Antibody-Mediated Rejection Criteria – an Addition to the Banff ‘97 Classification of Renal Allograft Rejection


aThe Johns Hopkins University School of Medicine, Baltimore, MD, USA
bMassachusetts General Hospital, Boston, MA, USA
cThe University of Alberta, Edmonton, Alberta, Canada
dThe University of Basel, Basel, Switzerland
eCatholic University of Louvain Medical School, Brussels, Belgium
fUniversity of Pittsburgh, Pittsburgh, PA, USA
gDavid Geffen School of Medicine at UCLA, Los Angeles, CA, USA
hVanderbilt University, Nashville, TN, USA
iUniversity of Leicester, Leicester, UK
jHealth Sciences Center, Winnipeg, Manitoba, Canada
kHôpital Européen Georges Pompidou, Paris, France
lUniversity of Helsinki, Helsinki, Finland
mUniversity of Iowa, Iowa City, Iowa, USA
nYale University, New Haven, CT, USA
oUniversity of Texas, Houston, TX, USA
pSt. Pauls Hospital, Vancouver, British Columbia, Canada
qNagoya City University, Nagoya, Japan
rUniversity of North Carolina, Chapel Hill, NC, USA
sUniversity of Vienna, Vienna, Austria
tUniversity of Barcelona, Barcelona, Spain
uCornell Medical Center, New York, NY, USA
vNational Hospital, Oslo, Norway
wRockyview General Hospital, Calgary, Alberta, Canada

*Corresponding author: Lorraine C. Racusen, lracusen@jhmi.edu

Introduction

The 1997 Revised Banff Classification of Renal Allograft Pathology (1) is used internationally for scoring and classifying rejection in kidney transplant biopsies. However, it deals with antibody-mediated rejection (AbAR) in an imprecise manner, categorizing AbAR by clinical presentation as ‘hyperacute’ and delayed forms only. This construct is not based on pathological findings, and does not take into account the burgeoning recent literature in this area as antibody-mediated rejection is increasingly recognized. At the 2001 Banff meeting, as the result of lively and in-depth discussions, the more detailed pathologic classification of antibody-mediated rejection outlined in this article was crafted. It is intended that the classification outlined here replace the original category 2 of the Banff ‘97 classification, leaving the remainder of the schema unchanged, except that the prior category of ‘Acute/Active Rejection’ be renamed ‘Acute/Active Cellular Rejection’.

The role of antibody in rejection of transplanted organs was the subject of spirited debate in the early days of transplantation, with Peter Gorer championing the role of antibody and Peter Medwar championing cell-mediated immunity. Following Gorer’s untimely death in 1961, the concept of antibody-mediated rejection faded into the background. However, by 1997 it was an entity recognized by most of the participants in international transplant meetings. Moreover, demonstration of the relative sensitivity and specificity of C4d staining in peritubular capillaries in identifying AbAR raised the hope that a rigorous morphological classification of antibody-mediated rejection could be devised.

Based on data from modern series, the incidence of antibody-mediated rejection is significant, reported at between 0 and 8% in renal allograft recipients in large centers (2–4). Increased rates of reported occurrence of AbAR reflect several forces: (i) increased recognition of
the condition, (ii) better techniques for detecting anti-donor antibody, (iii) increasing numbers of retransplanted patients, and (iv) increased transplantation across ABO and other immunologic barriers as the shortage of donor organs persists. Despite many advances, however, numerous transplantation centers can only speculate about the presence of anti-donor antibody based on panel reactive antibody (PRA) levels, since they lack the technical capability to measure donor-specific antibody (dsAb) directly. Advances in methods to detect HLA antibodies and determination of specificity, however, which are beyond the scope of this article, such as ELISA and flow-PRA, are making testing easier to use and more informative. Even with these techniques, it may sometimes be difficult to detect antibody, perhaps due to adsorption by the graft.

The identification of some relatively specific pathological markers of antibody-mediated rejection has helped revive interest in and increase recognition of AbAR. Halloran et al. in the early 1990s reported a triad of allograft dysfunction, neutrophils in peritubular capillaries, and antibody against donor class I HLA antigens which defined an entity discrete from classical acute rejection and from hyperacute rejection (5,6). Trpkov et al. later reported several specific morphological features in rejecting allografts that correlated with the presence of anti-class I antibody in the recipient (7). These features included severe vasculitis (Figure 1), glomerulitis (Figure 2), fibrin thrombi, fibrinoid necrosis, infarction and neutrophils in peritubular capillaries (Figure 3). There is some evidence suggesting that a monocyte/macrophage infiltrate in glomeruli and peritubular capillaries is specific for AbAR (Figure 2B), while glomerulitis with infiltrate of T cells can occur in C4d-negative biopsies.

Figure 1: Severe small vessel vasculitis with transmural lymphocyte infiltration (long arrow), fibrinoid necrosis (short arrow) and very swollen endothelial cells (near asterisk). (Original magnification 400×).

Figure 2: A. Glomerulitis, with infiltrating mononuclear cells, and swollen endothelial cells in capillaries. B. Immunoperoxidase stain for monocyte/macrophages (KP-1), demonstrating numerous positive cells in glomerular capillaries (long arrow) and peritubular capillaries (short arrow). (Original magnification 400×).

Figure 3: Margination of inflammatory cells in congested peritubular capillaries, including mononuclear cell and neutrophils. (Original magnification 400×).
Discussion

At Banff, Dr K. Morozumi reviewed experience with ABO-incompatible transplants at the Nagoya Daini Red Cross Hospital Kidney Center (8), confirming correlation of neutrophils in peritubular capillaries, with or without hemorrhage (Figure 4), edema, and microthrombi; glomerulitis with neutrophils; glomerular and arteriolar thrombi; and cortical necrosis/coagulative necrosis with AbAR due to ABO incompatibility. While suggestive, however, these morphological findings are not specific for rejection, may be absent in small biopsy samples, or may be difficult to recognize in the presence of concomitant cell-mediated rejection, which is not uncommon.

Within the past decade, reports have emerged regarding the usefulness of peritubular capillary staining for C4d as a marker of AbAR (see Figure 5). Feucht et al. in seminal and pioneering studies, reported capillary deposition of C4d, a terminal component of the complement cascade which binds covalently to capillary wall and therefore persists in graft tissue, in 51 of 93 biopsies from allografts with early graft dysfunction. One-year graft survival was 57% in grafts with generalized deposits, and 63% in those with focal deposition of C4d, contrasted to 90% survival in those that were C4d negative. Moreover, 3 of the 4 grafts that were C4d negative when biopsied during early dysfunction that subsequently failed showed C4d deposits in a second biopsy, and the authors proposed that C4d deposition was useful in assessing individual graft prognosis (9). At Banff, Dr Alex Magil summarized data from his group indicating that C4d is an important predictor of graft survival, and indeed has predictive value independent of numerous other morphological and clinical factors on multivariate analysis; these data have since been published (10). The C4d + group had more female patients, more patients with PRA > 30%, more with resistance to standard anti-rejection therapy, and a greater rate of graft loss (38 vs. 7%). C4d deposition was more frequently seen in cases of type II rejection (46%) compared to type I (24%). They were unable to explore directly the correlation of C4d staining with the presence of donor-specific HLA antibody in this retrospective study.

Dr Robert Colvin reviewed published studies from the Massachusetts General Hospital that were the first to show a clear correlation of peritubular capillary C4d staining with concurrent circulating anti-donor-specific antibody and with certain pathologic features (11). A follow-up larger study, done in 67 consecutive biopsies from patients with acute rejection in the first 3 months, has now been published (2). C4d staining was 95% sensitive and 96% specific for anti-donor antibodies; C4d staining was required to be bright and diffuse to be classified as positive. None of the morphologic features described by Trpkov et al. (7) were absolutely reliable markers for donor-specific Ab. However, as in that previous study, strong correlations were found with neutrophils in capillaries (peritubular and glomerular) and vascular/arterial fibrinoid necrosis. Colvin presented data demonstrating that, overall, these histologic features were 58–74% specific and 88–96% sensitive. C4d did not correlate with endarteritis or interstitial infiltrate. In addition, Crespo et al. reported that 37% of their cases of steroid-insensitive acute rejection had donor-specific antibody, and 95% of those were C4d positive on biopsy (4). One of 20 cases with antibody was C4d negative. Among the important points made were that: (i) acute humoral rejection may be manifested only by acute tubular injury without other evidence of rejection (seen in 10% of cases); (ii) acute humoral rejection commonly occurs in conjunction with acute cellular rejection (60% of cases); and (iii) acute humoral rejection, when present, has markedly poorer prognosis (27–40% 1-year graft loss) than acute cellular rejection.

Figure 4: Interstitial hemorrhage due to capillary disruption, with neutrophils and focal tubulitis. (Original magnification 400×).

Figure 5: Immunofluorescence stain showing bright linear peritubular capillary staining for C4d. (Original magnification 50×).
rejection without a humoral component (3–7% 1-year graft loss).

A large retrospective series of biopsies was presented by Dr Volker Nickeleit and Dr M.J. Mihatsch, including 398 biopsies from 265 recipients, all obtained in the setting of graft dysfunction; 113 native kidney and 12 baseline allograft control biopsies were all C4d negative. These investigators found a prevalence of 30% of biopsies that were C4d positive, defined as diffuse (18%) or focal (12%) positivity, with a median occurrence at 38 days. No deposition of immunoglobulin or other complement components was detected in these cases. In cases of serial biopsies, C4d-negative biopsies could become positive within as few as 4 days; positive biopsies could convert to negative within as few as 8 days. Of those that were C4d positive, 57% had glomerulitis, 45% had endarteritis, 41% had HLA-DR expression on tubular epithelium, and 43% had tubulointerstitial rejection. Stepwise regression analysis with C4d as a dependent variable demonstrated that tubular HLA-DR expression was the morphologic feature most closely linked with C4d, followed by glomerulitis. Stepwise regression did not show an association between C4d deposition and endarteritis or tubulointerstitial rejection. Stepwise regression analysis of the sensitivity and specificity of C4d staining on paraffin sections showed a high level of sensitivity (88%) in C4d-positive biopsies, with approximately 50% of C4d-negative biopsies having donor-specific antibodies. Specificity of C4d staining for the presence of anti-donor antibody was 93%, sensitivity 31%. Creatinine at 1 year and 1-year immunologic graft loss were highest in the C4d-positive group. In addition, 1-year creatinine and graft loss were higher in cases which were C4d negative but had antibody than in those which were antibody negative (14). Staining for C4d was comparable on frozen tissue using standard antibody and on paraffin-embedded tissue using their antibody in a group of index biopsies. The sensitivity of C4d-positive staining for presence of antibody reported in this study is low, which could reflect some differences in the two techniques and/or the very sensitive methods (flow-cross-match and flow-PRA analysis) used in this study. The antibody is now commercially available for use in paraffin sections [Biomedica/Vienna (www.biomedica.co.at)]. Additional validation studies are under way to confirm this new and useful tool for immunohistology of the renal allograft.

Identification of an antibody-mediated component to a rejection reaction is of more than theoretical interest, since there are now strategies to prevent or treat antibody-mediated rejection. Dr Robert Montgomery reviewed his center’s experience with use of plasmapheresis and IVIg to treat patients with post-transplant antibody-mediated rejection, all with identified anti-donor antibody (15). Anti-CD 20 therapy was also used as needed for rescue therapy. Many of these patients had multiple historical risk factors, and had Banff grades 2 and 3 rejection. Outcomes overall were good, with the loss of only one graft, and good function in remaining grafts. His group (15) and others (12,16,17) have also reported use of these protocols and/or immunoadsorption (3) to prevent and/or treat AbAR in sensitized patients. Dr Denis Glotz reviewed experience with IVIg to desensitize patients in anticipation of cadaveric transplantation. The strategy was successful in enabling allografting in a number of individuals who had waited for very prolonged periods for a suitable donor organ (18). Dr Morozumi discussed the preparative regimen used for their ABO-incompatible allografts, including double-filtration plasmapheresis, cycloporine, cyclophosphamide, and anti-lymphocyte globulin. End-points achieved by this regimen included marked reduction in antibody titers and in IgG and IgM, as well as increases in C3, C4 and CH50.

Effects of antibody as evidenced in the UNOS database were reviewed by Dr Michael Cecka. The half-life of allograft survival was 8.9 years among patients with PRA of 51–100, compared with 10.7 years for unsensitized recipients, suggesting that prior sensitization of the patient may affect long-term allograft survival. However, on multivariate analysis, sensitization accounted for 45% of variation in 1-year graft survival, but had a much smaller effect (0.4%) on variation in 5-year survival for those whose grafts survived the first year (19). The donor’s age, cause of death, HLA mismatch, and race had more impact on long-term survival than PRA. There was a linear correlation between PRA reactivity and delayed graft function (DGF), and DGF in turn was associated with decreased graft half-life. PRA also correlated with the incidence of early rejection, the occurrence of which also correlated with reduced graft survival when analyzed in paired donor kidneys.
Antibody-mediated rejection is not confined to renal allografts. Dr Michael Fishbein reviewed AbAR in heart allografts, the clinical manifestations of which include graft dysfunction, accelerated graft arteriosclerosis and death. Morphological manifestation of AbAR in the heart had been previously described by Hammond et al. (20), and included endothelial cell swelling, interstitial edema and hemorrhage, and neutrophils in and around capillaries. Immunofluorescence studies revealed immunoglobulin and complement deposition in small vessels, HLA DR expression and interstitial fibrinogen. They and others (21) have noted decreased graft survival and accelerated graft arteriosclerosis in AbAR. Fishbein et al. have also found prominent macrophage infiltrate in this setting. The UCLA group (22) found that female gender, increased PRA, positive cross-match, CMV positivity and OKT3 induction were risk factors for AbAR in the heart. Of note, biopsy features were not predictive of clinical dysfunction, and correlation with clinical findings was recommended. A review of the UCLA data-base revealed 11% of patients had documented AbAR (23).

Dr A. Jake Demetris reviewed historical data on AbAR in liver allografts. He described the relatively rare phenomenon of delayed graft function, with hemorrhage, thrombosis and bile duct complications which occasionally result from early AbAR in the liver transplant. On biopsy, there was bile duct proliferation, hepatocyte swelling and edema, sinusoidal polymorphonuclear cells, neutrophilic portal venulitis, arterial thrombosis, and fibrinoid changes. C4d staining has not been systematically studied. Liver transplants appear to be relatively resistant to AbAR. However, high-titer PRA and/or positive cross-match have been associated with decreased survival in 7 of 10 published studies (24).

**Table 1:** Antibody-mediated rejection (meeting criteria of C4d+ and with circulating anti-donor antibody)

| 1. ATN-like |
| 2. Capillary-glomerulitis, polymorphonuclear and/or mononuclear leukocytes in peritubular capillaries |
| 3. Arterial–transmural inflammation/fibrinoid change |

**Table 2:** Banff 97 diagnostic categories for renal allograft biopsies – update

1. **Normal, see Definitions**
2. **Antibody-mediated rejection**
   - Rejection due, at least in part, to documented anti-donor antibody (‘suspicious for’ if antibody not demonstrated); may coincide with categories 3, 4 and 5
   - Type (Grade)
     - I. ATN-like – C4d+, minimal inflammation
     - II. Capillary- margination and/or thromboses, C4d+
     - III. Arterial – v3, C4d+
3. **Borderline changes: ‘Suspicious’ for acute cellular rejection**
   - This category is used when no intimal arteritis is present, but there are foci of mild tubulitis (1–4 mononuclear cells/tubular cross-section) and at least i1; may coincide with categories 2 and 5
4. **Acute/active cellular rejection**
   - T-cell-mediated rejection; may coincide with categories 2 and 5
   - Type (Grade) Histopathological findings
     - IA Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of moderate tubulitis (>4 mononuclear cells/tubular cross section or group of 10 tubular cells)
     - IB Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of severe tubulitis (>10 mononuclear cells/tubular cross-section or group of 10 tubular cells)
     - IIA Cases with mild to moderate intimal arteritis (v1)
     - IIB Cases with severe intimal arteritis comprising >25% of the luminal area (v2)
     - III Cases with ‘transmural’ arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3)
5. **Chronic/sclerosing allograft nephropathy**
   - Fibrosing changes in the allograft, with or without features of true alloimmune injury to the graft; may coincide with categories 2,3, and 4
   - Grade Histopathological findings
     - Grade I Mild interstitial fibrosis and tubular atrophy without (a) or with (b) specific changes suggesting chronic rejection
     - Grade II Moderate interstitial fibrosis and tubular atrophy (a) or (b)
     - Grade III Severe interstitial fibrosis and tubular atrophy and tubular loss (a) or (b)
6. **Other**
   - Changes not considered to be due to rejection; may coincide with categories 2,3, 4, and 5

---

**Proposed Schema**

Following the formal presentations at Banff, Dr Robert Colvin drafted a proposal for pathological criteria for acute antibody-mediated rejection in kidney allografts and a proposed schema for AbAR, which were discussed in a plenary process led by Dr Kim Solez. The proposed schema which emerged from that consensus process is presented in Table 1, to replace the current category 2 in the Banff 97 schema.

**Criteria for acute antibody-mediated rejection in renal allografts include 3 cardinal features**

1. Morphologic evidence of acute tissue injury, such as:
   - (a) acute tubular injury, (b) neutrophils and/or mononuclear cells in peritubular capillaries and/or glomeruli, and/or capillary necrosis; or (c) intimal arteritis/fibrinoid necrosis/intramural or transmural inflammation in arteries.
2. Immunopathologic evidence for antibody action, such as:
   - (a) C4d and/or (rarely) immunoglobulin in peritubular capillaries or (b) immunoglobulin and complement in arterial fibrinoid necrosis.
3. Serologic evidence of circulating antibodies to donor HLA or other anti-donor endothelial antigens.

By immunostaining, C4d deposition in peritubular capillaries is the significant feature, not deposition in glomerular capillaries, arteries or arterioles. At this time, positive staining is defined as bright linear staining along capillary basement membranes typically involving over half of sampled capillaries. Focal/weak C4d capillary staining may be correlated with poor outcome and/or requirement for more aggressive immunosuppressive therapy (9,10,12) and suggestive for an antibody-mediated process; testing for anti-donor antibody should be performed, and the patient should be followed carefully. If there are morphological features suggesting AbAR and C4d is positive, but specific immunopathological evidence of immunoglobulin deposition or serologic evidence of anti-donor Ab is not available, the biopsy can be reported as suspicious for AbAR. If there are one or more morphological features of AbAR and serologic evidence of anti-donor antibody, the biopsy can be interpreted as suspicious for, or consistent with, AbAR. Recent data from Haas (25) indicate that in the kidney, in possible contrast to the heart (26), C4d is not deposited as a consequence of ischemia alone, but rather points to an antibody-mediated rejection, even in a setting of tubular injury and delayed graft function. Until a consistent correlation of C4d peritubular capillary staining and anti-donor antibody can be proven, however, all three criteria will be required for definitive diagnosis. These criteria will be revisited as the many ongoing and planned investigations in this area reach fruition.

In many cases, biopsies will meet the criteria for acute cellular rejection and the criteria 2 and 3 above, and should be classified as combined cellular and antibody-mediated rejection. They may well behave as acute antibody-mediated rejection (1), or at least require more aggressive anti-rejection therapy (10,12). Similarly, antibody-mediated rejection may coincide with chronic allograft nephropathy/chronic rejection. Most, if not all, cases with transmural arteritis/fibrinoid necrosis (v3) are associated with other evidence of antibody-mediated rejection (1,7). Whether the original Banff/CCTT type 3 category exists as a form of pure cellular rejection remains to be demonstrated. More investigation is needed in this area, though a significant percentage of cases with vascular fibrinoid necrosis were negative for C4d in one retrospective study (12). Since this lesion is rare, multicenter studies may be necessary to adequately address this issue.

The updated Banff schema, presented in Table 2, reflects this change in category 2, antibody-mediated rejection. Grading in all other categories remains the same. Often, more than one rejection category can be applied to a given allograft biopsy. The more sophisticated approach to the category of antibody-mediated rejection in this update of the Banff schema reflects increasing recognition and incidence of this important mechanism of immunologic allograft injury and loss. The schema will be revisited once again in the Seventh Banff Conference on Allograft Pathology, to be held in Aberdeen, Scotland, on June 14–18, 2003.

**Acknowledgments**


**References**

7. Tippett K, Campbell P, Pazderka F, Cocksfield S, Solez K, Halloran PF. Pathologic features of acute renal allograft rejection associated with