

Chronic Renal Allograft Dysfunction

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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. Chronic allograft nephropathy (CAN) is the histologic description of the fibrosis, vascular and glomerular damage occurring in renal allografts. Clinical programs rely on monitoring change in serum creatinine for identification of patients at risk of CAN, but this change occurs late in the course of the disease, and underestimates the severity of pathologic change. CAN has several causes: ischemia-reperfusion injury, ineffectively or untreated clinical and subclinical rejection, and superimposed calcineurin inhibitor nephrotoxicity, exacerbating pre-existing donor disease. Once established, interstitial fibrosis and arteriolar hyalinosis lead to progressive glomerulosclerosis over the subsequent years. There have been a number of approaches to treatment aimed at reducing the impact of CAN, mostly centered around avoidance of calcineurin inhibitors through their elimination in all, or just selected, patients. These immunosuppression strategies combine corticosteroids with azathioprine or mycophenolate mofetil, and/or sirolimus and everolimus. Late identification of CAN in individual patients has meant that strategies for intervening to prevent chronic renal allograft dysfunction and subsequent graft loss tend to be "too little and far too late."

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Chronic renal dysfunction is a prelude to the majority of graft failures. Despite the dramatic impact of modern immunosuppression and anti-infective prophylaxis on reducing acute graft loss (1), there has been little impact on long term graft loss (2). The major risks that renal transplant recipients face today are: death from vascular, malignant, or infectious disease; and loss of the allograft from chronic renal dysfunction (Figure 1) (1,3). The purpose of this review is to outline current assessment of renal dysfunction, define its causes, and describe the therapeutic strategies used to ameliorate chronic allograft dysfunction.

Measurement of Chronic Renal Dysfunction

The longitudinal measurement of serum creatinine is a very useful test for acute rejection or other treatable cause of acute impairment of kidney function. However, glomerular filtration rate (GFR) is a more important measure of renal function, though is clumsy and complex to measure accurately. While the inulin clearance rate is the historical gold standard measurement of GFR, alternative radiopharmaceuticals have been used, such as iodine-labeled iothalamate (5), ⁵¹Chromium ethylenediamine-tetra-acetic acid (EDTA) (6) and ^{99m}Technetium diethylene-triamine-penta-acetic acid (DTPA) (7,8). Each methodology has its own systemic biases ensuring that none of the "gold standards" are 24-carat gold. A simpler option is to calculate the GFR from more easily measured factors, such as weight,

height, and serum creatinine. Still the most widely accepted calculator is the Cockcroft-Gault equation (9), which overestimates GFR at low levels. Alternative equations have been developed in groups of normal individuals (9), those with chronic renal failure (10–19), or transplant recipients (20). The available formulae have been compared in different transplant populations (21–24) demonstrating advantages and disadvantages of each. The least useful assay in isolation, especially at levels of GFR between 70 and 30 ml/min, is the one that all transplant units currently rely upon: the serum creatinine measured in different laboratories by different methods over the years.

Tubular Function

The renal tubules not only perform most of the metabolic functions of the kidney but also bear the brunt of damage from allograft rejection and tubular nephrotoxins. It is thus, on one level, surprising that direct or indirect measurements of tubular function have not been developed. Two explanations are the inconvenience and expense of measurements of tubular function, and the considerable functional reserve of tubules. The most promising avenues of research have been proteomic (25) and genomic (26) studies of urine and its cellular debris, but neither has yet reached the clinic in applicable form.

Vascular Flow

Flow of blood is relatively easy to assess using Doppler ultrasound, which easily and accurately measures blood velocity, but cannot quantify the volume of blood flow. Doppler ultrasound has become a routine investigation in acute renal transplantation and has also been assessed in the management of chronic allografts. A high resistive index has been demonstrated to be a marker of poor prognosis in long-term renal

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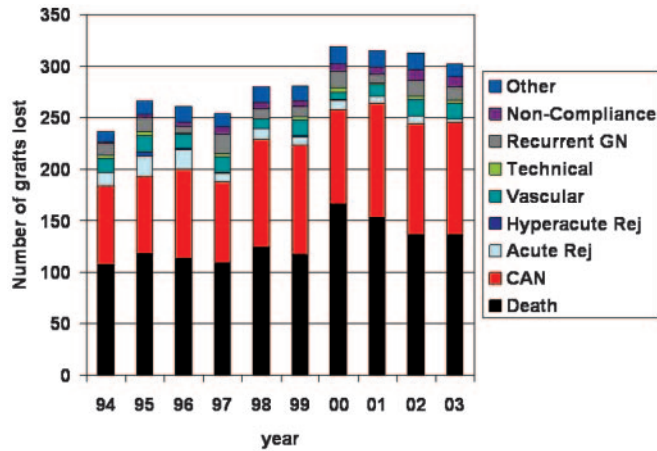


Figure 1. Causes of graft loss in Australia between 1994 and 2003, demonstrating the major causes to be death of the recipient and chronic allograft nephropathy. Vascular thrombosis and recurrent primary renal disease cause more graft losses than acute rejection (1).

allografts (27) with a value >0.80 (present in 20% of patients), giving a high relative risk of a reaching a composite endpoint of graft failure, death, or 50% decline in creatinine clearance (relative risk [RR] = 9.9), which was higher than any other single predictive factor tested. A more complex assessment of Doppler ultrasound cine-loops (28) demonstrated a clear relationship with histologic grading of chronic allograft nephropathy (CAN) using the Banff schema (29). Although these assessments show statistically significant correlations in grouped data, they may incorrectly assign the grade of CAN and are insensitive to milder degrees of damage. The Doppler ultrasound can provide useful diagnostic information about allografts with chronic dysfunction in a limited number of patients, but is not a reliable screening test for chronic allograft dysfunction.

Urinary Flow

Obstruction to urinary flow is a treatable cause of chronic allograft dysfunction. While acute and total ureteric obstruction is usually clinically obvious, diagnosis of partial obstruction is a much bigger challenge. An ultrasound will demonstrate hydronephrosis with good reliability in a well-hydrated patient, while an antegrade nephrostogram will detect the source of an obstruction. Mild degrees of hydronephrosis are, however, common after transplantation and determining functional significance is much more complex.

Renal transplants with relatively poor function do not give reliable excretion of contrast in radiographic imaging of the kidney and ureter. Radiolabeled diuretic renography provides one option for noninvasive diagnosis of partial obstruction using a variety of agents (30–32). ^{99m}Tc mercaptoacetyl-triglycine (MAG3) diuretic renography carries 92% sensitivity and 87% specificity for functional ureteric obstruction and may be the current agent of choice (33).

Evolution of Chronic Renal Dysfunction

The baseline GFR achieved in each renal transplant is determined by a variety of factors, including donor factors such as

type (living or deceased), age, prior disease, cold ischemia at time of surgery, and early posttransplant factors such as acute rejection and the use of nephrotoxic drugs. Thus, renal allograft recipients achieve very different levels of GFR in the early period after the transplant. The concept of “intercept” and subsequent “slope” of the decline in GFR was introduced by Hunsicker to describe the different ways in which renal allografts deteriorate (34). A kidney from an elderly deceased donor with significant ischemic damage and early rejection may achieve a maximum GFR of 30 ml/min. If the subsequent slope of decline in GFR is 2 ml/min per yr, then the GFR will reach 10 ml/min 10 yr after the transplant (Figure 2). This can be contrasted with the kidney from a young road trauma victim, which after transplantation yields a GFR of 70 ml/min, rising to 100 ml/min in the early months due to glomerular hyperfiltration. If this kidney also declines at a rate of 2 ml/min per yr, then a GFR of 10 ml/min may not be reached in the recipient’s lifetime, as it will take 45 yr. On the other hand, a rapid rate of decline—on the order of 10 ml/min—will cause the first example to fail in a couple of years and the second to fail in about 10 yr.

Thus, the intercept (GFR achieved by 6 mo) and the slope (the rate of chronic decline in GFR) combine to predict when each transplanted kidney will fail. This model is influenced by acute damaging events after transplantation and by the degree to which a kidney hyperfiltrates (as illustrated in Figure 3) before chronic damage to the renal tubules, interstitium and glomeruli intervene. The slope of GFR after 6 mo may be positive, with improving renal function, or negative in patients with chronic allograft dysfunction. A single-center analysis has shown that the average Cockcroft-Gault calculated creatinine clearance (CCI) 6 mo after transplantation was 64.6 ± 1.1 ml/min and was the same in patients transplanted in each year of the decade from 1990 to 2000 (35). The predictors of worse 6-mo CCI were kidneys from donors with low CCI who were older, female, and died from a cerebrovascular bleed. Recipient fac-

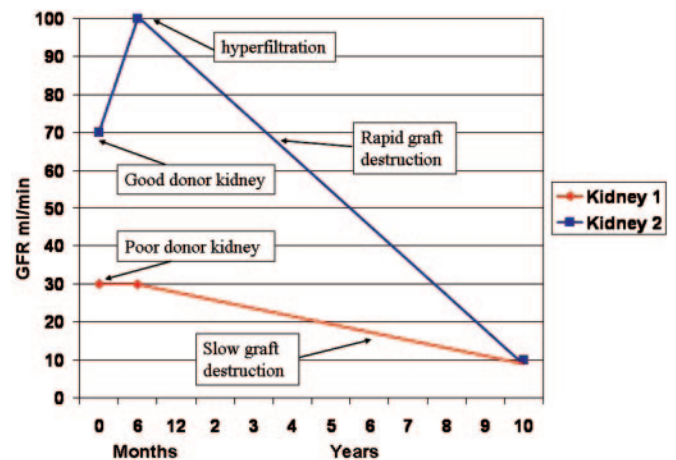


Figure 2. Model of the concept of “intercept” and “slope,” showing two different kidneys, both of which fail at 10 yr, kidney 1 through a low intercept and shallow slope and kidney 2 with a high intercept and rapid slope of decline.

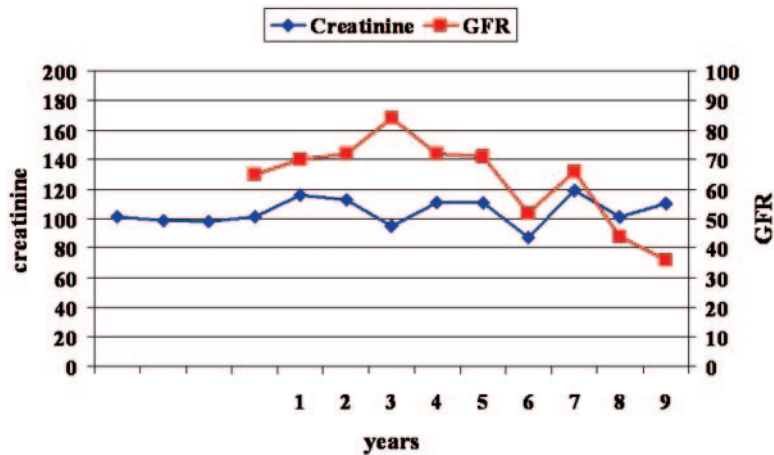


Figure 3. An example of a renal transplant recipient with a relatively stable serum creatinine (blue) over the first 10 yr, but with demonstrated changes in ^{99m}Tc-diethylene-triamine-penta-acetic acid (DTPA)–measured GFR (red), with a rise in GFR over the first 3 yr, perhaps due to glomerular hyperfiltration, followed by progressive decline in function.

tors that predicted worse function were old age, female sex, second or subsequent graft, long cold ischemia and delayed graft function, hypertension at 6 mo, and acute rejection episodes. In contrast to the factors that predicted the CCI at 6 mo, the variables associated with the subsequent decline in CCI over time by multivariate analysis were: any acute rejection episode, female recipients, hypertension at 2 yr, and the year of transplant. In the univariate analysis, the use of mycophenolate mofetil (MMF) (instead of azathioprine) and tacrolimus (TAC) (instead of cyclosporine A [CSA]) were also significantly associated with higher CCI. The fact that the slope of CCI was positive in more patients in the recent years of the study implied that better immunosuppression and transplant management may yield improved long-term graft survival rates. Importantly, the transplanted kidney, as in the example in Figure 3, was shown to retain the ability to both increase and decrease GFR with time, just as the remaining kidney does in living donors (36).

Differential Diagnosis of Chronic Renal Dysfunction

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. Clinical factors associated with chronic allograft dysfunction are shown in Table 1, while the differential diagnoses are shown in Table 2, the most important of which are discussed in more detail below. 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.

Recurrent and De Novo Glomerulonephritis

It has been well known since the early days of transplantation that some glomerular diseases may recur in the graft and

lead to chronic graft dysfunction (37). It was however regarded as a relatively rare phenomenon (38) as long as patients with anti-glomerular basement membrane antibodies were excluded from transplantation. The incidence of *de novo* glomerulonephritis was also regarded as rare and thus unlikely to cause significant numbers of graft losses (39). More recent analyses have challenged this view (40,41). In a study of US data (41), recurrence of glomerulonephritis was seen to increase as grafts and patients survived longer. The median graft survival with recurrence was 1360 versus 3382 d without recurrence ($P < 0.0001$), increasing the relative risk of graft failure by 1.9 (confidence interval, 1.57 to 2.40). An Australian study demonstrated that recurrent disease is the third largest cause of chronic allograft loss in patients with primary native glomerulonephritis, exceeded in impact only by chronic allograft nephropathy and death with a functioning graft (40). In that study, twice as many grafts were lost from recurrent glomerulonephritis (8% by 10 yr) as from acute rejection (4% by 10 yr). The risk was highest for patients with primary diagnoses of focal and segmental glomerular sclerosis, mesangiocapillary glomerulonephritis types I & III, Henoch-Schönlein purpura, IgA nephropathy, or membranous or anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. The risk was low in systemic lupus erythematosus, scleroderma, familial nephritis, anti-glomerular basement membrane-negative Goodpasture's syndrome, and non-IgA mesangioproliferative glomerular nephritis (40).

BK Virus Nephropathy

BK virus (BKV) is a common human polyomavirus, with antibody to it found in up to 80% of normal individuals. In immunosuppressed patients it is associated with ureteric ulceration, ureteric stenosis, cystitis, and renal allograft nephropathy (42). The development of BKV nephropathy appears to be increasing in incidence, between 1% and 5%, associated with the recent use of more powerful immunosuppression such as

Table 1. Factors reported to be associated with chronic allograft dysfunction.**Donor Factors:**Living *versus* deceased

Age

Female sex

Vascular disease

Glomerular disease

Cause of death

Nephron mass

Ischaemic time

Delayed graft function

Recipient Factors

Age

Female sex

African-American race

Cause of renal disease

Diabetes mellitus

HLA matching

Panel reactive antibodies

Blood pressure

Compliance with treatment

Renal function

Creatinine at 1, 6, 12 months

Change in serum creatinine between 6 and 12 months

GFR at 6 and 12 months

Change in GFR between 6 and 12 months

Renal Events

Acute rejection, especially vascular rejection and late rejection

CMV disease

BK virus nephropathy

Doppler ultrasound resistive index >0.80

Proteinuria >800 mg/24 h

Renal Histology in First Year

Subclinical rejection

Nephrocalcinosis

Arteriolar hyalinosis

Interstitial fibrosis

Tubular atrophy

C4d binding

Recurrent or de novo glomerulonephritis

TAC and MMF (43–45). Diagnosis can be made by histology (46), supported by detection of BKV in serum using the PCR test, immunocytochemistry for SV40 antigen, *in situ* hybridization, or electron microscopy (47). Screening for urinary “decoy” cells is sensitive but not specific, whereas BKV PCR quantitation may serve to identify viral replication reliably and guide therapy (44). The combination of BKV nephropathy and acute allograft rejection provides a difficult therapeutic problem: Whether to reduce immunosuppression to allow for control of viral replication or treat the acute rejection with increased therapy (48). Chronic renal dysfunction frequently accompa-

Table 2. Differential diagnosis of renal allograft dysfunction

Ureteric obstruction

Renal artery stenosis

Glomerulonephritis: recurrent and de novo

Infection:

polyomavirus nephropathy

recurrent pyelonephritis/vesico-ureteric reflux

Nephrotoxic agents

Late/recurrent acute rejection

non-compliance

iatrogenic

Chronic allograft nephropathy

sclerosing non-specific tubulo-interstitial damage

chronic cellular rejection

chronic humoral rejection

calcineurin inhibitor nephrotoxicity

transplant glomerulopathy

nies BKV nephropathy and graft loss occurs in approximately half of patients within 2 yr. A number of therapeutic strategies have been used to treat established disease, including reduction and/or switch of immunosuppressive agents (49,50), use of leflunomide (51), cidofovir (52), ciprofloxacin (53), and other agents known to have *in vitro* activity against the virus. The proliferation of alternative strategies stands testament to the weak evidence for efficacy of each of them.

Late or Recurrent Acute Rejection and the Role of Noncompliance

Late acute rejection is a powerful predictor of allograft dysfunction and late graft loss (54). A minority have immunological factors to explain late acute rejection, such as episodes that occur after conversion of immunosuppression (55), but more commonly patient noncompliance is the major cause. A recent meta-analysis demonstrated that nearly one quarter of patients were noncompliant with their prescribed medication and had a seven-fold risk of chronic graft loss (56). A classification of noncompliance and a recommendation that it be recognized and managed as a medical syndrome may help turn this increasingly serious problem into a therapeutic target (57).

Chronic Allograft Nephropathy

The classification of renal transplant biopsy findings was formulated through the early 1990s by a series of meetings in Banff (29,58–60), focusing initially on acute rejection. An alternative system of describing chronic renal allograft histology was created in Helsinki—the Chronic Allograft Damage Index (CADI) (61)—facets of which were subsequently incorporated into the Banff system.

CAN was defined histologically and the term was used to identify an entity that was restricted to renal transplants but, unlike the term “chronic rejection,” was independent of etiol-

ogy (29). This simple aim has caused confusion through its application to similar histologic appearances in donor biopsies before transplantation. Further confusion has been added within the Banff schema itself (29) by the attribution of immunological causation to some facets of CAN, such as vascular changes with disruption of the elastica, inflammatory cells in the fibrotic intima, and proliferation of myofibroblasts in the intima. A refinement of this approach has been to identify lesions that provide evidence for one etiology or another, such as association of fibrosis and tubular atrophy with nodular arteriolar hyalinosis implying calcineurin inhibitor (CNI) toxicity (62).

CAN is a histologic diagnosis and represents the final common pathway of renal allograft damage (29). The specific features are interstitial fibrosis and tubular atrophy, not because these are the only or even most important changes, but because they are widespread within the kidney and thus reproducible in small biopsy samples. Grading of CAN, from I to III, is based upon the severity of chronic interstitial fibrosis (ci0 to ci3) and tubular atrophy (ct0 to ct3). CAN grade I requires minor changes (equivalent to ci1, ct1); CAN grade II requires moderate changes (ci2/ct2, ci1/ct2, or ci2/ct1); and grade III requires severe changes (ci3/ct3, ci2/ct3, or ci3/ct2). If there are none of the changes of “chronic rejection” described above, the grade of CAN is qualified with an “a,” but if these changes are present then it is rated “b.”

Lack of reproducibility of some histologic reports has been a source of surprise to some clinicians who simultaneously accept differences of opinion about other facets of transplantation. A careful study of the reproducibility of protocol biopsies demonstrated relatively poor correlations between centers for reporting of acute histologic qualifiers under the Banff schema (63), although the particular statistical methodology may have contributed to this result. The study emphasized both the need for within-center clinicopathologic correlations and centralized pathology reading in multicenter studies. A better result was described in Canada for both changes of acute rejection ($\kappa = 0.77$) and for the reproducibility of chronic changes ($\kappa = 0.53$ to 0.65), with the exception of chronic glomerulopathy where there was poor correlation (64).

Much of the recent advances in knowledge of CAN has come from long-term protocol histology. Despite concern in some centers, modern biopsy techniques using ultrasound guidance and automated needles have made this a relatively safe procedure. A multicenter European study showed a single graft was lost and three patients required direct intervention for bleeding from 2127 biopsies (65). Data from “protocol-driven” histology has major methodologic superiority to “event-derived” data, because the answers emanating from the latter are driven by the assumptions inherent in the decision to biopsy the kidney.

So far, the most extensive published series of protocol biopsies addressing the causes and correlates of CAN has come from our group in Sydney (66–70). Recipients of simultaneous pancreas kidney transplants were biopsied annually for 10 yr, yielding approximately 1000 biopsies from 120 patients. The series from the Mayo Clinic has examined a larger number of predominantly living-donor kidney allograft recipients during

the first 2 yr after transplantation (71), whereas other studies have addressed components of CAN in the context of different immunosuppressive regimens (72–76).

The best controlled demonstration of the prevalence of CAN at 2 yr was from a US trial of CSA against TAC (72), where 72.3% and 62.0% of biopsies exhibited CAN, respectively. There was no difference in the chronic histology between the therapeutic arms, but CAN at 2 yr was associated with older donor age, early acute rejection, and episodes of acute CNI nephrotoxicity.

The longer-term histologic evolution of graft fibrosis has been described in the Sydney series (66). The results of this study have revealed the natural history of CAN in CNI-treated patients, its evolution, and in particular the intrarenal relationships between fibrosis, arteriolar damage, and glomerular damage. (Figure 4).

Renal allograft fibrosis occurred in two phases (68). Two thirds of the fibrosis present by 10 yr had already appeared by 1 yr, during which time interstitial fibrosis exceeded the development of tubular atrophy. This suggested that early interstitial fibrosis was related to factors other than simply the rate of tubular damage, with a demonstrated role for both ischemia-reperfusion injury and direct immune-mediated mechanisms in causing interstitial injury. Acute tubular necrosis is predictive of CAN and the use of MMF was protective (66). In a study from Hanover, Germany, 258 patients were biopsied at 6, 12, and 26 wk and grouped on the presence ($n = 70$) or absence ($n = 120$) of CAN at 26 wk (75). Risk factors for the development of CAN by 26 wk included a kidney from a deceased donor, a longer cold ischemic time, and acute rejection episodes. Interestingly, calculated GFR was already 8 to 10 ml/min lower at 6 and 12 wk in those that developed CAN by 26 wk than in those that did not, but without visible differences in the earlier biopsies.

Subclinical inflammation, scored under the Banff schema as rejection, but not associated with acute changes in creatinine, also lead to increased interstitial fibrosis and CAN within the first year (67), confirming earlier observations in a different cohort of renal transplants (77). Persistent subclinical rejection in sequential biopsies taken after the first year, although confined to only 6% of patients, lead to progressive increases in fibrosis and decline in renal function. Finally, the presence of inflammatory cells within areas of graft fibrosis, ignored in the Banff schema, was also associated with increases in the area of fibrosis in subsequent biopsies (68). These data demonstrated that untreated inflammatory cell infiltration in the allograft insidiously destroys tubules and fibroses the interstitium, unheralded by acute changes in serum creatinine.

Between 1 and 10 yr after transplantation, tubular atrophy and interstitial fibrosis progressed simultaneously, with features of chronic CNI nephrotoxicity dominant. Striped interstitial fibrosis and arteriolar hyalinosis with or without tubular calcification developed almost universally by 10 yr (70). Similar to the 2-yr US multicenter CSA/TAC trial (72), the rate of development and severity of chronic CNI nephrotoxicity were indistinguishable between TAC, and both sandimmune and neoral-microemulsion preparations of CSA (70).

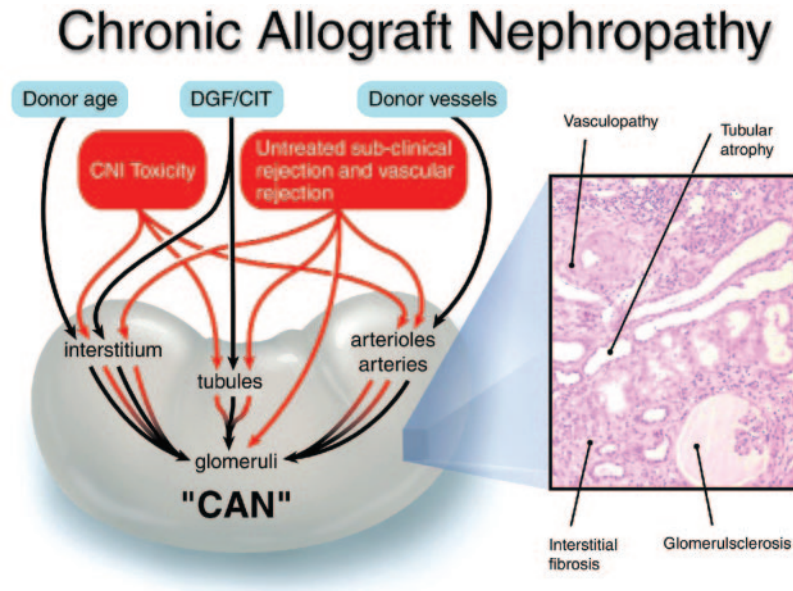


Figure 4. Factors that influence the development of the histologic features of chronic allograft nephropathy. Shown in black are the donor and transplant surgical factors, while the posttransplant factors are shown in red. Illustration by Josh Gramling—Gramling Medical Illustration.

Arteriolar hyalinosis most frequently occurred for the first time, sometimes transiently, between 3 and 12 mo after transplantation and was predicted by a 3-mo trough level of CSA > 200 ng/ml and by prior episodes of acute clinical nephrotoxicity, reducing in severity with CNI dose reduction (70). In the subsequent biopsies, arteriolar hyalinosis developed and persisted in approximately 75% of patients by 10 yr, occurring especially in those treated with >5 mg/kg per d of CSA in the first 5 yr. The classic appearance of nodular hyalinosis was seen in relatively few patients, usually making way for more severe and diffuse hyalinosis with vascular luminal narrowing and associated glomerular ischemia. Alternative explanations for arteriolar disease, such as impaired glucose tolerance, ischemic microvascular injury, and arterial hypertension were excluded in this cohort of patients. Indeed, arteriolar hyalinosis preceded hypertension in most patients and was present in 60% of normotensive and 68% of hypertensive patients.

Glomerulosclerosis represents the final and irreversible destruction of functioning nephrons and was seen in two phases in the long-term protocol biopsy series (69). An early phase of ischemic injury was followed by a period of 2 to 5 yr in which little glomerular loss occurred before progressive and severe glomerular destruction lead to chronic renal dysfunction. Early glomerular injury was correlated with subclinical rejection and weakly associated with cold ischemic time, while glomerular sclerosis at 1 mo was associated with CNI nephrotoxicity. The later phase of glomerular destruction followed earlier chronic interstitial fibrosis and tubular atrophy seen on the 1-yr biopsy, and was also associated with the severity of arteriolar hyalinosis. Thus it would appear that glomerulosclerosis, the harbinger of renal allograft dysfunction, resulted initially from interstitial fibrosis, with development of periglomerular fibrosis and at-

tubular glomeruli, and secondly from high-grade arteriolar hyalinosis leading to ischemic glomeruli.

Chronic Humoral Rejection and Transplant Glomerulopathy

Antibody mediated damage is a well-accepted mechanism of acute rejection, but was largely thought to be solved with identification of the HLA system and use of the crossmatch. There has been renewed interest in acute humoral rejection as its pathologic and clinical hallmarks such as peritubular capillary staining for C4d and donor-specific HLA antibodies have been defined (78,79). The role of antibody in CAN is unfolding, with anti-HLA antibody predicting poor long-term outcome (80) and the demonstration of C4d as a hallmark of CAN and precedent for transplant glomerulopathy (81–83). One possible mechanism is the demonstration that high titers of anti-HLA antibody activate endothelial cells and induce proliferation, possibly contributing to graft vascular disease (84).

Transplant glomerulopathy was first demonstrated in 1963 (85) and is characterized by enlarged glomeruli, mesangial matrix expansion, changes in mesangial cells, and splitting of the glomerular and peritubular basement membranes. Recent studies have demonstrated an association between anti-glomerular basement membrane antibody directed at the heparan sulfate proteoglycan, agrin (86). While glomerulopathy is one of the most inconsistently reported histologic findings (62,63), it undoubtedly contributes to chronic allograft dysfunction and loss in some patients (87).

The current understanding of the evolution of CAN is summarized in Figure 5. The transplanted kidney brings with it the donor's history of both acute and chronic disease, especially microvascular disease and interstitial fibrosis. Ischemia at the time of transplantation, followed by early

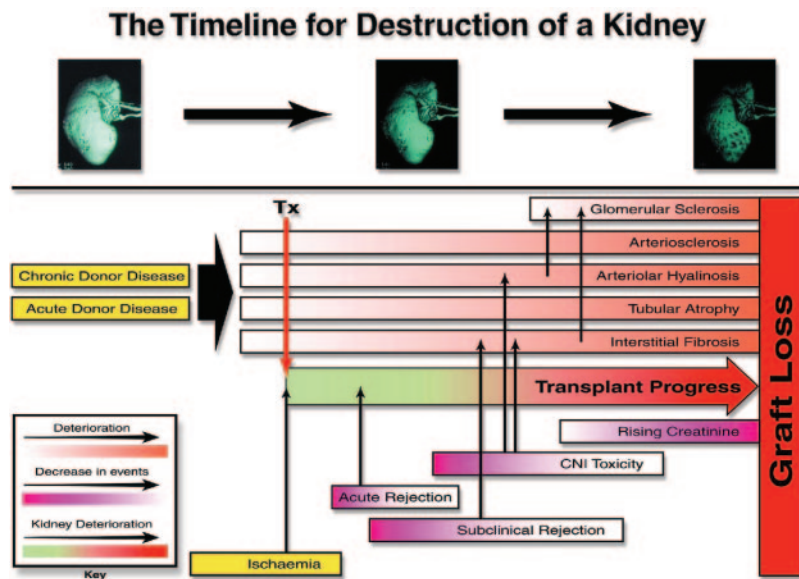


Figure 5. The relationships between factors that lead to chronic allograft dysfunction in the majority of grafts lost from chronic allograft dysfunction. Illustration by Josh Gramling—Gramling Medical Illustration.

rejection and CNI nephrotoxicity, compound the injury to the interstitium and tubules. Untreated and severe acute rejection, especially if humoral in origin and involving the vascular structures, is uncommon but particularly damaging. Subclinical rejection remains largely unidentified unless managed by protocol biopsy, and it is insidiously damaging. Beyond the early months, CNI toxicity becomes progressively more important as the source of renal injury with development of interstitial fibrosis and arteriolar hyalinosis both leading to progressive glomerulosclerosis. When the function of the remaining hyperfiltrating glomeruli is exhausted, the GFR falls, and it is only as the GFR falls below about 30 ml/min that a change in serum creatinine becomes clinically evident. Graft failure, once the creatinine has started to rise, is usually inevitable.

Therapeutic Strategies in Chronic Renal Dysfunction

Both preventative and therapeutic strategies will be needed to reduce the burden of chronic allograft dysfunction and loss. Preventative strategies that simultaneously provide effective long-term prevention of both clinical and subclinical rejection and avoid all nephrotoxic agents, remain an unachieved goal of research. Current understanding of the damaging events and agents, as well as clarity over the relationships of intrarenal pathology and their timing, may help in designing effective prevention.

One of the most effective clinical preventative strategies was proven by a trial in which subclinical rejection was identified and treated by protocol biopsy at 1, 2, and 3 mo (88). The results of this landmark trial have not been universally understood yet, but they have demonstrated a significant reduction in chronic interstitial fibrosis at 6 mo in the group managed by protocol biopsy. It is fully understand-

able that the difference in renal function was not seen for 2 yr, and one would expect it to take another 5 yr to show differences in graft survival. It has also been shown that subclinical rejection can be prevented in almost all patients in the first 3 to 6 mo through use of highly immunosuppressive protocols, such as induction regimens with an anti-thymocyte globulin, TAC, MMF, and corticosteroids (65,89). It is not yet clear which of these approaches yields the best long-term result, with reduction in subclinical rejection balanced by higher incidences of BKV nephropathy and post-transplant lymphoproliferative disease.

Therapeutic strategies to halt or reverse CAN have centered around avoidance of calcineurin inhibitors through their elimination in all, or just selected, patients. The long term agents relied upon for immunosuppression in these strategies combine corticosteroids with azathioprine or MMF and/or sirolimus.

Azathioprine has a long established role, resulting partly from reliance upon it until the mid-1980s. The longest-term renal transplant survivors in almost every unit in the world will still be treated today with azathioprine and prednisolone alone. They will have had stable renal function for up to 30 yr, but they will be the rare survivors from their cohort (1). There were a number of trials in the 1980s in which patients were treated with short-term CSA and then converted to azathioprine (90). Two of these studies were re-analyzed later, after as long as 15 yr (91,92). Both showed that the group with the best renal function and long-term survival were treated initially with CSA and then converted to azathioprine. Late attrition of grafts in the CSA-treated groups eventually dissipated the early survival advantage of the CNI over the anti-metabolite.

MMF has been used both to reduce the incidence of CAN and to treat patients with progressively falling GFR. A single-center, randomized trial of azathioprine ($n = 34$) versus MMF ($n = 37$) combined with CSA and corticosteroids, showed a reduction in CAN at 1 yr from 71% to 46% ($P = 0.03$) (93). A treatment strategy characterized as “the creeping creatinine study” randomized patients on any combination of CSA, azathioprine, and corticosteroids to either start MMF and stop the CSA, or to continue with CSA with or without dose reduction (94). Response was defined as stabilization or improvement in slope of $1/\text{creatinine}$ and occurred in 36 of 62 (58%) of patients switched to MMF and 19 of 60 (32%) patients of the continuation group ($P = 0.006$). The strategies of both *de novo* use and conversion to MMF can thus be seen to be superior to reliance on CSA and azathioprine, at least in the short-term.

Sirolimus, a target of rapamycin inhibitor (TORi), was shown to be both effective and non-nephrotoxic in two studies comparing it with cyclosporine in the context of either azathioprine (95) or MMF (96). It was disappointing to find that both sirolimus (97,98) and everolimus (99,100) caused an increase in CNI nephrotoxicity, perhaps through p-glycoprotein inhibition leading to intrarenal accumulation of CSA (101). Two alternative strategies have been examined to combine the immunosuppressive potency of the TORi and CNI without a nephrotoxic penalty.

The first approach was to combine sirolimus and CSA for 3 mo and then eliminate the CNI (102). This study has now reached 36 mo of follow up, with sustained improvements in renal function in the CSA elimination arm and better graft survival (103). The sirolimus group showed an improvement in the protocol biopsy CADI score from 12 to 36 mo, predominantly because of a reduction in tubular atrophy (72). Although the comparator arm uses combination CSA and sirolimus, which would now be expected to yield poor results, this cannot obviate significant improvements within the other arm. Similar histopathologic results have been seen in other smaller trials (104,105), supporting the view that the TORi lead to better long-term graft histology and reduced chronic allograft dysfunction.

Everolimus at doses of either 1.5 mg/d or 3 mg/d combined with low-dose CSA with or without the anti-IL2 receptor antibody basiliximab (100) showed 6-mo calculated GFR values between 62 and 67 ml/min, and 12-mo values between 64 and 67 ml/min (106). Thus the strategy of low- or very low-dose CNI combined with everolimus holds some promise, but long-term renal function and histology data will be needed to determine the impact on chronic allograft dysfunction.

The final strategic approach is to avoid the use of nephrotoxic CNI altogether. The number of non-nephrotoxic immunosuppressants under investigation has risen markedly in the past few years, including FTY720 (107) and Campath (108). Combination of MMF, sirolimus, corticosteroids and an IL2 receptor blocker has been tested successfully in a small single-center study (109). Calculated GFR at 12 mo was 81 ml/min in sirolimus-treated ($n = 31$) versus 61 ml/min in CSA-treated patients ($n = 30$). The 2-yr follow-up revealed better renal function and

histology, with normal renal biopsies in 66.6% (sirolimus) versus 20.8% (CSA) and CAN grade II and III in 12.5% versus 37.5% (75). Adverse events and tolerability may be the only factors limiting the wide application of this protocol.

Conclusions

The major determinant of chronic renal allograft dysfunction is development of CAN, which has several causes: ischemia-reperfusion injury, ineffectively or untreated clinical and subclinical rejection, and superimposed CNI 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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