Determinants of acute and chronic renal allograft injury

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Chronic Allograft Nephropathy

Synonyms
Chronic transplant nephropathy; chronic renal allograft dysfunction; chronic kidney rejection; interstitial fibrosis/tubular atrophy (IF/TA)

Definition
Chronic allograft nephropathy (CAN) is a histopathological syndrome characterized by interstitial fibrosis, tubular atrophy, vascular intima thickening and glomerulosclerosis of the transplanted kidney, independent of aetiology. The histological features of CAN are described to result from both immune and non-immune mediated damage to the grafted kidney and since these features can also be observed in native kidneys with end-stage renal disease, they are considered non-specific. CAN is therefore considered a final common pathway of renal parenchymal scarring in response to underlying diseases that include episodes of (sub)clinical cellular and antibody-mediated rejection, toxicity, infections and recurrence of the initial renal disease in the transplant (including diabetic and hypertensive nephropathy) (Paul, 1999). According to the Banff '97 working classification, CAN should be graded by the severity of interstitial fibrosis and tubular atrophy at the biopsy, because tubulointerstitial in contrary to vascular and glomerular lesions are least influenced by sampling error of the transplant biopsy (Racusen et al., 1999). In 2005, the Banff meeting report filed for an elimination of the term CAN in order to reduce the misconception that it can be diagnosed as a specific disease entity. Since CAN is solely a descriptive entity, the pathologist should always try to find the underlying causes for fibrosis that are potentially treatable. Besides the areas of fibrosis, on-going immune- and non-immune-mediated processes indicative of underlying pathology can be found in the glomeruli, the minor and major vasculature and the tubulointerstitium.

Clinically, CAN is characterized by a slowly declining renal function either or not accompanied by proteinuria. Various studies have demonstrated that serum creatinine or its derivative formulas to estimate the glomerular filtration rate have poor predictive value for features of CAN in the biopsy.
Various risk factors have been related to the development of CAN and definite graft failure. An important contributor to the risk of failure is the quality of the donor kidney at time of transplantation (Organ Procurement and Transplantation Network [OPTN] and Scientific Registry of Transplant Recipients [SRTR], 2012). The kidney donor risk index (KDRI) predicts allograft survival based on deceased donor and transplant risk factors. Donor parameters that contribute to a higher risk for graft failure include donor age, African American race, serum creatinine of the donor at time of nephrectomy, donor hypertension and pre-existing diabetes mellitus, a cerebrovascular accident or cardiac arrest as the cause of death, shorter length and a higher weight of the donor and a positive hepatitis C virus serology. Also a positive CMV serology contributes to the development of graft failure, probably due to reactivation of cellular immunity causing more acute rejection in affected recipients. Besides donor-specific parameters, also characteristics intrinsic to the transplantation procedure directly or indirectly contribute to graft failure. A higher number of human leukocyte antigen (HLA) mismatches between donor and recipient, especially on the HLA-B and HLA-DR locus, associates with lower graft survival. Kidneys procured from deceased donors that are kept in cold storage for longer periods of time have an unfavourable outcome as well (Organ Procurement and Transplantation Network [OPTN] and Scientific Registry of Transplant Recipients [SRTR], 2012). However, recent studies based on paired analysis of kidneys from a single donor into two different recipients with a difference in cold ischemia time showed a higher incidence of delayed graft function but no significant effect on late allograft outcome. Besides donor- and transplant-related risk factors, also characteristics of the recipients contribute to reduced allograft survival. Cardiovascular risk factors like hypertriglyceridaemia, hypertension, diabetes mellitus (either pre-existing or de novo) and smoking have a negative impact on graft outcome. When graft dysfunction develops after transplantation, either in the form of a reduced glomerular filtration rate or the development of de novo proteinuria (requiring the performance of a biopsy), graft outcome is worse as well. Graft biopsies that contain vascular rejection, i.e. Banff T cell-mediated rejection class IIA, IIB or III, have been shown to be less responsive to pulse steroid therapy and subsequently, graft outcome is worse. Fortunately with the current immunosuppressive regimens, the incidence of intimal arteritis has declined. Both over- and under-immunosuppression can have a negative impact on graft survival. Graft failure by over-immunosuppression can be
caused by calcineurin inhibitor toxicity and the increased risk of local or systemic infections like intragraft polyomavirus nephropathy or cytomegalovirus reactivation, while graft failure by under-immunosuppression can be facilitated via an increased risk of acute rejection.

Clinical features

Incidence
The incidence of CAN has been estimated on the basis of protocol renal allograft biopsies. In the US, the incidence of CAN was estimated to be around 70% at 2 years after transplantation. Based on the Banff '97 criteria, in Australia 94.2% and 24.7% of protocol renal allograft biopsies showed grade I and grade II/III CAN, respectively (Nankivell et al., 2003).

Age
The development of CAN is especially dependent on donor age, which is able to explain around 30% of the variance in graft outcome after one year. Conflicting results exist concerning the relationship between the recipient's age and CAN however, showing both younger and older recipients at risk. This discrepancy might be due to the inverse relationship of the recipient's age with the occurrence of acute rejection, which in turn is associated with the development of CAN.

Sex
Male recipients have a predisposition for the development of CAN.

Site
CAN is limited to the renal allograft. Within the renal allograft, the glomerulus, the tubulointerstitium and the allograft vasculature are all involved in CAN.

Treatment
There is currently no treatment for established CAN. Various interventions aim at the prevention of the development of CAN by intervening with its modifiable risk factors. Since episodes of acute rejection have been associated with CAN, effective immunosuppressive therapy aims at preventing its development. Besides clinically
evident rejection, also subclinical inflammation that can be found in protocol biopsies associated with later CAN. Multiple randomized-controlled trials have suggested that treatment of subclinical inflammation with a steroid pulse regimen in the early phase after transplantation (within 6 months after surgery) would result in lower CAN scores at later time-points.

Of the most effective immunosuppressive agents, calcineurin inhibitors (CNIs) are particularly toxic to the transplanted kidney. The exact mode of toxicity is still under debate. In vitro analyses showed that CNIs induced stress of the endoplasmic reticulum of tubular epithelial cells, resulting in epithelial-mesenchymal transition and cell death through apoptosis. In vivo, the use of CNIs related to vasoconstriction of the afferent vasculature of glomeruli and arteriolar hyalinosis. These processes are thought to cause glomerular collapse with ischemia of the renal parenchyma, which might be involved in the development of CAN. Various studies have investigated whether the use of CNIs related to the development of CAN, but thus far it has been difficult to correlate the dose or serum concentration of CNIs to graft outcome. Minimizing the exposure to CNIs is therefore being investigated as one of the strategies to prevent the development of CAN.

**Outcome**

CAN is believed to be a good surrogate outcome for definite graft failure due to its supposedly irreversible character. It has been estimated that the presence of CAN at 1-year protocol biopsies increases the hazard of graft failure by a factor 2.6 per category (< 5 % vs 6 - 25 % vs > 25 % IF/TA), an effect that not only accounted for deceased donor but also living donor kidneys. However, a variety of studies reported that solely the detection of low-grade fibrosis does not imply on-going scarring of the transplant parenchyma with an ever-declining renal function. An important indicator of a declining renal function with future graft loss is the presence of disease activity in viable non-fibrotic tissue.
**Macroscopy**

On gross examination, transplanted kidneys with CAN are normal to smaller in size, depending on the extent of fibrous tissue. In general, kidneys with CAN have a pale appearance. In rare cases, kidneys with CAN can be calcified. Obtaining percutaneous core needle biopsies can be difficult owing to the toughness of the tissue.

**Microscopy**

The features of CAN with the lowest inter-observer variability are interstitial fibrosis and tubular atrophy (IF/TA), which according to the Banff classification (Racusen et al., 1999) can be subdivided in less than 5 %, 6 – 25 %, 26 – 50 % or over 50 % of the tissue area involved in the process. The interstitium is fibrotic (Figure 1) either or not with infiltrates of a large variety of different effector and helper T and B lymphocytes, in a scattered pattern or aggregates can be found and even neogenesis of lymphoid structures has been observed at more advanced stages. Besides T and B lymphocytes, also plasma cells, monocytes, macrophages, mast cells, natural killer cells and granulocytes have been associated with underlying pathology and consequently with the progression of interstitial fibrosis. Tubular atrophy is characteristic of CAN. This state of the tubules is characterized by a small outer diameter with a thickened and wrinkled tubular basement membrane. Epithelial cells have a flattened and simplified appearance with loss of apical brush border and a lacking apico-basal polarity. The loss of the capsular vasculature during the transplantation procedure causes local ischemia resulting in the involvement of the subcortical area in the fibrotic process in most transplanted kidneys (Nickeleit, 2009). In case of calcineurin inhibitor toxicity, viable tubules might show uniformly sized clear small sized vacuoles (isometric vacuolization). In this case, very few infiltrated leukocytes can be seen (over-immunosuppressive state). The interstitial fibrosis that is observed in response to calcineurin inhibitor toxicity follows a more striped pattern and is patchy.
In the various stages of CAN, glomerular structures can have a variable appearance ranging from normal to global glomerulosclerosis (Figure 1). In early stages of CAN, active inflammation of the glomeruli can be observed. Glomerulitis is most often seen during antibody-mediated rejection, as there appears to be an association with the presence of donor-specific antibodies, but this lesion can also be seen during cellular rejection. At later stages, the glomeruli tend to be shrunken with a low cell count and an increased mesangial matrix. Transplant glomerulopathy (Figure 2), defined as capillary loop double contours, is probably the result of chronic antibody-mediated rejection that causes multiplication of the glomerular basement membrane. However, transplant glomerulopathy is not pathognomonic for chronic antibody-mediated rejection, since it is also observed in cases of hepatitis C infection and thrombotic microangiopathy, processes that are not mutually exclusive in transplant recipients. At later stages, also focal segmental glomerulosclerosis and global glomerulosclerosis can be detected. At this stage it is often impossible to determine whether the initial cause was chronic antibody-mediated rejection or the recurrence of focal segmental glomerulosclerosis in the transplanted kidney.
Like the changes in the glomeruli, also the minor and major vessels of the kidney have a variable appearance in the different stages of CAN. In areas of interstitial fibrosis, the peritubular capillary network has undergone regression and is mostly absent. Since the microcirculation is thought to be the major target of donor-specific antibodies, lesions representative of (possibly on-going) antibody-mediated rejection can be found in peritubular capillaries in areas free of interstitial fibrosis.

Acute antibody-mediated rejection is characterized by peritubular capillaritis with granulocytes, monocytes/macrophages and natural killer cells. Due to donor-specific antibody fixation to the endothelium of peritubular capillaries, also the complement cascade can be activated at the site. C4d staining of peritubular capillaries can be indicative of on-going antibody-mediated injury. Comparable to the chronic changes that are observed in glomeruli that have undergone antibody-mediated rejection, also multiplication of the basement membrane of peritubular capillaries can be observed. As a result of chronic T cell-mediated endarteritis, but occasionally also antibody-mediated endothelial injury, arterioles and bigger
arteries undergo intimal thickening (Figure 3). Deposition of type I and III collagens by myofibroblasts induces concentric or eccentric occlusion of the vascular lumen. When active vascular rejection is still on-going, lymphocytes can still be observed in the thickened intima. When infiltrating inflammatory cells are absent, intimal thickening can also have a hypertension-induced origin. In the latter case, an elastic tissue stain can visualize multiple layers of elastic lamellae (Nickeleit, 2009). The smaller arterioles of the transplanted kidney are generally the target of calcineurin inhibitor induced toxicity. Medial ballooning of smooth muscle cells with accompanying vasoconstriction resulting in narrowing of afferent glomerular arterioles relates to a reversible decrease in glomerular filtration rate can be a sign of early calcineurin inhibitor toxicity. In a later stage of calcineurin inhibitor toxicity, nodular hyalinosis of the arteriolar media is the result of replacement of necrotic smooth muscle cells (Figure 4). Arteriolar hyalinosis is not specific to calcineurin inhibitor toxicity since it can also be observed in patients with diabetic nephropathy. In patients where arteriolar hyalinosis is present that is not treated by reducing the dose of calcineurin inhibitor, the occlusion of the transplant vasculature can result in tissue hypoxia and the development of CAN.

Figure 3 | Vascular intimal thickening
As a response to chronic T cell-mediated, but probably also to antibody-mediated rejection, the intima of arterioles and bigger arteries undergo concentric or eccentric thickening and multiplication, which results in narrowing of the vascular lumen causing hypoxia. Besides chronic rejection, also damage mediated by hypertension can cause intimal thickening of the vasculature.
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Immunophenotype

Histological assessment with routine haematoxylin-eosin, Jones’ methenamine silver and periodic acid Schiff diastase stainings is sufficient to visualize CAN and immunohistochemistry does not aid in its detection. The prime cell-type involved in the production of extracellular matrix is believed to be the myofibroblast. The myofibroblast can immunohistochemically be detected by its intracellular stress fibers by staining of alpha-smooth muscle actin ($\alpha$-SMA). $\alpha$-SMA$^+$ cell can be found in the renal interstitium, the arterioles and the glomeruli. In order to detect the underlying cause of CAN, immunohistochemistry can be of use. The recurrence of initial renal diseases like immunoglobulin A nephropathy and lupus nephritis as well as antibody-mediated rejection can be detected by immunofluorescent staining of immunoglobulin subtypes and members of the complement cascade (C4d staining on peritubular capillaries for the detection of antibody-mediated rejection). Immunohistochemical staining of polyoma large-T antigen and in situ hybridization for BK virus DNA can help to detect underlying polyomavirus nephropathy (BK-virus

Figure 4 | Arteriolar hyalinosis
As a result from chronic calcineurin inhibitor toxicity, vascular smooth muscle cells within the media of arterioles are damaged, undergo cell death and are being replaced by hyaline. Arteriolar hyalinosis (yellow asterisk) is not pathognomonic for calcineurin inhibitor toxicity, since it is also observed in patients with diabetes mellitus that were not treated by calcineurin inhibitors. Narrowing of the lumen by nodular hyalinosis of arterioles can cause tissue hypoxia, which further contributes to the development of interstitial fibrosis.
nephropathy) at the biopsy. In the literature, a large effort has been made to identify inflammatory cell types that are related to a certain underlying pathological process, including T cell, B cell and macrophage subtypes, but due to the large variety in results at present, none has entered clinical practice protocols yet.

Molecular features

Molecular techniques, especially novel high-throughput screenings, have contributed to the understanding of progressive CAN. A wide range of different transcripts, mostly genes involved in innate and adaptive immunity as well as genes implicated in epithelial de-differentiation and tissue remodelling, has been correlated with the progression of CAN and definite graft failure. Besides evaluation of biopsy material, also serum and urine have been of major interest for the development of non-invasive methods to detect fibrosis. Non-invasive determination of CAN and its underlying pathology might allow for future elimination of biopsies for the prediction graft outcome. To date, none of the genes associated with the progression of CAN have been introduced in clinical practice on a routine basis yet.

Differential diagnosis

CAN is a descriptive entity, therefore no differential diagnosis is applicable. However, if non-fibrotic areas are present, the differential diagnosis of the underlying pathology includes T cell-mediated and/or antibody-mediated rejection, calcineurin inhibitor toxicity, viral nephropathy (polyomavirus nephropathy), local infarction due to thrombosis and recurrence of the initial renal disease in the transplanted kidney (anti-glomerular basement membrane disease, membranoproliferative glomerulonephritis, recurrent focal glomerulosclerosis, membranous glomerulonephritis, IgA nephropathy, diabetic nephropathy and hypertensive nephropathy).