Banff 07 Classification of Renal Allograft Pathology: Updates and Future Directions


1 Institute for Pathology, University of Vienna, Vienna, Austria
2 Department of Pathology, CHU Hotel Dieu, Nantes, France
3 Department of Cellular Pathology, John Radcliffe Hospital, Oxford, United Kingdom
4 Department of Nephrology, Hospital, Universitario de Bellvitge, Barcelona, Spain
5 Department of Medico-Diagnostic Sciences, University of Padua, Padua, Italy
6 Corresponding author: Kim Solez, Kim.Solez@ualberta.ca

The 9th Banff Conference on Allograft Pathology was held in La Coruna, Spain on June 23–29, 2007. A total of 235 pathologists, clinicians and scientists met to address unsolved issues in transplantation and adapt the Banff schema for renal allograft rejection in response to emerging data and technologies. The outcome of the consensus discussions on renal pathology is provided in this article. Major updates from the 2007 Banff Conference were: inclusion of peritubular capillaritis grading, C4d scoring, interpretation of C4d deposition without morphological evidence of active rejection, application of the Banff criteria to zero-time and protocol biopsies and introduction of a new scoring for total interstitial inflammation (ti-score). In addition, emerging research data led to the establishment of collaborative working groups addressing issues like isolated ‘v’ lesion and incorporation of omics-technologies, paving the way for future combination of graft biopsy and molecular parameters within the Banff process.

Key words: Acute allograft rejection, acute cellular rejection, acute rejection, allograft rejection, antibody-mediated rejection, Banff, Banff lesions, Banff schema, classification, chronic allograft nephropathy, chronic allograft rejection, genomic markers, GeneChip

Abbreviations: ABMR, antibody-mediated rejection; AB0I, AB0-incompatible; ATN, acute tubular necrosis; ±CM, positive HLA cross-match; DSA, donor specific antibody; FSGS, focal segmental glomerulosclerosis; IF, immunofluorescence; IF/TA, interstitial fibrosis and tubular atrophy; IHC, immunohistochemistry; PBT, pathogenesis-based transcript set; PTC, peritubular capillaries; Ptg, peritubular capillaritis; SCr, serum creatinine; SR, subclinical rejection; TCMR, T cell-mediated rejection; TG, transplant glomerulopathy.

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Peritubular Capillaritis, C4d Scoring and C4d Deposition Without Graft Pathology

Peritubular capillaritis

Ian Gibson (Winnipeg) reported on clinico-pathological implications and reproducibility of the previously described peritubular capillaritis (ptc) scores (1). Results from a collaborative study analyzing ptc in 688 biopsies were presented. In C4d+ biopsies, 78% had ptc; whereas in C4d–biopsies, 24% had ptc. The most common pattern was grade 2, focal (<50%), with a minority of neutrophils. Localization of cells in peritubular capillaries (PTC) can best be appreciated with a specific endothelial stain (2), but this is not a requirement for ptc scoring. The inter-observer reproducibility of the ptc scoring features was fair to moderate (weighted kappa 0.32–0.43) on PAS-stained slides.

Evelyne Lerut (Leuven) presented her retrospective study of C4d, C3d and ptc in 731 indication biopsies from renal allografts. Overall, 25% had C4d in PTC and 8% in glomerular capillaries alone by immunohistochemistry (IHC). C3d was rarely present without C4d (2–3%). Ptc was associated with C4d in 50%, versus 10% of those without C4d, but was not a risk factor for later graft loss. C4d in PTC correlated strongly with C4d in glomerular capillaries and with C3d. The late (>3 month) presence of C4d in the glomerular capillaries was an independent risk factor for late (>6 month) graft failure. In a prospective protocol biopsy study, ptc at 3 months predicted multilamination of PTC basement membranes and sub-clinical chronic antibody-mediated rejection (ABMR) at 1 year (3).

Subsequent discussion by participants led to a consensus for a ptc-scoring system, now recommended for routine clinical practice (see updates section below).

C4d scoring

Criteria for the diagnosis of acute and chronic ABMR were previously introduced (1,4), and require positive immunostaining for C4d and/or immunoglobulin in PTC. It is recommended that every renal allograft biopsy should be stained for C4d. Although a diffuse C4d staining (i.e. >50% of PTC stained) is defined as positive (4), the definition and clinical significance of ‘focal’ C4d staining remain debated issues.

Michael Mihatsch (Basel) reviewed his comparative analysis of paraffin-polyclonal antibody-IHC and the cryostat-monoclonal antibody-immunofluorescence (IF) techniques on C4d staining (5). IHC was less sensitive, by about one grade level: diffuse staining on IF was seen as focal on IHC (in ~25% of diffuse on IF) or focal became minimal. Of focal cases on IHC, about 60% became diffuse and 40% remained focal/minimal on IF. This result is similar to that reported by Nadasdy (6). These results formed the basis for adjustment of the C4d interpretation according to the technique (Figure 1).

Michael Mengel (Hannover/Edmonton) reviewed the variable criteria used for C4d grading. Although diffuse staining is widely considered >50% of PTC involved, focal staining is reported from 10 capillaries to 50% of PTC. Results from large biopsy series on paraffin sections showed that

<table>
<thead>
<tr>
<th>% biopsy area (cortex and/or medulla)</th>
<th>Significance and interpretation according to technique</th>
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<tbody>
<tr>
<td>C4d0 Negative: 0%</td>
<td>Neg         Neg</td>
</tr>
<tr>
<td>C4d1 Minimal: 1&lt;10%</td>
<td>Neg         Unknown</td>
</tr>
<tr>
<td>C4d2 Focal: 10-50%</td>
<td>Unknown     ? Pos</td>
</tr>
<tr>
<td>C4d3 Diffuse: &gt;50%</td>
<td>Pos         Pos</td>
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Figure 1: C4d scoring in peritubular capillaries (PTC) and influence of staining method. The interpretation of C4d staining should be adjusted for the applied technique. Immunohistochemistry (IHC) on paraffin section is usually less sensitive by about one grade level (i.e. diffuse staining on IF [cryosections] can be seen as focal on IHC [paraffin sections]). Therefore, the report should indicate the actual % of tissue involved and the potential clinical significance. For example, diffuse positive C4d by IF or IHC is highly correlated with circulating antidonor antibody. Focal positive C4d by IHC is possibly equivalent to diffuse positive IF, and should be retested on IF, if possible. However, for focal positive C4d by IF and for minimal C4d by IHC, the clinical significance is unknown.
focal C4d is associated with ptc/glomerulitis (7). In addition, some studies suggest that focal C4d cases may have an intermediate prognosis between diffuse and negative cases (8–10). However, the significance of these observations is limited by the lack of consensus criteria for focal staining, concurrent measurement of antibodies and small sample size. Thus prospective correlation of C4d cut-offs with detection of alloantibody and long-term outcome is needed. To make the results of such trials comparable, criteria for C4d scoring were discussed and defined (see updates section below).

**C4d deposition without graft pathology**

Protocol biopsies have revealed C4d along the PTC in 25–80% of AB0-incompatible (AB0I) renal allografts, with evidence of acute ABMR in only 4–12% (11,12). Furthermore, C4d deposition occurs in 2–26% of histologically normal AB0-compatible grafts, with the higher frequency found in HLA-presensitized patients (7,11). This ‘incidental’ C4d deposition does not necessarily portend acute ABMR, but the longer-term significance is unknown. In a small biopsy series from AB0-compatible grafts with normal histology, focal or diffuse C4d was detected in 5%. Although C4d positivity was not associated with rapid graft loss in this cohort, even in cases with unaltered immunosuppression, patients benefited from antirejection therapy or an increase of baseline immunosuppression (13). Robert Colvin (Boston) presented a draft written proposal that was discussed, refined and accepted, which recognizes this new diagnostic category (see updates below).

**Protocol Biopsies**

Protocol biopsies taken at the time of transplantation (‘zero-time’) and later have the potential to influence clinical management and are the standard of care in some centers (14–16). Protocol biopsies also provide a window into pathogenetic mechanisms that might not be appreciated if the graft is only examined after dysfunction develops.

Volker Nickeleit (Chapel Hill) presented his experience with 114 postperfusion, zero-time biopsies. Over 50% of biopsies showed arterial intimal fibroelastosis or arteriolar hyalinosis, and 18% showed Banff cv2 (fibrous intimal thickening) lesions. The incidence of cv2 was not different in live-donor and deceased donor kidneys, and increased with increasing donor age. Zero-time biopsy cv2-3 was associated with a higher recipient serum creatinine (SCR) 3 and 6 months posttransplant, but not with posttransplant hypertension, delayed graft function or graft loss during 12 months follow-up; similar data on the significance of donor arterial intimal fibroelastosis on long-term graft function have been reported by others previously (17,18).

Michael Mengel (Hanover/Edmonton) reported his studies on interstitial inflammation patterns considered as nonspecific in the Banff 97 classification, including nodular infiltrates and infiltrates in the sub-capsular cortex, peri-ventitial areas and in areas of interstitial fibrosis and tubular atrophy (IF/TA) (19). Findings from 1139 biopsies showed that inflammation in areas of IF/TA and the sum of persistent inflammation of any type were negative prognostic indicators for longer-term graft function. Several groups also report that the combination of IF/TA and interstitial inflammation is associated with later graft loss (20–22). In addition, correlating inflammation with transcriptome changes showed that all types of infiltrates contribute to an increased expression of rejection and injury-related gene sets (23,24). To allow assessment of the overall infiltrate in the Banff system, a consensus for scoring a ‘total i-score (ti)’, was developed (see updates below).

Edward Kraus (Baltimore) reported the frequency of subclinical rejection (SR) or diffuse C4d staining in protocol biopsies of AB0I or AB0-compatible renal allografts with positive HLA cross-match (+CM) (25). At 1 month, Banff IA occurred in 38% of +CM or AB0I grafts and then declined to 14% at 1 year, higher than the 3–6% observed in nonsensitized patients. The incidence of Banff IA was not correlated with the presence of donor-specific antibodies (DSA) at time of biopsy. Thirty percent of +CM biopsies were C4d+, with most also showing capillaritis. All C4d+ biopsies were associated with DSA at the time of biopsy, although 50% of biopsies from DSA+ patients were C4d−. Mark Haas (Baltimore) continued the discussion of +CM and AB0I grafts (11). The majority of C4d+ protocol biopsies in +CM met Banff criteria for ABMR. Overall, 12% of patients with +CM grafts had sub-clinical ABMR. Compared to patients with +CM grafts and no evidence of ABMR, patients with sub-clinical ABMR had significantly more graft scarring and transplant glomerulopathy (TG) after 1 year. In contrast, most C4d+ protocol biopsies of AB0I grafts showed no histologic evidence of ABMR or T-cell-mediated rejection (TCMR).

Daniel Seron (Barcelona) discussed the role of protocol biopsies in clinical trials. He found that IF/TA associated with transplant vasculopathy, SR or TG on biopsies done within the first year implies a poorer outcome than IF/TA without other lesions. Using receiver operating characteristic curves to predict graft survival, he showed that the predictive value of the above-mentioned histologic patterns is not inferior to renal function or acute rejection, suggesting their potential utility in the design of clinical trials (26).

Fernando Cosio (Rochester, MN) reviewed glomerular lesions in 613 protocol biopsies of (non-presensitized, ABO-compatible) renal allografts done at 1 year. Overall, 9% of biopsies showed glomerular lesions, including recurrent disease (1.6%), de novo focal–segmental glomerulosclerosis (FSGS) (3.0%) and TG (3.8%). FSGS was associated with a poor overall prognosis. The incidence of TG in these grafts steadily rose to ~20% by 5 years posttransplant. TG was strongly associated with the presence of antibodies to HLA-class II (risk factor 6.2), and to a lesser extent with
anti-class I (risk factor 1.6); patients having antibodies to both classes I and II had the highest risk (9.7). Overall, 50% of patients with acute ABMR developed TG despite treatment of the acute process. Glomerulitis correlated with the onset of TG. In 1 year biopsies with TG, 26% showed PTC C4d and 32% glomerular C4d.

Roslyn Mannon (Bethesda) and Brian Nankivell (Sydney) reviewed their experiences with molecular studies of protocol biopsies (27,28). Both emphasized that a histologically normal biopsy often has a rather abnormal molecular profile. Dr. Mannon reported on the use of low-density gene arrays that utilize real-time PCR technology. Using this type of array, protocol biopsies from recipients with stable function demonstrated increased expression of genes associated with T-cell activation compared to normal kidney, with a higher expression in SR and clinical TCMR (28). The expression of T-cell activation marker, T bet and costimulatory molecule CD152, was higher in overt TCMR than in SR. Brian Nankivell reviewed the potential pitfalls of gene chip studies of protocol biopsies and emphasized the need to develop molecular profiles for lesions other than rejection, especially calcineurin inhibitor toxicity. He and Phil Halloran (Edmonton) noted that cytokines are better detected by PCR than microarrays, as some probes used for microarrays are not optimal. Agreement on technical and mathematical methodologies will be needed for general clinical applications.

Mechanisms of Allograft Rejection

While the pathologic features of graft rejection have been recognized for a long time, the pathogenesis of both TCMR and ABMR is not fully understood. In this session, elegant mechanistic studies in human and experimental transplantation dealing with different aspects of graft rejection and response to injury were presented.

Banu Sis (Edmonton) emphasized that most of the current literature of ABMR is based on clinical observations, and experimental models and mechanistic studies of ABMR are lacking. Sis and colleagues developed a knock-out (Rag1) mouse kidney transplant model to explore the relationship between alloantibody, C4d deposition and associated transcriptome changes (29). In situ C4d deposition is alloantibody dependent, and develops as a continuous process, which starts as focal staining progressing to diffuse PTC staining after posttransplant day 7. However, they did not observe significant histologic lesions and/or transcriptome changes including expression of endothelial genes after treatment with a high dose of a donor-specific monoclonal anti-MHC antibody, although it triggered in situ C4d deposition. The tissue resistance to alloantibody-mediated injury in this in vivo model may be due to incomplete activation of complement system and/or increased activity of complement regulatory factors (30,31).

Heinz Regele (Vienna) described preliminary results of in vitro studies on the interaction of human capillary endothelium with anti-HLA antibodies and complement. Cultured endothelium was derived from renal allograft donor adrenals and aorta. Incubation with donor-reactive antibody resulted in the deposition of IgG on the endothelial cell surface and addition of normal serum led to the accumulation of C4d and C3d. C3d disappeared quickly, but IgG and C4d persisted longer, with a half-life of about 24 h. Eight patients were identified who were +CM to donor endothelial cells, but not lymphocytes. All had a benign course; 2 had biopsies, both C4d negative. Thus antiendothelial antibodies can exist without evident clinical consequences.

Neal Smith (Boston) reviewed findings in chronic ABMR in 102 renal allografts in nonhuman primates with C4d in PTC all had circulating DSA, and most later showed TG and transplant arteriopathy (89%). DSA was found in some recipients without C4d, which appeared later, sometimes without other evidence of graft pathology. Later biopsies showed TG, IF/TA. The presence of either C4d or DSA was associated with poorer graft survival. All C4d+ grafts failed in 3–27 months (32). The data support a four-stage sequence of evolution of chronic ABMR, beginning with DSA, followed by C4d in PTC, then graft pathology (TG, fibrosis) and finally graft dysfunction. No evidence for stable accommodation was found in these studies.

Gunilla Einecke (Edmonton) presented the gene expression of renal epithelium detected by microarrays during allograft rejection. In mouse kidney allografts, interstitial inflammation and tubulitis have been shown to be dependent on T cells, but independent of granzymes, perforin, B cells and antibody (32,33). Tubular epithelial molecular changes develop before tubulitis becomes apparent in routine histology (23,34) suggesting that alterations of gene expression in epithelium are a part of a stereotyped injury response evoked by the interstitial inflammation.

Philip Halloran (Edmonton) presented the emerging microarray findings in 143 human consecutive renal transplant biopsies for cause (35). Analysis of pathogenesis-based transcript sets (PBTs) indicated a large scale of disturbance in gene expression. The degree of disturbance across all biopsies was continuous rather than dichotomous, with many other forms of renal injury having disturbances in PBTs similar to but at a lower level than the rejection. PBT changes correlated with histopathologic lesions (I, t, v) and were highest in biopsies with clinical rejection episodes. Biopsies with low PBTs did not have rejection. Surprisingly, C4d+ ABMR biopsies had changes similar to TCMR in respect to a quantitatively similar inflammatory response. But, ABMR biopsies were discriminated by increased expression of genes related to endothelial cell activation (36). There was no discrimination by PBTs of the cut-off between t1 and t2 lesions. Furthermore, low PBTs were found in cases of isolated ‘v’ lesions (i.e. without other criteria for TCMR) classified as TCMR on the basis of...
The criteria for ptc score are given in Table 1. Biopsies with Scoring of ptc

| ptc 0 | No significant cortical ptc, or <10% of PTCs with inflammation |
| ptc 1 | ≥10% of cortical peritubular capillaries with capillaritis, with max 3 to 4 luminal inflammatory cells |
| ptc 2 | ≥10% of cortical peritubular capillaries with capillaritis, with max 5 to 10 luminal inflammatory cells |
| ptc 3 | ≥10% of cortical peritubular capillaries with capillaritis, with max >10 luminal inflammatory cells |

1It is recommended that one comment on the composition (mononuclear cells vs. neutrophils) and extent (focal, ≤50% vs. diffuse, >50%) of peritubular capillaritis.

vasculitis. In order to determine the significance of these relatively uncommon lesions, a collaborative multicenter study will be organized.

Finally, it was recommended that the Banff group actively participate in and encourage studies and workshops on gene expression in allograft biopsies from renal transplant recipients, to promote a consensus on the optimal tests and their meaning. When warranted by scientific studies, molecular measurements of clinical relevance can be incorporated into the Banff system.

The 2007 Updates on the Banff Classification

Scoring of ptc

The criteria for ptc score are given in Table 1. Biopsies with inflammatory cells in <10% of cortical PTC are scored as ptc0, regardless of the number of cells in the most severely involved PTC. If ≥10% of PTCs are inflamed, the ptc score is based upon the highest number of all types of luminal inflammatory cells in the most inflamed cross-sectioned PTCs in the cortex. The types of cells should be noted: only mononuclear cells, a minority (≤50%) of neutrophils or a majority (>50%) of neutrophils. The extent of capillaritis should also be noted: focal ≤50% versus diffuse >50% of PTCs involved. PTCs that are cut in a longitudinal plane of section should not be scored. PTC should not be scored in medulla, due to the association of vasa recta infiltrates with acute tubular necrosis (ATN), and in vessels surrounding nodular lymphoid aggregates (due to confusion with lymphatics). Areas of pyelonephritis, and adjacent to infarcts, should also be avoided for ptc scoring.

C4d scoring

Scoring of C4d staining is based on the percentage of stained tissue on IF/IHC that has a linear, circumferential staining pattern in PTC (Table 2, Figure 1). The minimal sample for evaluation is 5 HPF of cortex and/or medulla without scarring or infarction. Biopsies with IF/TA may have reduced PTC density that could affect the extent of staining (37). On IF, staining should be >1+ in intensity. The report should indicate the actual percentage of tissue involved and the potential significance (Figure 1). For example, diffuse+ IF/IHC is highly correlated with circulating DSA. Focal+ IHC is possibly equivalent to diffuse+ IF (but not in all), should be restained by IF, if possible, and be correlated with antibody status and clinical features. For focal+ IF, the evidence is still uncertain, but this finding is often associated with circulating antibodies, particularly in allograft biopsies with IF/TA. For minimal C4d by IHC, the clinical significance is unknown.

C4d deposition without morphological evidence of active rejection

By consensus the term ‘C4d deposition without morphologic evidence of active rejection’ is added to the Banff diagnoses under the antibody-mediated category (Table 3). The criteria for this diagnosis will be (i) presence of complement fixation (e.g. C4d) in PTC, (ii) lack of histologic evidence of acute or chronic rejection (cellular or humoral) with lack of glomerulitis (g = 0), TG (cg = 0), ptc (ptc = 0) and PTC basement membrane lamination (assessed by electron microscopy <5 layers), (iii) presence of DSA. If borderline inflammation (i) or ATN is present, the diagnosis is indeterminate, since this lesion might be related to antibody. Other potential causes of the ATN (e.g. ischemic injury, calcineurin inhibitor toxicity, etc.) should be ruled out. This condition was intentionally not termed ‘accommodation’ as described in xenografts (38), because the long-term outcome may not be benign (13). In the report a cautionary statement is strongly urged, for example: ‘The stability and long-term significance of C4d deposition in the absence of morphologic evidence of rejection have not been established. Continued clinical monitoring is advised’. If the graft is ABOI, it should be noted that ‘C4d deposition without morphological evidence of active rejection’ is common, but even in that setting may be clinically significant.

Scoring zero-time biopsies

These biopsies should be routinely scored using the same criteria as for protocol and indication biopsies. Notably, the criteria for cg should be the same in zero-time biopsies (where in most instances it would be zero), as in other
biopsies. In some cases the use of a table tracking changes in individual lesions over time, beginning with the zero-time biopsy, may be helpful. Reference to previous biopsies in reporting is a standard practice in pathology.

**Scoring of total inflammation in renal allograft (ti)**

A new lesion score, termed ‘ti’ (total interstitial inflammation), is added to the Banff schema. The significance of this score will be tested over the next 2 years; for now routine scoring of ‘ti’ would be optional (Table 4). The ti score uses the same semi-quantitative criteria used for determining the i score, except that all of the cortical tissue present, including the sub-capsular cortex, perivascular cortex and areas of IF/TA would be considered. Criteria for the i-score remain unchanged. Cortical nodular infiltrates will be included in the i or ti score depending on their localization.

**Alternate qualitative scoring for hyaline arteriolar thickening (aah scoring)**

Reproducibility of Banff arteriolar hyaline thickening (‘ah’) score is alarmingly low with a kappa of 0.18 (39).

| Table 4: Quantitative criteria for mononuclear cell interstitial inflammation (‘ti’) in total parenchyma (scarred and unscarred) scores—to be evaluated over next two years. Not incorporated into classification yet |
|----------------------|----------------------|
| ti0                  | No or trivial interstitial inflammation (<10% of parenchyma) |
| ti1                  | 10–25% of parenchyma inflamed |
| ti2                  | 26–50% of parenchyma inflamed |
| ti3                  | >50% of parenchyma inflamed |

1 The 2007 updates are underlined.

2 All existing scoring categories (g, t, v, i, cg, ct, ci, cv, ah, mm) remain unchanged (42)

3 Please refer to Table 2 and Figure 1.

4 Suspect for antibody-mediated rejection if C4d (in the presence of antibody) or alloantibody (C4d+) not demonstrated in the presence of morphologic evidence of tissue injury.
Therefore, more objective criteria for the assessment of hyaline arteriolar thickening are needed. Recently, a new quantitative scoring system for CNI-arteriolopathy by Michael Mihatsch has been proposed so that the severity of hyaline arteriopathy is quantified according to the presence of circular or noncircular involvement and the number of involved arterioles (40). Sis et al. (41) reported that the new criterion for hyaline arteriolar thickening (‘aah’) results in better inter-observer reproducibility (kappa 0.67), and is validated against graft function. This new scoring system was discussed at an international protocol biopsy meeting (15). The aah score will be evaluated over the next 2 years; for now routine scoring of “aah” would be optional (Table 5).

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