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REVIEW

Phenotypes of antibody-mediated rejection in organ transplants

Michael Mengel, Sufia Husain, Luis Hidalgo and Banu Sis

Department of Laboratory Medicine and Pathology, Alberta Transplant Applied Genomics Centre, University of Alberta, Edmonton, AB, Canada

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Correspondence
Michael Mengel, 3-126 Li Ka Shing Centre for Health Research Innovation, University of Alberta, Edmonton, AB, T6G 2S2, Canada.
Tel.: 780-492-5943; fax: 780-492-0145;
e-mail: mmengel@ualberta.ca

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Summary

Antibody-mediated hyperacute rejection was the first rejection phenotype observed in human organ transplants. This devastating phenotype was eliminated by reliable crossmatch technologies. Since then, the focus was on T-cell-mediated rejection and *de novo* donor-specific antibodies were considered an epiphenomenon of cognate T-cell activation. The immune theory was that controlling the T-cell response would entail elimination of antibody-mediated rejection (ABMR). With modern immunosuppressive drugs, T-cell-mediated rejection is essentially treatable. However, this did not prevent ABMR from emerging as a significant phenotype in all types of organ transplants. It became obvious that both rejection types require distinct treatment and thus reliable diagnosis. This is the current challenge. ABMR, depending on stage, grade, time course, organ type or prior treatment, can present with a wide spectrum of phenotypes. This review summarizes the current diagnostic consensus for ABMR, describes unmet needs and challenges in diagnostics, and proposes new approaches for consideration.

Introduction

Antibody-mediated rejection (ABMR) emerged as the major clinical challenge and recently was identified as the most frequent cause for renal allograft failure [1,2]. Donor-specific antibodies (DSAs) play a crucial role in the development of ABMR. The primary target of DSA is the endothelium of the microcirculation. Clinical management of ABMR is significantly different from T-cell-mediated rejection (TCMR) therefore accurate diagnosis of ABMR is crucial. The morphological spectrum of ABMR is heterogeneous and comprises a set of nonspecific morphological lesions which are nevertheless an essential part of current diagnostic criteria for ABMR along with C4d deposition in the microcirculation, circulating DSA, and clinical evidence of graft dysfunction. Chronic ABMR has been widely recognized in kidney transplants, but needs yet to be defined in other organ transplants. Despite being a significant contributor to late graft loss, it is often missed because of limitations of current diagnostic criteria. In particular, the inability of C4d to detect all phenotypes of ABMR and the limited specificity of DSA account for most missed ABMR cases.

The focus of this review is to discuss the spectrum of alloantibody-mediated phenotypes in allografts and the limitations of current diagnostic criteria in various organ transplants. The aim is also to present new concepts for more precise diagnosis of ABMR.

Acute ABMR in kidney allografts

Prior to the advent of crossmatch testing and effective immunosuppressive therapy, hyperacute rejection because of pre-existing DSA (blood group and/or HLA incompatibility) was a common and most feared phenotype of ABMR. Clinically, these patients presented with immediate

allograft dysfunction. Histology of hyperacutely rejected kidneys presents with neutrophils and platelet margination along the endothelium of the microcirculation, i.e. the peritubular capillaries (PTC) and the glomeruli. Microthrombi and hemorrhage with cortical necrosis develop in most cases [3,4]. However, with the introduction of sensitive crossmatch techniques, the hyperacute ABMR phenotype is essentially eliminated.

With the advent of potent T-cell-directed maintenance immunosuppression, ABMR emerged to play a significant role in the development of acute and chronic allograft dysfunction post-transplantation. In this context, two major phenotypes for acute ABMR need to be discriminated: phenotype 1 (early/acute) – ABMR developing in the presensitized, crossmatch positive, but de-sensitized patient early post-transplantation and phenotype 2 (late/chronic) – ABMR after *de novo* DSA developing late (>12 months) post-transplantation, mostly in relation to noncompliance [5]. Histologic features of acute anti-HLA antibody-mediated injury were first described in the early 1990s [6–8] as a triad of allograft dysfunction, neutrophilic peritubular capillaritis, i.e. microcirculation inflammation (MI), and *de novo* anti-donor class I HLA antibodies. Simultaneously, a pivotal study by Feucht *et al.* [9] pushed C4d into the limelight by describing a prognostic association between C4d deposition in peritubular capillaries and inferior graft survival. C4d is a breakdown product of activated C4, a component of the classical complement pathway. After activation, it binds covalently to the endothelium. Thus, the detection of C4d in the capillary wall lining provides *in situ* evidence for DSA acting on the allograft endothelium. The binding of complement-fixing alloantibody to endothelium causes (in addition to complement activation) lysis and activation of the endothelial cells, recruitment, and activation of leukocytes, which all eventually lead to acute graft tissue injury. In the late 1990s, Collins *et al.* [10] defined the relationship among DSA, histology of microcirculation injury, and C4d deposition in the peritubular capillaries in the clinical context of allograft dysfunction and thus acute ABMR.

At the beginning of the Banff process in 1991, ABMR was only marginally appreciated as the hyperacute phenotype with fibrinoid necrosis in large arteries [11]. Because TCMR was the dominating clinical problem in those days and a widespread assumption was that ABMR is an epiphenomenon of TCMR, no consensus could be generated to integrate detailed diagnostic criteria for ABMR until the 2001 Banff meeting. At that meeting the fundamental Banff diagnostic criteria for acute ABMR in kidney allografts were defined: (i) histologic evidence of tissue injury, (ii) immunopathologic evidence of antibody acting on the microcirculation, i.e. the detection of C4d in peritubular capillaries, and (iii) demonstration of circulating DSA [12] (Fig. 1). Cases that meet only two of the criteria are considered suspicious for ABMR. At the 9th Banff conference held in 2007, a scoring system for the peritubular capillaritis and for C4d staining was incorporated into the Banff classification [13]. It was acknowledged that C4d staining can be done using immunohistochemistry on paraffin sections or immunofluorescence on cryosections. It was also noted that staining on paraffin sections is less sensitive by about one grade [14]. Therefore, current Banff consensus is that only cases with diffuse C4d stain on frozen or diffuse or focal stain on paraffin sections are considered positive.

Potential limitations of histology for diagnosing ABMR

The specificity of ABMR-associated histologic lesions is limited: glomerulitis can occur in TCMR and very similar endocapillary hypercellularity can be seen in various types of recurrent/*de novo* glomerulonephritis [15,16]. Similarly, peritubular capillaritis can be seen in early biopsies with acute tubular necrosis or TCMR [15]. It has also previously been shown that Banff v1 and v2 lesions, i.e. intimal arteritis that is traditionally attributed to TCMR, can have an association with ABMR [17,18]. However, it may well be that a proportion of cases presents with simultaneous features of mixed TCMR and ABMR, in particular in the context of noncompliance. It can be expected that with under-immunosuppression the T-cell response can rebound faster and set the stage for ramping up a consecutive, overlapping *de novo* DSA response.

In addition, some of the ABMR lesions are of limited reproducibility between observers [19,20]. Preliminary data presented at the 2011 Banff meeting indicate that in particular glomerulitis scoring is difficult to be reproduced [21]. As a consequence collaborative efforts are currently underway to refine and standardize the criteria for glomerular lesion scoring in renal allograft biopsies.

Potential limitations of C4d for diagnosing ABMR

Several peculiarities and limitations of the C4d stain as a biomarker for ABMR were identified. Haas *et al.* [22] and Fidler *et al.* [23] reported C4d deposition along peritubular capillaries without graft pathology in 25–80% of ABO-incompatible of renal allografts, but only 4–12% developed acute ABMR. As a result, the term 'C4d deposition without morphologic evidence of active rejection' was coined at the Banff 2007 meeting [13]. C4d deposition was also observed in protocol biopsies from stable grafts in 2% of ABO-compatible transplants, not always associated with MI [24]. The long-term implications of C4d deposition without any morphologic evidence of rejection

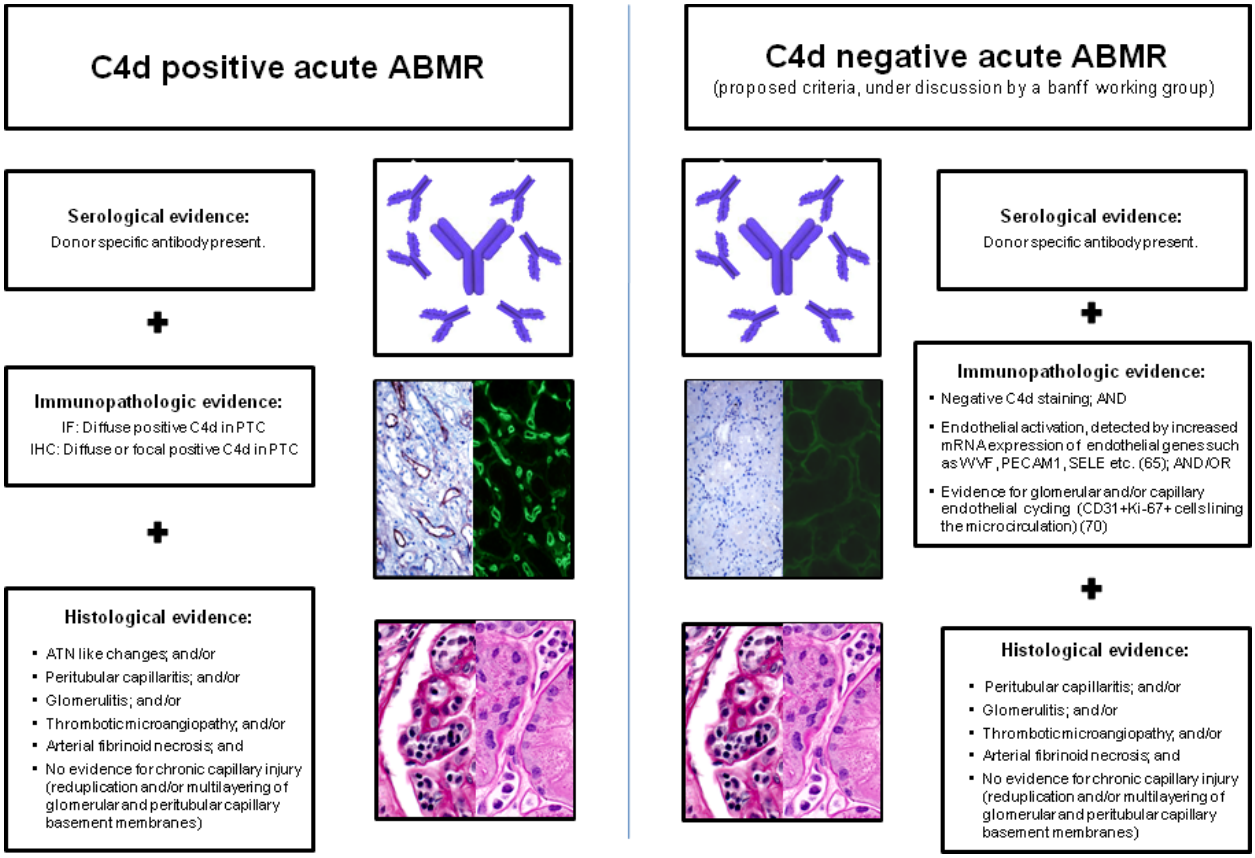

Figure 1 Diagnostic criteria for acute antibody-mediated rejection in kidney transplants. [Correction added after online publication 30 March 2012: reference citation numbers have been updated in 'Immunopathologic evidence' box]

are still under investigation, but data presented at the most recent Banff meeting (2011) indicated that at least in ABO-compatible transplants, some of the cases evolve into ABMR. Early subclinical MI correlates with an increased risk for later development of transplant glomerulopathy (TG). Subsequent development of histologic changes of chronic ABMR was also found to be associated with prior persisting subclinical interstitial inflammation [25], suggesting under-immunosuppression as a risk factor for the onset of ABMR.

In addition to focal linear C4d staining in PTC, non-typical C4d staining patterns have been described using immunohistochemistry in paraffin section: granular C4d deposits in PTC and staining outside of PTC in glomeruli and arterioles [26]. Although a close interrelationship between diffuse and focal linear, and linear endothelial C4d deposition in glomeruli and arterioles with glomerulitis and presensitization was observed, no such association was seen with granular/nonlinear C4d staining. But overall the strongest association with outcome was with diffuse linear C4d staining in PTC, re-emphasizing current Banff consensus for scoring C4d.

It is also well known that significant interlaboratory and interobserver variability can occur with immunohistochemical stains. Thus, the Banff group organized an international quality assurance trial with the aim of assessing the reproducibility of C4d stain on paraffin sections. Preliminary results from this trial were presented at the 2011 Banff meeting and indicated that a significant variability of C4d results between institutions exists. In addition to methodological issues with the C4d stain true biological C4d-negativity might occur in the setting of ABMR as further discussed below. In particular waxing and weaning of C4d in the microcirculation over time is a well described phenomenon in sequential biopsies from patients with ABMR [18,27,28].

Potential limitations of DSA testing for diagnosing ABMR

Limited sensitivity of antibody testing in the past could explain case in which C4d staining was seen without DSA detection in the serum. But today with available highly sensitive single antigen bead assays (SABs) on the Luminex

platform, the diagnostic value of detecting DSA is limited as not all patients with *de novo* DSA develop ABMR [29]. Despite many advantages, limitations are inherent to the technology. Ambiguity in determining thresholds makes reporting of low level HLA antibodies difficult [30,31] and the highest affinity DSA may be bound to the graft making it harder to detect in serum [32]. The completeness of donor HLA typing dictates the accuracy of DSA assignment. The Luminex platform can identify DSA to HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3/4/5, DQA1, DQB1, DPA1, and DPB1 alleles, yet many of these are not characterized in long-term transplant recipients. Interferences also arise in some sera where multiple, nonstandardized methods have been proposed [33,34]. These problems could translate to false-negative DSA assignments, thus preventing correct diagnosis of ABMR.

The clinical relevance of DSA may depend on the IgG subclass. Complement-fixing IgG subclasses (IgG3/IgG1) may be more damaging than noncomplement-fixing subclasses (IgG2/IgG4) [35]. The commercially available Luminex SAB assays do not differentiate between subclasses of IgG. However, new reagents can detect C1q-binding antibodies [36], C4d deposition on beads [37], and individual IgG subclasses. These Luminex SAB enhancements may aid in the detection of pathogenic DSA, thus minimizing false-positive DSAs.

Thus, the challenges in diagnosis, grading and staging of ABMR are reflected in the fact that diagnostic features are not specific and dependent on the time point in the course of the disease, whereas the dynamics of the disease course show significant individual variability. For example, protocol biopsies from sensitized patients with phenotype 1 ABMR were frequently C4d-negative despite the presence of DSA and MI [27,38].

Chronic ABMR in renal allografts

Smoldering or episodic alloantibody-mediated endothelial injury causes time-dependent structural changes in microvessels (glomeruli, PTC), arteries, and nephron loss (interstitial fibrosis and tubular atrophy). It is important to note that the term 'chronic' is not related to a certain time post-transplantation, but indicates morphological changes of capillary remodeling seen in the allograft because of antibody-mediated injury, e.g. duplication of the glomerular basement membrane and/or multilayering of PTC basement membrane.

A decade after the discovery of C4d as a biomarker, late graft losses because of antibody and morphological features of chronic ABMR were recognized [39,40]. Based on clinical observations, the diagnostic criteria for chronic ABMR were established by the Banff consensus in 2005 [41], including (i) TG (duplication or 'double contours' in glomerular basement membranes in the absence of immune complexes, cg-score >0), and/or peritubular capillary basement membrane multilayering (PTCML) with ≥5 layers and/or interstitial fibrosis/tubular atrophy, and/or fibrous intimal thickening in arteries; (ii) diffuse C4d staining by immunofluorescence or diffuse or focal C4d by immunohistochemistry in PTC; *and* (iii) circulating DSA (Fig. 2). Chronic ABMR injury is dominated by microcirculation damage, which is seen as reduplication and/or multilamination of basement membranes of glomeruli and peritubular capillaries. Much less specific features of chronic ABMR include arterial fibrous intimal thickening [42], interstitial fibrosis, tubular atrophy, and sometimes loss of PTC. Other morphologic findings of activity that may be seen in chronic ABMR are peritubular capillaritis, mostly with mononuclear inflammatory cells [40] and glomerulitis [43]. We observed peritubular capillaritis and glomerulitis in 70% and 35% of TG biopsies, respectively [44].

DSA, especially HLA class II antibodies, are capable of triggering insidious graft injury and thus represent one of the major causative factors of late kidney graft dysfunction and failure. In a leading study by Mauiyyedi *et al.* [39], C4d deposition in PTCs was observed in 61% of chronic rejection biopsies with TG and/or chronic arteriopathy, of which the majority had anti-HLA DSA (88%). Regele *et al.* [40] described C4d PTC positivity in 34% kidney allografts with chronic transplant dysfunction, TG, and PTCML. More interestingly, C4d staining predicted subsequent development of TG in 9 of 11 cases. Active antibody-mediated injury, whether there is C4d staining and/or MI (glomerulitis/peritubular capillaritis), is a harbinger of chronic ABMR pathology, especially if left untreated [38,45]. A model in nonhuman primates, suggested four stages in the progression of chronic ABMR, namely circulating DSA followed by C4d deposition, development of chronic tissue pathology (TG, fibrosis), and finally graft dysfunction [46]. We observed that more than 50% of TG cases were C4d-negative despite the fact that HLA alloantibodies, mostly class II, were detected in 73% of patients with TG [44]. The Mayo group reported the overall incidence of TG as 5–10% of crossmatch negative kidney transplants, but its cumulative incidence increased over time from 4% at 1 year to 20% at 5 years using protocol biopsies, suggesting many TG cases are subclinical [47]. The authors reported an association between TG and DSA, especially class II antibodies. Furthermore, the incidence of TG at 1 year was much higher in crossmatch positive kidney transplants than in nonpresensitized grafts (22% vs. 8%) [48], indicating a causal link between DSA and TG.

Chronic ABMR is a major cause of late kidney transplant failure [1,2,49]. Data from two major transplant

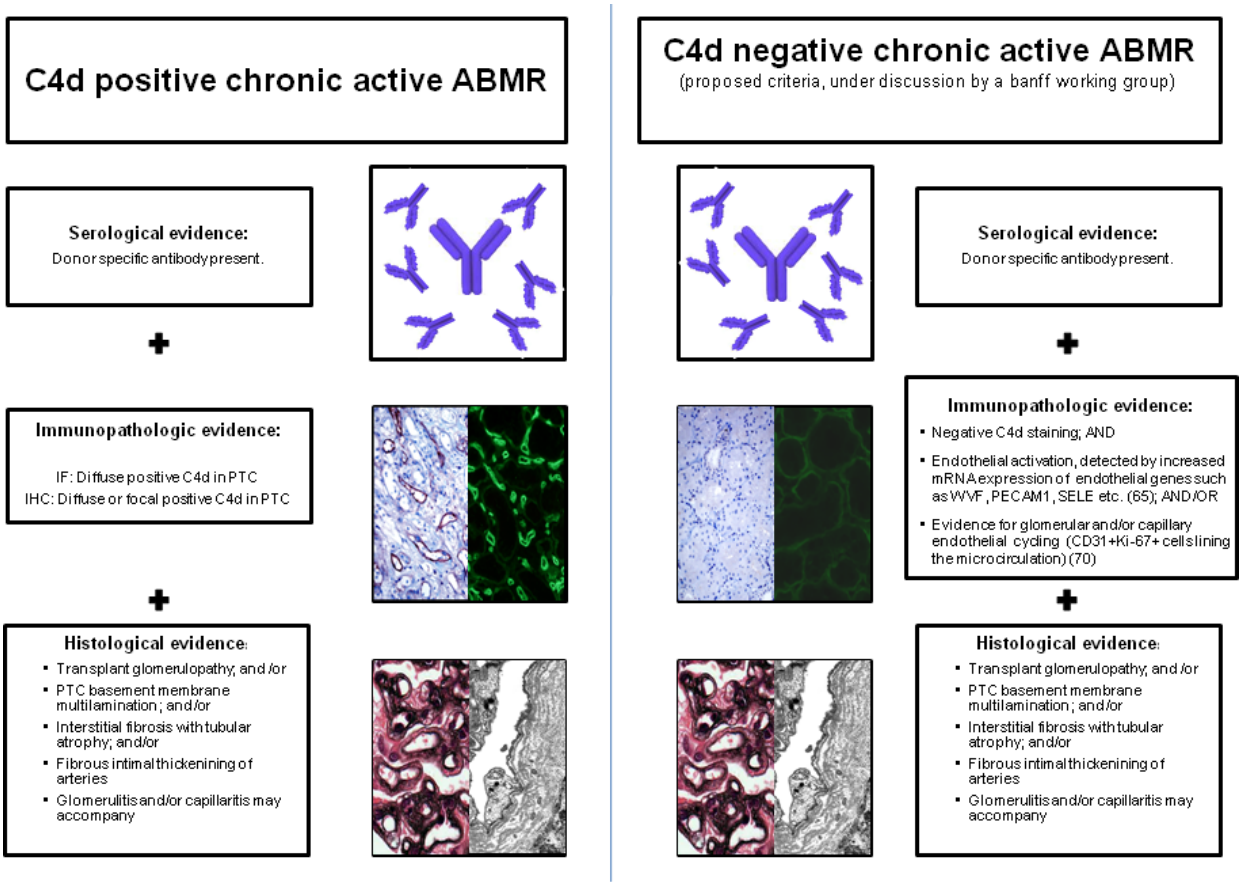

Figure 2 Diagnostic criteria for chronic active ABMR in kidney transplants. [Correction added after online publication 30 March 2012: reference citation numbers have been updated in 'Immunopathologic evidence' box]

centers (Mayo Clinic and Edmonton) and the multicenter DEKAF study independently indicated that most late kidney transplant losses have a specific etiology with ABMR being the leading cause, whereas idiopathic fibrosis or drug toxicity were very rarely responsible for renal allograft failure [1,2,50]. If microcirculation lesions and presence of HLA alloantibodies were used to define ABMR (regardless of the C4d status), 63% of the late kidney failures were attributed to antibody-mediated microcirculation injury, of which many were C4d-negative [1].

TG and PTCML tend to occur concomitantly, and both lesions show basement membrane remodeling, which are regarded as sequela of endothelial cell injury and repair [22,27,38,40,51,52]. Initially, Monga *et al.* [53,54] described PTCML in renal allografts in association with TG. Ivanyi *et al.* [55] reported significant PTCML with ≥5 layers in 28% of allograft biopsies and in 59% of failed transplant nephrectomy specimens with chronic rejection, respectively. We observed PTCML in 91% of TG biopsies [44]. However, PTCML was also increased in late biopsies without DSA, therefore showing an ambiguous relationship between the microcirculation deterioration lesions of the glomerulus (double contours, mesangial matrix increase) and the time-dependent scarring [17,44]. The precise definition of PTCML is critical when comparing studies describing associations with PTCML. For instance, Drachenberg *et al.* [56] showed that TG was mostly associated with severe PTCML (more than six layers), whereas lesser PTCML was observed in other conditions.

The current concept is that TG is a pathological pattern, rather than a disease, which may be caused by different etiologies: alloantibodies (major etiology) [38,44,47,48,51,57], autoantibodies [58,59], sequela of previous thrombotic microangiopathy [60], and HCV infection [60,61]. In a retrospective study, we did not find evidence for DSA in 27% of patients with TG and concluded that these cases may be a different disease or a stage in which DSA/C4d are undetectable [44]. Other groups also found no correlation between TG and C4d deposition [62,63]. Akalin *et al.* [64] showed glomerular infiltration by $CXCR3^+$ $ICOS^+$ activated T cells in grafts with TG, but not in fibrosis/atrophy alone, suggesting ongoing effector T-cell responses to glomerular antigens can result in TG. It should be noted, however, that these

studies may reflect intragraft effector mechanisms in the development of TG, i.e. complement, cytokines/chemokines, leuokocytes, increased coagulation/thrombotic microangiopathy, rather than extracting the underlying diseases. There is still an unmet need to better understand the non-ABMR etiologies associated with TG.

New concepts in ABMR

Recognition of C4d-negative active ABMR in kidney allografts

Evidence 1

ABMR is dominated by microvascular endothelial injury, which is mediated by complement and leukocytes recruited via complement split products and/or Fc receptors and/or platelets. In a retrospective study, we observed that more than 50% of TG cases with alloantibody were C4d-negative [44]. This earlier observation suggested that C4d is insensitive for chronic ABMR. We then pursued this finding by new studies using gene microarray assessment of biopsy tissues. In a large number of kidney transplant biopsies for clinical indications, the biopsies with high expression of endothelial transcripts and circulating alloantibody showed histopathologic lesions of ABMR (such as capillaritis, glomerulitis, TG, and fibrosis/atrophy), DSA, and poor outcomes [65]. Surprisingly, many of these chronic-active ABMR cases were missed by current Banff criteria: Only 40% of kidneys with high endothelial gene expression and chronic ABMR were diagnosed by C4d positivity. Thus, the current Banff terminology of chronic-active ABMR (positive C4d stain as the only sign of activity recognized by Banff) is challenged by the fact that cases with chronic damage, i.e. TG, can be molecularly active despite being negative for C4d stain or can show some of the histologic (MI) features of acute ABMR. Therefore, the term of active ABMR might be more appropriate.

In addition to limited sensitivity and reproducibility of the C4d stain as the potential sources for C4d-negative ABMR cases, recent evidence suggests that there are a number of pathways that could lead to complement independent antibody-mediated endothelial injury [66]. (i) DSA can cause endothelial activation in the absence of complement: release of VWF and externalization of P-selectin on endothelial cells were shown after stimulation by anti-HLA class I [67]; (ii) activated endothelial cells express/secrete proinflammatory molecules (i.e. E-selectin, P-selectin, ICAM-1, VCAM-1, CX3CL1) that increase leukocyte recruitment. Recruited and activated leukocytes secrete cytokines, which further increase endothelial activation; (iii) it has been shown that alloantibody indirectly induces platelet activation and adhesion *in vivo*, which then can recruit leukocytes [68]; (iv). leukocytes can damage endothelium via antibody-dependent cellular cytotoxicity by NK cells or monocytes/macrophages through Fc/Fc receptors. Hidalgo *et al.* [69] showed increased NK cell transcripts and NK cells within peritubular capillaries in clinical biopsies with C4d-positive or C4d-negative ABMR, supporting the involvement of NK cells in the pathogenesis of ABMR, such that DSA bound to endothelial cells triggers NK cell cytotoxic activity and release of interferon-gamma through engaging with low affinity Fc receptors.

Thus, it is obvious that C4d has low sensitivity for ABMR and many cases of active ABMR have been missed or misdiagnosed, and therefore not treated. Regarding the new diagnostic approaches to identify active ABMR, molecular tests (documented DSA and high endothelial or NK cell gene expression in biopsy tissue) are definitely on the horizon, but need validation and standardization (i.e. which transcripts, platform, thresholds) across multiple centers. Immunohistochemistry would be very helpful, but it is a difficult task to detect increased expression of endothelial proteins by staining, as many are constitutively expressed in renal microcirculatory endothelium. We have recently observed that microcirculation endothelial cycling is specifically increased in ABMR (C4d positive or negative) compared with other diseases, and a CD31/Ki-67 double immunostaining has a specificity of 95% for ABMR (Osasan *et al.*, manuscript in preparation) [70].

Evidence 2

A recent protocol study in highly presensitized patients by Loupy *et al.* [38] suggested that C4d has also low sensitivity for acute ABMR: 49% of presensitized kidney transplant patients developed subclinical C4d-negative ABMR (capillaritis plus glomerulitis plus DSA) (vs. 31% $C4d^+$ ABMR) at 3-month protocol biopsies, who later developed higher fibrosis, TG, and lower graft function at 1 year, when compared with presensitized patients without MI. The same group later reported that MI is associated with adverse outcomes and increased risk of progression to TG independently of C4d staining [27].

Nevertheless, these results are based on protocol biopsies from well functioning kidney grafts. There is still much debate as to whether or not C4d-negative acute ABMR truly exists in patients presenting with clinical problems. This is an open question to be answered by different transplant programs at the moment. In fact, C4d staining may have a higher sensitivity for early/acute ABMR than it has for chronic ABMR.

Evidence 3

A retrospective study by Haas and Mirocha [71] examined the specificity of early electron microscopic endothelial changes (glomerular endothelial swelling, subendothelial widening, and early glomerular basement membrane

duplication) for ABMR and subsequent development of overt TG. Of 98 renal allograft biopsies for cause carried out within 3 months of transplantation with available serologic data, 17 showed C4d-positive acute ABMR and 16 had MI (glomerulitis/capillaritis) and DSA with negative C4d. Ultrastructural changes were seen in 11 of 17 biopsies with C4d-positive acute ABMR, 8 of 16 with MI and DSA but no C4d, and 0 of 65 without histologic changes of ABMR and/or DSA. Among the patients with DSA and histologic changes of ABMR, 11 C4d-positive and 7 C4d-negative, treatment for ABMR after the early biopsy significantly reduced subsequent development of overt TG.

This study and others [15,27] demonstrate MI in the presence of DSA as an early sign of active antibody-mediated graft injury and if suppressed by intervention would potentially avoid future graft dysfunction and failure. As a response to this unmet need, a respective Banff Working Group was established at the Banff 2011 meeting with the aim of assessing the significance of MI and to construct and recommend evidence-based diagnostic criteria for C4d-negative ABMR [21] (Figs 1 and 2).

Phenotypes of ABMR in heart, pancreas, lung, and liver transplants

The basic principles for diagnosing ABMR are applicable to all types of organ transplants: (i) demonstration of circulating DSA; (ii) C4d deposition in the microcirculation of the allograft; (iii) antibody-mediated tissue pathology; and (iv) clinical evidence of graft impairment (Fig. 3). However, these features are not equally represented during episodes of ABMR in all types of transplants. This is presumably because of organ-specific differences in the microcirculation causing different degrees of resistance to antibody-mediated injury. Furthermore, because of differences in the anatomy and the material retrieved by biopsies, antibody-mediated pathology features are not equally reliably appreciable by histology. In addition, in all organs C4d, the presence of DSA, and respective histologic lesion are of limited specificity. These limitations and organ-specific particularities mean that, to date, the available data, and thus the status of diagnostic consensus classifications, is quite variable for the different types of organ trans-

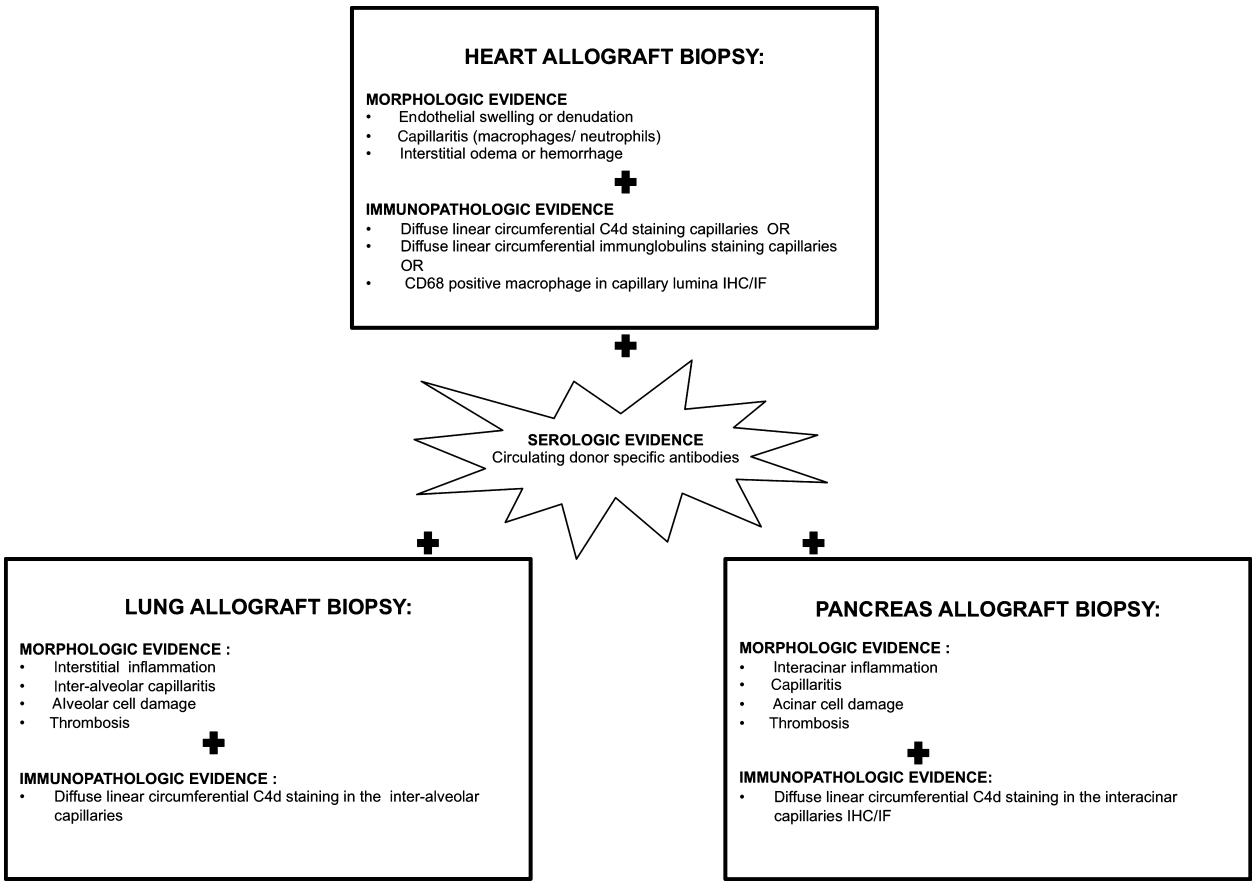

Figure 3 A triad of features required for the diagnosis of acute antibody mediated rejection in heart, lung, and pancreas allografts with allograft dysfunction.

Table 1. Current status of diagnostic consensus criteria for various phenotypes of ABMR in the different types of organ transplants.

	Kidney	Heart	Pancreas	Lung	Liver
Hyperacute ABMR	Yes	Yes	Yes	Yes	No
Acute ABMR	Yes	Yes	Yes	Under development	Under development
Chronic ABMR	Yes	No	No	No	No
C4d-negative ABMR	Under development	Yes	No	No	No

plants (Table 1). Constant collaborative efforts are underway to further refine diagnostic criteria for ABMR in the various types of organ transplants, of which the current status is summarized below.

ABMR in heart transplants

The diagnostic criteria for ABMR of heart transplants went through several refinements and currently include the following requirements: (i) clinical evidence of graft dysfunction; (ii) morphological evidence of acute capillary injury: (a) capillary endothelial swelling or denudation with congestion, (b) macrophages in capillaries, (c) neutrophils in capillaries and (d) interstitial edema and/or hemorrhage; (iii) immunopathologic evidence for antibody effects in the capillaries, (a) IgG, IgM, and/or IgA plus C3d and/or C4d or C1q demonstrated by immunofluorescence (b) CD68 positivity for macrophages in capillaries (identified using CD31 or CD34 stains), and/or C4d staining of capillaries (c) fibrin in vessels (optional); and (iv) serologic evidence of anti-HLA or other anti-donor antibody at time of biopsy [72,73].

Against the background of the emerging literature in this area, surveys and multicenter studies done under the leadership of the International Society of Heart and Lung Transplantation (ISHLT), the Association of the European Cardiovascular Pathologists, and the Banff Heart Group, these fundamental diagnostic criteria were extensively discussed at a recent ISHLT Consensus Conference [74]. It became obvious that a significant variance still exists in how different centers render the diagnosis of ABMR, interpret DSA findings, and stain and interpret C4d and other immune-pathological markers [75]. The current proposal by the ISHLT suggests that the combination of histopathologic and immunopathologic findings will be reported as ''pathologic ABMR'' (pABMR) ('p' for pathology). This was reiterated in light of the decision by clinicians that ABMR should be a diagnosis rendered by pathologists, without the requirements of clinical dysfunction or positive DSA, as currently required. It was emphasized that the proposed grading system represents an initial formulation that is intended to permit the accumulation of data and further refinement of the classification. The provisional categories for the reporting of ABMR are summarized in Table 2 [74]. No criteria are yet established for chronic ABMR in heart allografts.

ABMR in pancreas transplants

Consensus for diagnosing acute ABMR in pancreas allografts has recently been established [76]. The updated Banff Grading Schema emphasizes the need for clinicopathologic integration for the diagnosis of ABMR in pancreas allografts similar to renal allografts. The criteria for acute ABMR in a pancreatic allograft include (i) demonstration of circulating DSA, (ii) C4d staining in the interacinar capillaries, and (iii) histologic evidence of microvascular tissue injury (interacinar inflammation/capillaritis, acinar cell damage, e.g. swelling/necrosis/apoptosis/dropout, vasculitis and thrombosis). Analogous

Table 2. The provisional categories for the reporting of ABMR in heart allografts as proposed by the ISHLT (adapted from [74])

ISHLT class	Diagnostic category	Description
pABMR 0	Negative for pathologic ABMR	Both histologic and immunopathologic studies are negative.
pABMR 1 (H+)	Histopathologic ABMR alone	Histologic findings present and immunopathologic findings negative.
pABMR 1 (I+)	Immunopathologic ABMR alone	Histologic findings negative and immunopathologic findings positive.
pABMR 2	Pathologic ABMR	Both histologic *and* immunopathologic findings are present.
pABMR 3	Severe pathologic ABMR	This category recognizes the rare cases of severe AMR with histopathologic findings of interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis, and/or karyorrhexis and marked edema. The reported experience of the group was that these cases are associated with profound hemodynamic dysfunction and poor clinical outcomes.

to kidney transplants, all three of the mentioned components should be present to render a diagnosis of ABMR. In cases where only two components are present, the case should be labeled suspicious of ABMR. If only one element is present the recommendation is to monitor the patient closely with regular follow-up [76]. Currently studies are underway to assess the reproducibility of this new grading schema including the reproducibility of interpreting the C4d stain. No criteria for chronic ABMR in pancreas allografts have been established yet.

ABMR in lung transplants

The diagnostic criteria for ABMR in the lung transplants are still controversial and not yet completely defined [77]. However, the same principles apply. The presence of pulmonary intra-alveolar capillaritis and signs of capillary injury are considered most representative of antibody-mediated injury to lungs. But again, it is well known that capillary injury can also be seen in severe cell-mediated rejection, diffuse alveolar damage, and infections [78]. Furthermore, C4d and other complement components were detected in the microcirculation in various settings besides DSA [79]. Thus, current provisional ABMR criteria describe four putative stages of the humoral response to a lung allograft [78]: stage I – latent (circulating DSA, C4d-negative, no pathology, no graft dysfunction); stage II – silent (circulating DSA, C4d-positive, no pathology, no graft dysfunction); stage III – subclinical (circulating DSA, C4d-positive, tissue pathology, no graft dysfunction); stage IV – clinical (circulating DSA, C4d-positive, tissue pathology, graft dysfunction). A few studies indicate a potential relationship between ABMR and chronic rejection of lung allografts, i.e. the development of bronchiolitis obliterans syndrome [77,80].

At the 2011 Banff meeting, the lung session focused on reviewing available immunology, serology, and pathology data related to ABMR and revisited the above described gaps in the understanding of ABMR-related pathology [21]. The working group concluded to conduct a multicenter study with an aim to develop internationally acceptable recommendations for the pathologic diagnosis of ABMR in lung allografts.

ABMR in liver transplants

No specific consensus criteria yet exist for ABMR in liver transplants. Compared with kidney, relatively sparse data are available regarding the significance of DSA and ABMR. Recent studies describe an association between DSA and chronic (ductopenic) liver allograft rejection with inferior outcome [81,82]. Furthermore, robust associations between DSA and C4d deposition and increased rates of cellular rejection, and allograft dysfunction were recently identified [81,83–85]. However, C4d staining was also found to be positive in other insults to liver transplants such as cellular rejection, recurrent disease, or preservation injury [83]. Interestingly at the 2011 Banff meeting, preliminary data presented suggested that weaning protocols should be revisited in recipients showing DSA and diffuse $C4d^+$ staining. In these patients a higher failure rate of weaning protocols was observed. In addition, C4d positivity was observed more frequently in patients after weaning of immunosuppression suggesting that underimmunsuppression might represent a risk factor for *de novo* DSA and ABMR in liver allografts. Thus, upcoming goals of the Banff liver group include a better understanding of the ABMR phenotypes in liver transplant with the aim to develop consensus guidelines for interpreting C4d stains [21].

Conclusions

ABMR emerged as a significant disease in all types of organ transplants. It requires distinct treatment and thus reliable diagnosis. This represents the current challenge. ABMR, depending on stage, grade, time course, organ type or prior treatment, can present with a wide spectrum of phenotypes. Despite being a significant contributor to late graft loss, it is often missed because of limited specificity and sensitivity of current diagnostic criteria. Future collaborative efforts of the transplant community need to address this unmet need by pursuing new concepts for more precise diagnosis of ABMR, which ultimately will allow for further stratification of patients to personalized therapeutic interventions.

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References

1. 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.
2. Gaston RS, Cecka JM, Kasiske BL, *et al.* Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure 1. *Transplant* 2010; **90**: 68.
3. Colvin RB, Nickeleit V. Renal transplant pathology. In: Charles Jennette J, Olsen JL, Schwarz MM, Silva Fred C, eds. *Heptinstall's Pathology of the Kidney*, 6th edn. Philadelphia: Lippincott Williams & Wilkins, 2007: 1347–1490.

4. Williams GM, Hume DM, Hudson RP, Morris PJ, Kano K, Milgrom F. Hyperacute renal-homograft rejection in man. *N Engl J Med* 1968; **279**: 611.
5. Halloran PF, de Freitas DG, Einecke G, *et al.* An integrated view of molecular changes, histopathology, and outcomes in kidney transplants. *Am J Transplant* 2010; **10**: 2223.
6. Halloran PF, Wadgymar A, Ritchie S, Falk J, Solez K, Srinivasa NS. The significance of the anti-class I antibody response. I. Clinical and pathologic features of anti-class I-mediated rejection. *Transplant* 1990; **49**: 85.
7. Halloran PF, Schlaut J, Solez K, Srinivasa NS. The significance of the anti-class I response II. Clinical and pathologic features of renal transplants with anti-class I-like antibody. *Transplant* 1992; **53**: 550.
8. Trpkov K, Campbell P, Pazderka F, Cockfield S, Solez K, Halloran PF. Pathologic features of acute renal allograft rejection associated with donor-specific antibody. *Transplant* 1996; **61**: 1586.
9. Feucht HE, Schneeberger H, Hillebrand G, *et al.* Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 1993; **43**: 1333.
10. Collins AB, Schneeberger EE, Pascual MA, *et al.* Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 1999; **10**: 2208.
11. Solez K, Axelsen RA, Benediktsson H, *et al.* International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. *Kidney Int* 1993; **44**: 411.
12. Racusen LC, Colvin RB, Solez K, *et al.* Antibody-mediated rejection criteria – an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003; **3**: 708.
13. Solez K, Colvin RB, Racusen LC, *et al.* Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008; **8**: 753.
14. Seemayer CA, Gaspert A, Nickeleit V, Mihatsch MJ. C4d staining of renal allograft biopsies: a comparative analysis of different staining techniques. *Nephrol Dial Transplant* 2007; **22**: 568.
15. Sis B, Jhangri GS, Riopel J, *et al.* A new diagnostic algorithm for antibody-mediated microcirculation inflammation in kidney transplants. *Am J Transplant* 2011 (In press).
16. Batal I, Lunz III JG, Aggarwal N, *et al.* A critical appraisal of methods to grade transplant glomerulitis in renal allograft biopsies. *Am J Transplant* 2010; **10**: 2442.
17. Sis B, Einecke G, Chang J, *et al.* Cluster analysis of lesions in kidney transplant biopsies: Microcirculation changes, tubulointerstitial inflammation, and scarring. *Am J Transplant* 2010; **10**: 421.
18. Nickeleit V, Zeiler M, Gudat F, Thiel G, Mihatsch MJ. Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. *J Am Soc Nephrol* 2002; **13**: 242.
19. Furness PN, Taub N. International variation in the interpretation of renal transplant biopsies: Report of the CERT-PAP Project. *Kidney Int* 2001; **60**: 1998.
20. Gibson IW, Gwinner W, Brocker V, *et al.* Peritubular capillaritis in renal allografts: prevalence, scoring system, reproducibility and clinicopathological correlates. *Am J Transplant* 2008; **8**: 819.
21. Mengel M, Sis B, Haas M, *et al.* Banff 2011 Meeting Report: new concepts in antibody-mediated rejection. *Am J Transplant* 2012; **12**: 563.
22. 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.
23. Fidler ME, Gloor JM, Lager DJ, *et al.* Histologic findings of antibody-mediated rejection in ABO blood-group-incompatible living-donor kidney transplantation. *Am J Transplant* 2004; **4**: 101.
24. Mengel M, Bogers J, Bosmans JL, *et al.* Incidence of C4d stain in protocol biopsies from renal allografts: results from a multicenter trial. *Am J Transplant* 2005; **5**: 1050.
25. Moreso F, Carrera M, Goma M, *et al.* Early subclinical rejection as a risk factor for late chronic humoral rejection. *Transplant* 2012; **93**: 41.
26. Kikic Z, Regele H, Nordmeyer V, *et al.* Significance of peritubular capillary, glomerular, and arteriolar C4d staining patterns in paraffin sections of early kidney transplant biopsies. *Transplant* 2011; **91**: 440.
27. Loupy A, Hill GS, Suberbielle C, *et al.* Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). *Am J Transplant* 2011; **11**: 56.
28. Nickeleit V, Mihatsch MJ. Kidney transplants, antibodies and rejection: is C4d a magic marker? *Nephrol Dial Transplant* 2003; **18**: 2232.
29. Leffell MS, Zachary AA. Anti-allograft antibodies: some are harmful, some can be overcome, and some may be beneficial. *Discov Med* 2010; **9**: 478.
30. Bray RA, Nickerson PW, Kerman RH, Gebel HM. Evolution of HLA antibody detection: technology emulating biology. *Immunol Res* 2004; **29**: 41.
31. Gebel HM, Bray RA. The evolution and clinical impact of human leukocyte antigen technology. *Curr Opin Nephrol Hypertens* 2010; **19**: 598.
32. Martin L, Guignier F, Mousson C, Rageot D, Justrabo E, Rifle G. Detection of donor-specific anti-HLA antibodies with flow cytometry in eluates and sera from renal transplant recipients with chronic allograft nephropathy. *Transplant* 2003; **76**: 395.
33. Kosmoliaptsis V, O'Rourke C, Bradley JA, Taylor CJ. Improved Luminex-based human leukocyte antigen-specific antibody screening using dithiothreitol-treated sera. *Hum Immunol* 2010; **71**: 45.

34. Zachary AA, Lucas DP, Detrick B, Leffell MS. Naturally occurring interference in Luminex assays for HLA-specific antibodies: characteristics and resolution. *Hum Immunol* 2009; **70**: 496.
35. 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.
36. Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. *Hum Immunol* 2011; **72**: 849.
37. Bartel G, Wahrmann M, Exner M, *et al.* In vitro detection of C4d-fixing HLA alloantibodies: associations with capillary C4d deposition in kidney allografts. *Am J Transplant* 2008; **8**: 41.
38. 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.
39. 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.
40. Regele H, Bohmig 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.
41. Solez K, Colvin RB, Racusen LC, *et al.* Banff '05 Meeting report: Differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 2007; **7**: 518.
42. Hill GS, Nochy D, Bruneval P, *et al.* Donor-specific antibodies accelerate arteriosclerosis after kidney transplantation. *J Am Soc Nephrol* 2011; **22**: 975.
43. Sijpkens YW, Joosten SA, Wong MC, *et al.* Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy. *Kidney Int* 2004; **65**: 2409.
44. 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.
45. Lerut E, Naesens M, Kuypers DR, Vanrenterghem Y, Van Damme B. Subclinical peritubular capillaritis at 3 months is associated with chronic rejection at 1 year. *Transplant* 2007; **83**: 1416.
46. Jabs WJ, Sedlmeyer A, Ramassar V, *et al.* Heterogeneity in the evolution and mechanisms of the lesions of kidney allograft rejection in mice. *Am J Transplant* 2003; **3**: 1501.
47. Gloor JM, Sethi S, Stegall MD, *et al.* Transplant glomerulopathy: subclinical incidence and association with alloantibody. *Am J Transplant* 2007; **7**: 2124.
48. 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.
49. Sellares J, De Freitas D, Mengel M, *et al.* Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and non-adherence. *Am J Transplant* 2012; **12**: 388.
50. El ZM, Stegall MD, Lager DJ, *et al.* Identifying specific causes of kidney allograft loss. *Am J Transplant* 2009; **9**: 527.
51. Cosio FG, Gloor JM, Sethi S, Stegall MD. Transplant glomerulopathy. *Am J Transplant* 2008; **8**: 492.
52. Cosio FG, Lager DJ, Lorenz EC, Amer H, Gloor JM, Stegall MD. Significance and implications of capillaritis during acute rejection of kidney allografts. *Transplant* 2010; **89**: 1088.
53. Monga G, Mazzucco G, Novara R, Reale L. Intertubular capillary changes in kidney allografts: an ultrastructural study in patients with transplant glomerulopathy. *Ultrastruct Pathol* 1990; **14**: 201.
54. Monga G, Mazzucco G, Messina M, Motta M, Wuaranta S, Novara R. Intertubular capillary changes in kidney allografts: a morphologic investigation on 61 renal specimens. *Mod Pathol* 1992; **5**: 125.
55. Ivanyi B, Fahmy H, Brown H, Szenohradszky P, Halloran PF, Solez K. Peritubular capillaries in chronic renal allograft rejection: a quantitative ultrastructural study. *Hum Pathol* 2000; **31**: 1129.
56. 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.
57. Haas M, Montgomery RA, Segev DL, *et al.* Subclinical acute antibody-mediated rejection in positive crossmatch renal allografts. *Am J Transplant* 2007; **7**: 576.
58. Dinavahi R, George A, Tretin A, *et al.* Antibodies reactive to non-HLA antigens in transplant glomerulopathy. *J Am Soc Nephrol* 2011; **22**: 1168.
59. Joosten SA, Sijpkens YW, Van HVTrouw LA, *et al.* Antibody response against the glomerular basement membrane protein agrin in patients with transplant glomerulopathy. *Am J Transplant* 2005; **5**: 383.
60. Baid-Agrawal S, Farris III AB, Pascual M, *et al.* Overlapping pathways to transplant glomerulopathy: chronic humoral rejection, hepatitis C infection, and thrombotic microangiopathy. *Kidney Int* 2011; **80**: 879.
61. Cosio FG, Sedmak DD, Henry ML, *et al.* The high prevalence of severe early posttransplant renal allograft pathology in hepatitis C positive recipients. *Transplant* 1996; **62**: 1054.
62. 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.

63. Al Aly Z, Yalamanchili P, Cortese C, Salinas-Madriga L, Bastani B. C4d peritubular capillary staining in chronic allograft nephropathy and transplant glomerulopathy: an uncommon finding. *Transpl Int* 2005; **18**: 800.
64. Akalin E, Dikman S, Murphy B, Bromberg JS, Hancock WW. Glomerular infiltration by $CXCR3^+$ $ICOS^+$ activated T cells in chronic allograft nephropathy with transplant glomerulopathy. *Am J Transplant* 2003; **3**: 1116.
65. Sis B, Jhangri G, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial gene expression in kidney transplants with alloantibody indicates antibody-mediated damage despite lack of C4d staining. *Am J Transplant* 2009; **9**: 2312.
66. Sis B, Halloran PF. Endothelial transcripts uncover a previously unknown phenotype: C4d-negative antibody-mediated rejection. *Curr Opin Organ Transplant* 2010; **15**: 42.
67. Yamakuchi M, Kirkiles-Smith NC, Ferlito M, *et al.* Antibody to human leukocyte antigen triggers endothelial exocytosis. *Proc Natl Acad Sci USA* 2007; **104**: 1301.
68. Morrell CN, Murata K, Swaim AM, *et al.* In vivo platelet-endothelial cell interactions in response to major histocompatibility complex alloantibody. *Circ Res* 2008; **102**: 777.
69. Hidalgo LG, Sis B, Sellares J, *et al.* NK cell transcripts and NK cells in kidney biopsies from patients with donor-specific antibodies: evidence for NK cell involvement in antibody-mediated rejection. *Am J Transplant* 2010; **10**1812.
70. Osasan S, Sellares J, de Freitas DG, *et al.* Microcirculation endothelial cell cycling is selectively increased in antibody-mediated rejection of kidney transplants, but not in other diseases. *Am J Transplant* 2011; **11**: 193 [s2].
71. Haas M, Mirocha J. Early ultrastructural changes in renal allografts: correlation with antibody-mediated rejection and transplant glomerulopathy. *Am J Transplant* 2011; **11**: 2123.
72. Reed EF, Demetris AJ, Hammond E, *et al.* Acute antibody-mediated rejection of cardiac transplants. *J Heart Lung Transplant* 2006; **25**: 153.
73. Stewart S, Winters GL, Fishbein MC, *et al.* Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant* 2005; **24**: 1710.
74. Berry GJ, Angelini A, Burke MM, *et al.* The ISHLT working formulation for pathologic diagnosis of antibody-mediated rejection in heart transplantation: evolution and current status (2005-2011). *J Heart Lung Transplant* 2011; **30**: 601.
75. Rodriguez ER, Tan CD. Pathologic evaluation for antibody-mediated rejection: prognostic vs diagnostic markers? *J Heart Lung Transplant* 2011; **30**: 136.
76. Drachenberg CB, Torrealba JR, Nankivell BJ, *et al.* Guidelines for the diagnosis of antibody-mediated rejection in pancreas allografts – updated Banff grading schema. *Am J Transplant* 2011; **11**: 1792.
77. Glanville AR. Antibody-mediated rejection in lung transplantation: myth or reality? *J Heart Lung Transplant* 2010; **29**: 395.
78. Stewart S, Fishbein MC, Snell GI, *et al.* Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant* 2007; **26**: 1229.
79. Wallace WD, Reed EF, Ross D, Lassman CR, Fishbein MC. C4d staining of pulmonary allograft biopsies: an immunoperoxidase study. *J Heart Lung Transplant* 2005; **24**: 1565.
80. Saini D, Weber J, Ramachandran S, *et al.* Alloimmunity-induced autoimmunity as a potential mechanism in the pathogenesis of chronic rejection of human lung allografts. *J Heart Lung Transplant* 2011; **30**: 624.
81. Musat AI, Agni RM, Wai PY, *et al.* The significance of donor-specific HLA antibodies in rejection and ductopenia development in ABO compatible liver transplantation. *Am J Transplant* 2011; **11**: 500.
82. O'Leary JG, Kaneku H, Susskind BM, *et al.* High mean fluorescence intensity donor-specific anti-HLA antibodies associated with chronic rejection Postliver transplant. *Am J Transplant* 2011; **11**: 003A1868.
83. Ali S, Ormsby A, Shah V, *et al.* Significance of complement split product C4d in ABO-compatible liver allograft: Diagnosing utility in acute antibody mediated rejection. *Transpl Immunol* 2012; **26**: 62.
84. Kozlowski T, Rubinas T, Nickeleit V, *et al.* Liver allograft antibody-mediated rejection with demonstration of sinusoidal C4d staining and circulating donor-specific antibodies. *Liver Transpl* 2011; **17**: 357.
85. Lunz J, Ruppert KM, Cajaiba MM, *et al.* Re-examination of the lymphocytotoxic crossmatch in liver transplantation: can C4d stains help in monitoring? *Am J Transplant* 2012; **12**: 171.