

Diagnosis and prevention of chronic kidney allograft loss

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Abstract: Background: Kidney transplantation is the best possible treatment for many patients with end-stage renal failure. Chronic, progressive, and irreversible loss of a transplanted kidney function, previously named chronic allograft nephropathy (CAN), is the leading cause of chronic allograft failure among kidney transplant recipients and eventual allograft loss with return to dialysis is associated with increased mortality and morbidity. CAN is a generic term of all causes of chronic kidney allograft nephropathy associated with fibrosis. It is clinically characterized by a gradual worsening of renal function in the presence of arterial hypertension and low-grade proteinuria. Histological changes of CAN usually precede functional deterioration and include interstitial fibrosis/tubular atrophy accompanied by vascular changes and glomerulosclerosis. Both immunological and non immunological factors can be responsible for CAN. Immunological causes include chronic active antibody-mediated and T cell-mediated rejection. Non immunological factors include brain death in the donor, increasing donor age, ischemia-reperfusion injury, calcineurin inhibitor nephrotoxicity, hypertension, diabetes mellitus, hyperlipidemia, chronic obstruction and chronic viral infections. Even if the contributing factors to CAN can be identified, not all of them can be interrupted prior to and after grafting. Preventive strategies include improvements in medical and surgical strategies to reduce ischemia-reperfusion injury, strategies to minimize acute rejection and strategies aiming for HLA-matched transplants. Additional measures include tight control of blood pressure, proteinuria, lipids and glucose. Antivirus treatment, appropriate diet, weight control, no smoking and good compliance are also suggested in certain settings. **Conclusion:** Evidence-based treatment strategies for CAN are lacking, but several prevention and management strategies are recommended in clinical practice. Recommended proactive preventive measures are control of hypertension, proteinuria, dyslipidaemia, diabetes, smoking, and other comorbidities. Strategies to maintain transplant function and improve long-term graft survival are important goals of translational research.

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Introduction

Previous studies have indicated that the rate of decline in allograft function after kidney transplantation has decreased, suggesting that stable, long-term function may be achievable [1,2]. Therefore, it is important to assess the components of deteriorating graft that can be treated [3].

Despite advances in transplantation reducing early acute rejection rates to less than 15% and lifting 1-year graft survival higher than 90%, long-term graft attrition rates have remained unchanged at 4% loss per year [4]. Chronic allograft dysfunction (CAD), previously named chronic allograft nephropathy (CAN), is a multifactorial process associated with progressive fibrosis and tubular atrophy [5].

CAN is characterized by a relatively slow but variable rate of decline in renal function after first 3 months of RT, often in combination with proteinuria and hypertension [6]. CAN should be differentiated from other causes of transplant dysfunction such as

rejection (acute, subclinical, and chronic), calcineurin inhibitor (CNI) nephrotoxicity, glomerulonephritis (recurrent and de novo), nephrosclerosis (secondary to old donor age, recipient hypertension, hyperlipidaemia, and smoking), and others (ureteric obstruction, BK virus nephropathy, and transplant renal artery stenosis) [5, 7].

Schweitzer et al. from Minnesota in 1991 reported, in a cohort of 2396 patients over a period of 20 years (1970–1989), chronic rejection as the leading cause of graft loss following renal transplantation (RT) amounting to 24%, followed by death with functioning graft (18%), infection (13%), and acute rejection (11%) [8].

More lately, **Sijkens et al.** from Netherlands reported that 54 of the 654 (8%) RTs performed between 1983 and 1997 had histological evidence of CAN and CAN accounted for 37% of graft loss after first 6 months post-RT [9]. **Naesens et al.** have reported that the global burden of early chronic

histological damage within the first year after transplantation significantly affected the long-term survival of the allografts [10]. Currently, chronic antibody-mediated rejection from both anti-human leucocyte antigen (HLA) antibodies and non-HLA antibodies is being recognized as an important cause of CAN [11, 12].

This review was designed to elucidate diagnosis and prevention of chronic kidney allograft loss.

Risk factors for late graft loss:

The major causes of renal transplant loss are death from vascular, malignant or infectious disease, and loss of the allograft from chronic renal dysfunction associated with the development of graft fibrosis and glomerulosclerosis. CAN is the histologic description of the fibrosis, vascular and glomerular damage occurring in renal allografts [13-22].

Late loss of organ transplants is a major problem in transplantation [23, 24]. These factors can be separated into 3 clusters: 1) alloantigen-dependent-factors, 2) innate defense reaction to tissue damage that is present before transplantation or is a result of the ischemic injury at the time of transplantation, and 3) nonimmunologic factors, such as donor age, brain death, and other issues specific to the deceased donor, and posttransplantation factors in the recipient, such as viral infections (eg, BK polyoma virus, cytomegalovirus), hypertension, drug toxicity, such as calcineurin inhibitors, and hyperlipidemia [25-27].

Death with a functioning graft and CAN are the major causes of late graft loss. The prevalence of CAN is as high as 60–70% on protocol biopsies after the 1st year [28]. However, CAN defined by interstitial fibrosis and tubular atrophy is probably the result of several different immunologic and non-immunologic processes. Studies of the natural history of CAN have suggested that it may result from immunologic causes during the 1st year and non-immunologic causes, particularly calcineurin inhibitor (CNI) toxicity thereafter (Fig. 1 and Table 1). There is a growing need to discriminate among the different causes of CAN and to elucidate the pathogenesis of CAN [29].

1. Immune-mediated factors

Acute rejection has been recognized as one of the most important risk factors for chronic rejection [30]. Numerous studies indicated that acute rejection, the time of occurrence, and the number of episodes were all associated with an increased risk of graft loss, but less is known regarding the severity of rejection [31].

Factors contributing to ongoing alloimmune responses include breakdown in immunosuppression as a result of patient non compliance, therapeutic decisions to minimize exposure to complications of immunosuppressive drugs or increased HLA mismatches [32]. The deleterious long-term impact of

cytotoxic anti-HLA antibodies that develop after transplantation is another factor supporting immunological involvement in chronic rejection [33].

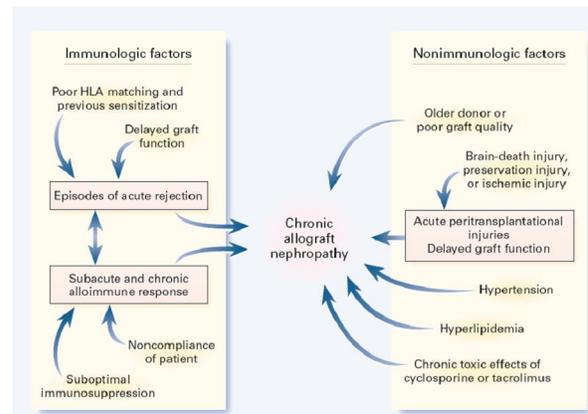


Figure 1: Causes and pathogenesis of chronic allograft nephropathy (CAN) (Fadili *et al.*, 2013)

2. Non-immune factors

The main non-immunologic factors include brain death in the donor, ischemia-reperfusion injury, calcineurin inhibitor toxicity, hypertension, diabetes mellitus (post-transplant or pre-existing), hyperlipidemia and Cytomegalovirus (CMV) infection [34].

Delaying the progression of renal fibrosis and preservation of allograft function should be the goal, which is being achieved through substitution with less nephrotoxic immunosuppressive agents and modification of risk factors, such as adequate control of hypertension, diabetes, hyperlipidaemia, proteinuria (angiotensin blockade), and infections (CMV, BKV, and urine tract infections (UTI)). Secondary CNI and steroid-sparing regimens were shown to reduce the progression of CAN [35]. Substitution of CNIs with sirolimus and mycophenolate mofetil leads to improvement and preservation of renal function in CAN cases [36, 37].

Kidney allografts are lost by both immune and nonimmune mechanisms, against a background of various donor and recipient risk factors. Early tubular injury occurs from ischaemia-reperfusion injury, severe acute rejection or subacute persistent rejection, or BKV infection in addition to donor disease, and is often accompanied by a destructive mononuclear infiltrate generating chronic interstitial fibrosis. Late nephron damage, with increasing glomerulosclerosis and micro vascular abnormalities, is associated with CNI nephrotoxicity, recurrent glomerulonephritis, and persistent chronic cellular rejection, with or without antibody-mediated rejection, hypertension, or late acute rejection [38].

Table 1: Causes and risks of graft loss

<p>Immunological risk factors</p> <ul style="list-style-type: none"> • Histocompatibility • Acute rejection episodes • Suboptimal immunosuppression • Subclinical rejections • Anti-donor antibodies • Noncompliance <p>Non-immunological risk factors</p> <ul style="list-style-type: none"> • Ischemia-reperfusion injury • Brain death • Infection (cytomegalovirus and BK virus) • CNI toxicity • Donor factors: age, hypertension, smoking, diabetes, gender, and reduced renal mass • Recipient factors: race, hypertension, smoking, diabetes, and hyperlipidemia
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On occasions, it is difficult to pinpoint a single etiological factor, as more than one factor is usually implicated in the pathogenesis of CAN [39].

The incidence of this disorder varies, ranging from 23% at 5 years after transplantation up to 60% of grafts at 10 years after transplant. CAN once established, is irreversible [28].

Risk factors implicated in graft loss:

Chronic allograft nephropathy (CAN) is characterized by a relatively slow but variable rate of decline in renal function after first 3 months of RT, often in combination with proteinuria and hypertension. CAN should be differentiated from other causes of transplant dysfunction such as rejection (acute, subclinical, and chronic), CNI nephrotoxicity, glomerulonephritis (recurrent and de novo), nephrosclerosis (secondary to old donor age, recipient hypertension, hyperlipidaemia, and smoking), and others (ureteric obstruction, BK virus nephropathy, and transplant renal artery stenosis) [39].

Arteriosclerosis and interstitial fibrosis in the allograft may also occur as a result of hypertension, recurrent pyelonephritis, and chronic cyclosporine or tacrolimus toxicity. The relative contribution of these various processes to the ultimate loss of any given allograft may be difficult to determine by pathological evaluation alone. The etiologically noncommittal term “chronic allograft nephropathy” was in fact coined to accommodate this difficulty [40].

The risk of graft loss has traditionally been divided into an early, high-risk period and a later period of constant low risk [41]. A major improvement in renal allograft survival in the past 20 years has been the relative elimination of the early-risk period [42].

Some of the risk factors have been identified for lower one-year deceased donor renal allograft

survival, including second or third transplant, prior sensitization with more than 50 % panel reactivity, the presence of delayed graft function (defined as the requirement for dialysis during the first week post transplantation), the frequency and severity of rejection episodes, donor age less than 5 or more than sixty years, more degrees of HLA mismatching, and allograft dysfunction at discharge (plasma creatinine level more than 2 mg/dL (176 mol/L) [43].

After one-year post transplantation, an increased risk of death was observed among patients over the age of 40, men, cadaveric donor recipients, those with diabetes or hypertension, and smokers. Although transplantation confers the highest survival benefit among all the different renal replacement therapies, renal allograft recipients still have a high mortality rate compared with population controls [44].

Recurrent episodes of acute tubular-interstitial rejection can explain the interstitial fibrosis and tubular atrophy observed in some cases. Cytokines released during episodes of rejection, including interleukin-1 (IL-1), fibroblast growth factor, and platelet derived growth factor, are likely to play a role in promoting the fibroblast and smooth muscle proliferation seen in allograft vessels. In cases with prior documented intimal arteritis, vessel thickening can be explained as a direct result of immunologic vascular injury. Graft atherosclerosis leads to ischemic glomerulopathy [45].

Once glomerulosclerosis has developed, the remaining glomeruli undergo compensatory hypertrophy, increased glomerular capillary hydraulic pressure, and increased glomerular filtration. These hemodynamic forces damage the glomerular capillary endothelium, cause mesangial expansion, and accentuate the evolution of chronic transplant glomerulopathy [45]. In support of this hypothesis, it has been shown experimentally that if the increase in glomerular filtration rate is prevented by putting animals on a severely protein restricted diet, the rate of progression of glomerular sclerosis in allograft kidneys is retarded [46, 47].

Arteriosclerosis and interstitial fibrosis in the allograft may also occur as a result of hypertension, recurrent pyelonephritis, and chronic cyclosporine or tacrolimus toxicity. The relative contribution of these various processes to the ultimate loss of any given allograft may be difficult to determine by pathological evaluation alone. The etiologically noncommittal term “chronic allograft nephropathy” was in fact coined to accommodate this difficulty [48].

A number of factors have been shown to influence short-term graft survival. These include delayed allograft function (DAGF), HLA antibodies, type of donor kidney, donor illness, medical center factors, and other factors [49].

1. Delayed allograft function and ischemia-reperfusion injury

Delayed graft function is one of the most important independent risk factors for the development of CAD [50]. Ischemia-reperfusion injury can be responsible for delayed graft function and can be associated with late graft dysfunction particularly when it is combined with acute rejection [51]. Tissue ischemia and reperfusion represent a complex interplay between biochemical, cellular, vascular endothelial and tissue-specific factors [52]. Ischemia-reperfusion injury has been shown to cause endothelial injury with consequent upregulation of adhesion molecules and infiltration of leucocytes and thus create a proinflammatory and profibrotic state within the graft [50].

2. HLA antibodies

Given the strong association of HLA antibodies with inferior graft function and survival, it is crucial to understand the mechanisms of HLA antibody-mediated graft injury. **Terasaki and Ozawa** suggested that the presence of HLA antibodies is associated with an increased risk of early graft loss [53]. Based upon data from nearly 5,000 patients, the frequency of HLA antibodies was 21 percent among renal transplant recipients. Over 2,000 patients were followed prospectively, with 91 grafts failing and 34 deaths. The risk of allograft failure at one year was significantly higher among those with HLA antibodies (6.6 versus 3.3 percent), as well as among those who developed such antibodies *de novo* (8.6 versus 3 percent) [54].

Although the mechanism by which HLA I antibodies promote inflammation and proliferation has been revealed by experimental models, the pathogenesis of HLA II antibodies is less defined [55]. In addition, such antibodies place patients awaiting transplantation at a significant disadvantage, as their waiting time for an allograft is markedly prolonged and they are at increased risk of both delayed graft function and rejection in the perioperative period. The presence of HLA antibodies also has an adverse effect upon long-term allograft survival [54].

3. Type of donor kidney

1) Donor age

Increasing donor age has been linked with an increased risk of CAD [56]. A donor age over 60 years or over 50 years but with vascular comorbidity reduced graft survival [34]. It is now hypothesized that the development of chronic allograft injury may be related to replicative senescence. The senescence hypothesis is based upon cellular exhaustion leading to endothelial and epithelial dysfunction and atrophy and thus persistence of profibrotic stimuli [57].

2) Donor source

The results observed with living-unrelated donors are better than with cadaveric HLA-matched donors [58]. Donor brain death is an independent factor for graft failure [59] and is associated with an increased risk of acute vascular rejection [60]. Brain death is often associated with severe hypotension, an increase in catecholamines, electrolytes abnormalities and intracranial hypertension that can favor the overproduction of cytokines and growth factors leading to overexpression of alloantigens on tubular and endothelial cells [61].

3) Donor organ quality and comorbidity

Most donors die from cerebrovascular events, which are frequently caused by underlying hypertension, diabetes and/or atherosclerosis that may also involve the kidney [62]. Donor diabetes mellitus, even lasting more than 10 years, is not necessary an overwhelming risk factor for graft and patient survival [63]. On the other hand, hypertension is a significant independent risk factor for graft survival, especially if it lasts for more than 10 years [64].

4. Calcineurin inhibitors (CNI) nephrotoxicity

Reports of cyclosporine A and tacrolimus nephrotoxicity are increasingly common late after transplantation [65]. Both cyclosporine and tacrolimus can cause renal and systemic vasoconstriction, through increased release of endothelin-1, activation of the renin-angiotension system, increased production of thromboxane A₂, and decreased production of vasodilators such as nitric oxide and prostacyclin [66]. At renal biopsy, calcineurin inhibitor nephrotoxicity is mainly expressed as progressive arteriolar hyalinosis and downstream glomerulosclerosis [34].

5. Cytomegalovirus (CMV) infection

CMV-seronegative recipients of seronegative grafts have a 10% higher graft survival rate than those receiving seropositive grafts [31]. CMV disease is frequent after transplantation and determines changes in immune cell function favoring acute rejection [67]. Chronic rejection is also accelerated by CMV infection which is associated with upregulation of TGFβ and platelet derived growth factor (PDGF) in endothelial cells and connective tissue growth factor within fibroblasts [68].

Pathology of can

The kidney affected by CAN looks pale and fibrotic with a dense, thickened, adherent capsule. Under light microscopy, characteristic changes are found in the glomerular, tubule interstitial, and microvascular compartments.

The most commonly reported pathological changes in progressive graft failure is chronic interstitial fibrosis and tubular atrophy, which is accompanied by vascular changes and glomerulosclerosis [34].

Both immunological and non-immunological factors may cause chronic allograft injury. Underlying pathophysiology can be detected histologically by typical glomerular and vascular lesions in order to assign a presumed etiology in 60% of chronic allograft biopsies [69]. With the recognition of the entity of chronic antibody-mediated rejection and based on new pathologic knowledge, the traditional CAN has been divided into three parts: (1) chronic active antibody-mediated rejection; (2) chronic active T cell-mediated rejection; (3) interstitial fibrosis and tubular atrophy with no evidence of any specific etiology [29].

The diagnosis of chronic rejection is usually reserved for cases in which there is evidence for a significant role of a host immune rejection of the graft, such as the presence of transplant glomerulopathy and graft atherosclerosis [70]. The presence of C4d deposits in the peritubular capillaries also indicates the presence of humoral rejection [71].

Reports of cyclosporine A (CsA) and tacrolimus (Tac) nephrotoxicity are increasingly common late after transplantation [72]. CsA-induced arteriolopathy is characterized by vacuolisation and necrosis of smooth muscle and endothelial cells with hyaline deposits, considered to be the most characteristic marker of CNI nephrotoxicity [73].

Non-immune chronic graft injury can be also induced by chronic obstruction of the ureter and chronic viral infections [29]. Chronic obstruction is characterized by marked tubular dilation and large Tamm-Horsfall protein casts with extravasation into the interstitium, and/or lymphatic channels. Chronic polyomavirus infection can lead to interstitial fibrosis and tubular atrophy with chronic inflammation and viral intranuclear inclusions [29].

Diagnosis of can

A. Clinical Diagnosis

The clinical diagnosis is usually suggested by gradual deterioration of graft function as manifested by slowly rising plasma creatinine concentration, increasing proteinuria, and worsening hypertension [74]; the diagnosis is confirmed pathologically with change involving all parts of the renal parenchyma, including the blood vessels, glomeruli, interstitium, and tubules [75].

B. Histopathologic and Radiological Diagnosis

Despite significant improvements in life expectancy of kidney transplant patients due to advances in surgery and immunosuppression, CAN remains a daunting problem. A complex network of cellular mechanisms in both graft and peripheral immune compartments complicates the non-invasive diagnosis of CAN, which still requires biopsy histology. This is compounded by non-immunological

factors contributing to graft injury. There is a pressing need to identify and validate minimally invasive biomarkers for CAN to serve as early predictors of graft loss and as metrics for managing long-term immunosuppression [76].

Histopathological evaluation of biopsy tissue is the gold standard for the diagnosis of CAN, while prediction of the onset of CAN is currently impossible. The pathologic changes of chronic renal allograft nephropathy involve all parts of the renal parenchyma including the blood vessels, glomeruli, interstitium, and tubules [73, 77].

Diagnostic biopsy should be considered after exclusion of reversible causes of dysfunction. Sometimes protocol biopsies are undertaken for surveillance of subclinical rejection, occurring with stable function [78]. Outpatient biopsies can be done under ultrasound guidance and local anaesthetic with an automated biopsy gun, with 1% risk of major complications (eg, macroscopic hematuria) and 0.03% risk of graft loss [79].

Biopsy samples should be obtained early, because severely damaged grafts lose their diagnostic specificity and respond poorly to treatment. Adequate tissue samples should ideally be processed for immunofluorescence, light and electron microscopy, and C4d staining. High throughput microarrays for genomics, transcriptomics, proteomics, and metabolomics analyzing DNA, RNA, protein and small metabolites, respectively, help to elucidate mechanisms of injury, promising additional diagnostic and prognostic information in the future [80-82].

A collaborative diagnosis between clinician (assessing immune risk, antibody status, previous rejections, treatment and compliance, donor quality, and renal function) and pathologist helps to best interpret findings. A specific diagnosis is an essential prerequisite for rational and specific treatment directed towards underlying pathophysiological causes [29, 81].

C. Biomarkers Diagnosis

Several biomarkers of CAN have been examined for early detection and prediction of CAN, which still remain in investigative stage. Chemokine (C-C motif) ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1), recruits monocytes, memory T cells, and dendritic cells to the sites of tissue injury, infections, and inflammation. Urinary CCL2 was measured and protocol biopsies performed prospectively in 111 RT recipients at 0, 6, and 24 months, which demonstrated urinary CCL2 at 6 months as an independent risk factor for subsequent development of IFTA at 24 months, both in univariate and multivariate analyses [83].

Proteomic analysis of blood samples using mass spectrometry has identified several unique signatures

of transcript and protein biomarkers with high predictive accuracies for mild and moderate/severe CAN, which can be used for proteogenomic classification of CAN based on peripheral blood profiling, although the validity remains to be proven [84, 85].

In 2003, Scherer *et al.*, in their genomics study using microarray technology, detected upregulation of several genes, which could predict the development of CAN. Those genes were APRIL (acidic protein rich in leucines), OBCML (opiate-binding protein-cell adhesion molecule-like), the tumour suppressor gene NPRL2, cytokeratin 15, homeobox gene B7, prolactin receptor, and guanine nucleotide-binding protein g7 [86]. The same group also demonstrated early changes in several transcriptomes post-RT, which could predict development of CAN and identify patients at risk [87].

Recently, Einecke *et al.* examined RT biopsy specimens that showed genes associated with graft failure were related to tissue injury, epithelial dedifferentiation, matrix remodelling, and TGF- β . In multivariate analysis, molecular risk score, peritubular capillary basement membrane multilayering, arteriolar hyalinosis, and proteinuria were independent predictors of graft loss [88].

More recently, Oetting *et al.* from Minnesota have investigated the effect of telomere length (TL) on the allograft survival and CAN by measuring TL in DNA isolated from peripheral blood in 1805 recipients and 1038 living kidney donors using the multiplexed monochrome quantitative polymerase chain reaction assay. They concluded that the CAN was not associated with shorter TL, although older donor chronological age was associated with increased risk of CAN [89].

Molecular profiling is a newer advancement in identifying molecular signatures related to CAN. Maluf *et al.* have identified calcineurin inhibitor toxicity at the molecular level as a nonimmunological factor involved in the progression to CAD [90].

Serum creatinine (sCr) concentration is a cheap and convenient marker of pathological processes, with excellent measurement precision (intra-laboratory variation 3–5%) and accuracy (10–20 μ mol/L), and modest biological variation. Routine daily monitoring of serum creatinine concentration begins immediately after transplantation (screening for acute tubular necrosis and rejection), and is reduced to monthly monitoring by 1 year in stable patients. The fairly consistent individual daily rate of creatinine-generation means that this measure is sensitive for relative changes in allograft function (25% rise above baseline value is significant) [43].

Patients with deteriorating or persistently raised sCr concentrations should be assessed for dehydration,

uncontrolled hypertension or sepsis (by clinical examination), calcineurin inhibitor nephrotoxicity, glomerular disease, and ureteric obstruction and vascular impairment. Acute and complete ureteric obstruction is rare, presenting with oligoanuria, dysfunction and hydronephrosis on ultrasound, and is confirmed and localized by antegrade or retrograde pyelography. Chronic or partial obstruction is diagnostically challenging because mild transplant hydronephrosis is common. Diuretic isotopic renography with 99m Tc mercaptoacetyltriglycine secreted by tubules despite poor function has a sensitivity of 92% and 87% specificity [91]. Initial decompression by ureteric stent is followed by corrective urological surgery. Whether untreated chronic transplant vesicoureteric reflux produces irreversible parenchymal scarring is uncertain [92, 93].

Differential Diagnosis

Table 2: Differential diagnosis of chronic allograft nephropathy (CAN)

<p>Structural or infective</p> <ul style="list-style-type: none"> • Ureteric obstruction • Lower urinary tract obstruction • Renal arterial stenosis • Recurrent pyelonephritis or vesicoureteric reflux • Polyoma (BK) virus nephropathy <p>Alloimmune injury</p> <ul style="list-style-type: none"> • Late acute rejection (iatrogenic or patient non-compliance) • Chronic cellular rejection • Chronic antibody-mediated rejection with transplant glomerulopathy <p>Other pathophysiology</p> <ul style="list-style-type: none"> • Non-specific sclerosing tubulointerstitial damage (formally designated as chronic allograft nephropathy) • Chronic calcineurin inhibitor nephrotoxicity • Thrombotic microangiopathy • Recurrent or de-novo glomerulonephritis • Poorly controlled hypertension • Metabolic disorders (eg, hypercalcaemia) • Concomitant drugs (eg, angiotensin-converting enzyme inhibitors, angiotensin receptor blocker, non-steroidal anti-inflammatory drugs, cyclooxygenase-II inhibitors) • Acute kidney injury associated with major medical illness

It is important to distinguish between factors that are associated with, or that correlate with, progressive allograft dysfunction or chronic graft failure and the pathophysiologic causes of renal allograft damage.

The kidney has a relatively stereotypic response to injury, thus histologic description alone may not help in understanding the cause of injury. However, longitudinal histologic studies are providing an understanding of the processes of chronic allograft damage and identifying strategies for prevention and treatment [34].

Preventive measures

Prevention and early intervention in CAN remain long-term unmet medical needs in renal transplantation, as CAN eventually affects the majority of CNI-treated renal transplant recipients. CAN is usually not being detected early enough for treatments to effectively stop progression and prevent graft loss. Therefore, in the absence of routine protocol biopsies in many transplant centres, there is a need to improve early detection of CAN through recognition of early clinical signs of renal damage such as changes in mGFR or eGFR, which alert physicians before changes in serum creatinine or proteinuria. In this way, early identification of risk factors before CAN occurs, along with earlier detection, may prompt early intervention, with clinicians making changes to immunosuppressive regimens that may improve the outcomes of CAN. The use of PSIs early post-transplant may aid the management of CAN through minimizing the use of CNIs, maintaining a low level of acute rejection and by reducing smooth muscle cell proliferation within the kidney. This approach is currently being investigated in a series of global clinical trials. In addition, a CNI-free regimen utilizing mycophenolate therapy alone, or in combination with a PSI, may result in an improvement in renal function and graft survival without increasing the risk of acute rejection in patients with CAN. Long-term data also demonstrate that, compared to a CNI and MPA based regimen, MPA and PSI combination therapy preserved renal function and resulted in fewer graft losses, further suggesting that this regimen is a viable therapeutic option in the management of CAN [34].

Even if the contributing factors to CAD can be identified, not all of them can be interrupted prior to and after grafting [94]. Improvements in medical and surgical strategies reduced the incidence of delayed graft function which is a key factor for CAD [95]. Most programs strive to minimize acute rejection rates based on the understanding that both clinical and subclinical rejections are major factors for the development of interstitial fibrosis and tubular atrophy [96]. Allocation strategies primarily aim for HLA-matched transplants that have an established superior long-term outcome compared with HLA-mismatched grafts [97].

Additional preventive measures include the pre-transplant identification of sensitized patients and pre-

treatment of sensitized recipients, because of the strong association between pre-sensitization and development of CAN or humoral-driven chronic rejection [98]. Various modalities have been used for pre-treatment of sensitized patients, including either plasmapheresis combined with intravenous immunoglobulins or rituximab [99]. After transplantation, a sufficient level of immunosuppression is definitely required to prevent the onset of acute rejection [94].

Some investigators reported that, in the long term, practically all cyclosporine A-treated transplant patients showed histologic signs of nephrotoxicity, but in spite of this the 10-year kidney graft survival was 95% [72]. Three main protocols have been investigated to prevent toxicity of calcineurin-inhibitors (CNI): CNI minimization, CNI withdrawal and complete avoidance [100]. 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 [101]. A CNI-free immunosuppression based on sirolimus, mycophenolate mofetil and steroids appeared to be effective but the available studies have only a short follow-up [102].

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 [103]. Significant reduction in proteinuria has been reported as a beneficial effect of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor antagonists in clinical transplantation [94]. The treatment of hyperlipidemia and hypertension is also warranted to prevent both progressive graft dysfunction and cardiovascular disease [104]. Antivirus treatment, diet, weight control, no smoking and good compliance are also suggested in certain settings [94].

Recommended proactive preventive measures are control of hypertension, proteinuria, dyslipidaemia, diabetes, smoking, and other comorbidities. Strategies to maintain transplant function and improve long-term graft survival are important goals of translational research [34].

Despite advances in transplantation reducing early acute rejection rates to less than 15% and lifting 1-year graft survival higher than 90%, long-term graft attrition rates have remained unchanged at 4% loss per year [105].

Early diagnosis of CAN through protocol biopsies and institution of appropriate immunosuppressive regimens and treatment of subclinical rejection is essential to prevent late diagnosis of CAN [34, 106].

Management

Considering the variety of the causes of CAD, individualization of its management is very important. The general approach should be attempting to minimize the risk of acute rejection by choosing an appropriate regimen, considering patient and transplant-related characteristics [107].

Studies such as the Symphony trial [24] suggested that a combination of tacrolimus and mycophenolate mofetil as maintenance agents provide lower risk of rejection, while considering their limitations could help choosing the appropriate maintenance regime [107].

Screening for BK virus reactivation and preemptive reduction in immunosuppression could reduce the chance of chronic changes. Close screening of high-risk patients for cytomegalovirus infection and using preemptive treatment or universal prophylaxis to reduce the risk of cytomegalovirus infection could reduce the risk of direct or indirect injury and subsequent irreversible fibrosis. Patients should be educated about the importance of biopsy, and nephrologists need to realize the value of performing biopsies in patients with increased serum creatinine or proteinuria [107].

Although protocol biopsies may not provide information that could impact the management in low-risk compliant patients, they could be valuable in high-risk patients by helping adjustment of the immunosuppressive regimen. Optimal control of hyperglycemia and hypertension are essential in reducing the risk of CAD [107].

Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are safe in kidney transplant recipients and should be considered for blood pressure control, particularly in the presence of proteinuria. Although there is no strong data supporting their beneficial effect on improving graft survival, patient survival may improve [107].

Aggressive treatment of traditional risk factors for cardiovascular disease, which is the major cause of death in patients with functioning graft, is strongly recommended. Early calcineurin inhibitor conversion to sirolimus has been shown to improve the graft function in short-medium term and should be considered in properly chosen patients. Late conversion in patients with an estimated glomerular filtration rate more than 40 mL/min and minimal proteinuria is also advisable [108].

Posttransplant monitoring for development of Donor-specific antibodies (DSAs) could identify patients at risk for the adverse long-term outcome. Although we are currently unable to identify the characteristics of the antibodies that cause chronic antibody-mediated rejection (ABMR) and do not have specific therapeutic agents for its treatment, by identifying these at-risk patients, closer clinical

monitoring and optimizing their maintenance immunosuppressive regimen could help to improve the long-term outcome [109].

Conclusions

CAD remains one of the major causes of chronic graft loss. The etiology of CAD includes both immune and non-immune causes. To date, evidence-based treatment strategies for CAD are lacking, but several prevention and management strategies are recommended in clinical practice. The major determinant of CAN, which has several causes: ischemia reperfusion injury, ineffectively or untreated clinical and subclinical rejection, and superimposed CNIs nephrotoxicity exacerbating pre-existing donor disease. Interstitial fibrosis and arteriolar hyalinosis, once established, lead to progressive glomerular sclerosis over the subsequent years, with decline in GFR eventually manifesting in a rising serum creatinine. If clinical programs continue to rely on measurement of serum creatinine for identification of patients at risk of CAN, then strategies for intervening to prevent chronic renal allograft dysfunction and subsequent graft loss will be too little and far too late.

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