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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 $\mu\text{mol/l}$, 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 $\mu\text{mol/l}$ 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 $\mu\text{mol/l}$ her creatinine level gradually increased again and she developed 3.7 g/day of

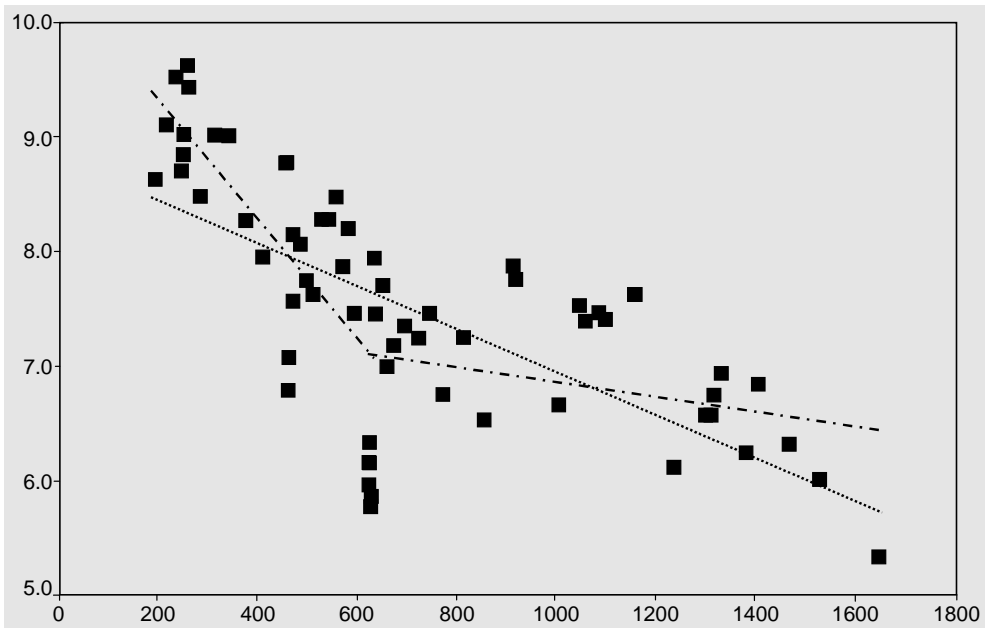


Figure 1 Transplant recipient with graft loss from chronic rejection. Gradual decline in renal function, expressed as a negative slope of the reciprocal creatinines (1000/serum creatinine) over time (days post-transplantation) curve.

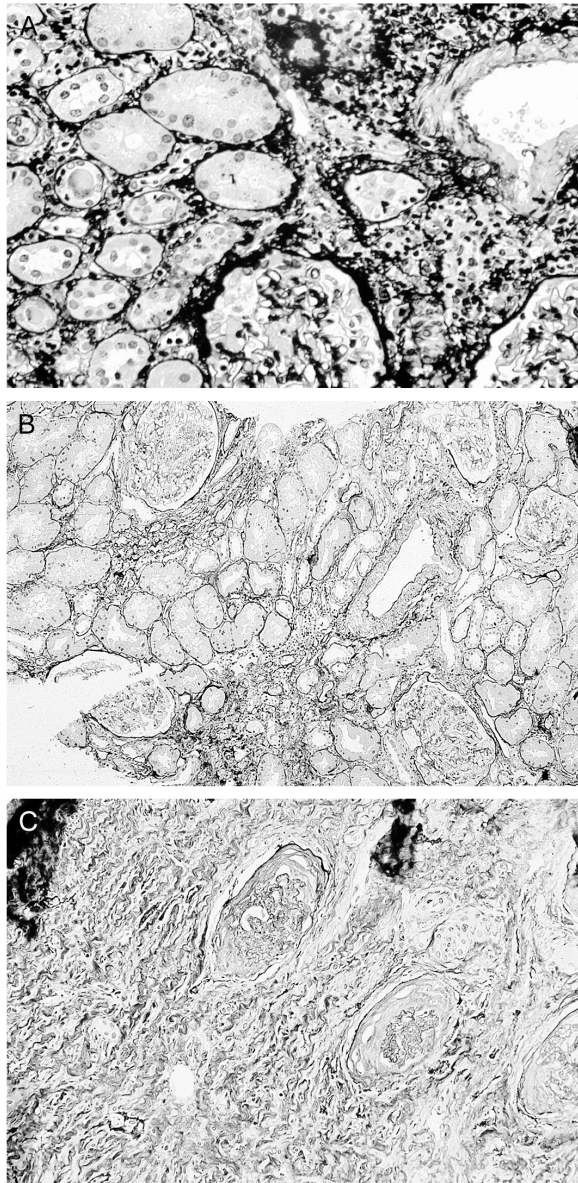


Figure 2 Three consecutive biopsies of a transplant recipient with graft loss from chronic interstitial rejection. (A) Acute rejection episode, one and a half years after transplantation, following prednisone withdrawal. The histology is characterized by an interstitial infiltrate and tubulitis. The 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 hyperparathyroidism 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

Table 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% luminal 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 luminal area*
cv3	Severe vascular changes with >50% narrowing of vascular luminal 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 cytokeratin 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 IV α 3 and laminin β 2 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 hypertriglyceridemia in 30 to 40% of transplant patients (117). Hypertriglyceridemia 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 smoke 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 incomppliance 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

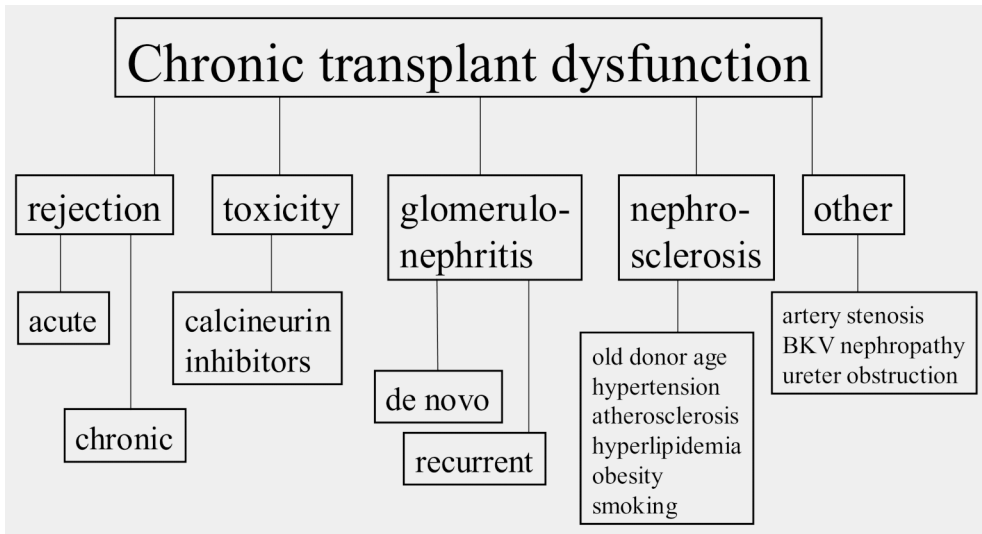


Figure 3 Differential diagnosis of chronic transplant dysfunction

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 hemorrhagic 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). Rapamycin (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 sequelae 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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