Chronic Allograft Nephropathy (CAN) – An Update

Thomas Oates, Behdad Afzali and David Goldsmith
Renal Unit, NGH, Guy’s Hospital, London, UK

Abstract

We feel that CAN may be minimized but not completely avoided while we rely on substantial numbers of marginal/cadaveric donors, whilst immunosuppression is imperfectly monitored and individualized, and whilst scrupulous attention to “medical matters” such as blood pressure and anemia, remain subordinated to prevention of acute rejection, and “creatinine watching”. Most of the risk factors for the development of CAN are those for the development of cardiovascular disease and minimizing risks for one will minimize risks for the other (after all, the commonest cause of graft loss is death with a functional graft from cardiovascular events). This is just the same as aggressively treating potential cardiovascular pathology in chronic kidney disease, hoping that both the circulation and the kidney will benefit. It will be immensely important for there to be more reliable and earlier warning signs of CAN, whether through immunologic, genomic or proteomic approaches.

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Corresponding author: David Goldsmith, david.goldsmith@gstt.nhs.uk

Key words


Correspondence to:
David Goldsmith
Renal Unit, 6th Floor, NGH
Guy's Hospital
London SE1 9RT, UK
E-mail: david.goldsmith@gstt.nhs.uk
Introduction

Our first problem, and one we cannot solve (but do read on, please!) is the vexed issue of definition and nomenclature. The latest (8th) Banff on Allograft Pathology was held in Edmonton, Canada, 15-21 July, 2005 (and the proceedings of these deliberations were published in 2007)\(^1\). Major outcomes included the elimination of the non-specific term “chronic allograft nephropathy” (CAN) from the Banff classification for kidney allograft pathology, and the recognition of the entity of chronic antibody mediated rejection. This newly revised Banff classification system has renamed chronic allograft nephropathy, “interstitial fibrosis and tubular atrophy, without evidence of any specific etiology”. This was done because the widespread and poorly-reproducible use of the term chronic allograft nephropathy was thought to be acting to undermine attempts to determine the underlying cause(s) of the histologic lesions.

So, we should not in theory even be discussing the catch-all term “CAN” (or its very many synonyms: chronic rejection, transplant nephropathy, chronic allograft damage, chronic renal allograft dysfunction, transplant glomerulopathy, or chronic renal allograft nephropathy. But we have no choice but to discuss this entity/entities, as CAN/interstitial fibrosis and tubular atrophy, without evidence of any specific etiology is one of the most important reasons for the loss of allograft function beyond 12 months of engraftment, and the return of transplanted patients to renal dialysis programs, and their earlier demise. Renal allograft failure is a surprisingly common cause of end-stage renal disease requiring renal replacement therapy, accounting for 25-30% of patients awaiting renal transplantation. Similarly, over 20% of kidney transplants performed in the USA go to patients who have failed one or more renal allograft(s).

Definition

Originally coined fifteen years ago in 1991 as a more generic alternative to the then popular, but profoundly misleading, term “chronic rejection”, acceptance of the term “CAN” did succeed in reversing the misconception that all late scarring of the graft was due to alloimmune injury/rejection. However, there are now over 600 PubMed citations using the term, many fostering and actively nurturing the misconception that CAN is a specific disease rather than just another term for nonspecific parenchymal scarring (see below). In the Banff consensus report are outlined targeted alterations in the Banff schema replacing “CAN” as a diagnostic term. The rationale for this update of the Banff schema is the misusage of “CAN” as a generic term for all causes of chronic renal allograft dysfunction with fibrosis that inhibits the accurate diagnosis and appropriate therapy. In order to treat something, we first must have a definitive diagnosis, which is not artificial but rather specifies the underlying disease processes\(^1\).

CAN - Clinical features, diagnosis, pathology, differential diagnosis

Chronic allograft nephropathy is a poorly understood pathologic process that is defined as renal allograft dysfunction (occurring at least three months posttransplant) in the absence of active acute rejection, acute drug toxicity (principally calcineurin inhibitors), or other diseases. There is no doubt that chronic calcineurin inhibitor (CNI) usage is associated with a marked tendency to CAN on renal allograft biopsy\(^2\).

The clinical diagnosis is usually suggested by gradual deterioration of graft function as manifested by slowly rising plasma creatinine concentration, increasing proteinuria (but rarely causing nephrotic-range
proteinuria), and worsening hypertension. However, the reliance on these clinical features commonly results in the late identification of chronic renal allograft nephropathy, frequently culminating in allograft loss. Reliance on loss of renal function as part of a definition of a pathologic process is profoundly unsatisfactory, with much effort either from protocol biopsies, or from novel genetic or proteomic markers, directed towards earlier diagnosis³.

The pathologic changes of CAN involve all parts of the renal parenchyma, including the blood vessels, glomeruli, interstitium, and tubules. The vessel walls are thickened by the subintimal accumulation of loose and then organized connective tissue, variable mononuclear cellular infiltration, proliferation of myofibroblasts, and disruption and duplication of the internal elastic lamina. Immunofluorescence staining in chronic renal allograft nephropathy may be positive for IgG, C3, and fibrin. The glomerular capillary walls are thickened, with an occasional double-contour appearance. Proteinuria is common when this feature is pronounced on biopsy. The glomeruli may also be enlarged and show a lobular pattern; segmental or, in severe cases, global sclerosis also may be seen. Electron microscopy may show mesangial cell interposition and subendothelial accumulation of electron-lucent material. The interstitium shows variable degrees of patchy fibrosis and focal cellular infiltrates with lymphocytes and plasma cells, associated with a variable degree of tubular atrophy and tubular cell dropout. Peritubular capillary basement membrane splitting and lamination may be relatively specific; these abnormalities, which are only observed by electron microscopy, are found in 60% of patients⁴.

Histopathologic evidence of CAN correlates with adverse long-term outcomes, including elevated concentrations of serum creatinine and lower rates of graft survival. The outcomes of 280 transplant patients, in whom 282 protocol biopsies were obtained three months after transplantation, were the following. At 10 years after surgery, allograft survival was 95% among those without chronic nephropathy (175 patients), 82% for those with chronic nephropathy but without vasculopathy (87 patients), and 41% for those with both chronic nephropathy and vasculopathy (21 patients)⁵.

The evaluation of progressive allograft dysfunction is complex but very important. Some of the different pathologic processes and diseases are listed in table 1.

### Pathogenesis of CAN

There is of course no single cause of CAN. There are in fact very many potential causes, acting often in concert. There is an important distinction to be made between immunologic-mediated causation, and non-immune-mediated causation²,⁶,⁷, but of course, it is likely that both of these pathways are involved either simultaneously or sequentially in the majority of patients, and that these pathways act in an interdependent way to

<table>
<thead>
<tr>
<th>Table 1. Different causes for progressive allograft dysfunction</th>
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<tr>
<td>CAN</td>
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<tr>
<td>BK virus nephropathy</td>
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<tr>
<td>De novo/recurrent glomerular pathology</td>
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<tr>
<td>(Late) acute rejection</td>
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<tr>
<td>Renal artery stenosis</td>
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<td>Ureteric/other obstruction</td>
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Note: - Proteinuria, reduced glomerular filtration rate, hypertension, diabetes, and hyperlipidemia are common to many of the above. - Renal allograft biopsy appearances of hepatitis C/mesangiocapillary glomerulonephritis can mimic CAN.
accelerate the ageing processes that even under ideal circumstances cause slow progressive loss of allograft function.

**Immune pathogenesis**

All aspects of the immune system may be involved in immunologic CAN – cell-mediated, humoral responses against alloantigens (major and minor), inflammatory cytokines and the innate immune response. Patients with a better human leukocyte antigen (HLA) match fare better than those with a less favorable match over time: a retrospective study in which, among over 4000 recipients of HLA-identical sibling transplants, patients with no panel reactive antibodies (PRA) had significantly higher 10-year survival (72%) versus those with either 1-50% PRA (63%) or greater than 50% PRA (56%).

Acute rejection (early, recurrent and often associated with low levels of immunosuppressants in the early posttransplant period) is an important risk factor for later CAN and worse renal function.

The immunosuppressive regimen used can also have a profound bearing on whether CAN will develop. If there is aggressive or repeated early acute rejection this can lead to progressive renal fibrosis and, for example, intermittently and especially persistently low CNI levels are associated with this. But equally, long-term exposure to high levels of CNI is now strongly implicated in later-developing CAN. There is an increasingly rich literature on limiting CNI exposure. The principal evaluated strategy is to limit exposure to a CNI, particularly ciclosporin. This involves either a decrease or withdrawal of ciclosporin therapy in patients with or without renal dysfunction, or the administration of immunosuppressive regimens that do not include ciclosporin.

Immunosuppressive therapy is generally ineffective in patients with established nephropathy, except for those in whom the precipitating cause is inadequate immunosuppression due to noncompliance or aggressive drug tapering. Intravenous pulse or oral pulse corticosteroid administration may be beneficial if there is evidence of active acute rejection on allograft biopsy.

Many of the current ongoing trials have as their primary or secondary goals the limiting of long-term ciclosporin/CNI exposure. In one example, 150 renal transplant recipients were randomly assigned to one of three maintenance immunosuppressive regimens: tacrolimus plus sirolimus; tacrolimus plus mycophenolate mofetil (MMF); and ciclosporin (Neoral®, Novartis) plus sirolimus. Patients in each arm also received induction therapy with daclizumab and maintenance therapy with methylprednisolone. Interim analysis at three years found that patient and allograft survival were similar in all three groups.

Similar findings showing better protection from chronic renal dysfunction with tacrolimus- or sirolimus-based regimens than with a ciclosporin-based regimen were reported in two prospective protocol biopsy studies. With the sirolimus study, there was a significantly higher serum creatinine level, lower glomerular filtration rate, and increased prevalence of histologic findings of CAN with ciclosporin than that observed with the sirolimus-based regimen at two years. At five years, the sirolimus-based regimen continued to be associated with a higher glomerular filtration rate.

The comparison of sirolimus and tacrolimus has not been yet subjected to a study with three or five-year outcomes, so it is premature at this stage in our opinion to overinterpret shorter-term, often surrogate out-
comes (promising though these may be). In general though, it is becoming clearer that long-term exposure to CNI in stable transplant patients can be reduced or eliminated, and by so doing, CAN may be prevented or ameliorated\textsuperscript{18}.

Nonimmune pathogenesis

Table 2 lists the main nonimmune pathogenetic mechanisms that have been linked to the development of CAN. It should be noted that many of these derive from cross-sectional studies with limited prospective information. Also, very few interventional data with hard, as opposed to surrogate, endpoints are available.

A detailed exposition of each of the above factors, many of which occur together, is beyond the scope of this chapter. Clearly there are well-known and well-practiced interventions for many of the above factors, including angiotensin converting enzyme inhibitor and angiotensin receptor blocker-based antihypertensive regimens\textsuperscript{19}, anemia treatments, and mainly statin-based lipid-lowering therapies.

It must not be forgotten that in many cases the immunosuppressant choices themselves play a role in the development or severity of raised blood pressure, glucose or cholesterol, further muddying waters that are hardly crystal-clear at the outset.

A realistic “synthesis scenario”

Possibly the most influential and insightfully informative study of the last five years to appear shedding new light on CAN was that of Nankivell, et al. from the Westmead unit in Sydney, Australia\textsuperscript{2}. These workers recruited 120 recipients of kidney/pancreas transplantation who underwent sequential protocol biopsies over a 10-year posttransplantation period. Immunosuppressive therapy consisting of cyclosporin/tacrolimus, prednisone, and azathioprine/MMF was used over this time period.

Based upon the time posttransplantation, two types of histologic injury (early and late) could be distinguished. Early damage, which was observed within one year posttransplantation, resulted primarily from immunologic factors (under-immunosuppression), such as severe acute rejection and persistent early subclinical rejection, as well as from ischemic injury. After one year, damage was increasingly characterized by progressive high-grade arteriolar hyalinosis with vessel narrowing, glomerulosclerosis, and additional tubulointerstitial injury. This was thought to principally be the result of calcineurin injury, as by 10 years the likelihood of CAN was high.

At 10 years, severe allograft nephropathy was present in 60% of patients, with glomerulosclerosis being observed in almost 40% of glomeruli\textsuperscript{2}. Although the generalizability of these results to all renal transplant recipients is unclear, these find-
ings suggest that different treatment strategies for chronic nephropathy based in part upon time posttransplantation (prevention of rejection in the first year and, in stable patients, limiting calcineurin exposure in the subsequent years) may prove effective. Of course, this needs to be tested rigorously, and this is a major challenge for the transplantation community.

The early posttransplant period is thought to be dominated by the direct alloresponse (directly alloreactive CD4 T-cells are very high in frequency and very vigorous), whereas exhaustion of donor antigen presenting cells over time leads to a decrease in direct priming of recipient T-cells and an increase in indirect allorecognition. The indirect pathway is the one which is primarily responsible for chronic rejection. Regulatory T-cells have a predominant effect on the indirect pathway so we would expect their influence to reduce the tendency to reject over time, except of course that CNI are directly toxic to regulatory T-cells. Rapamycin, on the other hand, is a facilitator to regulatory T-cells, so there is a good scientific basis for a switch from CNI to mammalian target of rapamycin (mTOR) inhibitors (and probably, by the same token, MMF) with time.

Attempts to begin to test this out have been made – 84 patients with biopsy-proven CAN were randomly assigned to either MMF plus a reduction in the CNI, or immediate CNI withdrawal plus rapamycin initiation. At 24 month follow-up, a second biopsy was performed in 25 patients (10 and 15 in CNI reduction and withdrawal arms, respectively). Significantly better allograft survival was observed with CNI withdrawal plus rapamycin versus the CNI reduction group, while biopsy grading worsened and remained stable in the reduction and withdrawal groups, respectively. Similarly, the “creeping creatinine” study by Dudley, et al. showed benefit of CNI withdrawal and/or elimination using MMF. Clearly, one must not, in one’s enthusiasm to remove CNI, expose patients to the risks of acute or chronic humoral rejection by under-immunosuppression. It is a delicate balancing act, with careful individualization of treatment based on the most thorough risk analysis for each patient; e.g. in someone with a history of aggressive early rejection we might expect to have to wait longer before commencing CNI elimination, and to perform the minimization or elimination protocol more slowly.

Zero “tolerance” for CAN

There are international studies being conducted at present aiming to identify the “fingerprints of tolerance” (e.g. The EU and International Tolerance Network studies) to help identify those patients in whom a degree of tolerance to their graft has developed and in whom immunosuppression can be minimized or withdrawn. This would, if successful, at least in some patients reduce their risk of allograft, or cardiovascular damage, and malignancy from immunosuppression.

To B-iopsy or not to B-iopsy: that is the question

We have agreed and seen that the diagnosis of what we call CAN is made as a clinical combination of risk factors, changing renal function, and a supportive renal biopsy (as much to exclude other important causations for allograft dysfunction). On this basis, and on the background of decades of attempts to predict or understand renal outcomes before too much time has elapsed, people have advocated the use of protocol biopsies in the routine clinical management of engrafted patients. But is there evidence to support this?
Two recent papers\textsuperscript{21,22} have nicely summarized what are, to us, a delicately balanced series of arguments. Certainly, the use of protocol biopsies in a well-managed clinical service seems safe, but should of course be continuously audited. However, until we have well-designed and well-conducted clinical trials which show improved outcomes for decisions which have been made as a result of protocol biopsy derived information, we must be cautious. Knowing how best to respond to borderline infiltrates, for example, may depend as much on the clinical context (low versus high immunologic risk) as any features seen on the biopsy. If the rates of sub-clinical and clinical/borderline rejection are low, as they might be in many low-risk patients, the added value of protocol biopsies may be modest or absent. It may be that several cohorts of patients (delayed graft function, marginal donor, high immunologic risk) would benefit most from a protocol biopsy service, but this benefit may not extend to all. The demands on histopathology to deliver objective, structured, and comparative biopsy reports should also not be underestimated\textsuperscript{21,22}.

**New “kids on the (tissue) block”**

If we return to the beginning, and the 2005 Banff conference, one of the two main outcomes was the recognition of chronic antibody mediated rejection. Detection of the complement split product C4d in renal allograft biopsies is emerging as an important adjunctive tool to help understand the alloimmune response and, in particular, to diagnose antibody mediated rejection. After an antigen-antibody complex fixes complement, a cascade of events follows, with activation of several classical complement proteins. The complement protein C4 is split into C4a and C4b. The C4b is then converted to C4d. A unique feature of C4d is that it binds covalently to the endothelial and collagen base-membranes, thereby avoiding removal and raising the possibility of serving as an immunologic hallmark of complement activation and antibody activity. This concept has revolutionized our understanding of chronic rejection.

In normal kidneys, C4d is detectable in the glomerular mesangium and at the vascular pole. In transplanted kidneys, the reason for the specificity of C4d staining in the peritubular capillaries is not clear.

The latest Banff diagnostic criteria have changed the antibody mediated rejection category to reflect the finding of C4d in some cases of chronic allograft dysfunction, suggesting that antibody mediated rejection is a likely contributor to some forms of CAN\textsuperscript{1}. The association of C4d deposition in the peritubular capillaries with features of chronic renal allograft injury was evaluated in a retrospective study – the presence of C4d was evaluated in controls and cases that met current histologic criteria for CAN and had frozen tissue\textsuperscript{23}. In the majority of the patients with chronic allograft glomerulopathy and C4d\textsuperscript{*} biopsies, donor-specific antibodies could also be detected. Conversely, donor-specific antibodies were not detected in the patients with CAG+/C4d\textsuperscript{−} biopsies. The one-year post-biopsy graft survival improved from 40% historically to 100% in patients that were CAG+/C4d\textsuperscript{*}, after the institution of a protocol for conversion to tacrolimus and MMF in this population. In another study of 543 patients it was found that proteinuria and decreased allograft function and survival correlated with the development of donor and non-donor specific HLA antibodies posttransplantation as well as the presence histologically of C4d and transplant glomerulopathy\textsuperscript{24}.

At the moment we feel it would be fair to say that this mechanism, chronic humoral-mediated rejection, is another “immune-medi-
ated” process by which chronic interstitial, glomerular and vascular fibrosis and sclerosis/obliteration can take place.

Table 3 illustrates the need for a combination of therapeutic interventions to prevent or to minimise CAN. We feel that CAN may be minimized but not completely avoided while we rely on substantial numbers of marginal/cadaveric donors, whilst immunosuppression is imperfectly monitored and individualized, and whilst scrupulous attention to “medical matters” such as blood pressure and anemia, remain subordinated to prevention of acute rejection, and “creatinine watching”. Most of the risk factors for the development of CAN are those for the development of cardiovascular disease and minimizing risks for one will minimize risks for the other (after all, the commonest cause of graft loss is death with a functional graft from cardiovascular events). This is just the same as aggressively treating potential cardiovascular pathology in chronic kidney disease, hoping that both the circulation and the kidney will benefit. It will be immensely important for there to be more reliable and earlier warning signs of CAN, whether through immunologic, genomic or proteomic approaches.

References