How Do We Interpret the Presence of Donor-Specific Antibodies When There Is No Rejection?

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Pretransplant measurement of donor-specific human leukocyte antigen (HLA) antibodies (DSAs) in kidney transplantation has been a mainstay of immunologic risk assessment since the 1970s, starting with the original T-cell Cytotoxic Crossmatch. Testing has become progressively more sensitive through modifications in the assay and the addition of flow cytometry. The newest iteration of antibody assessment involves mixing patient sera with HLA peptides attached to polystyrene beads and measuring the antibody binding using the Luminex platform. This technique provides detailed information about breadth of anti-HLA antibodies that could lead to rejection, as well as semi-quantitative information about the quantity of each antibody specificity. As experience in the anti-HLA Luminex testing has grown, it has become clear that not all antibodies are created equal.

Although many patients with pretransplant anti-donor HLA antibodies detected using this technology develop antibody-mediated rejection (AMR), most do not. Certain characteristics of the antibody, its quantity, whether it is able to bind complement, its IgG subtype, whether the epitope is expressed in tissue, among other factors, likely contribute to the difference in the outcomes our patients experience.

With the success and ease of using anti-HLA bead technology in the pretransplant setting, many transplant centers have moved toward using it at follow-up visits as a biomarker. In transplantation, there has always been an interest in finding tests that identify patients at risk for rejection and patients in whom we can safely reduce immunosuppression. Among other features, a good biomarker should be reproducible within the same patient and over time, be comparable between institutions to allow comparison and research, should be relatively easily to obtain (e.g., blood and urine specimens are simpler and lower risk to obtain than allograft biopsies), and the turnaround time for the assay should be rapid enough to be clinically applicable. An even better marker would identify a problem or, in the case of transplant, an immunologic pathway that is modifiable through treatment. Ideally, it would not only identify a treatment pathway but also provide meaningful data early enough in the course of the disease process to allow intervention before a significant amount of injury has been done.

Serum creatinine is the most commonly used biomarker in kidney transplantation, as it is easy to obtain, inexpensive, widely available, and reproducible across different laboratories given the standardization of the assay, and its reflection of allograft function. However, it fails many of the other aspects, including not identifying a cause of the underlying dysfunction and being insensitive in the setting of early damage. A few of the assays currently being studied include gene expression profiling of circulating immune cells, measuring cytokine levels in blood or serum, and donor-derived cell free DNA tests.

At the heart of the desire to use anti-HLA antibody screens in the postoperative period is the recognition that chronic active AMR is the dominant cause of late allograft failure.¹ Patients with de novo antibody development leading to chronic active AMR have worse outcomes than patient with preformed DSA at the time of transplant.² These antibodies are frequently resistant to treatment with therapies that have been shown to be effective for the treatment of early acute AMR. It is possible, but not proven, that detection of late AMR earlier in its course, before the elevation in creatinine or the development of proteinuria (frequently a sign of
transplant glomerulopathy in these patients), would allow intervention at a time when the injury is preventable and the antibodies are at levels that are amenable to treatment. It is important to remember that activation of the T-cell compartment of the immune system is required for the generation of anti-HLA antibodies (Figure 1). Patients who have anti-HLA antibodies generally have a higher risk for cellular rejection as well as AMR. Development of de novo anti-HLA antibodies after transplantation also may be seen as a marker of relative under-immunosuppression.

The question of what to do when you have detectable antibody and a negative biopsy is the question underlying the article by Parajuli et al. From 2013 to 2016, they performed 1102 renal allograft biopsies. The study cohort was made up of 587 patients who had no acute changes, of whom 192 had detectable DSA. Although the DSA-negative cohort included only for-cause biopsies, 40% of the DSA-positive group underwent renal biopsy only for DSA monitoring (detected pretransplant, de novo during follow-up screening, or a documented 50% increase in prior antibody-strength mean fluorescence intensity). During the follow-up period of 33.1 ± 16.0 months, 34 of 192 in the DSA-positive group developed subsequent rejection versus 42 of 395 in the DSA-negative group, which was not statistically significantly different. But the strongest risk factor for the development of subsequent rejection was the presence of the de novo DSA. More importantly, there were no differences in graft failure between the groups: 12% of patients in the DSA-positive group failed compared with 15% in the DSA-negative group. It appears that despite being associated with a higher rejection rate, de novo DSA was NOT a predictor of graft failure.

Although reassuring, this needs to be interpreted cautiously. (i) The patients in the DSA-negative group were all undergoing renal biopsy for cause: there was something wrong with the organ that may not have been captured in the pathology but affected allograft outcome; and these patients started with a lower eGFR and more proteinuria, both predictors of future allograft failure regardless of DSA status. To have the same graft survival as patients starting with sicker organs may not be such a good thing. (ii) The cutoff level of mean fluorescence intensity that was considered clinically significant, a positive anti-HLA antibody level, was low when compared with other labs; only 80 of the 192 patients started with a mean fluorescence intensity >2000. However, even patients with a higher mean fluorescence intensity that most would agree were clinically significant did not correlate with future risk of graft failure, as long as the index biopsies were normal. (iii) Finally, the duration
of follow-up was relatively short, and perhaps the patients with DSA did not have enough time to develop disease progression.

How can we incorporate these results into clinical practice? It seems that patients with preformed DSA but negative biopsies have a course that is similar to those without antibodies. There are likely many different mechanisms for these antibodies that do not cause tissue injury despite being measurable, as suggested previously. Those with de novo antibodies appear to be more likely to be in response to exposure to the engrafted tissue.

The immunology of these antibodies and recent findings suggest that such patients should be followed more closely for the possibility of developing rejection. Further, additional developments in understanding the biology of DSA and the mechanism of antibody-mediated injury by the current group and other centers will likely help us further risk-stratify these patients.

**DISCLOSURE**

All the authors declared no competing interests.

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