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TRANSPLANTATION:

Complementing donor-specific antibody testing

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Abstract

New data suggests that among kidney transplant recipients, those whose serum contains donor-specific antibodies that bind C1q fare the worst. Although these findings are intriguing, several unanswered questions remain. Changing practice to include a C1q binding assay as standard of care in kidney transplantation would be premature.

Antibodies that bind to donor HLA are pathogenic mediators of allograft injury and loss in kidney transplant recipients. Measurements of the presence of donor-specific anti-HLA antibodies (DSAs) in the serum of kidney transplant recipients are increasingly used by clinicians to assess the risk of acute and chronic allograft injury. In a new study, Loupy and colleagues investigated whether the identification of DSAs that bind to C1q—a molecule that initiates complement activation—adds additional prognostic value to these assessments.¹

In 1969, Terasaki and colleagues showed that the presence of donor antibodies in recipient serum that were capable of binding to donor cells and triggering complement-dependent cytotoxicity *in vitro* (a positive crossmatch) was strongly predictive of hyperacute kidney transplant rejection in humans.² This discovery led to the widespread clinical use of crossmatch testing to stratify risk and guide decision-making regarding donor and recipient compatibility. Crossmatch-testing methodology has evolved dramatically in the past few decades and the newer flow cytometry assays are able to detect low-titer antibodies that bind to donor cells and confer an increased risk of post-transplant rejection.³

A substantial breakthrough in the field occurred with the advent of single antigen bead (SAB) technology, which has enabled antibodies that bind to individual HLA allelic variants to be identified at a higher sensitivity and specificity than was previously achievable. Purified HLA molecules are covalently bound to inert microspheres, which are then mixed

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Conflicts of interest

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with patient serum. HLA-specific antibodies that bind to the HLA-coated beads can be detected using a flow cytometer or luminex machine. Using this strategy, multiple studies have demonstrated that the presence of pre-transplant and/or *de novo* post-transplant DSAs, particularly those that bind class II HLA molecules, confers an increased risk of late graft failure⁴⁻⁶ Nonetheless, a subset of patients with DSAs that can be detected using SAB approaches do not lose their allografts,³⁻⁵ raising the possibility that antibody characteristics other than specificity might determine their pathogenicity. As complement activation initiated by C1q cross-linking of IgG bound to the allograft is a crucial effector function of alloantibodies, investigators hypothesized that DSAs that are capable of binding C1q (C1q-positive DSAs) might confer the highest risk of graft injury.^{5, 7} The latest iteration of the SAB technology can identify specific anti-HLA antibodies that bind C1q. However, the impact of C1q-positive DSAs on graft survival has not been fully addressed in large scale studies.

To address this issue, Loupy *et al.* analyzed serum samples from 1,016 patients who received a kidney transplant between 2005 and 2011.¹ Samples were obtained pre-transplantation, at 12 months post-transplantation (coincident with a surveillance biopsy) and at the time of for-cause biopsies for suspected rejection. The researchers determined the presence of donor-specific anti-HLA antibodies in the serum samples using SAB assays and tested the positive samples for C1q binding. They correlated the results with allograft survival (independent of acute rejection) during a median follow-up of 4.8 years.

The researchers identified 45 patients who had C1q-positive DSAs before transplantation. Of the 196 patients who had C1q-negative DSAs pre-transplantation, 58 patients developed C1q-positive DSAs after transplantation. The presence of DSAs before transplantation conferred an approximately threefold increased risk of graft failure. However, C1q status before transplantation had no additional discernible effect on this risk. Analysis of serum samples obtained at the time of for-cause biopsies showed strong correlations between the presence of DSAs and microvascular inflammation, intragraft C4d staining (a marker of complement activation), transplant glomerulopathy (a manifestation of chronic antibody-mediated injury), interstitial inflammation and tubulitis. The presence of C1q-positive DSAs was associated with substantially worse pathology than was the presence of C1q-negative DSAs. Graft survival at 5 years was lower in patients with DSAs than in those without DSAs (83% versus 94%; $P < 0.001$). However, in patients with C1q-positive DSAs after transplantation, graft survival at 5 years was only 54%. Patients who were C1q-negative before transplantation and C1q-positive after transplantation had the highest risk of graft loss.

Although these findings suggest a dramatic effect of C1q-positive DSAs on allograft survival, enthusiasm should be tempered by two important caveats. First, C1q binding might simply be an indirect measure of the strength of the serum anti-HLA antibody (or of the detecting antibody), which is otherwise masked by interfering proteins found in the plasma.^{6,8} In order to bind C1q, antibodies must be present at sufficiently high concentrations and be of the subtypes IgG1, IgG3 or IgM, with SAB detecting IgG1 and IgG3 in a majority of sera.⁵ The mean fluorescence intensity (MFI) of staining using SAB has been shown to qualitatively reflect antibody concentration or titer.⁹ An MFI $> 10,000$

correlates strongly with C1q positivity,^{7, 10} and C1q-positive DSAs and any DSA with MFI>12,000 have equivalent effects on graft survival.⁵ Peri-transplant changes from C1q-negative to C1q-positive status, which were associated with poor prognosis in the current study,¹ were previously associated with significant increases in MFI.¹⁰ Thus, the association between C1q-positive DSAs and shortened graft survival reported by Loupy *et al.*¹ might be explained by the strength of the DSA. This issue was not specifically addressed by the researchers. C1q values relative to MFI were examined categorically at MFI thresholds of 2,000 and 6,000. Either of these thresholds is likely too low to detect significant relationships with a C1q-positive status. Even so, two-thirds of C1q-positive DSAs had MFI >6,000 compared with only 10% of C1q-negative DSAs, consistent with the hypothesis that testing for C1q positivity detects higher titer DSAs. Second, anti-class II HLA antibodies have been shown to be associated with shorter graft survival than are antibodies reactive to class I HLA.^{4, 5} Loupy and colleagues did not analyze whether C1q-positive status was independent of reactivity to class II HLA, further limiting the ability to reach definitive conclusions.

Although the findings of this new study are intriguing, the clinical utility of a C1q-binding test in kidney transplant recipients remains unclear. The response of C1q-positive DSAs to treatment and the impact of such treatment on graft outcome are not known. When deciding whether to implement routine C1q DSA testing, transplant programs will need to consider the burden of extra testing as opposed to using MFI data that is likely already available from their HLA laboratories. The cost of additional testing is high and we believe that the data from Loupy *et al.* do not support changing current practice to include routine C1q DSA testing of transplant recipients or of patients on the pre-transplant waitlist. However, further study, ideally in the context of prospective clinical trials, is required to better define the prognostic utility of this potentially informative innovation in DSA testing.

Biographies

Kathryn J. Tinckam, MD FRCPC is the Co-Director of the University Health Network HLA Laboratory, and a renal transplant physician. Her clinical interest is management of acute and chronic antibody mediated rejection in kidney, heart and lung transplantation. Her research focuses on the impact and treatment of HLA antibodies and in standardization of HLA testing platforms.

Peter S. Heeger, MD, Professor of Medicine and Director of Transplant Research at Icahn School of Medicine at in New York is an NIH-funded physician-scientist. He leads the Clinical Trials in Organ Transplantation consortium, conducting trials to assess the utility of noninvasive biomarkers to predict outcomes in transplant recipients. His basic research interests are mechanisms of allograft injury and tolerance, with a focus on complement and T lymphocytes.

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