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Diagnostic challenges in chronic antibody-mediated rejection

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Abstract

Measurement of glomerular and peritubular capillaritis in kidney transplant biopsy samples identifies allograft dysfunction associated with alloantibodies. Sis *et al.* show that this technique has a higher sensitivity but lower specificity than the current diagnostic criteria using peritubular capillary C4d deposition, and that capillaritis is an independent predictor of progression to graft failure.

The majority of cases of late renal allograft dysfunction and loss are caused by the presence of donor-specific antibodies (DSAs) to HLA antigens.¹ This complication makes it imperative to develop better methods of diagnosing antibody-mediated rejection at an early stage. A major step forward in this area was the application of a stain developed by Feucht to detect the complement fragment C4d in biopsy samples from patients with transplant glomerulopathy or arteriopathy. The triad of peritubular capillary C4d deposition, transplant glomerulopathy or arteriopathy and presence of DSAs was used to define chronic humoral rejection, which is now officially known in the Banff classification as chronic active antibody-mediated rejection (CAMR).² In a paper from 2002, Regele *et al.* noted that peritubular capillaritis and glomerulitis with mononuclear cells were also strongly associated with C4d-positive CAMR;³ this finding was confirmed in a later study by Gibson and colleagues.⁴ Halloran's group have long associated peritubular capillaritis and glomerulitis with neutrophils with DSA-positive acute rejection, and have been long-time advocates of the importance of DSA. Although detection of C4d has been the cornerstone in the diagnosis of antibody-mediated rejection for over 10 years, it has become clear that some cases of antibody-mediated rejection with DSAs, which are otherwise pathologically similar, do not have detectable C4d in the peritubular capillaries or have only very low levels. These C4d-negative cases can be manifested by peritubular capillaritis and/or increased endothelial activation.^{5,6} Sis *et al.* have therefore developed an algorithm to improve diagnostic accuracy in the presence of variable C4d deposition.⁷

Sis and colleagues examined 329 biopsy samples from patients with graft dysfunction, and scored glomerulitis, peritubular capillaritis and C4d deposition in peritubular capillaries according to the Banff categories, correlating these results with the presence of DSAs by a single-antigen bead assay at the time of biopsy.⁷ C4d was negative or minimally positive (<10% of peritubular capillaries) in many of the cases with DSA (65%), although the stain remained highly specific (98%) for the presence of DSA. The investigators found that in the

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Competing interests

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first year after transplantation, peritubular capillaritis and glomerulitis were often not associated with DSA (27%), and they recommended C4d staining as the more definitive test for antibody-mediated rejection in the first year after transplantation. After 1 year, however, aggregate capillaritis (both in peritubular capillaries and glomeruli) was more often associated with DSA (79%), although the specificity (90%) was inferior to using C4d staining.

The authors developed a decision tree based on these results to predict DSA status. The analysis started by determining whether glomerulitis (at least one glomerulus with inflammatory cells) or peritubular capillaritis (>10% of peritubular capillaries with more than two leukocytes) was present, time post-transplantation (>351 days or >466 days), and finally, whether >10% of peritubular capillaries were C4d-positive. Sis *et al.* then validated the decision-tree approach and compared it with C4d staining alone for prediction of DSA. The decision tree was more sensitive than was C4d (59% versus 35%, respectively), but was less specific (90% versus 98%, respectively). Taken together, these opposing performance characteristics led to similar accuracy in predicting DSA (80% versus 76%, respectively). Notably, glomerulitis or peritubular capillaritis predicted a worse outcome in patients overall, which correlated directly with more extensive intracapillary inflammation in biopsy samples. Univariate correlation with C4d staining and outcome was not reported, but the authors stated that it had no additional predictive value after cases were sorted by capillaritis.

How will these results inform pathological interpretation of renal biopsy samples? First, they will focus more attention on evaluating peritubular capillaritis and glomerulitis, which is unfortunately a notoriously difficult assignment. Reported interobserver agreement for glomerulitis ranges from poor to moderate, with only moderate interobserver agreement for peritubular capillaritis.⁴ This variation is partly due to the difficulty in identifying peritubular capillaries in routine sections and the tedium of counting cells in hundreds of capillaries. Counting cells in glomeruli is easier, but no specific threshold for cell number exists in the Banff classification for a glomerulus affected by glomerulitis. A quantitative immunohistochemical strategy to identify and quantify intracapillary cells from amidst interstitial inflammation could improve the rigor of capillaritis and glomerulitis scoring.

The lack of specificity is the second problem for evaluation of peritubular capillaritis or glomerulitis. Here, defining specific cellular mediators of CAMR could be useful. Sis and colleagues show increased numbers of intraglomerular CD56⁺ natural killer (NK) cells in four of six cases of CAMR. Although this sample is hardly large enough to provide convincing results, other data point to a role for NK cells in CAMR, including increased levels of NK-cell-specific transcripts⁸ and experimental studies in mice which show that CAMR is dependent on NK cells and not complement fixation.⁹ Unfortunately, NK cells are exceedingly difficult to detect in tissue as their phenotype varies with activation (activated NK cells lose CD56 expression) and few markers are specific to NK cells. Nonetheless, using a combination of stains to detect markers of activated NK cells, such as CD16 and transcription factor T-bet, is quite feasible.

The low sensitivity of C4d staining leads Sis *et al.* to dismiss its value. In our experience, however, the sensitivity of C4d staining is much higher, at least 60%. The reasons for this difference are unknown, but could depend on the patient population, the indications for biopsy, the sensitivity of the DSA test and perhaps the sensitivity of the C4d technique itself. We use a triple-layer immunofluorescence technique, which is regarded as the most sensitive.

We certainly agree that a small number of CAMR cases have little or no detectable C4d, even with the most sensitive techniques. What is the explanation? Negative C4d could be the result of a fluctuation in deposition owing to DSA titer, alterations in endothelial HLA regulation or complement clearance, or an inability of DSA subclass to fix complement. The latter is of particular interest as there is evidence for at least three independent mechanisms of injury in CAMR: first, classic complement fixation, as shown by the beneficial effects of anti-C5 therapy in antibody-mediated rejection in presensitized patients; second, antibody-mediated rejection mediated by NK cells independent of complement, as shown in murine studies with some support from observational studies in humans; and third, endothelial activation by antibody alone, best shown in endothelial cell cultures (Figure 1).¹⁰ These mechanisms are likely to each have their own pathological signature in the biopsy sample, including C4d deposition, accumulation of NK cells and markers of endothelial activation. Identification of mechanisms of injury in CAMR will also be critical for future therapeutic implications. In addition to strategies to reduce circulating DSAs, complement-dependent CAMR could be addressed with complement inhibitor therapy, whereas Fc-receptor-dependent and NK-cell-dependent CAMR might require cell-directed or Fc inhibitor strategies.

The original formulation of the diagnostic criteria for antibody-mediated rejection had three elements: first, tissue injury such as transplant glomerulopathy and multi-lamination of peritubular capillary basement membranes; second, evidence that the DSA was interacting with the tissue; and third, demonstration of circulating DSAs. At the time of the publication of these criteria, the best marker of antibody interaction with tissue was C4d deposition in peritubular capillaries, but there was an expectation that new techniques would be developed. The work of Sis *et al.*⁷ and Loupy *et al.*⁵ argue effectively that the second criteria could be fulfilled by assessing peritubular capillaritis or endothelial gene expression. Adding criteria for antibody interaction with the tissue should be relatively easy once a reliable and efficient methodology is established.

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Figure 1.

Mechanisms of donor-specific antibody-mediated endothelial injury in renal allografts. Anti-MHC antibodies can cause direct injury to the capillary endothelium, or indirect injury via complement fixation or recruitment of inflammatory cells with Fc receptors. Endothelial injury and cellular recruitment could be important mediators in cases with donor-specific antibodies that lack C4d deposition. Capillaritis has been proposed as a sensitive marker for the presence of donor-specific antibodies and late allograft rejection.⁷ Abbreviations: MHC, major histocompatibility complex; NK, natural killer cell; Poly, polymorphonuclear cell.