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Transplant glomerulopathy

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In the renal allograft, transplant glomerulopathy represents a morphologic lesion and not a specific diagnosis. The hallmark pathologic feature is glomerular basement membrane reduplication by light microscopy or electron microscopy in the absence of immune complex deposits. Transplant glomerulopathy results from chronic, recurring endothelial cell injury that can be mediated by HLA alloantibodies (donor-specific antibodies), various autoantibodies, cell-mediated immune injury, thrombotic microangiopathy, or chronic hepatitis C. Clinically, transplant glomerulopathy may be silent, detectable on protocol biopsy, or present with overt manifestations, including up to nephrotic range proteinuria, hypertension, and declining glomerular filtration rate. In either case, transplant glomerulopathy is associated with reduced graft survival. This review details the morphologic features of transplant glomerulopathy found on light microscopy, immunofluorescence microscopy, and electron microscopy. The pathophysiology of the causes and risk factors are discussed. Clinical manifestations are emphasized and potential therapeutic modalities are examined.
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Transplant glomerulopathy is an important cause of proteinuria, rising creatinine, hypertension, and shortened allograft survival. Transplant glomerulopathy is defined as reduplication/multilammination of the glomerular basement membrane as observed by light (Figure 1) and/or electron microscopy (Figure 2) in a kidney allograft in the absence of immune deposits. By this definition, transplant glomerulopathy is a morphologic description of histologic or ultrastructural alterations and not a specific clinicopathologic entity.[1] Multiple pathophysiologic mechanisms result in development of this lesion, all related to chronic, repeated endothelial cell injury. Most notable is antibody-mediated rejection. Other reported causes include autoantibodies, cell-mediated rejection, thrombotic microangiopathy, and hepatitis C virus.

The development of glomerular disease in a transplant, manifested by proteinuria and elevated serum creatinine, has been well described for over 50 years.[2,3] The term transplant glomerulopathy was first used in the setting of apparent rejection (called rejection transplant glomerulopathy) by Busch *et al*[4] in 1971. This early report stressed repetitive endothelial cell injury as a prime pathogenic mechanism. Zollinger *et al*[5] in 1973 described in detail the lesion they termed 'transplant glomerulopathy,' and they attempted to differentiate it from recurrent or *de novo* inflammatory glomerulonephritides. The characteristic changes in the lamina rara interna of the glomerular basement membrane were stressed. In 1985 Maryniak *et al*[6] described the evolution of this lesion over time. The descriptions and pictures presented in these early publications represented the current pathologic criteria for diagnosing transplant glomerulopathy.

In this review, we describe in detail the morphologic features of transplant glomerulopathy found on light, immunofluorescence, and electron microscopies. The pathophysiology of the causes and risk factors are discussed. Clinical manifestations are emphasized and potential therapeutic modalities are examined.

Light microscopy

The hallmark of transplant glomerulopathy is duplication of the glomerular basement membrane. To diagnose transplant glomerulopathy, the Banff '97 criteria required ≥10% of capillary loops in the most affected glomerulus to evidence double contours.[7] Banff 2015 (Table 1) now requires double contours in only a single capillary loop as the minimal light microscopic finding (cg1b).[8,9] An increase in mesangial matrix may be present but is less specific and is not required for diagnosis. To satisfy the Banff 2015 criterion for mesangial matrix increase ('mm'), the

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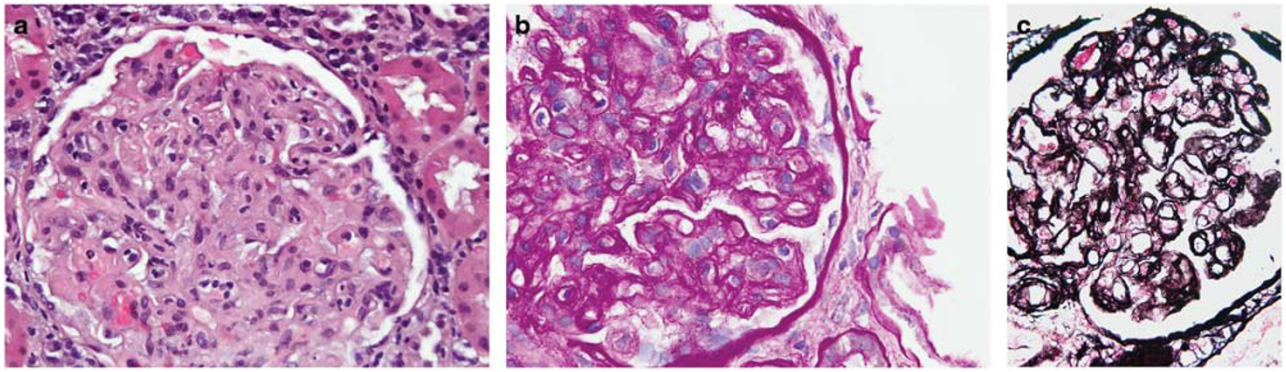

Figure 1 Transplant glomerulopathy—light microscopy. (**a**) The glomerulus has patent capillary loops with thickened basement membranes. The refractile appearance of the basement membranes suggests duplication. Epithelial and endothelial cells are swollen. The mesangial regions are mildly expanded by an increase in cells and matrix. Hematoxylin and eosin (H&E), ×40. (**b**) Silver stain on the same glomerulus shows patent capillary loops with thickened basement membrane with duplication in ≥10% of the patent loops. Jones methenamine silver, ×40. (**c**) The periodic acid Schiff (PAS) stain on the glomerulus demonstrates capillary basement membrane duplication and widening of the subendothelial compartment. The mesangial regions show an increase in matrix. PAS, ×40.

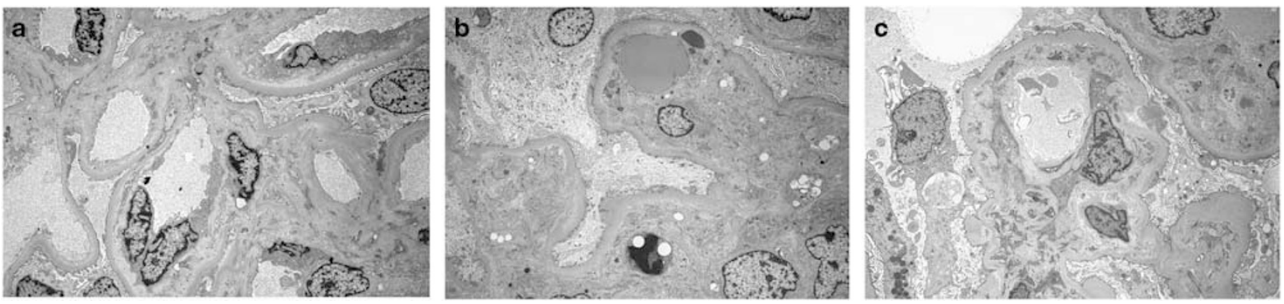

Figure 2 Transplant glomerulopathy—electron microscopy. (**a**) Transplant glomerulopathy with widening of the subendothelial space as a result of edema and amorphous electron-dense material (**b**) Transplant glomerulopathy widening subendothelial space as a result of cell debris, fragmented red blood cells (consistent with a thrombotic microangiopathy), a macrophage, and amorphous proteinaceous debris. (**c**) Transplant glomerulopathy with prominent reduplication of the basement membrane. Original magnification, ×4000.

Table 1 Transplant glomerulopathy per 2015 Banff (Adapted from Am J Transplantation, 2017;17: 28–41)

Quantitative criteria for scoring degree of double contours in diagnosing TG	
cg0	No GBM double contours by LM or EM (no TG)
cg1a	No GBM double contours by LM but GBM double contours in at least 3 glomerular capillaries by EM with associated endothelial/subendothelial changes
cg1b	GBM double contours in 1–25% of capillary loops by LM in the most affected glomerulus
cg2	GBM double contours in 26–50% of capillary loops by LM in the most affected glomerulus
cg3	GBM double contours in ≥50% of capillary loops by LM in the most affected glomerulus
Criteria required for diagnosis of chronic active antibody-mediated rejection as the cause of TG	
The presence of DSA	
Evidence of an antibody interaction with capillary endothelium (any of the following):	
C4d positivity	
g+ptc ≥ 2 (g ≥ s0 if ACMR, borderline, or infection)	
Increased expression of gene transcripts indicative of endothelial injury by a validated assay	

ACMR, acute cell-mediated rejection; cg, transplant glomerulopathy score; DSA, donor-specific antibodies; EM, electron microscopy; g, glomerulitis score; GBM, glomerular basement membrane; LM, light microscopy; ptc, peritubular capillaritis score; TG, transplant glomerulopathy.

expansion must be moderate, defined as mesangial matrix between adjacent capillaries exceeding the width of two mesangial cells in a minimum of two lobules. Mesangiolysis may be present, as well as glomerulosclerosis, the latter mimicking focal segmental glomerulosclerosis. Fibrin deposition can occur.[6] Microvascular inflammation, including glomerulitis (g) and/or peritubular capillaritis (ptc) (Figure 3), may be observed, especially in cases mediated by an alloimmune mechanism.

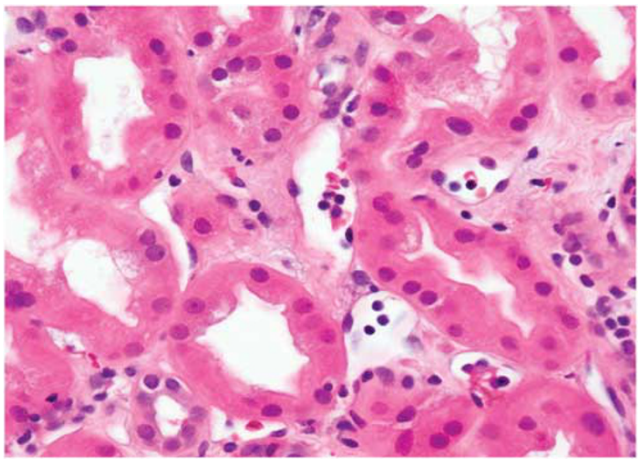

Figure 3 Peritubular capillaritis (ptc). The peritubular capillaries are dilated and contain lymphocytes. Ptc2. Hematoxylin and eosin (H&E), ×40.

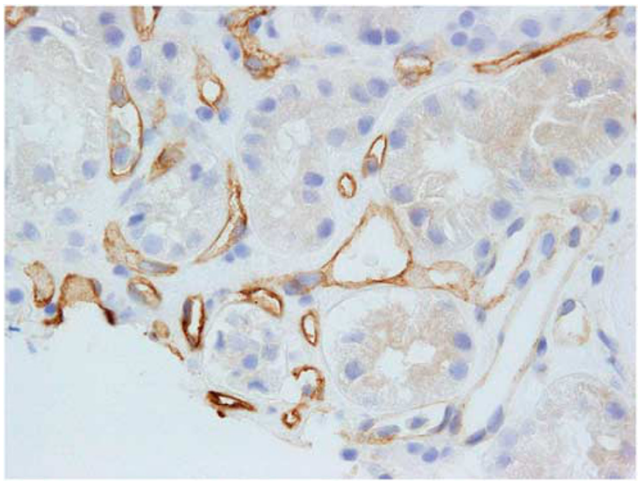

Figure 4 Immunohistochemical staining for C4d. The peritubular capillaries show diffuse positivity for C4d deposition by immunohistochemical staining. The staining is moderate to strong with a circumferential pattern. Greater than 50% of the peritubular capillaries were affected (C4d3). Original magnification ×40

Immunoflourescence microscopy

By definition, transplant glomerulopathy is distinguishable from the recurrent and *de novo* glomerulonephritides. Immunoflourescence for IgG, IgA, IgM, C1q, C3, κ, and λ is negative, with the exception of nonspecific staining for IgM that probably represents physiologic catabolism of IgM-containing immune complexes normally formed *in vivo*.[10]

C4d

Critical to evaluating kidney transplant biopsies is determining the endothelial staining of C4d involving peritubular capillaries. Whereas tubular basement membranes and arterioles may occasionally stain positive for C4d, such staining is considered nonspecific and not part of current diagnostic criteria.

The C4 component of complement is activated following C1q activation by antigen–antibody complexes or may be directly activated by polysaccharides through the mannose-binding lectin pathway. In either case, C4d, a C4 split product without known function, can be covalently bound to tissues, and is assumed to represent a 'footprint' of prior antibody activity.[11] C4d positivity may remain after initiating factors (such as HLA antibodies) have resolved or are removed.[11] It is now standard practice to stain all kidney transplant biopsies for C4d to determine humoral alloreactivity with complement fixing antibodies. C4d evaluation can be accomplished by direct immunofluorescence microscopy on frozen tissue or by immunohistochemistry on fixed tissue (Figure 4). The immunoflourescence technique is reported to be more sensitive.[12] There is poor interobserver and interlaboratory reproducibility with immunohistochemistry resulting in an interinstitutional κ score of only 0.17.[13]

As not all donor-specific antibodies are pathogenic, their presence alone is insufficient for diagnosis of antibody-mediated rejection. Evidence of repeated endothelial cell–antibody interaction is required by Banff criteria to diagnose either acute antibody- or chronic active antibody-mediated rejections. Peritubular capillary C4d positivity satisfies this requirement. The one major exception to this rule is C4d positivity in ABO-incompatible kidney transplantation, where C4d positivity on the order of 80–90% is reported, usually without histologic evidence of rejection.[14,15] In this setting, C4d staining has been termed 'accommodation' and is not considered pathologic in the absence of HLA donor-specific antibodies. Diffuse peritubular capillary C4d positivity without concurrent rejection was even associated with reduced scarring on subsequent biopsies in one study of 33 ABO-incompatible transplants.[16] For ABO-incompatible allografts, other evidence of antibody interaction with the endothelium is required to diagnose antibody-mediated rejection. In all other situations, when transplant glomerulopathy coexists with both donor-specific antibodies against HLA antigens and positive peritubular capillary C4d staining, a definite diagnosis of chronic active antibody-mediated rejection can be made as the cause of the transplant glomerulopathy by Banff criteria.

It is well described that C4d positivity can be evanescent, with successive biopsies changing from C4d-positive to C4d-negative in days to weeks, and *vice versa*.[17,18] Both C4d-negative acute antibody-mediated rejection and chronic active antibody-mediated rejection are well described. With donor-specific antibody-positive and C4d-negative, chronic active antibody-mediated rejection can be diagnosed by Banff 2015 if either g+ptc $\geq$ 2 (with requirement

for g ≥ 0 when associated borderline or acute cell-mediated rejection exists) or there is increased expression of gene transcripts indicating endothelial cell injury (known as ENDATS). On the other hand, C4d-positive but HLA donor-specific antibody-negative biopsies with appropriate tissue injury, for example, glomerulitis acutely or transplant glomerulopathy chronically, are considered only suspicious for humoral causation by Banff. Others, however, advocate that microvascular inflammation with C4d positivity can rule in antibody-mediated rejection despite HLA donor-specific antibody negativity, at least in the acute situation.[19]

By current Banff criteria, either focal (≥10% up to 50% peritubular capillary staining, termed C4d2) or diffuse (≥50% peritubular capillary staining, termed C4d3) by immunoflourescence are considered positive for diagnosing antibody-mediated rejection, while staining of 1–10% peritubular capillaries (C4d1) is not. Separating C4d1 (considered negative) from C4d2 (considered positive) may be technically difficult because of peritubular capillary dropout when significant interstitial fibrosis exists. In this circumstance, double immunofluorescence with C4d and CD34 can facilitate more accurate assessment of the true percent positive.[20] By less sensitive immunohistochemistry, anything ≥ 0% peritubular capillary staining is considered positive. These thresholds for positivity with either immunoflourescence or immunohistochemistry represent a reduction compared with prior Banff publications. A recent single center series demonstrated a doubling of cases diagnosed with chronic active antibody-mediated rejection (36% *vs* 18%) using the newer criteria for C4d positivity along with the g+ptc ≥ 2 criteria in C4d-negative cases.[21] The new criteria significantly predicted the combined endpoint of doubling of serum creatinine or graft loss, whereas prior criteria did not. Of note, this positive association with outcome was significantly related only to the lowered C4d threshold and not to the inflammatory component (g+ptc ≥ 2) of the new criteria.

The percentage of cases of transplant glomerulopathy that are C4d positive varies widely in the literature. Some studies used immunoflourescence, others immunohistochemistry, and some both methods. Specific criteria for positivity varied, as many studies were published before the 2013 and 2015 Banff updates. Regele *et al*[18] found 67% of 58 biopsies to be C4d positive by immunofluorescence and Mauiyyedi *et al*[22] found 61% of 38 cases positive by immunohistochemistry. In contrast, Al Aly *et al*[23] used immunoflourescence and found 0 of 20 patients with transplant glomerulopathy had positive staining. Akalin *et al*[24] studied 36 patients by immunohistochemistry and found only 4 with diffuse and 1 with focal (10–50% peritubular capillaries) staining, although the number with ≥ 0–10% peritubular capillary positivity, now considered positive by current Banff criteria using immunohistochemistry, was not noted. Sis *et al*[25] used immunoflourescence on 50 biopsies with transplant glomerulopathy and found diffuse (≥50% peritubular capillaries) positivity in 13 and focal positivity in 5 others (1–50% peritubular capillaries, not 10–50% as currently specified by Banff for immunoflourescence). Gloor *et al*[26] used immunoflourescence and found 13 of 51 patients positive without specifying the extent. Hayde *et al*[27] used immunohistochemistry and found 7 of 46 transplant glomerulopathy biopsies to be C4d positive. Overall ~20–25% of biopsies with transplant glomerulopathy are C4d positive. If donor-specific antibodies are detectable, chronic active antibody-mediated rejection is the cause of the transplant glomerulopathy.

Biopsies with transplant glomerulopathy are not uncommonly C4d positive in the absence of detectable HLA donor-specific antibodies in ABO-compatible transplants, which is deemed 'suspicious' for chronic active antibody-mediated rejection. The donor-specific antibodies may exist in the serum, but at a lower level than detectable by current methodology or below an arbitrary cutoff. The antibodies may be of high affinity and totally absorbed on the allograft.[28,29] The antibodies may be directed against donor-recipient loci not previously typed, such as DP. The antibodies may be directed against non-HLA antigens, such as MHC class I chain-related genes A and B (MICA and MICB). The alloantibodies may react with epitopes contained within donor antigens, although not donor specific in terms of antigen.[30] Finally, the alloantibody response may wane over time, such that particular donor-specific antibodies are no longer being produced. Theoretically, antibodies may not even be involved at all, with activation of C4 via the lectin pathway, although clinical support for this does not exist to our knowledge.

In the case of chronically failing allografts, both donor-specific antibody-positive/C4d-negative and donor-specific antibody-negative/C4d-positive scenarios are not uncommon, and both may indicate an antibody-mediated cause. In a series of 173 patients with late graft dysfunction, 31 were donor-specific antibody positive/C4d negative and 28 were donor-specific antibody negative/C4d positive as compared with 40 donor-specific antibody positive/C4d positive.[31] The results are similar if transplant glomerulopathy is specifically considered. For example, in a case series of 71 patients with transplant glomerulopathy, Lesage *et al*[32] noted that 8 were donor-specific antibody positive/C4d negative and 6 were donor-specific antibody negative/C4d positive, and only 12 were donor-specific antibody positive/C4d positive.[32] The long-term outcomes for all three groups were similar and significantly worse than those with both Cd and donor-specific antibody negativity. Likewise, Shimizu *et al*[33] found that 12 of 50 biopsies with transplant glomerulopathy were donor-specific antibody positive/C4d negative and 7 were donor-specific antibody negative/C4d positive, although only 17 of 37 patients received ABO-

compatible grafts clouding the issue. In a recent case series of 92 patients with transplant glomerulopathy, 35 were considered only suspicious for chronic active antibody-mediated rejection based on either donor-specific antibody positive/C4d negative or donor-specific antibody negative (or not available)/C4d positive, compared with 34 definite chronic active antibody-mediated rejection.[34] In C4d-positive cases without donor-specific antibody (or any HLA antibodies), non-HLA antibodies should be considered. If non-donor-specific HLA antibodies are present, epitope analysis searching for antibodies against donor-specific epitopes by HLA Matchmaker (http:HLAMatchmaker.com) may be indicated.[35]

Normal human glomeruli will often stain positive for C4d by light microscopy, predominantly in the mesangial regions,[10] although there may be subendothelial staining as well.[10] Immune complex-mediated glomerulonephridites (either in a native kidney or a kidney allograft) stain positive for C4d in a granular or pseudolinear manner, including membranous nephropathy and immune complex-mediated membranoproliferative glomerulonephritis, where there can be intense capillary wall staining.[36] Thrombotic microangiopathy in the native kidney also frequently stains positive for C4d in glomeruli.[37] The C3 glomerulopathies appear to stain negative for C4d,[36] owing to alternate pathway activation of complement.

Glomerular staining for C4d in kidneys allografts is not uncommonly found, although reports of frequency are quite variable (as low as 10% to as high as 49%) .[17,38,39] Reports of glomerular C4d staining specifically in transplant glomerulopathy have also been variable, ranging from a frequency of 12%[18] to as high as 100%.[39–41] Gasim *et al*[41] recently postulated that glomerular C4d staining indicated structural glomerular basement membrane modification, specifically duplication of the glomerular basement membrane by light or electron microscopy, as may occur in transplant glomerulopathy in kidney allografts or thrombotic microangiopathy in native kidneys.[41] The concurrent presence of peritubular capillary C4d staining was indicative of a humoral pathophysiology in the allografts and was associated with positive glomerular staining even in the absence of detectable glomerular basement membrane duplication. Glomerular C4d positivity is not part of current Banff criteria.

Electron microscopy

The ultrastructural features of transplant glomerulopathy include endothelial cell swelling and/or vacuolization, loss of endothelial fenestrations, subendothelial widening of the lamina rara interna with electron-lucent or flocculent material, cell debris, and reduplication or multilamination of the lamina densa (see Figure 2). Fibrin may be present. These findings are indistinguishable from those of any thrombotic microangiopathy. Electron-dense deposits are usually not present, and these changes strongly suggest a recurrent or *de novo* immune complex glomerulonephritis, especially immunoglobulin-mediated (type 1) membranoproliferative glomerulonephritis or a C3 glomerulopathy. Importantly, these latter two conditions would have characteristic immunofluorescence findings separating them from transplant glomerulopathy. Of note, however, sparse deposits may occasionally be found in transplant glomerulopathy, presumably resulting from nonspecific deposition of immune reactants such as IgM and C3.

In a seminal paper, Wavamunno *et al*[42] profiled with protocol biopsies the ultrastructural changes occurring over time in seven patients who eventually developed transplant glomerulopathy by light microscopy as compared with eight controls who did not. Endothelial cell swelling and vacuolization, as well as widening of the lamina rara interna, were detectable within 1 to 3 months and were significantly greater than in controls. Glomerular basement membrane duplication was obvious within the first year, although endothelial fenestration was not significantly reduced until 3 years. Light microscopic changes diagnostic of transplant glomerulopathy appeared only after 2.3 years. Similarly, Haas and Mirocha[43] found the combination of endothelial swelling, subendothelial widening, and glomerular basement membrane reduplication together within the first 3 months post-transplantation in 11 of 17 biopsies with C4d-positive acute antibody-mediated rejection and 8 of 16 with C4d-negative acute antibody-mediated rejection compared to 0 of 65 without acute antibody-mediated rejection and/or donor-specific antibody. Individually, however, these changes were found in 5 of 17 with cellular rejection and 4 of 10 with calcineurin inhibitor toxicity. On follow-up biopsies of 18 patients (11 C4d positive), 8 developed overt transplant glomerulopathy by light microscopy, a result that appeared to be inhibited by treatment of the acute rejection.

Based on these findings, transplant glomerulopathy is now diagnosable by the most recent Banff criteria purely by electron microscopy in the absence of double contours by light microscopy. This requires glomerular basement membrane duplication in at least three glomerular capillaries (with associated endothelial changes and/or lamina rara interna electron-lucent widening) and is designated cg1a by Banff 2015.

Similar to the glomerular capillary bed, endothelial cell activation and damage with basement membrane changes occur in the peritubular capillary bed in transplant glomerulopathy. In fact finding such changes is sufficient to satisfy Banff criteria for tissue injury in diagnosing chronic active antibody-mediated rejection, even in the absence of glomerular changes. Such peritubular capillary changes frequently do coexist, however, with transplant glomerulopathy irrespective of the underlying

etiology. Monga *et al*[44] initially reported peritubular capillary basement membrane multilayering by electron microscopy (Figure 5) in all 14 patients with transplant glomerulopathy, as well as in all 38 specimens with transplant glomerulopathy in a subsequent study,[45] and they suggest that their presence can be used to infer current or future transplant glomerulopathy, when glomeruli are not present in the specimen.[45] Although clearly found in association with transplant glomerulopathy in cases of antibody-mediated rejection, numerous authors have emphasized that peritubular capillary basement membrane multilayering is not specific for an antibody cause. Other etiologies include postinfectious glomerulonephritis, lupus nephritis, cyclosporine nephrotoxicity, and obstructive uropathy.[46–48]

In properly interpreting peritubular capillary basement membrane multilayering pathologically, several issues require consideration: the minimal circumference of a given capillary that must be involved to consider it positive, for example, $\geq 60\%$, 100%; the cutoff for positivity, for example, ≥ 3 layers, ≥ 5 layers; how many peritubular capillaries should be studied, for example 10, 25, and so on; and finally, whether only the three most affected capillaries should be graded as opposed to evaluating all that are available. For example, Iványi *et al*[49] considered circumferential changes to involve $\geq 75\%$ of the peritubular capillary circumference, and they evaluated the mean number of layers in the entire specimen. In contrast, Liapis *et al*[50] scored biopsies based only on the three most affected capillaries. The most severe category required 1 capillary with ≥ 7 circumferential layers, with 5 or more in the remaining 2. This study served as the basis of current Banff criteria. These severe changes were rare in native kidneys with the exception of advanced thrombotic microangiopathy, and they were not specific for chronic active antibody-mediated rejection in the transplant specimens. Severe changes were also found in acute cell-mediated rejection, chronic active cell-mediated rejection, and calcineurin inhibitor nephrotoxicity. The positive predictive value for the most severe changes indicating antibody causation was only 49%.

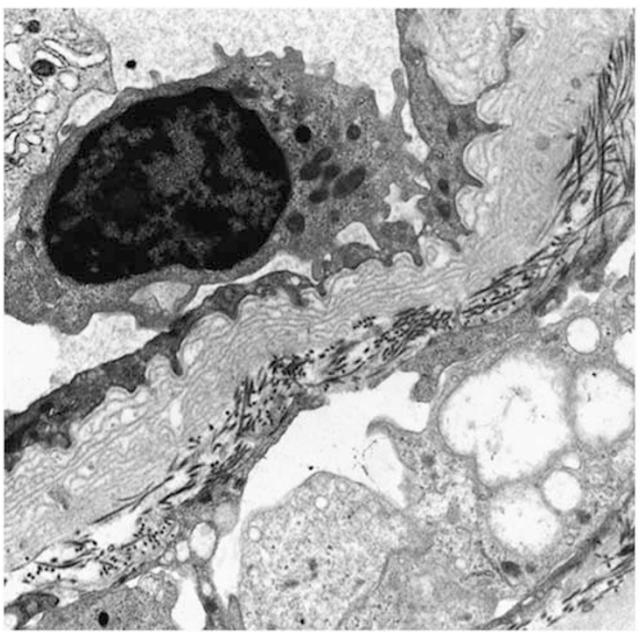

Figure 5 Electron microscopy showing peritubular capillary basement membrane multilayering (PTCBMML). Prominent reduplication of the basement membrane of a peritubular capillary. Original magnification ×4000

Current Banff criteria for using peritubular capillary basement membrane multilayering to diagnose chronic active antibody-mediated rejection require ≥ 7 layers in 1 capillary plus ≥ 5 layers in 2 additional capillaries, but it is not specified what percentage of circumference must be involved to be considered positive or how many capillaries should be studied to exclude the diagnosis.

Several recent studies addressed the utility of assessing the mean peritubular capillary basement membrane multilayering in the entire sample or a fraction thereof .[51] de Kort *et al*[52] found that a mean of 2.5 layers/peritubular capillary significantly predicted future development of transplant glomerulopathy. Evaluation of only 10 peritubular capillaries was sufficiently sensitive to calculate means. Additionally, in patients with 2 or more biopsies evaluated by electron microscopy, the presence of at least 1 biopsy with a mean peritubular capillary basement membrane multilayering of ≥ 2.5 resulted in a significantly greater chance of developing transplant glomerulopathy than if consistently ≤ 2.5. Others have also found that 10 peritubular capillaries is a sufficient number to evaluate for prognostic purposes.[53]

Pathogenesis of transplant glomerulopathy

Chronic, repetitive glomerular endothelial cell injury and activation result in the pathologic findings diagnostic of transplant glomerulopathy. When antibodies are involved, complement activation may mediate endothelial damage, although antibodies can activate and injure endothelial cells in the absence of complement. Numerous inflammatory cell types may be involved, including monocytes/macrophages, lymphocytes, NK cells, and neutrophils. Various cytokines may participate as well. Five major, often interrelated, causes of endothelial injury have been described in association with transplant glomerulopathy: alloantibodies, autoantibodies, cell-mediated injury, thrombotic microangiopathy, and hepatitis C. These will be discussed in detail.

Although the endothelium may be initially injured, it has been hypothesized that podocyte depletion may be the final common pathway resulting in proteinuria, reduced glomerular filtration rate,

and allograft loss.[54] In a recent study, patients with transplant glomerulopathy had 10 to 20 times increased urinary podocin/creatinine ratios, a marker of the urinary podocyte number, as compared with those with stable function.[54] A subset of these patients developed transplant glomerulopathy early (within 2 years) with reduced podocyte number per glomerulus without significant change in size, resulting in reduced podocyte density. A second subset developed transplant glomerulopathy much later (~10 years) and had both reduced podocyte number/glomerulus and significant glomerular enlargement, resulting in an even more marked reduction in podocyte density. Podocyte density significantly correlated with proteinuria, glomerular filtration rate, and glomerulosclerosis. Experimental evidence that podocyte damage can follow primary endothelial cell injury comes from a murine model of adriamycin nephrosis.[55]

Antibody-mediated rejection

By far the most widely recognized cause of transplant glomerulopathy is chronic antibody-mediated injury from donor-specific HLA alloantibodies. Based on current Banff criteria, transplant glomerulopathy represents sufficient histologic evidence of chronic tissue injury to satisfy the morphologic component for a diagnosis of chronic active antibody-mediated rejection by Banff criteria.[9] The progression of chronic antibody-mediated damage to transplant glomerulopathy has been demonstrated in Cynomolgus monkeys.[56] The clinical evidence in support of antibody-mediated causation is overwhelming. TG can develop in association with either preformed donor-specific antibody or *de novo* donor-specific antibody. In a study of 1-year protocol biopsies, Gloor *et al*[57] found transplant glomerulopathy in 22% of 37 HLA-incompatible (cross-match positive), 13% of 24 ABO-incompatible, and 8% of 198 conventional live donor transplants. By multivariable analysis, transplant glomerulopathy was significantly associated with prior acute antibody-mediated rejection (odds ratio 17.5, $P < 0.0001$), and 44% of such patients developed transplant glomerulopathy. Similarly, Loupy *et al*[58] found that 43% of patients with pretransplant donor-specific antibody positivity and subclinical acute antibody-mediated rejection on a 3-month protocol biopsy developed transplant glomerulopathy by 1 year. Weibe *et al*[59] studied 315 patients without preformed donor-specific antibody. Using routine surveillance serum screening and protocol biopsies, they determined that *de novo* donor-specific antibody developed in 47 patients (15%). Transplant glomerulopathy was not significantly detected before antibody development, but was significantly present afterwards in the 29 with clinical renal dysfunction. Other studies demonstrate the association of *de novo* donor-specific antibody and transplant glomerulopathy.[60]

Additional evidence supports donor-specific antibody causation. In a study of 598 conventional transplants, 73 developed transplant glomerulopathy over 5 years, with a significant association by multivariable analysis with HLA class II level, HLA class II donor-specific antibody level, and prior acute antibody-mediated rejection.[61] Sis *et al*[25] coined the term 'ABCD Tetrad' of late antibody-mediated rejection: 'A' for donor-specific antibody, 'B' for peritubular capillary basement membrane multilayering, 'C' for C4d positivity, and 'D' for reduplication of the glomerular basement membrane (transplant glomerulopathy). In their series of 53 biopsies (41 patients) with transplant glomerulopathy, 60% had donor-specific antibody, 91% had peritubular capillary basement membrane multilayering, and 36% were C4d positive. Many other series showed similar results, with a majority or significant minority of transplant glomerulopathy cases having evidence of HLA antibody involvement, either as donor-specific antibody positive and/or C4d positive.[18,22,27,32,34,38,62] The importance of this antibody-mediated mechanism has been stressed and discussed in detail in recent reviews on transplant glomerulopathy .[63,64]

Perhaps, more important than donor-specific antibody directed against donor serologic antigens may be antibodies reacting with donor-specific epitopes not present in the recipient.[65,66] Such epitopes may be shared among HLA antigens and hence not be recognized simply by considering donor serologic antigens. For example, in a study of 17 allograft nephrectomy specimens, ~20% of HLA antibodies were directed against donor-specific alleles, whereas ~80% were directed against donor specific epitopes.[30] Similarly, Lachmann *et al*[67] performed epitope analysis on 9 patients with allograft nephrectomies and identified 25 donor–recipient epitope mismatches. Out of a total of 243 class I anti-HLA antibodies in these 9 patients that were not donor-specific antibody, 125 reacted with donor epitopes and were considered donor epitope-specific antibodies. Sapir-Pichhadze *et al*[68] compared the DRB1, 3, 4, and 5 and DQ epitope mismatches of 52 donor–recipient pairs where transplant glomerulopathy developed with that of 104 controls without transplant glomerulopathy. By multivariable analysis, the OR for developing transplant glomerulopathy significantly increased by 25% for each additional 10 DRB1, 3, 4, and 5+DQ eplet mismatches and when comparing either of the higher two tertiles with the lowest tertile. This held for DRB alone but not for DQ alone.

Non-HLA antibodies

Non-HLA antigens may elicit antibody responses that contribute to renal allograft dysfunction and TG, either alone or in conjunction with anti-HLA antibodies. Some are polymorphic and elicit

alloresponses. Others are cryptic autoantigens that may be expressed on the cell surface following injury and result in loss of tolerance and autoantibody-mediated injury.[69]

The most polymorphic non-HLA alloantigens are the MICA and MICB. At least 100 MICA and 40 MICB alleles have been described (accessible at http://hla.alleles.org/nomenclature/stats.html) and to which an alloantibody response may occur.[70] Anti-MICA antibodies may be detectable pretransplantation and/or develop post-transplantation. They can be autoreactive, donor derived, or have unrelated third-party specificity. The effect of MICA/B sensitization on transplant outcome remains uncertain.[71] Their role specifically in transplant glomerulopathy has not been addressed to our knowledge.

Autoantibodies are now recognized as important mediators of acute and chronic allograft damage in kidney and other solid organ transplants. Naturally occurring IgM autoantibodies have been well described and may react with apoptotic neoantigens facilitating senescent cell clean-up.[72,73] More recently, however, it has been shown that IgG autoantibodies normally number in the thousands and are of uncertain physiologic significance.[74] Their numbers increase with age and male sex, although they may be decreased in certain disease states. Numerous autoantibodies in the setting of kidney and other solid organ transplantation have been described,[71] resulting in both acute[75] and chronic injury.[76] Some well-studied antigenic determinants include the antiangiotensin II type 1 receptor,[77,78] glutathione-*S*-transferase T1,[79] and vimentin.[80]

Some autoantibodies have been described specifically in association with transplant glomerulopathy. Joosten *et al*[81] compared 16 patients with TG to 16 controls with chronic allograft changes but without transplant glomerulopathy. Serum IgG from 11 of the 16 transplant glomerulopathy patients reacted with human glomerular basement membrane, 7 of which also reacted with purified heparin sulfate proteoglycans, predominantly agrin. Only 3/16 controls reacted with glomerular basement membrane ($P = 0.0044$). Interestingly, patients with antiglomerular basement membrane antibodies had significantly more prior acute rejections than the 5 without these antibodies, yet only 6 of the 11 had concurrent anti-HLA antibodies. Of note, agrin is also expressed in the basement membranes of lung, skin, and muscle. Interestingly, there is no clinical evidence of disease in these organs associated with these antibodies. This same group had previously used a murine model of transplant glomerulopathy and found the predominant antibody to be directed against the heparin sulfate proteoglycan perlecan.[82] In humans, Yang *et al*[83] showed that pretransplant autoantibodies against LG3, a C-terminal fragment of perlecan, significantly increased the risk for delayed graft function and reduced 1-year allograft function following delayed graft function, although transplant glomerulopathy was not specifically addressed.

Dinavahi *et al*[84] used a protein microarray analysis to study autoantibody repertoires pre- and post-transplantation from patients with and without transplant glomerulopathy. Transplantation *per se* routinely resulted in changed autoantibody repertoires irrespective of transplant glomerulopathy with unique profiles for individual patients. Pre- and post-transplantation peroxisomal-*trans*-2-enoyl-CoA-reductase IgG autoantibodies strongly associated with transplant glomerulopathy, a result confirmed by ELISA and in a validation set.

Angaswamy *et al*[85] compared 26 patients with transplant glomerulopathy to 10 stable controls with normal histology. Although only 16 transplant glomerulopathy patients had anti-HLA antibodies (12 donor-specific antibodies), 22 of 26 had autoantibodies (both IgM and IgG) against the kidney-restricted antigens collagen-IV and fibronectin giving an OR of 22 ($P = 0.001$). Six of the 10 HLA-negative patients were autoantibody positive. Four of 18 transplant glomerulopathy patients had these autoantibodies pretransplantation.

Jackson *et al*[75] used a protein array analysis of eluates from 10 endothelial cell cross-match-positive but HLA-negative kidney transplant recipients to identify 4 endothelial cell antigenic targets: endoglin, Fms-like tyrosine kinase-3 ligand, EGF-like repeats and discoidin I-like domains 3, and intercellular adhesion molecule 4. Sera from 150 additional kidney transplant recipients were tested by ELISA for autoantibodies against these 4 antigens, 36 of which tested strongly positive against all 4 antigens. The transplant glomerulopathy score was significantly higher in this group of 36, even restricted to those with no/low HLA donor-specific antibody.

Cell-mediated rejection

Many cases of transplant glomerulopathy lack evidence of antibody involvement. Akalin *et al*[24] found that only 5 of 36 biopsies with transplant glomerulopathy were C4d positive, and only 36% of 28 tested patients were donor-specific antibody positive. In fact, 15 of the 28 patients lacked any HLA antibodies by Luminex Flow Beads. Lesage *et al*[32] found that 45 of 71 patients with transplant glomerulopathy lacked both donor-specific antibody and C4d staining. Of 46 patients with TG in one series, 25 were donor-specific antibody negative and C4d negative.[27] In another series of 45 transplant glomerulopathy biopsies, 26% lacked both donor-specific antibody and C4d staining.[25] Torres *et al*[62] evaluated the late (≥6 months) allograft biopsies of 59 patients and found 17 with transplant glomerulopathy satisfying Banff 2013 criteria for chronic active antibody-mediated rejection, as compared to 12 that were both donor-specific antibody and C4d negative.

There is a substantial body of evidence supporting cell-mediated rejection in the pathogenesis of

transplant glomerulopathy. Transplant glomerulitis, a precursor of transplant glomerulopathy, may be composed of predominantly T cells in C4d-negative biopsies with acute rejection, as opposed to C4d-positive biopsies where monocytes predominate.[86] Batal *et al*[87] found that 47% of 32 biopsies with donor-specific antibody-negative, C4d-negative acute cell-mediated rejection had glomerulitis ($g \geq 0$), as did 16 of 26 borderline, donor-specific antibody-negative, C4d-negative biopsies. Glomerulitis correlated with subsequent development of transplant glomerulopathy, although data were not provided specifically for donor-specific antibody-negative, C4d-negative, $g \geq 0$ cases. Similarly, in a series of 44 patients with acute cell-mediated rejections, 9 had $g \geq 0$.[88] In a series of late C4d-negative biopsies of donor-specific antibody-negative patients, 5 of 18 with acute cell-mediated rejections had $g \geq 0$, as did 1 of 10 with borderline lesions. Furthermore, 3 of 18 with acute cell-mediated rejections also had concurrent transplant glomerulopathy, as did 1 of 10 with borderline lesions.[89]

Cell-mediated rejection more commonly precedes transplant glomerulopathy than does antibody-mediated rejection in some reports. In a series of 55 patients with transplant glomerulopathy, 6 had prior acute cell-mediated rejection, 9 had prior borderline cell-mediated rejection, but only 3 had antibody-mediated rejection.[26] In another series of 37 patients with transplant glomerulopathy who had available rejection history, 20 had prior acute rejections, of which 74% were cell-mediated rejection and 26% were antibody-mediated rejection.[25] Of note, both of these studies were published before recognition of C4d-negative antibody-mediated rejection. It is probable that some of the cases considered cell-mediated rejection were mixed cell and antibody mediated. Furthermore, it should be acknowledged that cell-mediated rejection is a defined risk factor for future production of *de novo* DSA,[59] which could mediate the development of TG through antibody-mediated rejection.

Other evidence for T-cell involvement exists. Akalin *et al*[90] compared 16 patients with non-specific, chronic nephropathy to 5 with transplant glomerulopathy. All transplant glomerulopathy cases stained positive for CD3 and ICOS, present on activated CD4 and CD8 T cells (also on resting B cells, which were not present), as well as for the chemokine receptor CXCR3 (present on activated, effector T cells) and its ligands Mig and IP-10. Biopsies with chronic nephropathy lacking transplant glomerulopathy did not have these findings.

Naïve CD4 T cells can be directed to several lineages, including Th1, Th2, Th17, and T-regulatory cells. Th1 CD4 cells express the transcription factor T-bet and interferon-γ. Homs *et al*[91] found significantly increased expression of T-bet and interferon-γ mRNA in renal biopsies of patients with transplant glomerulopathy as compared to those without transplant glomerulopathy. Sun *et al*[92] also found significantly increased T-bet expression in glomerular and peritubular capillaries of patients with transplant glomerulopathy, which correlated with CD4, CD8, and CD68 infiltration. The Th2 transcription factor GATA-3 was rarely found. In contrast, Sun *et al*[93] found predominant intraglomerular T-bet expression in acute antibody-mediated rejection as compared with acute cell-mediated rejection. Yadav *et al*[94] found significantly greater T-bet-positive mononuclear cell infiltration of transplant glomerulopathy biopsies compared with biopsies with nonspecific, chronic changes without transplant glomerulopathy, although all of the transplant glomerulopathy patients were donor-specific antibody positive and C4d positive. Overall, these results support a role for effector T cells in mediating transplant glomerulopathy, either alone or in conjunction with antibody-mediated damage.

Thrombotic microangiopathies

The thrombotic microangiopathies may produce a morphologic picture by either light or electron microscopy indistinguishable from transplant glomerulopathy (Figure 6). Thrombotic microangiopathy represents a syndrome characterized pathologically by arteriolar and/or capillary thrombosis with associated vessel wall changes, including fibrinoid necrosis acutely and myointimal proliferation ('onion skinning') chronically.[95] Red blood cell fragments may be visible within the damaged vessel wall. Typically, a microangiopathic hemolytic anemia is concurrently present, characterized by thrombocytopenia, anemia with schistocytes on peripheral blood smear, reduced or absent haptoglobin, indirect hyperbilirubinemia, and elevated LDH. The thrombotic microangiopathys may be primary, including hereditary or acquired causes, or they may be secondary to various other conditions (Table 2). Disease may be limited clinically to a specific organ, such as native kidneys or a kidney allograft, or may be systemic when associated with a microangiopathic hemolytic anemia or multiple organ involvement. Similar to transplant glomerulopathy, the thrombotic microangiopathys are initiated by endothelial cell injury from any cause as listed in Table 2.

Following kidney transplantation, thrombotic microangiopathy may occur *de novo* or as recurrent disease with approximately equal frequency. Using a registry analysis of kidney transplant recipients, Reynolds *et al*[96] estimated *de novo* occurrence in 0.8% and a recurrence rate of 29%. Although most cases developed in the first 3 months, new cases continued to develop through the 3 years of follow-up. Single center series found incidence rates averaging about 5%,[97–99] although prevalence rates as high as 12% have been reported.[100] Recurrent thrombotic microangiopathy is an indication for genetic testing for complement regulatory protein mutations and possibly for treatment with eculizumab.[101]

Development of *de novo* post-transplantation should prompt evaluation for antibody-mediated

rejection.[98] By Banff 2015, acute thrombotic microangiopathy is one of the defining morphologic features of acute tissue injury sufficient to diagnose acute antibody-mediated rejection in the absence of another cause for the thrombotic microangiopathy. In a single center series of 59 patients with *de novo* thrombotic microangiopathy, 55% were C4d positive, indicating antibody-mediated rejection was the most common cause.[98] In a series of 1073 allograft biopsies, 37 had thrombotic microangiopathy.[102] The incidence of C4d positivity in those biopsies with thrombotic microangiopathy was identical compared with the overall population (16%). This result was confounded, however, by time of biopsy: the odds ratio for having a thrombotic microangiopathy if C4d positive was 3.8 for biopsies within 90 days, compared with 0.2 if taken ≥ 90 days. C4d-positive thrombotic microangiopathies were more likely to have glomerular thrombi and extensive microvascular inflammation, especially neutrophilic.

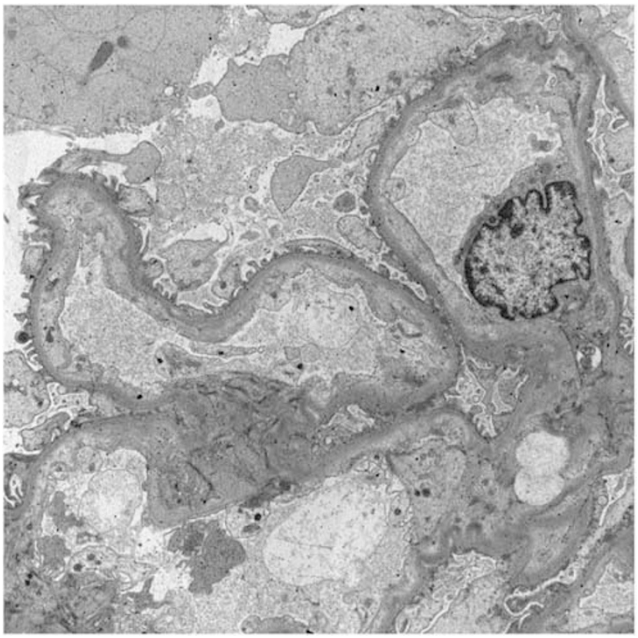

Figure 6 Thrombotic microangiopathy (TMA) in a native kidney. Electron micrograph of a glomerulus from a patient with bone marrow-associated TMA. There is prominent widening of the subendothelial space by edema, amorphous proteinaceous deposits, and cell debris. The endothelial cells are swollen and have lost their fenestrations.

Calcineurin inhibitor toxicity is a well-described cause of thrombotic microangiopathy both in native kidneys[97,103–105] and kidney allografts.[106–108] Viral infections are associated with *de novo* thrombotic microangiopathy, including CMV,[109] parvovirus B19,[110] hepatitis C (with associated antiphospholipid antibodies),[111] and influenza.[112] Whether all patients with *de novo* thrombotic microangiopathy should have screening for genetic mutations in complement regulatory proteins is uncertain at this time,[113] but may be a consideration especially with subsequent recurrence.

The pathologic identification of thrombotic microangiopathy as the cause of transplant glomerulopathy is most challenging when disease is allograft limited. By light microscopy, thrombotic microangiopathy is suggested by fibrin thrombi, RBC

Table 2 Classification of thrombotic microangiopathy (TMA)

	Type	*Defect*
Primary TMA		
Hereditary Primary TMA	ADAMTS13 deficiency	Reduced ADAMTS13 levels
	Complement-mediated TMA	Mutation in CFH, CFI, CD46, CFB, C3
	Metabolism-mediated TMA	*MMACHC* gene with cobalamin deficiency
	Coagulation-mediated TMA	Thrombomodulin, plasminogen, and DGKE
Acquired primary TMA		
	ADAMTS13 autoantibody	Reduced ADAMTS13 activity
	Shiga-toxin-mediated TMA	Shiga-toxin-mediated endothelial injury
	Immune drug-mediated TMA	Quinine-induced antibodies
	Toxic drug-mediated TMA	Endothelial injury as with VEGF inhibition, CNIs
	Complement-mediated TMA	Antibodies against CFH
Secondary TMA		
Metastatic cancer, usually adenocarcinoma		
Malignant hypertension		
Hematopoietic stem cell transplantation		
Antiphospholipid antibodies		
Systemic sclerosis		
Preeclampsia		
Infections, for example, HIV, CMV, fungal, bacterial		
Disseminated intravascular coagulation		

CFB, complement factor B; CFH, complement factor H; CFI, complement factor I; CMV, cytomegalovirus; CNI, calcineurin inhibitor; DKGE, diacylglycerol kinase ε; MMACHC, methylmalonic aciduria and homocystinuria type C protein gene; VEGF, vascular endothelial growth factor. Adapted from JN George and CM Nester. Syndromes of thrombotic microangiopathy. *N Engl J Med* 2014; 371: 654–666.

fragments, and myointimal proliferation with onion skinning involving arterioles. If just endothelial changes are present, the distinction from other causes of transplant glomerulopathy cannot be made. Similarly, by electron microscopy the changes of early transplant glomerulopathy are identical to early thrombotic microangiopathy. The presence of RBC fragments or fibrin thrombi support thrombotic microangiopathy as the cause of the transplant glomerulopathy. Both transplant glomerulopathy and thrombotic microangiopathy stain positive for C4d in glomeruli, although peritubular capillary staining for C4d indicates antibody-mediated transplant glomerulopathy.

Current Banff criteria for transplant glomerulopathy require absence of evidence of chronic thrombotic microangiopathy. Many published series of transplant glomerulopathy exclude cases with other evidence of thrombotic microangiopathy besides transplant glomerulopathy. Typically, this consists of detecting RBC fragments in vessel walls or glomerular capillaries, or onion-skin lesions of arteriole intima. By contrast, Baid-Agarwal *et al*[100] did not make this distinction, and in a series of 25 cases of transplant glomerulopathy, 8 had other evidence diagnostic of thrombotic microangiopathy. Sreedharanunni *et al*[99] evaluated 266 indication biopsies with glomerular pathology, of which 107 satisfied Banff 2009 criteria for transplant glomerulopathy (double contours in $\geq$10% of capillary loops in most affected glomerulus by light microscopy) and 91 had glomerular thrombotic microangiopathy (fibrin thrombi acutely, and subendothelial fibrin, endothelial swelling, mesangial hypercellularity, mesangiolysis, collapse, and small crescents chronically) with or without vascular thrombotic microangiopathy. Another 23 had only vascular thrombotic microangiopathy. Of the 114 biopsies with any thrombotic microangiopathy, 21 (18%) had coexisting transplant glomerulopathy by their definitions.

Hepatitis C virus infection

The most common glomerulopathy associated with hepatitis C virus infection is type I membranoproliferative glomerulonephritis, usually with low complement, type II cryoglobulinemia, and IgM rheumatoid factor.[114–116] In the kidney allograft, hepatitis C virus can cause either recurrent or *de novo* type I membranoproliferative glomerulonephritis. Chronic membranoproliferative glomerulonephritis in the allograft may be difficult to distinguish from transplant glomerulopathy by light microscopy, but these lesions should be readily distinguished by detecting immune complexes by immunofluorescence and electron microscopies, along with the associated serological findings such as low complement and serum cryoglobulins, which may[117] or may not be present.[118]

Other renal lesions associated with hepatitis C virus in native kidneys include membranous nephropathy,[119] fibrillary glomerulonephritis,[120] thrombotic microangiopathy,[121] and collapsing glomerulopathy following therapy with interferon[122] or pegylated interferon.[123] Kidney allografts may be similarly affected, including reports of thrombotic microangiopathy,[111] fibrillary glomerulonephritis,[124] and collapsing glomerulopathy.[125]

Gallay *et al*.[126] first noted the association of hepatitis C virus with transplant glomerulopathy in a report of two cases that concurrently had features of membranoproliferative glomerulonephritis. The altered morphological appearance of membranoproliferative glomerulonephritis that may result from immunosuppression following transplantation was noted, highlighting the difficulty in distinguishing this lesion from transplant glomerulopathy. Cosio *et al*[127] found 9 of 27 (33%) patients with transplant glomerulopathy to be hepatitis C virus positive, compared with only 1.5% of 105 transplant patients without transplant glomerulopathy ($P=0.0004$), although immunofluorescence and electron microscopies were not routinely performed, possibly resulting in misclassification of hepatitis C virus-related membranoproliferative glomerulonephritis as transplant glomerulopathy. Others have found a significant association as well.[26,100] Baid-Agrawal *et al*[100] described a case series of 25 patients with transplant glomerulopathy. Twelve were C4d positive supporting an antibody-mediated cause. Nine (33%) were hepatitis C virus positive (3 of which were C4d positive), a rate significantly higher than the 7% prevalence in 29 controls with calcineurin inhibitor toxicity and no transplant glomerulopathy. Interestingly, five of the nine patients had concurrent thrombotic microangiopathy, as did three other hepatitis C virus-negative patients, highlighting the potential overlapping pathophysiology of hepatitis C virus and thrombotic microangiopathy in causing non-antibody-mediated transplant glomerulopathy. In contrast, Cruzado *et al*[117] noted a similar prevalence of transplant glomerulopathy in patients with (5 of 44, 11.4%) or without hepatitis C virus (6 of 52, 11.5%) using light microscopy and immunofluorescence microscopy in all specimens, with electron microscopy if indicated.

The pathophysiologic link between hepatitis C virus and transplant glomerulopathy remains unclear. Baid-Agrawal *et al*[100] proposed several potential mechanisms. Transplant glomerulopathy may represent an atypical morphologic presentation of type 1 membranoproliferative glomerulonephritis, with altered immune complex deposition resulting from antigen–antibody imbalance secondary to chronic immunosuppression. Hepatitis C virus may upregulate the alloimmune response based on concurrent C4d positive and microvascular inflammation. Hepatitis C virus may cause transplant glomerulopathy as a result of the induction of antiphospholipid antibodies.[103]

Irrespective of the development of transplant glomerulopathy, hepatitis C virus can be effectively treated post-transplantation with direct acting antiviral agents.[125] Sustained virologic response rates of ~90% have been reported. Given the known association of hepatitis C virus with adverse patient and allograft survival, as well as the recently demonstrated efficacy and safety of direct acting anti-viral therapy of hepatitis C virus in transplant recipients,[125,128–130] in our opinion treatment should be considered in all viremic transplant patients irrespective of transplant glomerulopathy.

Clinical manifestations

TG may be clinically silent. In one series of 55 patients with transplant glomerulopathy, 27 (49%) were detected by protocol biopsy.[26] The prognosis of these patients was no better than those with clinically evident disease. Early clinical manifestations include low-level proteinuria and/or subtle decline in glomerular filtration rate. As the lesion progresses, however, hypertension may develop, glomerular filtration rate declines, and proteinuria increases, not uncommonly into the nephrotic range.[131]

Transplant glomerulopathy is associated with reduced allograft survival in numerous studies.[26,32,61,131,132] Attempts have been made to refine the prognostic implications of transplant glomerulopathy based on associated pathologic or clinical features. Concurrent peritubular capillary C4d positivity is associated with worse graft survival in some studies of transplant glomerulopathy,[32,61,132] but not in others.[133] Dobi *et al*[134] evaluated 59 patients with transplant glomerulopathy and found that concurrent intimal arteritis resulted in significantly shorter graft survival. When occurring late, microvascular inflammation (g and ptc) was shown by multivariable analysis to predict graft survival independent of transplant glomerulopathy in one study of 251 allograft recipients.[88] In another study of 33 transplant glomerulopathy patients, however, the degree of microvascular inflammation did not correlate with survival.[135] Concurrent hepatitis C virus may[100,136] or may not[34] significantly affect graft survival with transplant glomerulopathy. In an earlier study, Banfi *et al*[131] found that proteinuria ≥ 2.5 g/day significantly impacted graft survival in 28 patients with transplant glomerulopathy (graft survival 8% *vs* 67% if lower proteinuria, $P < 0.005$). Patri *et al*[34] studied 92 patients with transplant glomerulopathy and developed a prognostic index for graft survival based on the ci, ct, and ti Banff scores along with serum creatinine and proteinuria that was validated in an external cohort of transplant glomerulopathy patients.

Treatment

There is no specific treatment proven to work for transplant glomerulopathy. All patients should have general supportive measures given to any patient with chronic kidney disease. These include salt and protein restriction, cessation of smoking, weight loss if obese, control of blood pressure, renin–angiotensin system blockade, lipid control, mineral and bone disorder therapy, and control of anemia. Specific therapy may be offered on an individual basis, based on underlying cause and stage of progression. If a cell-mediated alloresponse is involved, optimization of immunosuppression with tacrolimus, mycophenolate, and steroids is recommended.

In the case of HLA alloantibody-mediated transplant glomerulopathy, the best approach to treatment is prevention. That would start by optimal HLA matching and avoiding transplantation if donor-specific antibodies are detectable, both of which are problematic given the shortage of organs. Whether epitope matching would reduce development of *de novo* donor-specific antibody and subsequently transplant glomerulopathy remains to be proven.[66] If donor-specific antibodies are detected, either preformed or *de novo*, close clinical surveillance as well as serial titration and protocol biopsy may identify the earliest stages and potentially prevent progression. There is some evidence that early, for example, at cg1a, appropriate treatment may forestall progression.[43]

It may become possible to determine patients destined to develop antibody-mediated transplant glomerulopathy before any microscopic changes appear by using a variety of microarray analyses of biopsy material. Validated ENDATs indicating endothelial cell injury satisfy current Banff criteria for antibody–endothelial interaction in diagnosing acute antibody-mediated rejection and chronic active antibody mediated. This is an active area of research by several groups,[137,138] and was highlighted at the 2015 Banff meeting.[9] Algorithms have been proposed to identify antibody-mediated injury as opposed to cell-mediated mechanisms by microarray analyses of transcript expression.[139] Whether treatment based on such analyses can prevent development of transplant glomerulopathy and/or improve allograft survival remains to be determined.

Eculizumab initially showed promise in preventing transplant glomerulopathy in sensitized recipients.[140] Longer term follow-up showed it was not effective, with the possible exception of those attaining persistently low B-cell flow crossmatches.[141] In a retrospective analysis, Orandi *et al*[142] found 4 of 5 HLA-incompatible live donor transplant recipients with acute antibody-mediated rejection given eculizumab plus splenectomy to have functioning grafts free of transplant glomerulopathy on 12-month biopsy, as compared with none of 5 given eculizumab alone and 4 of 14 given splenectomy alone. A pilot randomized controlled trial suggested stabilization of glomerular filtration rate with eculizumab.[143] A pilot randomized clinical trial in 18 patients with acute antibody-mediated rejection showed a trend for benefit with a human C1

esterase inhibitor,[144] and none of 7 treated patients had transplant glomerulopathy on 6-month biopsy as compared with 3 of 7 given placebo. Choi *et al*[145] reported a single center series of 36 patients with donor specific antibodies and chronic antibody-mediated rejection including transplant glomerulopathy who had failed standard of care were treated with monthly doses of tocilizumab, a monoclonal anti-IL-6 receptor antibody. They found stabilization of renal function without significant adverse events.

Standard treatment of antibody-mediated rejection should be considered for transplant glomerulopathy induced by alloantibodies if the lesion is early (cg1a or cg1b) and/or there is evidence of active microvascular inflammation, that is, g+ptc $\geq$ 2. In support, a retrospective, single center study of 33 patients with transplant glomerulopathy found that treatment with intravenous immunoglobulin and rituximab stabilized the serum creatinine, but only in the subgroup with significant microvascular inflammation (ptc $\geq$ 2 or g+ptc $\geq$ 4).[135] In other studies, the Banff microvascular inflammation scores and/or extent of peritubular capillary inflammation ($\geq$50% versus $\leq$ 50%) were significantly related to allograft survival and development of transplant glomerulopathy,[88,146,147] further supporting treatment based on microvascular inflammation. Additional potential therapies besides intravenous immunoglobulin and rituximab[148–151] for antibody-mediated transplant glomerulopathy include plasma exchange or immunoadsorption, high-dose steroids, and bortezomib, but data from randomized controlled trials are lacking. The use of such agents for autoantibody-mediated transplant glomerulopathy remains unstudied.

In transplant glomerulopathy patients with active hepatitis C virus, oral anti-viral therapy is required. Other active viruses such as cytomegalovirus should also be treated. In those with recurrent thrombotic microangiopathy or *de novo* thrombotic microangiopathy with complement regulatory protein mutations, eculizumab is indicated.[152] Eculizumab may also be indicated for recurrent thrombotic microangiopathy secondary to antiphospholipid antibodies.[153,154] For thrombotic microangiopathy ascribed to calcineurin inhibitors, as a minimum, there should be dose reduction or possibly discontinuation. Sirolimus is not recommended, as it has been similarly associated with thrombotic microangiopathy. Other calcineurin inhibitor-sparing agents, such as belatacept, require further study.

Conclusion

Transplant glomerulopathy represents a morphologic lesion and not a specific diagnosis. The hallmark pathologic feature is endothelial cell injury with glomerular basement membrane reduplication by light or electron microscopy in the absence of immune complex deposits. C4d may stain peritubular capillaries in cases mediated by alloantibodies, and glomeruli may also stain positive. Clinically, transplant glomerulopathy may be silent, detectable on protocol biopsy. Overt clinical manifestations include up to nephrotic range proteinuria, hypertension, and declining glomerular filtration rate. Either way, transplant glomerulopathy is clearly associated with reduced graft survival. Transplant glomerulopathy results from chronic, recurring endothelial cell injury potentially mediated by HLA alloantibodies, various autoantibodies, cell-mediated injury, thrombotic microangiopathy and/or hepatitis C virus. Multiple causes may exist in a single patient. Other than nonspecific supportive care, the only proactive therapy must be directed at the specific underlying cause.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Filippone EJ, Farber JL. The specificity of acute and chronic microvascular alterations in renal allografts. Clin Transplant 2013;27:790–798.
2 Hamburger J, Crosnier J, Dormont J. Experience with 45 renal homotransplantations in man. Lancet 1965;285:985–992.
3 Porter KA, Dossetor JB, Marchioro TL, *et al.* Human renal transplants. I. Glomerular changes. Lab Invest 1967;16:153–181.
4 Busch GJ, Galvanek EG, Reynolds ES Jr. Human renal allografts: analysis of lesions in long-term survivors. Hum Pathol 1971;2:253–298.
5 Zollinger HU, Moppert J, Thiel G, *et al.* Morphology and pathogenesis of glomerulopathy in cadaver kidney allografts treated with antilymphocyte globulin. Curr Top Pathol 1973;57:1–48.
6 Maryniak RK, Roy First M, Weiss MA. Transplant glomerulopathy: evolution of morphologically distinct changes. Kidney Int 1985;27:799–806.
7 Racusen LC, Solez K, Colvin RB, *et al.* The Banff 97 working classification of renal allograft pathology. Kidney Int 1999;55:713–723.
8 Haas M, Sis B, Racusen LC, *et al.* Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014;14:272–283.
9 Loupy A, Haas M, Solez K, *et al.* The Banff 2015 kidney meeting report: current challenges in rejection classification and prospects for adopting molecular pathology. Am J Transplant 2017;17:28–41.
10 Zwirner J, Felber E, Herzog V, *et al.* Classical pathway of complement activation in normal and diseased human glomeruli. Kidney Int 1989;36:1069–1077.
11 Cohen D, Colvin RB, Daha MR, *et al.* Pros and cons for C4d as a biomarker. Kidney Int 2012;81:628–639.
12 Batal I, Girnita A, Zeevi A, *et al.* Clinical significance of the distribution of C4d deposits in different anatomic compartments of the allograft kidney. Mod Pathol 2008;21:1490–1498.

13 Mengel M, Chan S, Climenhaga J, *et al.* Banff initiative for quality assurance in transplantation (BIFQUIT): reproducibility of C4d immunohistochemistry in kidney allografts. Am J Transplant 2013;13:1235–1245.
14 Haas M, Rahman MH, Racusen LC, *et al.* C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. Am J Transplant 2006;6:1829–1840.
15 Setoguchi K, Ishida H, Shimmura H, *et al.* Analysis of renal transplant protocol biopsies in ABO-incompatible kidney transplantation. Am J Transplant 2008;8: 86–94.
16 Haas M, Segev DL, Racusen LC, *et al.* C4d deposition without rejection correlates with reduced early scarring in ABO-incompatible renal allografts. J Am Soc Nephrol 2009;20:197–204.
17 Nickeleit V, Zeiler M, Gudat F, *et al.* Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. J Am Soc Nephrol 2002;13:242–251.
18 Regele H, Böhmig GA, Habicht A, *et al.* Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. J Am Soc Nephrol 2002;13:2371–2380.
19 Sapir-Pichhadze R, Curran SP, John R, *et al.* A systematic review of the role of C4d in the diagnosis of acute antibody-mediated rejection. Kidney Int 2015;87:182–194.
20 Jen K, Nguyen TB, Vincenti FG, *et al.* C4d/CD34 double-immunofluorescence staining of renal allograft biopsies for assessing peritubular capillary C4d positivity. Mod Pathol 2012;25:434–438.
21 De Serres SA, Noel R, Cote I *et al.* 2013 Banff criteria for chronic active antibody-mediated rejection: assessment in a real-life setting. Am J Transplant 2013;16:1516–1525.
22 MauiyyedI S, Pelle PD, Saidman S, *et al.* Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. J Am Soc Nephrol 2001;12: 574–582.
23 Al Aly Z, Yalamanchili P, Cortese C, *et al.* C4d peritubular capillary staining in chronic allograft nephropathy and transplant glomerulopathy: an uncommon finding. Transplant Int 2005;18:800–805.
24 Akalin E, Dinavahi R, Dikman S, *et al.* Transplant glomerulopathy may occur in the absence of donor-specific antibody and C4d staining. Clin J Am Soc Nephrol 2007;2:1261–1267.
25 Sis B, Campbell PM, Mueller T, *et al.* Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. Am J Transplant 2007;7:1743–1752.
26 Gloor JM, Sethi S, Stegall MD, *et al.* Transplant glomerulopathy: subclinical incidence and association with alloantibody. AmJ Transplant 2007;7: 2124–2132.
27 Hayde N, Bao Y, Pullman J, *et al.* The clinical and genomic significance of donor-specific antibody-positive/C4d-negative and donor-specific antibody-negative/C4d-negative transplant glomerulopathy. Clin J Am Soc Nephrol 2013;8:2141–2148.
28 Heinemann FM, Roth I, Rebmann V, *et al.* Characterization of anti-HLA antibodies eluted from explanted renal allografts. Clin Transplant 2006;371–378.
29 Billen EV, Christiaans MH, Lee J, *et al.* Donor-directed HLA antibodies before and after transplantectomy detected by the luminex single antigen assay. Transplantation 2009;87:563–569.
30 Milongo D, Kamar N, Del Bello A, *et al.* Allelic and epitopic characterization of intra? Kidney allograft anti-HLA antibodies at allograft nephrectomy. Am J Transplant 2017;17:420–431.
31 Gaston RS, Cecka JM, Kasiske BL, *et al.* Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. Transplantation 2010;90: 68–74.
32 Lesage J, Noel R, Lapointe I, *et al.* Donor-specific antibodies, C4d and their relationship with the prognosis of transplant glomerulopathy. Transplantation 2015;99:69–76.
33 Shimizu T, Tanabe T, Shirakawa H, *et al.* Clinical and pathological analysis of transplant glomerulopathy cases. Clin Transplant 2012;26:37–42.
34 Patri P, Seshan SV, Matignon M, *et al.* Development and validation of a prognostic index for allograft outcome in kidney recipients with transplant glomerulopathy. Kidney Int 2016;89:450–458.
35 Lachmann N, Schonemann C, El-Awar N, *et al.* Dynamics and epitope specificity of anti-human leukocyte antibodies following renal allograft nephrectomy. Nephrol Dial Transplant 2016;31: 1351–1359.
36 Sethi S, Nasr SH, De Vriese AS, *et al.* C4d as a diagnostic tool in proliferative GN. J Am Soc Nephrol 2015;26:2852–2859.
37 Chua JS, Baelde HJ, Zandbergen M, *et al.* Complement factor C4d is a common denominator in thrombotic microangiopathy. J Am Soc Nephrol 2015;26: 2239–2247.
38 Hayde N, Bao Y, Pullman J, *et al.* The clinical and molecular significance of C4d staining patterns in renal allografts. Transplantation 2013;95:580–588.
39 Sijpkens YW, Joosten SA, Wong M-, *et al.* Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy. Kidney Int 2004;65:2409–2418.
40 Verghese PS, Reed RC, Lihong B, *et al.* The clinical implications of the unique glomerular complement deposition pattern in transplant glomerulopathy. J Nephrol 2016; doi: 10.1007/s40620-016-0365-7.
41 Gasim AH, Chua JS, Wolterbeek Ron, *et al.* Glomerular C4d deposits can mark structural capillary wall remodeling in thrombotic microangiopathy and transplant glomerulopathy: C4d beyond active antibody mediated injury. Transpl Int 2017;31:1351–1359.
42 Wavamunno MD, O'Connell PJ, Vitalone M, *et al.* Transplant glomerulopathy: ultrastructural abnormalities occur early in longitudinal analysis of protocol biopsies. Am J Transplant 2007;7:2757–2768.
43 Haas M, Mirocha J. Early ultrastructural changes in renal allografts: correlation with antibody-mediated rejection and transplant glomerulopathy. Am J Transplant 2011;11:2123–2131.
44 Monga G, Mazzucco G, Novara R, *et al.* Intertubular capillary changes in kidney allografts: an ultrastructural study in patients with transplant glomerulopathy. Ultrastruct Pathol 1990;14:201–209.
45 Monga G, Mazzucco G, Messina M, *et al.* Intertubular capillary changes in kidney allografts: a morphologic investigation on 61 renal specimens. Mod Pathol 1992;5:125–130.

46 Drachenberg CB, Steinberger E, Hoehn-Saric E, *et al.* Specificity of intertubular capillary changes: comparative ultrastructural studies in renal allografts and native kidneys. Ultrastruct Pathol 1997;21:227–233.
47 Gough J, Yilmaz A, Miskulin D, *et al.* Peritubular capillary basement membrane reduplication in allografts and native kidney disease: a clinicopathologic study of 278 consecutive renal specimens. Transplantation 2001;71:1390–1393.
48 Iványi B, Kemeny E, Szederkenyi E, *et al.* The value of electron microscopy in the diagnosis of chronic renal allograft rejection. Mod Pathol 2001;14:1200–1208.
49 Iványi B, Fahmy H, Brown H, *et al.* Peritubular capillaries in chronic renal allograft rejection: a quantitative ultrastructural study. Hum Pathol 2000;31:1129–1138.
50 Liapis G, Singh HK, Derebail VK, *et al.* Diagnostic significance of peritubular capillary basement membrane multilaminations in kidney allografts: old concepts revisited. Transplantation 2012;94:620–629.
51 Roufosse CA, Shore I, Moss J, *et al.* Peritubular capillary basement membrane multilayering on electron microscopy: a useful marker of early chronic antibody-mediated damage. Transplantation 2012;94: 269–274.
52 de Kort H, Willicombe M, Brookes P, *et al.* Peritubular capillary basement membrane multilayering in renal allograft biopsies of patients with *de novo* donor-specific antibodies. Transplantation 2016;100: 889–897.
53 Dobi D, Bodo Z, Kemeny E, *et al.* Peritubular capillary basement membrane multilayering in early and advanced transplant glomerulopathy: quantitative parameters and diagnostic aspects. Virchows Arch 2016;469:563–573.
54 Yang Y, Hodgin JB, Afshinnia F, *et al.* The two kidney to one kidney transition and transplant glomerulopathy: a podocyte perspective. J Am Soc Nephrol 2015;26:1450–1465.
55 Sun YBY, Qu X, Zhang X, *et al.* Glomerular endothelial cell injury and damage precedes that of podocytes in adriamycin-induced nephropathy. PLoS ONE 2013;8:e55027.
56 Smith RN, Kawai T, Boskovic S, *et al.* Four stages and lack of stable accommodation in chronic alloantibody-mediated renal allograft rejection in cynomolgus monkeys. Am J Transplant 2008;8:1662–1672.
57 Gloor JM, Cosio FG, Rea DJ, *et al.* Histologic findings one year after positive crossmatch or ABO blood group incompatible living donor kidney transplantation. Am J Transplant 2006;6:1841–1847.
58 Loupy A, Suberbielle-Boissel C, Hill GS, *et al.* Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. Am J Transplant 2009;9:2561–2570.
59 Wiebe C, Gibson IW, Blydt-Hansen T, *et al.* Evolution and clinical pathologic correlations of *de novo* donor-specific HLA antibody post kidney transplant. Am J Transplant 2012;12:1157–1167.
60 Einecke G, Sis B, Reeve J, *et al.* Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. Am J Transplant 2009;9: 2520–2531.
61 Issa N, Cosio F, Gloor J, *et al.* Transplant glomerulopathy: risk and prognosis related to anti-human leukocyte antigen class II antibody levels. Transplantation 2008;86:681–685.
62 Torres IB, Salcedo M, Moreso F, *et al.* Comparing transplant glomerulopathy in the absence of C4d deposition and donor-specific antibodies to chronic antibody-mediated rejection. Clin Transplant 2014; 28:1148–1154.
63 Husain S, Sis B. Advances in the understanding of transplant glomerulopathy. Am J Kidney Dis 2013;62: 352–363.
64 Remport A, Ivanyi B, Mathe Z, *et al.* Better understanding of transplant glomerulopathy secondary to chronic antibody-mediated rejection. Nephrol Dial Transplant 2015;30:1825–1833.
65 Filippone EJ, Farber JL. Humoral immunity in renal transplantation: epitopes, cw and DP, and complement-activating capability—an update. Clin Transplant 2015;29:279–287.
66 Filippone EJ, Farber JL. The humoral theory of transplantation: epitope analysis and the pathogenicity of HLA antibodies. J Immunol Res 2016;2016: 10.1155/2016/5197396.
67 Lachmann N, Schonemann C, El-Awar N, *et al.* Dynamics and epitope specificity of anti-human leukocyte antibodies following renal allograft nephrectomy. Nephrol Dial Transplant 2016;31:1351–1359.
68 Sapir-Pichhadze R, Tinckam K, Quach K, *et al.* HLA-DR and -DQ eplet mismatches and transplant glomerulopathy: a nested case–control study. Am J Transplant 2015;15:137–148.
69 Cardinal H, Dieudé M, Hébert M. The emerging importance of non-HLA autoantibodies in kidney transplant complications. J Am Soc Nephrol 2017;28: 400–406.
70 Filippone EJ, Farber JL. Humoral immune response and allograft function in kidney transplantation. Am J Kidney Dis 2015;66:337–347.
71 Sigdel TK, Sarwal MM. Moving beyond HLA: a review of nHLA antibodies in organ transplantation. Hum Immunol 2013;74:1486–1490.
72 Fu M, Fan PS, Li W, *et al.* Identification of polyreactive natural IgM antibody that recognizes late apoptotic cells and promotes phagocytosis of the cells. Apoptosis 2007;12:355–362.
73 Peng Y, Kowalewski R, Kim S, *et al.* The role of IgM antibodies in the recognition and clearance of apoptotic cells. Mol Immunol 2005;42:781–787.
74 Nagele EP, Han M, Acharya NK, *et al.* Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. PLoS ONE 2013;8:e60726.
75 Jackson AM, Sigdel TK, Delville M, *et al.* Endothelial cell antibodies associated with novel targets and increased rejection. J Am Soc Nephrol 2015;26: 1161–1171.
76 Sigdel TK, Li L, Tran TQ, *et al.* Non-HLA antibodies to immunogenic epitopes predict the evolution of chronic renal allograft injury. J Am Soc Nephrol 2012;23:750–763.
77 Dragun D, Müller DN, Bräsen JH, *et al.* Angiotensin II type 1—receptor activating antibodies in renal-allograft rejection. N Engl J Med 2005;352:558–569.
78 Reinsmoen NL, Lai C, Heidecke H, *et al.* Anti-angiotensin type 1 receptor antibodies associated with antibody mediated rejection in donor HLA antibody negative patients. Transplantation 2010;90: 1473–1477.
79 AlvarezMarquez A, Aguilera I, Gentil MA, *et al.* Donor-specific antibodies against HLA, MICA, and GSTT1 in

patients with allograft rejection and C4d deposition in renal biopsies. Transplantation 2009;87:94–99.
80 Besarani D, Cerundolo L, Smith JD, *et al.* Role of anti-vimentin antibodies in renal transplantation. Transplantation 2014;98:72–78.
81 Joosten SA, Sijpkens YWJ, Van Ham V, *et al.* Antibody response against the glomerular basement membrane protein agrin in patients with transplant glomerulopathy. Am J Transplant 2005;5:383–393.
82 Joosten SA, Van Dixhoorn MGA, Borrias MC, *et al.* Antibody response against perlecan and collagen types IV and VI in chronic renal allograft rejection in the rat. Am J Pathol 2002;160:1301–1310.
83 Yang B, Dieudé M, Hamelin K, *et al.* Anti-LG3 antibodies aggravate renal ischemia? Reperfusion injury and long-term renal allograft dysfunction. Am J Transplant 2016;16:3416–3429.
84 Dinavahi R, George A, Tretin A, *et al.* Antibodies reactive to non-HLA antigens in transplant glomerulopathy. J Am Soc Nephrol 2011;22:1168–1178.
85 Angaswamy N, Klein C, Tiriveedhi V, *et al.* Immune responses to collagen-IV and fibronectin in renal transplant recipients with transplant glomerulopathy. Am J Transplant 2014;14:685–693.
86 Magil AB. Infiltrating cell types in transplant glomerulitis: relationship to peritubular capillary C4d deposition. Am J Kidney Dis 2005;45:1084–1089.
87 Batal I, Lunz JG III, Aggarwal N, *et al.* A critical appraisal of methods to grade transplant glomerulitis in renal allograft biopsies. Am J Transplant 2010;10:2442–2452.
88 Sis B, Jhangri GS, Riopel J, *et al.* A new diagnostic algorithm for antibody-mediated microcirculation inflammation in kidney transplants. Am J Transplant 2012;12:1168–1179.
89 Zhao X, Huang MD, Randhawa S, *et al.* Rejection of the renal allograft in the absence of demonstrable antibody and complement. Transplantation 2017;101:395–401.
90 Akalin E, Dikman S, Murphy B, *et al.* Glomerular infiltration by CXCR3+ ICOS+ activated T cells in chronic allograft nephropathy with transplant glomerulopathy. Am J Transplant 2003;3:1116–1120.
91 Homs S, Mansour H, Desvaux D, *et al.* Predominant Th1 and cytotoxic phenotype in biopsies from renal transplant recipients with transplant glomerulopathy. Am J Transplant 2009;9:1230–1236.
92 Sun Q, Zhang M, Xie K, *et al.* Endothelial injury in transplant glomerulopathy is correlated with transcription factor T-bet expression. Kidney Int 2012;82:321–329.
93 Sun Q, Cheng D, Zhang M, *et al.* Predominance of intraglomerular T-bet or GATA3 may determine mechanism of transplant rejection. J Am Soc Nephrol 2011;22:246–252.
94 Yadav B, Prasad N, Agrawal V, *et al.* T-bet-positive mononuclear cell infiltration is associated with transplant glomerulopathy and interstitial fibrosis and tubular atrophy in renal allograft recipients. Exp Clin Transplant 2015;13:145–151.
95 George JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med 2014;371:654–666.
96 Reynolds JC, Agodoa LY, Yuan CM, *et al.* Thrombotic microangiopathy after renal transplantation in the United States[1]. Am J Kid Dis 2003;42:1058–1068.
97 Schwimmer J, Nadasdy TA, Spitalnik PF, *et al. De novo* thrombotic microangiopathy in renal transplant recipients: a comparison of hemolytic uremic syndrome with localized renal thrombotic microangiopathy. Am J Kid Dis 2003;41:471–479.
98 Satoskar AA, Pelletier R, Adams P, *et al. De novo* thrombotic microangiopathy in renal allograft biopsies? Role of antibody-mediated rejection. Am J Transplant 2010;10:1804–1811.
99 Sreedharanunni S, Joshi K, Duggal R, *et al.* An analysis of transplant glomerulopathy and thrombotic microangiopathy in kidney transplant biopsies. Transplant Int 2014;27:784–792.
100 Baid-Agrawal S, Farris AB 3rd, Pascual M, *et al.* Overlapping pathways to transplant glomerulopathy: chronic humoral rejection, hepatitis C infection, and thrombotic microangiopathy. Kidney Int 2011;80:879–885.
101 Noris M, Remuzzi G. Thrombotic microangiopathy after kidney transplantation. Am J Transplant 2010;10:1517–1523.
102 Meehan SM, Kremer J, Ali FN, *et al.* Thrombotic microangiopathy and peritubular capillary C4d expression in renal allograft biopsies. Clin J Am Soc Nephrol 2011;6:395–403.
103 Shulman H, Striker G, Kennedy M, *et al.* Nephrotoxicity of cyclosporin a after allogeneic marrow transplantation. N Engl J Med 1981;305:1392–1395.
104 Bonser RS, Adu D, Franklin I, *et al.* Cyclosporin-induced haemolytic uremic syndrome in liver allograft recipient. Lancet 1984;324:1337.
105 Galli FC, Damon LE, Tomlanovich SJ, *et al.* Cyclosporine-induced hemolytic uremic syndrome in a heart transplant recipient. J Heart Lung Transplant 1993;12:440–444.
106 Young BA, Marsh CL, Alpers CE, *et al.* Cyclosporine-associated thrombotic microangiopathy/hemolytic uremic syndrome following kidney and kidney–pancreas transplantation. Am J Kid Dis 1996;28:561–571.
107 Van Buren D, Van Buren CT, Flechner SM, *et al. De novo* hemolytic uremic syndrome in renal transplant recipients immunosuppressed with cyclosporine. Surgery 1985;98:54–62.
108 Carson JM, Newman ED, Farber JL, *et al.* Tacrolimus-induced thrombotic microangiopathy: natural history of a severe, acute vasculopathy. Clin Nephrol 2012;77:79–84.
109 Java A, Edwards A, Rossi A, *et al.* Cytomegalovirus-induced thrombotic microangiopathy after renal transplant successfully treated with eculizumab: case report and review of the literature. Transplant Int 2015;28:1121–1125.
110 Murer L, Zachello G, Bianchi D, *et al.* Thrombotic microangiopathy associated with parvovirus B 19 infection after renal transplantation. J Am Soc Nephrol 2000;11:1132–1137.
111 Baid S, Pascual M, Williams WW, *et al.* Renal thrombotic microangiopathy associated with anticardiolipin antibodies in hepatitis C-positive renal allograft recipients. J Am Soc Nephrol 1999;10:146–153.
112 Asaka M, Ishikawa I, Nakazawa T, *et al.* Hemolytic uremic syndrome associated with influenza A virus infection in an adult renal allograft recipient: case report and review of the literature. Nephron 2000;84:258–266.
113 Le Quintrec M, Lionet A, Kamar N, *et al.* Complement mutation-associated *de novo* thrombotic microangiopathy following kidney transplantation. Am J Transplant 2008;8:1694–1701.
114 Johnson RJ, Gretch DR, Yamabe H, *et al.* Membranoproliferative glomerulonephritis associated with

hepatitis C virus infection. N Engl J Med 1993;328: 465–470.
115 Johnson RJ, Gretch DR, Couser WG, *et al.* Hepatitis C virus-associated glomerulonephritis. effect of alpha-interferon therapy. Kidney Int 1994;46:1700–1704.
116 Sabry AA, Sobh MA, Irving WL, *et al.* A comprehensive study of the association between hepatitis C virus and glomerulopathy. Nephrol Dial Transplant 2002;17:239–245.
117 Cruzado JM, Carrera M, Torras J, *et al.* Hepatitis C virus infection and *de novo* glomerular lesions in renal allografts. Am J Transplant 2001;1:171–178.
118 Roth D, Cirocco R, Zucker K, *et al. De novo* membranoproliferative glomerulonephritis in hepatitis C virus-infected renal allograft recipients. Transplantation 1995;59:1676–1682.
119 Stehman-Breen C, Alpers CE, Couser WG, *et al.* Hepatitis C virus associated membranous glomerulonephritis. Clin Nephrol 1995;44:141–147.
120 Rosenstock JL, Markowitz GS, Valeri AM, *et al.* Fibrillary and immunotactoid glomerulonephritis: distinct entities with different clinical and pathologic features. Kidney Int 2003;63:1450–1461.
121 Wong W, Denton M, Rennke HG, *et al.* Hepatitis C, proteinuria, and renal insufficiency. Am J Kid Dis 2004;44:924–929.
122 Kanungo S, Tamirisa S, Gopalakrishnan R, *et al.* Collapsing glomerulopathy as a complication of interferon therapy for hepatitis C infection. Int Urol Nephrol 2010;42:219–222.
123 Berdichevski RH, De Carvalho EM, Edelweiss MI, *et al.* Collapsing glomerulopathy after hepatitis C pegylated interferon treatment. recovery of renal function with high-dose steroid treatment. NDT Plus 2010;3:564–566.
124 Filippone EJ, Chmielewski C, Gulati R, *et al. De novo* fibrillary glomerulonephritis (FGN) in a renal transplant with chronic hepatitis C. Case Rep Transplant 2013;2013:978481.
125 Lin MV, Sise ME, Pavlakis M, *et al.* Efficacy and safety of direct acting antivirals in kidney transplant recipients with chronic hepatitis C virus infection. PLoS ONE 2016;11:e0158431.
126 Gallay BJ, Alpers CE, Davis CL, *et al.* Glomerulonephritis in renal allografts associated with hepatitis C infection: a possible relationship with transplant glomerulopathy in two cases. Am J Kid Dis 1995;26: 662–667.
127 Cosio FG, Roche Z, Agarwal A, *et al.* Prevalence of hepatitis C in patients with idiopathic glomerulopathies in native and transplant kidneys. Am J Kidney Dis 1996;28:752–758.
128 Kamar N, Marion O, Rostaing L, *et al.* Efficacy and safety of sofosbuvir-based antiviral therapy to treat hepatitis C virus infection after kidney transplantation. Am J Transplant 2016;16:1474–1479.
129 Sawinski D, Kaur N, Ajeti A, *et al.* Successful treatment of hepatitis C in renal transplant recipients with direct-acting antiviral agents. Am J Transplant 2016;16:1588–1595.
130 Lubetzky M, Chun S, Joelson A, *et al.* Safety and efficacy of treatment of hepatitis C in kidney transplant recipients with directly acting antiviral agents. Transplantation 2017;101:1704–1710.
131 Banfi G, Villa M, Cresseri D, *et al.* The clinical impact of chronic transplant glomerulopathy in cyclosporine era. Transplantation 2005;80:1392–1397.
132 Kieran N, Wang X, Perkins J, *et al.* Combination of peritubular C4d and transplant glomerulopathy predicts late renal allograft failure. J Am Soc Nephrol 2009;20:2260–2268.
133 Kamal L, Broin P, Bao Y, *et al.* Clinical, histological, and molecular markers associated with allograft loss in transplant glomerulopathy patients. Transplantation 2015;99:1912–1918.
134 Dobi D, Bodo Z, Kemeny E, *et al.* Morphologic features and clinical impact of arteritis concurrent with transplant glomerulopathy. Pathol Oncol Res 2016;22:15–25.
135 Kahwaji J, Najjar R, Kancherla D, *et al.* Histopathologic features of transplant glomerulopathy associated with response to therapy with intravenous immune globulin and rituximab. Clin Transplant 2014;28: 546–553.
136 Sun Q, Huang X, Jiang S, *et al.* Picking transplant glomerulopathy out of the CAN: evidence from a clinicopathological evaluation. BMC Nephrol 2012;13:128.
137 Halloran PF, Famulski KS, Reeve J. Molecular assessment of disease states in kidney transplant biopsy samples. Nat Rev Nephrol 2016;12:534–548.
138 Gupta A, Broin PO, Bao Y, *et al.* Clinical and molecular significance of microvascular inflammation in transplant kidney biopsies. Kidney Int 2016;89: 217–225.
139 Halloran PF, Venner JM, Famulski KS. Comprehensive analysis of transcript changes associated with allograft rejection: combining universal and selective features. Am J Transplant 2017;17:1754–1769.
140 Stegall MD, Diwan T, Raghavaiah S, *et al.* Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. Am J Transplant 2011;11:2405–2413.
141 Cornell LD, Schinstock CA, Gandhi MJ, *et al.* Positive crossmatch kidney transplant recipients treated with eculizumab: outcomes beyond 1 year. Am J Transplant 2015;15:1293–1302.
142 Orandi B, Zachary A, Dagher N, *et al.* Eculizumab and splenectomy as salvage therapy for severe antibody-mediated rejection after HLA-incompatible kidney transplantation. Transplantation 2014;98:857–863.
143 Kulkarni S, Kirkiles-Smith NC, Deng YH, *et al.* Eculizumab therapy for chronic antibody-mediated injury in kidney transplant recipients: a pilot randomized controlled trial. Am J Transplant 2017;17: 682–691.
144 Montgomery RA, Orandi BJ, Racusen L, *et al.* Plasma-derived C1 esterase inhibitor for acute antibody-mediated rejection following kidney transplantation: results of a randomized double-blind placebo-controlled pilot study. Am J Transplant 2016;16: 3468–3478.
145 Choi J, Aubert O, Vo A, *et al.* Assessment of tocilizumab (anti-interleukin-6 receptor monoclonal) as a potential treatment for chronic antibody-mediated rejection and transplant glomerulopathy in HLA-sensitized renal allograft recipients. Am J Transplant 2017;17:2381–2389.
146 Kozakowski N, Herkner H, Bohmig GA, *et al.* The diffuse extent of peritubular capillaritis in renal allograft rejection is an independent risk factor for graft loss. Kidney Int 2015;88:332–340.
147 Kozakowski N, Eskandary F, Herkner H, *et al.* Diffuse extent of peritubular capillaritis in late antibody-mediated rejection: associations with levels of

donor-specific antibodies and chronic allograft injury. Transplantation 2017;101:395–401.
148 Fehr T, Rusi B, Fischer A, *et al.* Rituximab and intravenous immunoglobulin treatment of chronic antibody-mediated kidney allograft rejection. Transplantation 2009;87:1837–1841.
149 Rostaing L, Guilbeau-Frugier C, Fort M, *et al.* Treatment of symptomatic transplant glomerulopathy with rituximab. Transpl Int 2009;22:906–913.
150 Billing H, Rieger S, Susal C, *et al.* IVIG and rituximab for treatment of chronic antibody-mediated rejection: a prospective study in paediatric renal transplantation with a 2-year follow-up. Transpl Int 2012;25:1165–1173.
151 Smith RN, Malik F, Goes N, *et al.* Partial therapeutic response to rituximab for the treatment of chronic alloantibody mediated rejection of kidney allografts. Transpl Immunol 2012;27:107–113.
152 Zuber J, Le Quintrec M, Krid S, *et al.* Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. Am J Transplant 2012;12: 3337–5 4.
153 Canaud G, Kamar N, Anglicheau D, *et al.* Eculizumab improves posttransplant thrombotic microangiopathy due to antiphospholipid syndrome recurrence but fails to prevent chronic vascular changes. Am J Transplant 2013;13:2179–2185.
154 Lonze BE, Zachary AA, Magro CM, *et al.* Eculizumab prevents recurrent antiphospholipid antibody syndrome and enables successful renal transplantation. Am J Transplant 2014;14:459–465.