibrosis and tubular atrophy with or without fibrous intimal thickening of arteries (4,5). Immunofluorescence microscopy is negative or shows mesangial granular deposits of IgM with greater intensity than C3 (1,6). Electron microscopy reveals reduplication of the GBM and subendothelial accumulation of electron-lucent material, distinguishing CTG from recurrent MPGN (6). Marked reduplication of peritubular capillary (PTC) basement membranes is strongly associated with CTG (7). Acute transplant glomerulitis (ATG), characterized by mononuclear cell infiltrate and endothelial cell enlargement, may precede CTG (1,4,8).
Recently, it has been suggested that allograft glomerulopathy should be separated from chronic rejection, as its pathogenesis is not understood (5). Furthermore, a new classification of renal allograft rejection incorporates cellular and humoral mechanisms of injury (9,10). CTG has been associated with circulating anti-donor HLA antibodies and the deposition of the complement split product C4d in PTC, suggesting antibody-mediated injury (11,12). Regele et al produced a polyclonal anti-C4d antibody that, in contrast to monoclonal antibodies, can be used on paraffin sections and does not stain normal glomeruli (13). However, glomerular deposits of C4d could be detected in only a minority of CTG biopsies (12).
The aim of the current study was to determine the incidence, risk factors, clinical characteristics and prognostic factors of CTG in comparison with chronic rejection without CTG. Furthermore, circulating anti-donor HLA antibodies and glomerular deposition of C4d were assessed to determine whether humoral immunity is involved in the development of CTG.

Patients and methods

Patients

All 1111 cadaveric (n=832), living related kidney (n=163) and simultaneous kidney pancreas (n=116) transplants done at the Leiden University Medical Center between January 1983 and January 2001 with at least 6 months of graft function were reviewed. There were 168 repeat transplants. Patients were followed to graft loss, death, or January 1, 2002. The initial immunosuppressive

Table 1. Patient characteristics of chronic transplant glomerulopathy and chronic rejection

	CTG (n=18)	Chronic rejection (n=108)	*P*
Recipient factors			
Age *years*	40 ± 11	41 ± 12	0.70
Gender *% female*	39	34	0.71
Cigarette smoking *%*	47	49	0.91
Peak panel reactive antibodies *%*	32 ± 29	29 ± 30	0.72
Current panel reactive antibodies *%*	18 ± 30	12 ± 23	0.33
Donor factors			
Age *years*	31 ± 15	41 ± 15	<0.01*
Gender *% female*	44	44	0.98
Transplant factors			
Year: 1983-89/1990-95/1996-2001 *%*	56/44/0	53/36/11	0.32
Living *%*	11	8	0.70
Pancreas *%*	11	6	0.48
Previous *%*	17	19	0.85
Cold ischemia time *hours*	25 ± 11	26 ± 11	0.79
Delayed graft function *%*	24	24	0.99
New immunosuppressive agents *%*	0	8	0.32
HLA CREG mismatches	1.3 ± 1.4	1.9 ± 1.8	0.16
HLA-A-B broad mismatches	1.4 ± 1.0	1.7 ± 1.9	0.51
HLA-DR broad mismatches	0.5 ± 0.7	0.5 ± 0.6	0.95
HLA-A-B-DR broad mismatches	1.9 ± 1.2	2.0 ± 1.5	0.70
Acute rejection episodes			
Type: none/interstitial/vascular *%*	33/33/28	23/49/21	0.59
Number: one/two/three or more *%*	17/28/22	21/32/29	0.51
First: < two month/> two months *%*	56/11	56/26	0.21
Last: < three months/> three months *%*	50/17	34/47	0.04*
Characteristic at time of biopsy			
Time from transplantation to biopsy *yr*	7.5 ± 3.2	3.2 ± 3.0	<0.01*
New immunosuppressive agents *%*	67	24	<0.01*
Serum creatinine *µmol/l*	254 ± 107	247 ± 96	0.78
Creatinine clearance *ml/min*	35 ± 17	37 ± 18	0.66
Serum albumin *mmol/l*	38 ± 6	40 ± 5	0.09
Serum cholesterol *mmol/l*	5.5 ± 1.1	6.3 ± 1.5	0.03*
Proteinuria *g/24h*	3.1 ± 3.4	1.8 ± 2.1	0.03*
Proteinuria: <0,5/0,5-2,0/>2,0 g/24h *%*	6/44/50	36/35/29	0.03*
Systolic blood pressure *mm Hg*	159 ± 17	150 ± 20	0.11
Diastolic blood pressure *mm Hg*	87 ± 10	89 ± 14	0.54
Number of antihypertensive drugs	2.4 ± 1.1	1,8 ± 1,1	0.01*
Minimum proteinuria *g/24h*	1.4 ± 2.1	1.0 ± 1.6	0.36
Systolic bp @ minimum proteinuria *mm Hg*	144 ± 19	147 ± 21	0.58
Diastolic bp @ minimum proteinuria *mm Hg*	85 ± 11	85 ± 12	0.88

Abbreviations: CREG, cross-reactive group; bp, blood pressure; *P value < 0.05

regimen consisted of prednisone and cyclosporine and/or azathioprine. In 1996 Sandimmune was changed to Neoral and mycophenolate mofetil was added as a third baseline drug.

Histopathology

We reviewed the biopsies that were taken beyond six months post-transplantation for clinical indications, including declining renal function or significant proteinuria. Recurrent (n=56) or de-novo glomerulonephritis (n=11) and predominant cyclosporine toxicity (n=53) were excluded by clinical and histological means. Cyclosporine nephrotoxicity was characterized by arteriolar hyalinosis and stabilization of renal function after dose reduction or switch to mycophenolate (14).

In 130 cases the histology indicated a diagnosis of chronic allograft nephropathy, chronic rejection alone or together with transplant glomerulopathy. All biopsies were re-examined and if transplant glomerulopathy was present, sections were scored according to the Banff '97 schema (4) by a single pathologist (HB). Eighteen patients met the definition of CTG according to the following criteria: 1) Light microscopy showing 'double contours' of the glomerular basement membrane in at least 10% of the most severely affected tuft (4). 2) Immunofluorescence negative or showing scant depositions of IgM with greater intensity than C3 (6). 3) Recipient's original renal disease other than MPGN and absence of hepatitis C seropositivity at time of transplantation (6,15).

Four patients had ATG defined by mononuclear cell infiltration and endothelial cell enlargement in the absence of double contours of the GBM (4,16). Finally, chronic rejection was defined in 108 patients as chronic allograft nephropathy without predominant clinical and histological signs of transplant glomerulopathy, cyclosporine toxicity or recurrent disease.

Clinical data and anti-donor HLA antibodies

Clinical information was obtained from hospital charts and laboratory records. Donor and recipient variables as well as transplant and clinical parameters were recorded as shown in table 1. We studied the impact of HLA mismatches as broad antigens and as cross-reactive groups (CREG) of MHC class I (17). The clinical parameters were obtained at the time of biopsy and at the time of the minimal level of proteinuria following biopsy. Finally, the last measured serum creatinine was collected from all patients with a functioning transplant until death or end of follow-up.

In 13/18 CTG patients, sera were available at time of biopsy. The presence of circulating anti-donor HLA class I and class II antibodies were assessed by ELISA and flow cytometer crossmatch.

C4d staining

Eleven biopsies from 18 patients with CTG and 3 of 4 biopsies showing ATG were available for immunohistochemistry. Fourteen patients with chronic

Figure 1 Cumulative incidence to biopsy showing chronic transplant glomerulopathy (dashed line) and chronic rejection (solid line). Log rank test: P=0.004

rejection without glomerular lesions were randomly selected as control. Polyclonal rabbit anti-C4d antibody (Biomedica, Vienna, Austria), kindly provided by Heinz Regele, was used on paraffin sections as recently described (12,13). In brief, 2-µm sections were deparaffinized andendogenous peroxidase activity was blocked with hydrogen peroxide. Antigen retrieval was carried out by pressure-cooking for 10 min at 10 bar in citrate-buffer(pH 6.0). After overnight incubation with polyclonal anti-C4d antibody (1:250), bound IgG was visualized using HRP-conjugated goat anti-rabbit immunoglobulins absorbed for human IgG. Finally, sections were stained with tyramid-FITC. Glomerular staining was considered positive if one or more glomeruli showed C4d deposits in the capillary wall. PTC staining was scored positive if 25% or more of the PTC was strongly positive.

Study design and statistical analysis

Demographic and clinical data of patients with CTG or chronic rejection were

compared using the independent samples t-test for continuous variables and Chi-square test for categorical variables. A p value < 0.05 was considered significant. The cumulative incidence of CTG was determined by the ratio of cases and total number of patients in the cohort. The time between transplantation and the diagnosis of CTG and chronic rejection was compared using the Kaplan-Meier actuarial method.

To identify the risk factors of CTG or chronic rejection the groups were compared with 739 patients with stable function defined as a last serum creatinine of less than 120% compared to the value at 6 months post-transplantation. The individual effect of the variables on the time to biopsy, graft failure or end of follow-up was evaluated with the use of the Cox proportional hazard model. Significant predictors ($P < 0.05$) in univariate analysis were fitted into a multivariate model according to a forward selection, likelihood ratio test.

To assess prognostic factors associated with graft failure, outcome was defined as return to dialysis or as a last serum creatinine concentration of more than 150% compared to the value at time of biopsy. Uni- and multivariate Cox regressions were used to evaluate the relationship between the biopsy variables and the time between diagnosis and graft failure. Cumulative survival rates were computed by the Kaplan-Meier method.

Results

Features of chronic transplant glomerulopathy and chronic rejection

The mean interval between transplantation and a histological diagnosis of CTG / chronic rejection or end of follow-up was 6.9 ± 4.8 years. Eighteen patients out of a cohort of 1111 transplants developed CTG, leading to a cumulative incidence of 1.6%. Chronic rejection was diagnosed in 108 patients (9.7%). Considering these 126 patients together, the percentage of CTG in chronic rejection was 14%.

Table 1 shows the clinical data of 18 patients with CTG in comparison with 108 patients with chronic rejection without CTG. Donor age was significantly lower in the CTG group. Mean peak and current panel reactive antibodies were not significantly different between the two groups. There was no difference in the number or type of acute rejection episodes but in the CTG group there were significantly fewer acute rejection episodes beyond three months. Biopsies showing CTG were obtained at 7.5 ± 3.2 years in contrast to 3.3 ± 3.0 years in the chronic rejection group (figure 1). At the time of biopsy, the immunosuppressive regimen consisted in 67% of the newer drugs, i.e. Neoral in 12 patients and mycophenolate mofetil as third drug in one patient, compared

to 24% in the chronic rejection group. Patients with CTG had a mean serum creatinine concentration of 254 ± 107 µmol/l, corresponding to a creatinine clearance of 35 ± 17 ml/min which is comparable with the chronic rejection group. The mean albumin concentration was 38 ± 6 g/l and proteinuria 3.1 ± 3.4 g per day which was significantly higher than the mean proteinuria of 1.8 ± 2.1 g of the chronic rejection group. Four out of 18 (22%) CTG patients had nephrotic syndrome defined by proteinuria of more than 3.5 g per day and an albumin level of less than 35 g/l, in contrast to 5 out of 111 (4%) cases with chronic rejection. Systolic blood pressure was higher in CTG than in chronic rejection despite a higher number of antihypertensive drugs. Graft survival plotted beginning at 6 months after transplantation (figure 2A) and at time of biopsy (figure 2B) was not significantly different between the two groups.

Characteristics of chronic transplant glomerulopathy

The features and outcome of the patients with CTG (1-18) are shown in table 2. Renal biopsies were performed because of chronic transplant dysfunction; 16 patients had a decline in renal function, and 16 had more than 1 gram proteinuria per day; hypertension (>140/90) was present in 14 cases, despite antihypertensive medication. 11 biopsies were adequate and 7 marginal according to the Banff '97 criteria, i.e. all specimens showed at least 7 glomeruli and 1 artery. The diagnosis of CTG was based on the extent of double contours of the GBM in the most severely affected glomerulus (5), which was present

Table 2. Characteristics and outcome of patients with chronic transplant glomerulopathy (1-18) and acute transplant glomerulitis (a-d)

		Chronic transplant dysfunction					Banff '97 score				Follow-up	
#	Disease	Time	Creat	Cl	Prot	BP	G	CG	CV	CAN	Time	Outcome
		years	*µmol/l*	*ml/min*	*g/24 h*	*mmHg*					*years*	
1	ADPKD	2.6	234	25	5.2	180/105	3	3	0	2	4.3	dialysis
2	DM I	2.8	475	16	15.2	175/80	2	2	1	2	2.9	dialysis
3	GN	3.1	506	22	2.8	160/85	3	3	2	2	3.2	dialysis
4	CIN	4.6	224	21	3.2	125/55	1	3	1	3	5.6	decline†
5	ADPKD	4.7	217	31	0.2	180/95	2	1	1	1	10.0	stable
6	DM I	5.2	406	15	1.0	145/90	2	2	3	1	5.8	stable
7	Alport	5.7	153	44	3.6	160/100	2	3	2	1	8.0	decline†
8	CIN	7.1	203	34	1.2	160/90	2	3	1	2	11.2	stable
9	CIN	8.3	169	49	6.0	155/95	2	2	3	2	17.8	dialysis
10	GN	8.4	112	85	4.8	160/90	2	3	0	2	12.5	stable†
11	CIN	8.6	225	18	1.7	190/90	3	2	3	3	8.8	stable
12	ADPKD	9.4	188	57	1.9	165/85	3	1	1	1	11.4	stable
13	CIN	9.4	168	48	0.7	160/85	0	3	1	1	12.2	dialysis
14	GN	9.7	250	41	2.0	140/85	3	3	3	2	10.6	stable
15	HT	9.9	236	30	2.0	160/85	2	1	2	2	10.6	dialysis
16	DM I	11.3	270	29	2.7	130/90	2	1	0	2	12.7	decline
17	CIN	12.3	220	35	1.0	140/85	3	3	1	3	14.9	stable
18	HT	12.5	313	24	1.2	170/80	3	3	2	2	13.8	stable
a	DM II	0.6	154	38	11.2	155/95	2	0	3	1	1.6	dialysis
b	GN	1.2	264	20	2.7	140/100	2	0	3	3	1.5	dialysis
c	GN	1.9	447	16	7.6	170/95	3	0	3	1	2.1	dialysis
d	HT	3.3	227	45	2.7	145/95	2	0	1	2	3.9	dialysis

Time: time since transplantation, ADPKD: autosomal dominant polycystic kidney disease, DM: diabetes mellitus, GN: glomerulonephritis, CIN: chronic interstitial nephritis, HT: hypertension, G: glomerulitis, CG: glomerulopathy, CV: fibrous intimal thickening,, CAN: chronic allograft nephropathy (grade), BP: blood pressure, prot: proteinuria, creat: creatinine, cl: clearance, †: died

in 10-25% of capillary loops in 4, 26-50% in 4 and more than 50% in 10 cases (figure 3). Seventeen out of 18 cases showed variable increase in mesangial cellularity. Fibrous intimal thickening of arteries was absent in 3, mild in 7, moderate in 4 and severe in 4 cases. CAN, based on the extent of interstitial fibrosis and tubular atrophy, was graded I, II, III in 5, 10 and 3 cases, respectively. Immunofluorescence revealed IgM in peripheral capillary loops and mesangial regions in 10 patients and weaker reactions to C3, IgG and IgA in 5, 4 and 3 patients, respectively. Circulating anti-donor HLA antibodies at the time of biopsy were found in 5/13 patients tested. These antibodies were directed to class I, class II and class I+II in respectively 1, 3 and 1 cases. Six patients lost their grafts and returned to dialysis. Three patients had graft failure defined by a creatinine rise of more than 50% at the end of follow-up. Renal function was

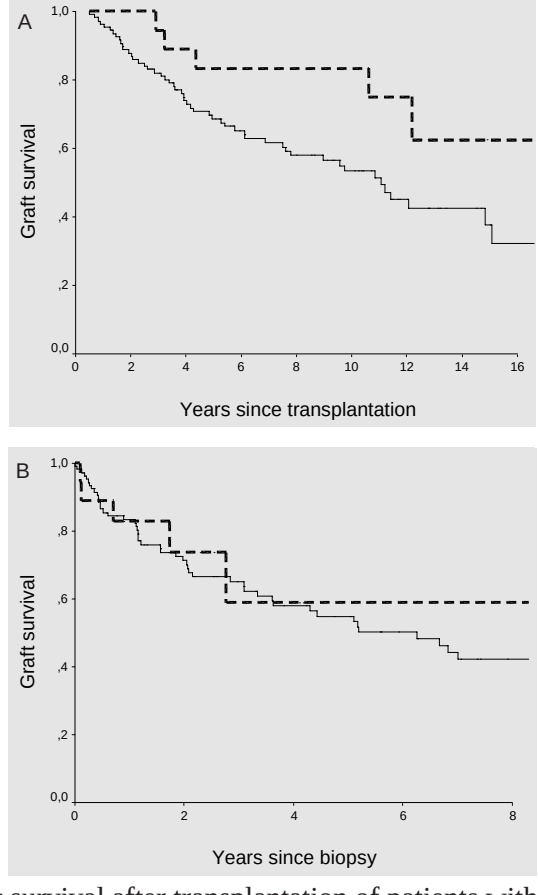

Figure 2 (A) Graft survival after transplantation of patients with CTG (dashed line) and chronic rejection (solid line). Log rank test: P = 0.11. (B) Graft survival after diagnosis of CTG (dashed line) and chronic rejection (solid line). Log rank test: P = 0.99

stable in the other 9 patients.
Four patients (a-d) had evidence of ATG (table 2). The percentage panel-reactive antibodies at time of transplantation was 17, 32, 5 and 6%. The patients had either 2 or 3 acute rejection episodes. Biopsies showing ATG were obtained at 0.6, 1.2, 1.9 and 3.3 years after transplantation. The biopsies showed glomerular infiltration by neutrophils and endocapillary proliferation. Extracapillary proliferation was present in one patient (figure 3A). There were no signs of tubulitis or vasculitis, but chronic vascular and tubulointerstitial changes were present in 2 and 4 patients, respectively. Antibodies to HLA class I+II of the donor were present in one of the two patients with serum available. Graft loss

Table 3. Cox analysis of risk factors of chronic transplant glomerulopathy and chronic rejection in comparison with 739 patients with a stable function

	CTG (n=18)		Chronic rejection (n=108)	
	RR	95% CI	RR	95% CI
Recipient factors				
Age *10 years*	0.83	0.57-1.23	0.83	0.71-0.97*
Gender *female*	1.17	0.45-3.03	1.20	0.81-1.77
Cigarette smoking	0.89	0.21-3.73	1.67	1.14-2.45*
Peak panel reactive antibodies *10%*	1.10	0.96-1.29	1.12	1.06-1.20*
Current panel reactive antibodies *10%*	1.20	1.02-1.40*	1.14	1.04-1.23*
Donor factors				
Age *10 years*	0.77	0.53-1.12	1.26	1.10-1.44*
Gender *female*	0.87	0.34-2.19	0.95	0.65-1.38
Transplant factors				
Year: 90-95 versus 83-89	1.52	0.53-4.32	0.62	0.41-0.95*
Year: 96-01 versus 83-89	na	na	0.37	0.20-0.70*
Living	0.66	0.15-2.88	0.52	0.26-1.02
Pancreas	2.50	0.56-11.3	0.70	0.33-1.51
Previous	1.13	0.33-3.91	1.43	0.88-2.32
Cold ischemic time *hour*	1.01	0.97-1.05	1.02	1.00-1.04*
Delayed graft function *%*	1.08	0.35-3.32	1.18	0.75-1.84
New immunosuppressive agents	0.04	0.00-85.3	0.30	0.15-0.63*
HLA CREG mismatches	0.93	0.66-1.30	1.12	1.00-1.26*
HLA-A-B mismatches	0.95	0.60-1.50	1.06	0.93-1.20
HLA-DR mismatches	1.70	0.54-2.52	0.95	0.69-1.30
HLA-A-B-DR mismatches	1.00	0.70-1.43	0.98	0.86-1.12
Acute rejection episodes				
Type: interstitial	8.21	0.91-74.3	3.59	2.32-5.77*
Type: vascular	3.36	1.02-10.9*	3.60	2.04-6.34*
Number	1.63	1.07-2.98*	1.87	1.58-2.13*
First episode within two months	7.93	0.97-64.7	3.06	1.85-5.07*
First episode after two months	10.7	0.66-171	13.5	7.57-23.9*
Last episode within three months	7.34	0.88-61.2	1.97	1.15-3.39*
Last episode after three months	12.6	1.13-139*	16.4	9.72-27.6*

CREG: cross-reactive group, na: not applicable, *P value < 0.05

Table 4. Prognostic factors of graft failure from chronic transplant glomerulopathy and chronic rejection

	CTG (*N* = 18)		Chronic rejection (n = 108)	
	RR	95% CI	RR	95% CI
Time since transplantation *yr*	0.71	0.51-0.98*	0.97	0.89-1.07
New immunosuppressive agents	0.48	0.12-1.94	1.07	0.51-2.22
Serum creatinine *10 µmol/l*	1.15	1.04-1.29*	1.05	1.03-1.08*
Endogenous creatinine clearance *10 ml/min*	0.41	0.16-1.06	0.74	0.61-0.89*
Serum albumin *g/l*	0.82	0.70-0.97*	0.94	0.89-0.99*
Proteinuria *g/24h*	1.29	1.01-1.64*	1.32	1.19-1.48*
Minimum proteinuria *g/24h*	1.45	1.08-1.95*	1.77	1.46-2.15*
Systolic bp *10 mm Hg*	1.08	0.63-1.88	1.15	1.01-1.29*
Systolic bp @ minimum prot. *10 mm Hg*	1.24	0.84-1.82	1.40	1.23-1.60*
Diastolic bp *10 mm Hg*	0.67	0.36-1.27	1.17	1.01-1.34*
Diastolic bp @ mininum prot. *10 mm Hg*	2.14	0.94-4.89	1.38	1.08-1.77*

bp: blood pressure, prot: proteinuria, **P* value < 0.05

occurred in all patients, within one year after diagnosis.

Risk factors of chronic transplant glomerulopathy and chronic rejection

The univariate effects of the various risk factors of CTG or chronic rejection in comparison with patients with a stable function are shown in table 3. Multivariate analysis revealed that current panel reactive antibodies, RR 1.23, 95% CI 1.05-1.45 per 10% increase, *P*=0.01 and last acute rejection episodes beyond 3 months, RR 7.6 (1.8-31.7), *P*=0.006 were independently associated with CTG.

Chronic rejection without CTG was independently predicted by cigarette smoking, RR 1.80 (1.21-2.67), *P*=0.004, peak panel reactive antibodies, RR=1.10 (1.03-1.18) per 10% increase, *P*=0.004, donor age, RR 1.24 (1.07-1.43), *P*=0.009, Neoral and mycophenolate mofetil based regimens, RR 0.46 (0.21-0.99), *P*=0.05 and especially a last acute rejection episode beyond 3 months, RR 14.5 (8.3-25.1), *P*=0.0001.

Glomerular and peritubular C4d staining in transplant glomerulopathy

C4d stained positive in the glomeruli of 10/11 CTG biopsies and in 3/3 biopsies showing ATG. Fig. 3 shows the light and immunofluorescence microscopy of three biopsies with ATG, CTG and chronic rejection without glomerular lesions, respectively. Patient *a* (A/D) had ATG, consisting of extensive endo- and extracapillary proliferation at 6 months post-transplantation (table 2). Patient *11* (B/D) developed CTG with reduplication of the GBM at 8.5 years. The glomeruli stained positive for C4d in a granular, segmental capillary pattern.

Panel C/E shows a normal glomerulus without C4d deposits in the biopsy of a patient with chronic rejection at 4 years. C4d staining of PTC was also positive in 4/10 CTG and 3/3 ATG cases with glomerular C4d deposits. Anti-donor HLA antibodies were detected in 3/10 CTG and 1/2 ATG cases. Glomerular and PTC C4d deposits were found in one of the 14 control patients with chronic rejection.

Factors predicting graft failure

Table 4 shows factors prognostic for graft failure or loss of renal function in patients with CTG and chronic rejection. Graft failure from CTG, occurring in 9 of the 18 patients, was independently correlated with the minimum 24 hours proteinuria (RR=2.21 per g/day, 95% CI 1.21-4.04, P=0.01). Fifty-one patients in the chronic rejection group (46%) lost their graft and 16 (14%) had a rise of serum creatinine of more than 50% at the end of follow-up. Therefore, 67 patients (60%) experienced graft failure. This was predicted by serum creatinine (RR 1.07, 1.04-1.10 per 10 µmol/l, P<0.001) at time of diagnosis, minimum 24-h proteinuria (RR 1.55, 1.24-1.93 per g/l, P<0.001) and the associated systolic blood pressure (RR 1.24, 1.06-1.45 per 10 mmHg, P=0.006) in multivariate analysis.

Discussion

We studied the clinical and immunohistochemical characteristics of 18 patients with CTG. The cumulative incidence of CTG was 1.6%, which is somewhat lower compared to the incidence of 1.9-7% reported in other series (1-3). As biopsies are not invariably obtained in patients with declining graft function, and CTG has been reported in protocol biopsies the incidence may have been underestimated (18).

CTG presents as chronic transplant dysfunction, a clinical syndrome consisting of an increased serum creatinine concentration, elevated blood pressure and proteinuria. Diagnostic biopsies were performed later compared with patients with chronic rejection without CTG, i.e. 7.5 versus 3.2 years, confirming that the clinical manifestations of CTG tend to develop late after transplantation (3). At the time of diagnosis, patients with CTG had a similar degree of renal dysfunction, but on average the serum albumin level was lower and proteinuria more severe than patients with chronic rejection, compatible with glomerular injury. We documented an increased systolic blood pressure and use of antihypertensive drugs in patients with CTG. However, more CTG patients used cyclosporine (Neoral) at time of biopsy, an agent with increased bioavailability that accounted for increased blood pressure and proteinuria after

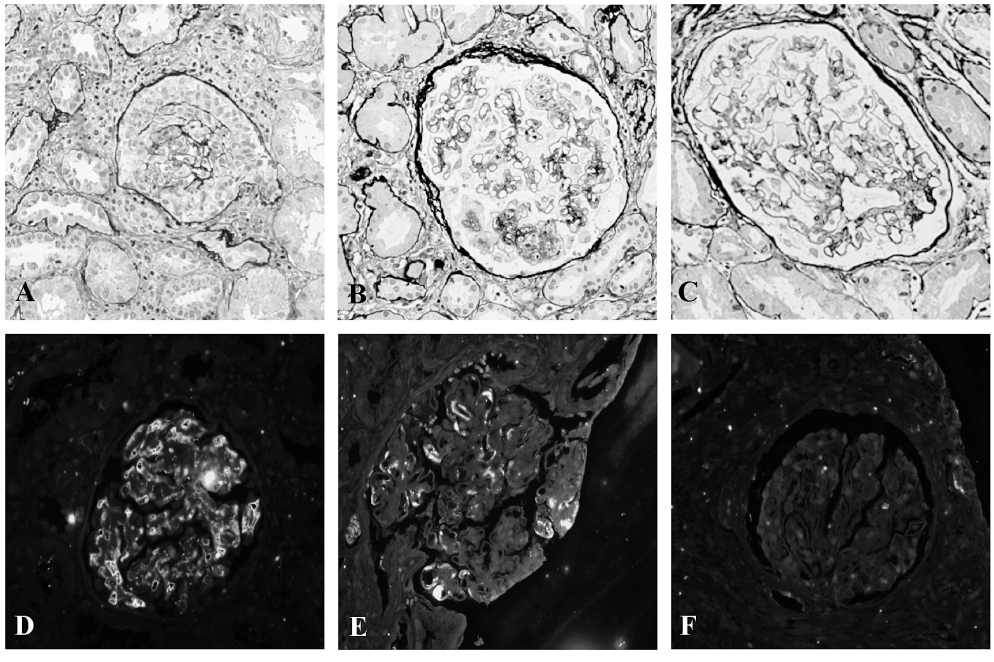

Figure 3 Light microscopy (A-C) (silver staining) and immunofluorescence for C4d (D-F) on paraffin sections. A/D: Transplant glomerulitis with endo- and extracapillary proliferation and segmental capillary staining of C4d. B/E: Chronic transplant glomerulopathy with reduplication of the GBM and capillary staining of C4d. C/F: Chronic rejection without glomerular lesions and negative C4d staining.

conversion from Sandimmune in the Leiden cohort of renal transplants (14). Double GBM contours are a key light microscopic feature of CTG and were required for the diagnosis of CTG in our study. We observed in all but one patient a variable degree of glomerulitis in the glomerular tuft, a feature characteristic of ATG (4,16). We confirm earlier results from our center and from others that chronic vascular changes did not parallel CTG (3,19). Ten patients showed no or minor obliteration of the arterial lumen, indicating that ischemia is not a likely mechanism responsible for CTG. The observed chronic tubulointerstitial changes are most likely related to previous acute rejection episodes (20).

Late ATG, observed in 4 patients, occurred earlier than CTG and was associated with severe proteinuria and subsequent rapid graft failure. Messias et al examined early glomerulitis, that occurred in 28/63 (44%) patients with acute rejection within 3 months (16). Due to a strong association with vascular rejection there was no independent effect on graft survival (16). In our opinion, early glomerulitis at time of an acute rejection episode should be distinguished from late onset transplant glomerulitis.

Assessment of risk factors revealed that both CTG and chronic rejection were strongly related with pre-transplant sensitization and late acute rejection episodes. These factors were also present in patients with ATG. In the study of Messias et al, a significantly higher percentage of patients in the early glomerulitis group was highly sensitized pre-transplantation, had retransplants and had delayed graft function compared to the nonglomerulitis group (16). Preformed anti-HLA antibodies are related to acute humoral rejection and also increase the risk of chronic rejection (21,22). In comparison with transplants with stable function both CTG and chronic rejection were associated with late acute rejection episodes. We found earlier that acute rejection episodes beyond 3 months have a detrimental impact on long-term outcome and are associated with CREG mismatches (17,23). At this time post-transplantation, indirect allorecognition, i.e. activation of T helper cells by donor MHC molecules presented by recipient antigen presenting cells, may trigger the production of antibodies that may mediate chronic rejection (11,24). Therefore, both pre-existing and newly formed antibodies post-transplantation may increase the risk of chronic rejection and CTG.

Because of this risk profile of CTG we decided to investigate C4d deposition in the transplants. We used a polyclonal anti-C4d antibody suitable for detection of glomerular C4d on paraffin sections (12,13). Glomerular deposits of C4d were present in 10/11 biopsies with CTG and in 3/3 cases showing ATG. Peritubular staining for C4d was positive in respectively 4/10 and 3/3 cases with glomerular C4d deposits. Absence of glomerular C4d staining in all but one tested patients with chronic rejection without CTG suggest that this antibody might be useful in characterizing late transplant glomerulopathy. Endothelial deposition of the complement split product C4d in PTC has been established as a marker for both acute and chronic humoral rejection defined by the presence of anti-donor HLA antibodies (9). Absence of concomitant capillary immunoglobulin staining in C4d positive biopsies has been explained by less covalent binding compared to C4d (9). Biopsies, taken within the first 3 months that have C4d in the PTC, show neutrophilic glomerulitis in 55% versus 4% in C4d negative acute rejection while the histology of C4d positive chronic rejection was reported as similar compared compared to their C4d negative counterparts (11,25). Recently, C4d deposition in PTC was detected on paraffin sections in 34% of 213 late biopsies and found to be associated with tubular basement membrane multilayering and CTG (12). However, in contrast to our data glomerular C4d staining was observed in only 12% of the CTG biopsies which is difficult to explain unless their criteria for the diagnosis of CTG were less strict (12). In biopsies with unaffected glomeruli but positive C4d in PTC progression to CTG could be observed in follow-up biopsies (12). Ongoing

humoral rejection may link early glomerulitis and late transplant glomerulopathy.

The evidence for humoral rejection in late transplant glomerulopathy suggests that antibodies directed against donor HLA antigens play a role. In a series of chronic humoral rejection, 15 out of 17 patients with C4d in the PTC had anti-donor HLA antibodies (11). We could detect anti-donor HLA antibodies in 3/10 CTG cases with glomerular C4d deposits suggesting that a tissue specific response might also be involved. However, we cannot exclude that the levels of circulating anti-HLA antibodies are undetectable due to absorption by antigens in the graft. In an experimental model, we found circulating and kidney graft bound IgG antibodies against the GBM in rats with CTG. Using proteomic techniques the heparan sulphate proteoglycan perlecan and the α1 chain of collagen VI in association with the α5 chain of collagen IV were identified as the antigens recognized by the antibodies (26). We hypothesize that similar responses against glomerular antigens are also present in patients with transplant glomerulopathy.

Humoral rejection warrants a specific therapeutic strategy. In chronic rejection decrease of anti-donor HLA antibodies and C4d deposition can be induced by rescue therapy with tacrolimus and mycophenolate mofetil (27). Furthermore, patients who stay on mycophenolate for a prolonged period of time have a lower risk of late acute rejection episodes and CAN (28,29). In our series, only one patient with CTG in the absence of C4d deposits used mycophenolate mofetil at time of diagnosis. As this agent was only introduced in our centre in 1997, longer follow-up is needed to determine whether this agent might reduce the incidence of CTG as well.

The prognosis of CTG was related to the time of diagnosis and level of proteinuria. However, once the diagnosis has been made we found a similar graft survival rate compared to patients with earlier diagnosed chronic rejection. In both groups outcome correlated with proteinuria, in the chronic rejection group together with concomitant systolic blood pressure and renal function at time of diagnosis. These results support recent evidence that renoprotection could be achieved when long-lasting ACE inhibition results in persistent reduction in proteinuria (30,31). ACE inhibitors and angiotensin II antagonists are well tolerated in transplant recipients with CAN and are associated with stabilization of renal function (32,33).

In conclusion, CTG may present years after transplantation. Sensitization and late acute rejection episodes were identified as risk factors strongly suggesting an underlying immunological mechanism. The presence of glomerular C4d deposits supports a role for humoral immune responses in the pathogenesis. CTG should be considered as a manifestation of chronic rejection.

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9

Summary and discussion

Summary and discussion

This thesis is based on a case history of a young woman who lost her renal transplant from presumably chronic rejection (CR). She received a transplant from a cadaveric young male donor and was treated with maintenance prednisone and cyclosporine. She had 4% panel-reactive antibodies at the time of transplantation and there was a 1-2-0 HLA-A,-B,-DR mismatch. After an excellent first transplant year she participated in a randomized trial and was assigned for prednisone withdrawal. After a few weeks, an acute rejection episode (ARE) was diagnosed which responded on treatment with methylprednisolone, albeit without complete recovery of renal function. Gradually she developed proteinuria and a decline in renal function consistent with chronic transplant dysfunction (CTD). A biopsy demonstrated chronic allograft nephropathy (CAN) without signs of vasculopathy, glomerulopathy or cyclosporine toxicity. Renal dysfunction was progressive, despite treatment with a converting enzyme inhibitor; six years after transplantation she had to resume hemodialysis therapy.

What is CR? What are its clinical and histological manifestations? Did this patient loose her transplant from CR? What are the positive arguments to make the clinical diagnosis CR? To answer these questions we studied the epidemiological, clinical and histopathological features of CR in the Leiden cohort of renal transplants. The terms CR, CAN and CTD has often been used as synonym leading to ambiguity and even the proposal to abandon the term CR. We favour the following definitions *(chapter 1).* CR is an alloantigen-dependent process leading to CAN and CTD ultimately resulting in failure of the transplant. CAN is a descriptive term of the characteristic pathology consisting of fibrous intimal thickening of arteries, interstitial fibrosis and tubular atrophy. CTD is a clinical syndrome characterized by a slowly rising plasma creatinine concentration, increasing proteinuria and worsening hypertension. All definitions contain the adjective “chronic” that conveys different meanings. It describes late and lasting in time, i.e. beyond 3 months post-transplantation (CTD), persistent or recurrent injury (CR), and scarring and atrophy as pathological features (CAN). Because all these meanings apply in most patients undergoing CR, the term “chronic” is appropriate. In the Banff ’97 classification of renal allograft nephropathy CAN has been graded by the severity of interstitial fibrosis and tubular atrophy. De novo vasculopathy or glomerulopathy supports the histological diagnosis of CR. CR should be differentiated from other causes of CTD, such as chronic calcineurin inhibitor (CNI) nephrotoxicity, recurrent or de novo glomerulonephritis, nephrosclerosis, transplant renal artery stenosis and BK virus nephropathy, entities which lead

to CTD and CAN and may occur alone or together. In the case report, the clinical diagnosis of CR could be made despite the lack of specific histological findings. The presence of immunological risk factors, a biopsy showing CAN, absence of other causes of CTD, and subsequent graft loss were considered as positive arguments supporting CR. This thesis focuses on the risk factors of graft loss from CR *(chapter 2 and 3)*, prediction of graft failure *(chapter 2,4 and 5)*, cyclosporine nephrotoxicity *(chapter 6)*, the different histological manifestations of CR *(chapter 7 and 8)*, and one of the mechanisms of CR *(chapter 8)*.

To evaluate the risk factors of CR, we studied all 654 cadaveric renal transplants performed in Leiden between 1983 and 1996 that had survived for more than six months *(chapter 2)*. Biopsies obtained beyond 6 months were reviewed blinded for clinical information and scored according the Banff '97 classification. A total of 224 grafts were lost, mainly from patient death with a functioning transplant (53%). After exclusion of other causes, 82 transplants (36%) were lost from presumably CR and used as outcome variable (figure 1). In 62 out of these 82 cases graft histology was available showing CAN in absence of other causes of CTD. Recipient, donor, transplantation and clinical variables were collected and assessed as risk factors using uni- and multivariate Cox regression analysis. The impact of HLA matching was studied in detail at

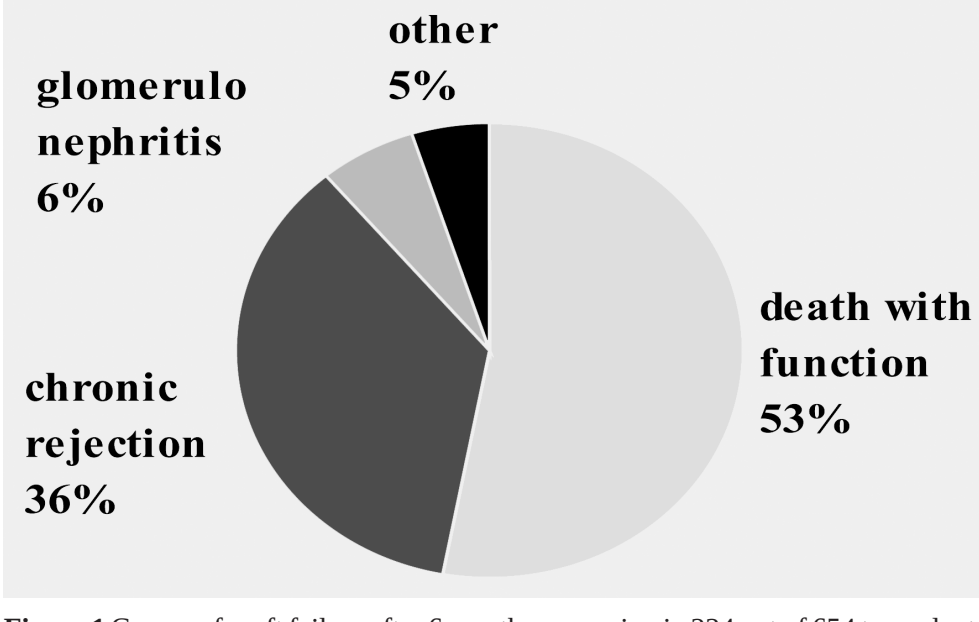

Figure 1 Causes of graft failure after 6 months, occurring in 224 out of 654 transplants between 1983 and 1996.

the level of broad and split antigens. Furthermore, MHC class I antigens were assigned to one or more public epitopes, the so-called cross reactive groups (CREG). Not only was the degree of mismatching between donor and recipient studied but also the effect of sharing HLA-antigens. The term 'shares' was used for the number of corresponding HLA antigens between donor and recipient. HLA mismatches were not related with graft loss but in contrast, sharing of HLA antigens, especially CREG, improved long-term graft survival. The discrepancy between mismatches and shares could be explained by cross-tabulation, demonstrating a non-reciprocal relationship. A higher percentage of homozygosity of donors compared to recipients, as result of selection on mismatches, appeared to be responsible for this finding. Hypothetically, more shared antigens between donor and recipient may down regulate the response to alloantigens. As known from the literature, ARE had a strong impact on graft survival. We extended earlier data from our centre that acute vascular rejection has also a detrimental effect on late outcome. Furthermore, the number and timing of ARE were of importance. Young recipients were at risk likely as result of an increased immune responsiveness and possibly non-compliance. Old donor age influenced outcome, with increased immunogenicity, and reduction in renal mass as possible mechanisms. Finally, smoking and presumably the presence of atherosclerosis in recipients with nephrosclerosis as baseline disease increased the risk of graft loss from CR. The reported recipient was young, shared only one CREG with her donor and experienced a late ARE, three factors explaining her risk to develop CR.

The late ARE was considered the most important risk factor of CR in this patient. Between 1983 and 1996, ARE occurred frequently, allowing epidemiological studies. In 384 of 654 transplant recipients (59%), one or more treated ARE were documented. However, not all these ARE had an adverse outcome. In most studies, early and late ARE are divided by the onset of the first ARE. However, we observed more contrast in prognosis when the onset of the last treated ARE was used as the time factor *(chapter 2)*. The last ARE occurred in 297 of 384 transplant recipients (77%) within 3 months and in 87 of 384 (23%) after 3 months *(chapter 3)*. Ten-year graft survival rates censored for causes of graft loss other than CR were 94%, 86%, and 45% for patients without ARE, with early ARE, and with late ARE, respectively. In the latter group, prognosis did not depend on the presence of previous early ARE. Applying multivariate logistic regression analysis, the predictor variables of the two groups were compared with transplants without ARE. Delayed graft function, and HLA-DR mismatches were independent risk factors for ARE within 3 months. In this immediate period after transplantation donor dendritic cells are present in the graft. Allo-class II antigens expressed by these cells are

recognized by T helper cells of the recipient in the so called direct pathway. The HLA-DR effect diminishes over time once the passenger cells disappear and donor-specific T helper cell hyporesponsiveness has been induced. In contrast to early ARE, young recipient age, old donor age, female donor gender, and CREG mismatches were associated with ARE beyond three months. These data strongly link late ARE with CR suggesting similarities in the pathogenesis with a role of the indirect pathway allorecognition. Hereby, recipient T cells recognize alloantigens that are shed from the graft, processed and presented by recipient antigen presenting cells. Interaction of helper T cells with B cells might be relevant for the induction of anti-donor antibodies possibly leading to subsequent chronic humoral rejection. Late rejection activity through the physiological indirect pathway might happen even under current immunosuppressive treatment. This explains the relatively minor change in long-term outcome despite significant reduction in the incidence of ARE and the increased impact of ARE on chronic allograft failure in recent time.

Risk factors may operate differently in the process of graft attrition *(chapter 4).* They can influence the input and the pace of deterioration in function. Therefore, we compared the risk factors of a low intercept, defined as a creatinine clearance lower than 50 ml/min at 6 months, and of a negative slope of the reciprocal creatinine concentrations after 6 months. Two hundred of 654 grafts (31%) failed to reach optimal function because of old donor age, female gender of the donor, histoincompatibility, delayed graft function or ARE in the first 6 months. Forty-four percent (288/654) of all grafts displayed progressive deterioration of function over time, which was not related with donor factors and delayed graft function. However, the association with younger recipient age, sensitization, class I histoincompatibility, baseline immunosuppression and late ARE suggests an underlying immunological process. The influence of histoincompatibility and late ARE on both the intercept and the slope supports the link between late ARE and CR. The rate of decline is worse for grafts with a creatinine clearance of < 50 ml/min compared with grafts with a clearance of > 50 ml/min at 6 months posttransplantation. The negative impact of proteinuria and hypertension at six months on the slope is compatible with their role as progression factors.

To improve the prediction of late graft failure we developed a model with time dependent renal function covariates *(chapter 5).* In contrast to time-fixed covariates, time-dependent covariates are measured repeatedly over time, where the number of observations and the time between the observations may vary between patients. All available serum creatinines taken beyond 6 months were used to obtain several time-dependent covariates. In a multivariate Cox proportional hazards model, cadaveric compared to living renal transplantation,

a lower reciprocal creatinine (RC) beyond 6 months and a lower ratio between RC and the RC at 6 months were independently associated with graft failure from CR or recurrent disease. This model was significantly better compared to a model with only time fixed parameters and therefore allows updates in prognosis during follow-up. In other words, renal dysfunction and a sharper decline in renal function beyond 6 months is more accurate predictor of subsequent graft failure compared to risk factors available at 6 months post-transplantation.

In our cohort, cyclosporine toxicity is the most important differential diagnosis of CR especially because of its therapeutic implications. In 1995-1996 we had to convert Sandimmune to Neoral, a micro-emulsion form of cyclosporine with a better bioavailability. Furthermore, a once-daily regimen with 24-hour target levels of 100 µg/l was changed to a twice-daily dosage aiming at a 12-hour target of 150 µg/l. Several patients developed a gradual decline in renal function and we decided to perform a retrospective cohort study to assess cyclosporine toxicity *(chapter 6).* Of 212 patients with a stable graft function pre-conversion clinical parameters at 1 and 12 months post-conversion were compared with those at time of conversion. The mean cyclosporine trough level rose from 87 ì g/l at the time of conversion to 139 µg/l at 12 months post-conversion whereas the daily drug dose increased over the same period from 233 mg to 252 mg. Mean serum creatinine increased by 10% from 135 to 148 µmol/l. Cyclosporine nephrotoxicity was defined in 42 patients (20%) as a significant decline of the reciprocal of the serum creatinine concentration over time post-conversion in the absence of other obvious causes for declining graft function. Biopsies performed in 10/42 patients showing arteriolar hyalinosis and stabilisation of renal function after dose reduction or switch to mycophenolate mofetil supported the diagnosis. The increased exposure to cyclosporine allowed a risk assessment of nephrotoxicity. Cyclosporine dose and trough level did not predict nephrotoxicity but the use of beta blockers or calcium channel blockers reduced the risk of nephrotoxicity, independent from their effect on blood pressure. Both drugs may counteract cyclosporine induced vasoconstriction, which is mediated by symphaticus activation. As result of this study, we abandoned the target drug levels and reduced the cyclosporine dose on clinical grounds. Area under the curve (AUC) monitoring using 0, 2 and 3 hour blood samples is currently under evaluation to improve immunosuppressive drug dosing.

Chronic allograft nephropathy (CAN) is defined and graded in the Banff ’97 scheme by the severity of interstitial fibrosis and tubular atrophy. It has been denoted that the diagnosis of chronic rejection requires typical vascular lesions, consisting of fibrointimal thickening *(chapter 1)*. As illustrated in the case

report we observed several patients who developed CAN without vascular changes or signs of cyclosporine toxicity and questioned the arguments for CR in this group of patients *(chapter 7)*. Therefore, we categorized CAN according the Banff CV score in a group with and without transplant vasculopathy and assessed their risk factor profiles. Fifty-four transplants had CAN defined by a significant decline in renal function together with interstitial fibrosis and tubular atrophy without signs of cyclosporine nephrotoxicity or recurrent disease. Using the Banff CV score, 23 of 54 cases (43%) had a chronic vasculopathy score of 0 or 1 whereas 31 cases (57%) had a CV score of 2 or 3. Applying multivariate logistic regression, predictor variables of the two groups were compared with 231 transplants with a stable function for at least five years. Late ARE after three months post-transplantation was the strongest risk factor for both forms of CAN. CAN with vasculopathy was also associated with transplants performed in the 1980s, and with creatinine clearance at 6 months. In contrast, young recipient age and pre-sensitization were the other independent risk factors for CAN without vasculopathy suggesting an immune pathogenesis. Disruption of the tubular basement membrane, herniation of tubular cells with differentiation into myofibroblasts, as consequence of persistent tubulitis has been shown by others to link ARE with chronic interstitial rejection in the absence of arterial injury. As demonstrated in humoral ARE, pre-sensitization might also correlate with humoral CR, but the presence of donor specific antibodies and C4d deposits in PTC, an in-situ marker for humoral immunity, was not assessed in this study. However, in the transplant recipient reported at the start of this thesis, C4d staining was retrospectively performed and appeared to be negative, suggesting dominance of the cellular immune response. Compatible with the findings in this patient, we conclude that vascular lesions are not a condition sine qua non for the diagnosis of CR. Finally, we studied the epidemiology and (immuno-) histological features of 18 patients with chronic transplant glomerulopathy (CTG) *(chapter 8)*. These patients had biopsies taken at 7.5 ± 3.5 years, on average 4 years later compared to 108 patients with CR without these glomerular lesions. Review of the histology revealed influx of mononuclear cells in the glomeruli in addition to the double contours of the glomerular basement membrane (GBM) together with a nonspecific immunofluorescence pattern. Multivariate analysis in comparison with 739 patients with a stable function revealed pre-transplant sensitization and ARE beyond 3 months as independent risk factors of CTG, similar to the risk profile of CR. Polyclonal anti-C4d antibody was used on paraffin sections for detection of C4d, a marker of humoral immunity. C4d stained positive in the glomeruli of 10/11 biopsies showing CTG in contrast to 1/14 positive biopsies in control patients with chronic rejection without CTG.

The risk factors and the presence of glomerular C4d deposits suggest a role of humoral immunity in the development of CTG. Additional results revealed that patients with glomerular C4d deposits had concomitant PTC deposits in 4/10 and donor specific anti HLA antibodies in 3/10 patients leaving the question open whether there are also tissue-specific antibodies involved. In a Fisher to Lewis rat model of CTG used in our centre IgG antibodies against the glomerular basement membrane (GBM) were found. Perlecan was identified as one of the antigens recognized. In clinical CTG, 9/13 sera contained antibodies directive to a non-Goodpasture antigen of the GBM. Preliminary results demonstrated that these antibodies might be reactive to agrin, a heparan sulphate proteoglycan with similar functions as perlecan. All C4d positive patients had anti-HLA and/or anti-GBM antibodies. Concerning the immunological risk factors, glomerular C4d deposits and presence of antibodies we concluded that CTG has to be considered a manifestation of humoral CR.

In this thesis, we assessed the risk profiles of different manifestations of CR. Table 1 summarizes the most important predictors of CR, defined by a significant decline in renal function and histological features of CAN in the absence of chronic cyclosporine nephrotoxicity and glomerulonephritis. Independent of the presence of early ARE, recipients with ARE occurring beyond three months had the highest risk to develop one of the CR manifestations. The development of CAN could be explained by a fibrogenic response to intermittent or persistent tubulointerstitial injury. This unfavourable ARE correlated with CREG mismatches. Therefore, we conclude that histoincompatibility results in CR via late ARE. Pre-sensitization increased the risk of CR independent of late ARE. It correlated with chronic interstitial rejection and transplant glomerulopathy, perhaps mediated by circulating donor-specific antibodies post-transplantation. The glomerular deposits of C4d provided support of humoral CR. Tissue-specific antibodies might be involved in CR, explaining the differences in histological presentation. Fibrous intimal thickening was associated with older donor age, putting the specificity of chronic

Table 1. Independent risk factors of 54 cases with chronic rejection in comparison with 231 transplants with a stable function

	OR (95% CI)	*P*
Recipient age (per 10 years increase)	0.71 (0.53-0.96)	0.025
Smoking cigarettes	2.14 (1.17-3.92)	0.006
Panel reactive antibodies (per 10% increase)	1.28 (1.09-1.48)	0.002
Last ARE > 3 months versus no ARE	12.6 (4.36.-36.7)	<0.001
Dipstick proteinuria >1+ @ 6 months (%)	4.77 (1.12-20.3)	0.034
ECC @ 6 months (10 ml/min increase)	0.78 (0.64-0.93)	0.006

Abbreviations: OR: odds ratio, CI: confidence interval, ARE: acute rejection episode, ECC: endogenous creatinine clearance

vascular lesions in doubt. On the other hand, early acute vascular rejection, in itself associated with HLA-DR mismatches, was also correlated with transplant vasculopathy suggesting a transition of the acute into the chronic form. The association of both class I and class II MHC mismatches and graft outcome warrants the current practice of HLA matching. Computer-based algorithms, such as HLAMatchmaker, that determine donor-recipient compatibility at the molecular level may better prevent CR despite a reduction in immunosuppressive drugs. Immune monitoring of both the cellular and humoral response post-transplantation may tailor treatment in the individual patient. We found that smoking cigarettes at time of transplantation increased the risk on CR by a factor 2. This habit should be stopped with the maximum aid of supportive care. Renal dysfunction, proteinuria and hypertension at 6 months post-transplantation and at time of the diagnostic biopsy were found as progression factors. Early and tight control of blood pressure and proteinuria, preferably with angiotensin converting enzyme and/or angiotensin II receptor blockers should prevent or postpone premature graft failure from CR.

This thesis concludes with the answers to the fundamental questions raised. What is CR? The CR concept includes a persistent or recurrent cellular and/or humoral alloimmune process resulting in a response to tissue injury. All anatomical compartments of the renal cortex can be affected by CR leading to transplant vasculopathy and/or glomerulopathy, and chronic interstitial rejection. Why does CR occur? CR may develop in patients with coexisting immunological risk factors and suboptimal immunosuppression. Young recipient age, histoincompatibility, sensitization, and especially late ARE explained the presence of CR in the case report. In patients with chronic transplant dysfunction, transplant physicians should take these risk factors into account as diagnostic tests influencing the chance on CR. Smoking, hypertension and proteinuria act as modifiable factors that accelerate progression. Multifactorial prevention and intervention strategies directed to these risk and progression factors are an achievable goal in our daily practice to prevent premature graft loss from CR.

10

Samenvatting

Samenvatting

Dit proefschrift start met de ziektegeschiedenis van een jonge vrouw die haar niertransplantaat verliest door chronische afstoting (CA). Ze werd in 1992 getransplanteerd met een nier afkomstig van een jonge man, overleden door een verkeersongeval. Ten tijde van transplantatie had ze 4% antistoffen tegen een panel van cellen met verschillende HLA antigenen. De donornier had 1-2-0 HLA-A,-B en DR mismatches. Ze werd behandeld met prednison en cyclosporine. De start was goed zonder perioden met acute afstoting. Na een half jaar had ze een uitstekend transplantaat functie zonder proteïnurie. Anderhalf jaar na transplantatie deed ze mee aan een onderzoek waarbij de prednison gestopt werd. Hierop ontstond een acute afstoting reactie *(hoofdstuk 1, figuur 2A)* die met succes behandeld werd met methylprednisolon. In het jaar daarop ging de nierfunctie geleidelijk achteruit *(hoofdstuk 1, figuur 1)* en werd proteïnurie vastgesteld. Een biopsie in 1995 liet gebieden van fibrose en tubulusatrofie zien *(hoofdstuk 1, figuur 2B)*. Glomeruli en bloedvaten waren niet afwijkend. Met een angiotensine converting enzym (ACE)-remmer nam de proteïnurie tijdelijk af. In 1997 verslechterde de nierfunctie verder en nam de proteïnurie weer toe. Een nieuw biopt van het transplantaat toonde zeer ernstige interstitiële fibrose en glomerulosclerose *(hoofdstuk 1, figuur 2C)*. In 1998, 6 jaar na transplantatie, moest ze opnieuw behandeld worden met hemodialyse.

Wat is CA en hoe kan het zich manifesteren? Wat zijn bij deze patiënt de positieve argumenten om de diagnose CA te stellen? Om deze vragen te beantwoorden onderzochten we de klinische, histologische en epidemiologische verschijnselen van CA in het Leidse cohort van niertransplantaties. Omdat de aanwezigheid van CA niet eenvoudig is vast te stellen is de term door velen vervangen door de neutralere term chronische transplantaat (allograft) nefropathie (CAN). Aan het begin van dit proefschrift *(hoofdstuk 1)* werden de definities van de verschillende begrippen uiteengezet. CA is een persisterende of intermitterende afweerreactie tegen vreemde antigenen die leidt tot CAN en functieverlies met uiteindelijk transplantaatverlies. CAN staat voor de aanwezigheid van schade in het biopt die bestaat uit intima fibrose van arteriën, interstitiële fibrose en tubulus atrofie. CTD staat voor chronisch transplantaat disfunctie, een syndroom dat geleidelijk nierfunctieverlies, stijging van de bloeddruk en ontstaan van proteïnurie behelst. Het woord ‘chronisch’ in deze definities heeft verschillende betekenissen, die te maken hebben met tijd (laat of langzaam), het onderliggende proces (voortdurend of terugkerend) en met schade in het biopt (fibrose en atrofie). Deze omschrijvingen correleren met respectievelijk de begrippen CTD, CA en CAN. Het feit dat CA in de regel

leidt tot CAN en CTD is een illustratie dat alle drie betekenissen van 'chronisch' meestal naast elkaar van toepassing kunnen zijn. Bovendien is het begrip 'chronisch' breed ingeburgerd in het medische jargon en wordt ook door patiënten juist verstaan. Voor de pathologische veranderingen in een niertransplantaat is in 1991 de Banff classificatie opgesteld *(hoofdstuk 1)*. In de herziening van 1997 *(hoofdstuk 1, tabel 1)* is CAN ingedeeld op basis van de ernst van interstitiële fibrose en tubulus atrofie. In een noot wordt vermeld dat de aanwezigheid van transplant vasculopathie, dat is nieuw ontstane vaatwand verdikking en/of transplant glomerulopathy, gekenmerkt door verdubbeling van de glomerulaire basaal membraan, nodig zijn voor het stellen van de pathologische diagnose CA. Klinisch moet CA onderscheiden worden van andere oorzaken van CTD *(hoofdstuk 1, figuur 3)*. De differentiaal diagnose bestaat vooral uit een laat optredende acute afstoting, toxiciteit van de calcineurine remmers cyclosporine en tacrolimus en terugkeer van de oorspronkelijke nierziekte. Net als in eigen nieren kan in een transplantaat nier nefrosclerose ontstaan. Dit is een multifactorieel bepaalde aandoening die laat na transplantatie kan optreden bij langer bestaande hypertensie, hyperlipidemie, overgewicht, roken, atherosclerose in de ontvanger en een oude donor leeftijd. Bij ernstige vernauwing van de arterie iliaca of de transplantaatarterie kan ischemische schade ontstaan. De laatste jaren werd bekend dat bij gebruik van sterke immunosuppressieve middelen het BK virus, een polyomavirus, op kan vlammen en nefropathie kan veroorzaken. Alle genoemde aandoeningen leiden tot CAN en kunnen naast elkaar voorkomen. In de beschreven patiënt werd de diagnose CA gesteld ondanks de afwezigheid van de meer specifieke vasculaire en glomerulaire afwijkingen. De aanwezigheid van immunologische risicofactoren, CAN in het biopt en progressief nierfunctieverlies waren samen met de afwezigheid van andere oorzaken van CTD de positieve argumenten voor CA. Dit proefschrift had als doel de risicofactoren voor CA vast te stellen *(hoofdstuk 2, 3, 4, 7 en 8)*, transplantaat verlies beter te leren voorspellen *(hoofdstuk 2, 4 en 5)*, en om cyclosporine toxiciteit *(hoofdstuk 6)* en de verschillende presentatievormen van CA *(hoofdstuk 7 en 8)* in kaart te brengen. De resultaten van de diverse studies moeten bijdragen aan het inzicht in het ontstaan van CA.

De risicofactoren voor CA werden vastgesteld in een bestand van 654 niertransplantaties, uitgevoerd in Leiden tussen 1983 en medio 1996, die minstens 6 maanden functioneerden *(hoofdstuk 2)*. Alle late (> 6 maanden) biopten werden geblindeerd voor de klinische informatie opnieuw gescoord volgens de Banff classificatie. Gedurende de follow-up tot 1 januari 1997 trad in 224 gevallen transplantaat verlies op, vooral doordat de patiënt overleed met een nog functionerende nier (53%). Na uitsluiting van andere oorzaken

kon aangenomen worden dat 82 transplantaatnieren (36%) door CA verloren waren gegaan. Deze groep vormde het primaire eindpunt in de risicofactor analyse. Alle relevante gegevens van ontvanger, donor, transplantatie en het beloop in de eerste 6 maanden werden verzameld en getest als risicofactor voor transplantaat verlies door CA met behulp van het Cox model. De invloed van HLA matching en acute afstoting op CA werd in detail bestudeerd. Het HLA systeem bestaat uit vele antigenen die op basis van kleine verschillen verder uitgesplitst kunnen worden. Er zijn echter ook overeenkomstige aminozuurvolgordes tussen de verschillende HLA–A en –B (klasse I) antigenen die eenzelfde antistofreactie oproepen en daarom kruisreagerende groepen (CREG) worden genoemd. De HLA verschillen tussen donor en ontvanger bleken niet gecorreleerd te zijn met CA. De HLA overeenkomsten tussen donor en ontvanger, vooral op het niveau van de CREG waren wel van invloed op het optreden van CA. De verklaring voor deze discrepantie werd gevonden door een kruistabel te maken tussen de HLA verschillen en de overeenkomsten. Door een hoger percentage homozygoten in de donoren, het gevolg van het beleid van Eurotransplant om de ontvanger te selecteren op basis van zo min mogelijk verschillen, bleek de relatie tussen verschillen en overeenkomsten niet wederkerig aan elkaar te zijn. Klaarblijkelijk is de mate van overeenkomst tussen donor en ontvanger van belang voor het verminderen van de afweerrespons tegen de verschillen. Acute afstoting is in de literatuur bekend als de belangrijkste risicofactor voor CA. Een eerdere studie uit Leiden had al aangetoond dat een acute vasculaire afstoting een slechte prognose heeft. Deze bevinding werd bevestigd met het verhoogde risico van acute vasculaire afstoting op laat transplantaatverlies. Ook het vaker en het late optreden van acute afstoting bleek van belang te zijn voor het risico op CA. Jongere transplantatiepatiënten hebben door een sterker afweersysteem, maar ook omdat ze meer geneigd zijn hun medicijnen slechter in te nemen, een verhoogd risico op CA. De relatie tussen oude donoren en transplantaatverlies is te verklaren door de afgenomen hoeveelheid functionerende nefronen en het feit dat ze vaker een acute afstoting doormaken waarbij het weefselherstel waarschijnlijk minder gunstig verloopt. Verder werd vastgesteld dat rokers en patiënten met nefrosclerose als basislijden, wellicht door meer atherosclerose en hypertensie in de ontvanger, hun transplaat eerder verliezen. De beschreven patiënt was jong, had maar 1 uit een maximum van 8 CREG die overeenkwam met die van de donor, en maakte een late acute afstoting door; drie risicofactoren die het optreden van CA kunnen verklaren.

Volgens deze eerste studie is bij de patiënt de late acute afstoting waarschijnlijk de sterkste risicofactor voor CA geweest. In de studie periode kwam acute afstoting nog veel voor, in 384 van de 654 transplantaties (59%). Niet elke

acute afstoting leidt echter tot CA en transplantaat verlies. De eerste studie liet zien dat zowel het aantal, het type en het tijdstip van optreden van belang zijn. Wij vonden het meeste contrast in prognose door te kijken naar het tijdstip van de laatste in plaats van de eerste afstoting *(hoofdstuk 2)*. In 297 van de 384 transplantaties (77%) traden de afstotingen alleen vroeg op, dat wil zeggen binnen 3 maanden na transplantatie. In de overige 87 gevallen (23%) vond de laatste afstoting plaats na 3 maanden *(hoofdstuk 3)*. Tien jaar na transplantatie, waarbij andere oorzaken van transplantaatverlies dan CA beschouwd waren als einde follow-up, functioneerden nog 94, 86 en slechts 45% van de transplantaten met achtereenvolgens geen, dan wel vroege of late acute afstoting. In de groep met late afstoting was de prognose niet afhankelijk van afstoting in de eerste 3 maanden. De groepen met vroege en late afstoting werden vergeleken met de groep zonder afstoting door middel van logistische regressie. Een slecht op gang komende transplantaat functie en HLA-DR mismatches waren de onafhankelijke risicofactoren voor vroege afstoting. Dit past bij het idee dat vroeg na transplantatie de afweer reactie zich volgens de zogenaamde directe route vooral richt tegen HLA-DR moleculen op dendritische cellen van de donor die met het transplantaat meekomen. Naarmate deze donorcellen verdwijnen, dooft dit type afweerreactie uit. Late afstoting was geassocieerd met andere risicofactoren: een jonge leeftijd van de ontvanger, een donornier afkomstig van een oudere persoon of een vrouw, en met CREG mismatches. Dit risicoprofiel komt goed overeen met die van CA hetgeen duidt op een sterke relatie tussen late acute afstoting en CA met een rol voor de afweerreactie volgens de indirecte route. Hierbij worden vreemde antigenen uit het transplantaat door dendritische cellen van de ontvanger verwerkt en als eiwitfragmenten gepresenteerd aan T cellen. Dit kan leiden tot een cellulaire afweerreactie of via de productie van antistoffen tot een humorale vorm van CA. Late afstoting kan alleen ontstaan via deze fysiologische route die minder gevoelig is voor immunosuppressieve behandeling in vergelijking met de directe route. Dit verklaart het gegeven dat de enorme afname van het percentage acute afstoting met het gebruik van sterkere middelen in de afgelopen jaren niet gepaard is gegaan met een evenredige afname van CA.

De gevonden risicofactoren kunnen op verschillende manieren bijdragen aan nierfunctie verlies. Ze kunnen invloed hebben op een slechte uitgangswaarde en op de mate van nierfunctieverlies. Daarom werden de risicofactoren voor een lage intercept, gedefinieerd als een klaring van minder dan 50 ml/min op 6 maanden vergeleken met de factoren die bijdragen aan een negatieve helling van de 1000/creatinine waarden in de loop van de tijd meer dan 6 maanden na transplantatie *(hoofdstuk 4)*. Tweehonderd van de 654 patiënten (31%) hadden een suboptimale functie op 6 maanden. Dit bleek samen te hangen met een donor nier van een oudere persoon of een vrouw, histoincompatibiliteit, een slecht

op gang komende transplantaat functie en acute afstoting. Vierenveertig procent (288/654) van de transplantaten toonde een significant nierfunctieverlies na 6 maanden. De associatie met een jonge leeftijd van de ontvanger, sensitisatie, histoincompatibiliteit, immunosuppressie, en late acute afstoting suggereren een onderliggend immunologisch proces. De invloed van HLA matching en late afstoting op zowel de intercept als de helling ondersteunen de gevonden relatie tussen late acute afstoting en CA. De negatieve invloed van hypertensie en proteïnurie op nierfunctieverlies na 6 maanden passen bij hun rol als progressiefactor.
Om de voorspelling op laat transplantatieverlies te verbeteren werd het eerder gebruikte multivariate Cox model met variabelen die bekend zijn op 6 maanden na transplantatie *(hoofdstuk 2)* uitgebreid met tijds-afhankelijke variabelen *(hoofdstuk 5)*. Dit zijn parameters die gemeten worden met een wisselend interval tussen de bepalingen en bij elke patiënt in wisselende mate. Wij gebruikten hiervoor alle creatinine waarden afgenomen na 6 maanden. De reciproque creatinine (1000/creatinine) werd gebruikt als maat voor de absolute nierfunctie en de ratio tussen de actuele en reciproque creatinine op 6 maanden als maat voor het nierfunctieverlies. In het nieuwe model met deze parameters werd transplantaatverlies door CA of recidief nierziekte beter voorspeld dan het model met variabelen die op 6 maanden bekend zijn. Het beloop van het serum creatinine, als maat voor absolute nierfunctie en nierfunctieverlies, is de beste indicator voor toekomstig transplantaatverlies. Dergelijke modellen zouden gebruikt kunnen worden om op basis van actuele gegevens de prognose beter te kwantificeren.
Cyclosporine toxiciteit is de belangrijkste differentiaal diagnose van CA, vooral vanwege de therapeutische consequenties. In 1995 en 1996 werd bij alle patiënten Sandimmune vervangen door Neoral, een micro-emulsie vorm van cyclosporine met een verbeterde biologische beschikbaarheid. Omdat de dosering veranderde van eenmaal naar tweemaal daags werd de streef dalspiegel verhoogd van 100 (24 uur) naar 150 µg/l (12 uur). Omdat bij diverse patiënten pas na verloop van tijd nierfunctieverlies optrad werd besloten om de gevolgen van deze conversie in de hele groep te onderzoeken *(hoofdstuk 6)*. Van 212 patiënten met een stabiele nierfunctie voor conversie, werden de klinische gegevens van 12 maanden na de conversie vergeleken met de waardes ten tijde van de switch. De gemiddelde dalspiegel steeg van 87 naar 139 µg/l en de dagdosis van 233 naar 252 mg. Het creatinine nam met 10% toe van 135 naar 148 µmol/l. De toegenomen cyclosporine expositie gaf de gelegenheid om de risicofactoren voor nefrotoxiciteit na te gaan. Tweeënveertig patiënten (20%) hadden significant nierfunctieverlies na de conversie. Biopsieën, gedaan bij 10/42 patiënten, toonden arteriolaire hyalinose en glomerulosclerose. Nefrotoxiciteit bleek niet gerelateerd te zijn aan dosis of dalspiegel. Het gebruik van betablokkers of calcium entry blokkers beschermde

tegen toxiciteit, onafhankelijk van hun bloeddruk verlagende effect, mogelijk omdat beide middelen vasoconstrictie tegengaan. Naar aanleiding van deze studie werd bij klinische tekenen van toxiciteit, zoals nierfunctieverlies, hypertensie, hypercholesterolemie, jicht en tandvleeshyperplasie, de cyclosporine dosis verlaagd onafhankelijk van de dalspiegel. De verwachting is dat periodieke monitoring van de cyclosporine opname door metingen van de serumspiegel, voor en 2 en/of 3 uur na inname van een dosis, toxiciteit beter kan voorkomen.
In de Banff classificatie wordt CAN ingedeeld naar de ernst van chronische tubulointerstitiele schade, waarbij wordt vermeldt dat pas van CA kan worden gesproken bij aanwezigheid van intimafibrose van de arteriën. Omdat in een aantal gevallen, waaronder de beschreven patiënt, deze vaatafwijkingen niet aanwezig waren, ontstond de vraag of CA kan optreden zonder transplant vasculopathie *(hoofdstuk 7)*. Vierenvijftig patiënten met CAN, gedefinieerd door significant nierfunctieverlies zonder tekenen van cyclosporine toxiciteit of recidief nierziekte, werden in twee groepen verdeeld op basis van de Banff CV score. Drieëntwintig patiënten (43%) zonder of met slechts lichte intima fibrose en 31 patiënten (57%) met matige tot ernstige intima fibrose werden vergeleken met 231 patiënten met een stabiele functie van meer dan 5 jaar. Transplant vasculopathie werd voorspeld door late afstoting, acute vasculaire afstoting, transplantatie in de tachtiger jaren maar ook door hogere donor leeftijd. Bij CAN zonder belangrijke vasculopathie waren een jonge leeftijd van de ontvanger, de aanwezigheid van panel reactieve anti-HLA antistoffen voor transplantatie en vooral late acute afstoting de risicofactoren die een immunologische pathogenese aannemelijk maken. Met deze gegevens kon de conclusie getrokken worden dat transplant vasculopathie geen conditie sine qua non is voor CA.
Tenslotte werden de klinische, epidemiologische en histologische verschijnselen van 18 patiënten met chronische transplant glomerulopathie (CTG) bestudeerd afkomstig uit een uitgebreid cohort van 1111 transplantaties *(hoofdstuk 8)*. Deze diagnose werd gemiddeld 7,5 jaar na transplantatie gesteld, 4 jaar later dan een controle groep van 108 patiënten met CA zonder CTG. Bestudering van de histologie toonde naast verdubbeling van de glomerulaire basaal membraan (GBM) influx van mononucleaire cellen en een aspecifiek immunofluorescentie patroon. In vergelijking met 739 patiënten met een stabiele nierfunctie bleek dat panel reactieve anti-HLA antistoffen voor transplantatie en late acute afstoting de onafhankelijke risicofactoren zijn die duiden op een immunologische pathogenese. Parafine coupes werden gekleurd met een polyclonaal antilichaam gericht tegen C4d, een afbraak product van complementfactor C4, dat covalent bindt aan weefsel en daarmee een aanwijzing is voor humorale afstoting. In 10/11 biopsieën werden glomerulaire C4d neerslagen

gevonden in een capillair patroon in tegenstelling tot 1/14 biopsieën met CA zonder CTG. Vier van de 10 patiënten met glomerulaire C4d neerslagen hadden ook deposities in peritubulaire capillairen en in 3/10 werden donor specifieke anti-HLA antistoffen gevonden. Dit zou een aanwijzing kunnen zijn voor het bestaan van weefsel specifieke antilichamen. Omdat in een ratten model voor CTG door medewerkers van ons laboratorium antilichamen gevonden waren tegen antigenen uit de GBM werd dit ook bij patiënten met CTG nagegaan. In 9/13 sera bevonden zich ook antilichamen tegen GBM isolaten en in 2/10 controles. Voorlopige resultaten wijzen op een reactie met agrine, een heparan sulfaat proteoglycaan. Alle C4d positieve patiënten hadden antistoffen tegen HLA of GBM. Op basis van de gevonden risicofactoren en glomerulaire C4d deposities luidt de slotsom dat CTG een manifestatie is van CA.

In dit proefschrift hebben we ons vooral gericht op de risicofactoren voor CA *(hoofdstuk 9, tabel 1)*. Uit de studies kwam naar voren dat patiënten die een acute afstoting doormaken meer dan 3 maanden na transplantatie de grootste kans hebben op CA, ongeacht de uitingsvorm. Deze late afstoting loopt via de indirecte route die een indolenter en meer voortdurend karakter heeft dan vroege afstoting via de directe route. Het gegeven uit de literatuur dat persisterende tubulitis via herniatie van tubuluscellen met transdifferentiatie naar myofibroblasten kan leiden tot interstitiële fibrose in CAN heeft het inzicht in het ontstaan van CA verder vergroot. Jonge ontvangers krijgen door een sterker afweersysteem vaker late afstoting en ook onafhankelijk hiervan CA. Histoincompatibiliteit op CREG niveau predisponeert voor late afstoting en CA. Onafhankelijk van late afstoting is sensitisatie een risicofactor voor CA. Dit geldt vooral voor chronische interstitiële afstoting en CTG en is wellicht gemedieerd via een humorale afweerreactie na transplantatie. De glomerulaire C4d deposities in CTG vormden de beste aanwijzing voor een humoraal mechanisme waarbij er aanwijzingen zijn dat antilichamen gericht zijn tegen het GBM eiwit agrine. Transplant vasculopathie was geassocieerd met donor leeftijd en kan daarom niet beschouwd worden als een voor CA specifieke afwijking. Acute vasculaire afstoting, vaker voorkomend bij HLA-DR verschillen, was voornamelijk in de jaren 80 een risicofactor voor chronische vasculaire afstoting. Uit dit onderzoek blijkt dat klasse I en II histoincompatibiliteit via respectievelijk late en vasculaire acute afstoting bijdraagt aan CA. HLA matching is daarom waardevol in het voorkomen van CA, zeker als het mogelijk wordt om bij een donoraanbod ontvangers te selecteren op HLA antigenen, die geen of weinig afweer oproepen. De verwachting is dat de monitoring van donor specifieke cellulaire en humorale afweer na transplantatie een belangrijk hulpmiddel wordt om de immunosuppressieve behandeling op de individuele patiënt af te stemmen. Verder werd gevonden dat

rokers een twee maal zo grote kans hebben op CA. Intensieve counseling is nodig om (toekomstige) transplantatiepatiënten van deze gewoonte af te helpen. De studies bevestigden dat ook bij transplantatiepatiënten de prognose in belangrijke mate bepaald wordt door nierfunctie, bloeddruk en proteïnurie zowel na 6 maanden als ten tijde van het diagnostische biopt bij CTD. Vroege en scherpe controle van bloeddruk en proteïnurie, bij voorkeur met ACE- en angiotensine II receptor remmers moeten een bijdrage kunnen leveren aan het voorkomen of uitstellen van nierfalen door CA.

Dit proefschrift eindigt met de antwoorden op de gestelde vragen. CA is een voortdurende of recidiverende cellulaire en/of humorale immuun respons met een fibroserende ontstekingsreactie op weefselschade. CA presenteert zich klinsich als CTD en manifesteert zich histologisch als CAN met of zonder transplant vasculopathie en/of glomerulopathie. CA ontstaat in patiënten met een immunologisch risicoprofiel en suboptimale immunosuppressieve medicatie. Jonge leeftijd van de ontvanger, sensitisatie, histoincompatibiliteit en vooral late acute afstoting zijn de belangrijkste risicofactoren zoals die ook bij de beschreven patiënt voorkwamen. Deze risicofactoren zouden bij patiënten met CTD beschouwd moeten worden als diagnostische tests die de kans op CA bepalen. Roken, hoge bloeddruk en proteïnurie zijn beïnvloedbare factoren die de progressie van nierfunctieverlies bevorderen. Preventie en interventie strategieën gericht op de nu bekende risico- en progressiefactoren moeten vroegtijdig transplantaatverlies door CA steeds beter kunnen voorkomen.

Nawoord

In september 1996 begon ik op de afdeling Nierziekten met de opleiding tot nefroloog. Het was vanzelfsprekend om de kliniek te gaan combineren met de wetenschap. Onder leiding van professor van Es startte ik met het onderzoek 'chronische afstoting na niertransplantatie'. Dit onderwerp was mede ingegeven door Hans de Fijter die op de klinische relevantie van dit probleem had gewezen. De centrale vraagstelling werd duidelijk vanuit de individuele patiëntenzorg op de polikliniek Niertransplantatie. Waarom ging bij een te groot aantal patiënten na verloop van tijd de transplantaatfunctie verloren? Omdat in de biopten van deze patiënten meestal chronische schade werd gezien zonder duidelijke afstotingsverschijnselen werd dit fenomeen chronische transplant disfunctie of chronische transplant nefropathie genoemd. Als internist was het mij duidelijk dat dit geen diagnose is maar een syndroom waar een differentiaal diagnose bij hoort. Ik moest dus op zoek gaan naar de positieve argumenten voor het bestaan van chronische afstoting. Hiervoor was onderzoek nodig naar de risicofactoren en de histologische afwijkingen bij patiënten met chronische transplant disfunctie. Het eerste jaar van het onderzoek werd besteed aan het opbouwen van een uitgebreid bestand met patiëntengegevens. De basis hiervoor kwam uit het bestand van Leidse transplantatie patiënten dat door Marko Mallat beheerd wordt. Voor het verkrijgen en analyseren van de andere data werd samengewerkt met verschillende afdelingen binnen het LUMC. Voor het onderzoek was het essentieel om goed te kijken naar biopten die laat na transplantatie verkregen waren. Via Jan Anthonie Bruijn van de afdeling Pathologie kreeg ik in de persoon van Folkert van Kemenade een maatje. Samen hebben we heel wat dagen biopten gescoord volgens de Banff classificatie. Zo werd de groep patiënten met 'chronische afstoting' vastgesteld. Met Ilias Doxiadis, Peter de Lange en Frans Claas van de Immunohematologie kon vervolgens de invloed van Het Leidse Antigeen (HLA) op chronische afstoting worden bestudeerd. Voor het vaststellen van de risicofactoren kreeg ik de hulp van Koos Zwinderman van de Medische Statistiek, die mij de kneepjes van de multivariaat analyse leerde. Verder maakte hij van elke patiënt een grafiek met alle creatinines vanaf 6 maanden na transplantatie waarmee patiënten met nierfunctieverlies, de 'progressors', werden geïdentificeerd. Samen met Mark de Bruijne werd ook nog de invloed van het nierfunctieverloop op transplantaatverlies gemodelleerd. Epidemioloog Rudi Westendorp gaf waardevolle adviezen voor de studieopzet. Met de komst van Leen Paul kreeg het onderzoek verdere verdieping. Allereerst werd het 'intercept en slope' concept uitgewerkt. Vervolgens werd in het laboratorium Nierziekten door Simone Joosten onderzoek voortgezet met een door hem meegenomen rattenmodel voor chronische afstoting. De resultaten die zij hiermee verkreeg

konden in essentie gereproduceerd worden bij patiënten waarmee het inzicht in het ontstaan van transplant glomerulopathie vergroot werd. Met grote inzet verzamelde studente Man-Chi Wong de klinische gegevens voor dit laatste project. Mede door de technische ondersteuning van Klaas van der Ham is dit proefschrift meer geworden dan de som der delen.
Met het onderzoek zijn de risicofactoren en de verschillende manifestaties van chronische afstoting goed in kaart gebracht. Door de resultaten zijn we beter in staat de diagnose 'chronische afstoting' te stellen en zijn we ons meer bewust van de maatregelen die nodig zijn om voortijdig transplantaatverlies te voorkomen. Dit zou tevens een gunstig effect moeten sorteren voor patiënten op de wachtlijst voor niertransplantatie. Zo wordt met dit onderzoek hopelijk een bijdrage geleverd aan een goede levenskwaliteit van de individuele transplantatiepatiënt.
Ik zie uit naar de verdediging van het promotieonderzoek. Net zoals in het dagelijks werk staan Marijke van Gurp en Paul van der Boog daarbij als gewaardeerde paranimfen aan mijn zijde. Naar de toekomst gekeken blijft voor mij de uitdaging van de elkaar stimulerende academische trias 'kliniek, onderwijs en wetenschap' bestaan. De inspiratie hiervoor heb ik leren ervaren in het contact met fellows, assistenten en studenten.
Feitelijk was al het extra werk wat nodig was voor deze promotie moeilijk te rijmen met de verantwoordelijkheid voor een groot gezin. Zonder de onevenredig grote inzet van Marie-Christine was de voltooiing dan ook niet mogelijk geweest. Ik hoop dat de afronding van dit onderzoek juist voor haar en de jongens een promotie zal betekenen.

Curriculum vitae

1960	Geboren te Utrecht
1979	Eindexamen Atheneum, college Hageveld, Heemstede
1987	Artsexamen, Universiteit Leiden
1987 - 1988	Militaire Bloedtransfusiedienst Amsterdam en afdeling Weefseltypering, Centraal laboratorium van de Bloedtransfusiedienst, Amsterdam
1989 - 1990	Assistent Interne Geneeskunde, afdeling Hemodialyse (Dr A.P. Roodvoets), Kennemer Gasthuis, Haarlem
1990 - 1994	Opleiding Interne Geneeskunde (Dr E.J. Buurke), Westeinde Ziekenhuis, Den Haag
1994 - 1996	Opleiding Interne Geneeskunde (Prof. Dr. A.E. Meinders), Leids Universitair Medisch Centrum (LUMC)
1996	Registratie als internist
1996 - 2000	Opleiding Nierziekten (Prof. Dr. L.A. van Es en Prof. Dr. L.C Paul), afdeling Nierziekten, LUMC
2000	Registratie als internist-nefroloog
2000 - heden	Sectiehoofd polikliniek Nierziekten en Niertransplantatie, afdeling Nierziekten, LUMC

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