Utility of HLA Antibody Testing in Kidney Transplantation

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ABSTRACT

HLA antigens are polymorphic proteins expressed on donor kidney allograft endothelium and are critical targets for recipient immune recognition. HLA antibodies are risk factors for acute and chronic rejection and allograft loss. Solid-phase immunoassays for HLA antibody detection represent a major advance in sensitivity and specificity over cell-based methods and are widely used in organ allocation and pretransplant risk assessment. Post-transplant, development of de novo donor-specific HLA antibodies and/or increase in donor-specific antibodies from pretransplant levels are associated with adverse outcomes. Although single antigen bead assays have allowed sensitive detection of recipient HLA antibodies and their specificities, a number of interpretive considerations must be appreciated to understand test results in clinical and research contexts. This review, which is especially relevant for clinicians caring for transplant patients, discusses the technical aspects of single antigen bead assays, emphasizes their quantitative limitations, and explores the utility of HLA antibody testing in identifying and managing important pre- and post-transplant clinical outcomes.


A major stimulant of allograft rejection is recipient T cell recognition of human leukocyte antigens (HLA) in the donor kidney. Preformed donor–specific HLA antibodies (DSA) resulting in hyperacute rejection were first detected in 19691 by the complement-dependent cytotoxicity crossmatch assay (CDC-XM); widespread application of this test detected higher titer DSA and reduced hyperacute and early accelerated rejection episodes.2 Evolution to flow cytometry cross-matching (FCXM) offers improved sensitivity for low titer but nonetheless, pathogenic antibodies.3–9 Newer immunoassays (using ELISA plates or microsphere technology), where purified HLA antigens are covalently bound to a solid-phase platform, have further improved sensitivity and specificity of HLA antibody detection.10 Despite advancements in technology, newer solid-phase assays have a number of interpretive considerations that must be appreciated by clinicians in order to more appropriately apply test results to patient care.

This review for transplant clinicians first discusses the analytic and technical parameters and quantitative considerations of contemporary HLA antibody testing methods, with an emphasis on the commonly used single antigen beads (SAB) and then explores the application and utility of SAB testing pre- and post-transplant. Non-HLA antibodies, although potentially determinants of outcomes, are outside the scope of this review and will be discussed only briefly.11–14

CELL-BASED ASSAYS: DETECTING HLA ANTIBODIES BEFORE SOLID-PHASE ASSAYS

Panel-reactive antibody (PRA) testing, in general, estimates the percentage of potential donors to whom a recipient has HLA antibodies and approximates the risk of positive crossmatch. Early PRA assays used panels of 25–60 real donor cells selected to represent the common HLA phenotype distribution of the potential deceased donor population, which were then tested for complement-dependent cytotoxicity with recipients’ sera. Results were subject to change with different donor cells in the panel, were less sensitive for rare HLA antigens, and detected only higher titer antibodies. Although still used in conjunction with other assays, cell panels are no longer used in isolation for PRA determination. The donor–specific CDC-XM assay detects high-titer DSA required to bind complement for demonstration of antibody presence, whereas lower titer DSA may be detected by FCXM, with a positive result requiring only antibody binding and not complement activation. All cell-based assays are subject to false-positive results caused by autoantibodies...
and non-HLA antibodies as well as false-negative results when the antibody is very low titer but still has clinical relevance. Cell-based assays are now routinely augmented by solid-phase assays, with significantly improved sensitivity and specificity. For a more detailed review of HLA techniques and interpretation, we refer to other publications.

**SOLID-PHASE ASSAYS**

Solid-phase assays, by comparison, are more sensitive for lower titer antibodies and permit more precise determination of the specific HLA antigens and alleles to which they bind. Furthermore, complementing traditional cell–based methods with solid-phase assays offers the potential to better discriminate immunologically relevant positive XMs from false-positive results.

To perform these assays, recipient serum is incubated with purified HLA antigens presented on a solid-phase platform (commonly microparticle beads). A fluorescent–conjugated anti-human IgG is added, which binds to and detects HLA antibody on its antigen target when the beads are analyzed on a flow cytometer or Luminex® platform. The latter platform generates a semiquantitative output for each bead of mean fluorescence intensity (MFI), which is compared with negative control MFI to determine if HLA antibody is present (Figure 1A). It has proven difficult to align MFI measurements within and between laboratories notwithstanding the increased reporting of this metric in published research. HLA antibody can also be detected in an indirect ELISA; however, this is less sensitive than bead platforms. Subsequent discussion will be restricted to bead-based assays. From a technical perspective, SAB assays allow identification of HLA antibodies for all common and many rare antigens and alleles at up to 11 HLA loci. SAB assays are rapid (3–6 hours), with up to 100 unique antigen beads able to be tested in a single reaction chamber, and high throughput, with additional multiplexing ability to test many patients simultaneously.

Solid-phase bead assays may be supplied as pooled antigen panels, single-patient phenotypes, or SAB. Pooled panel beads with many different class I or II HLA antigens on a bead yield a positive/negative result and are used for screening. Phenotype (also called ID) beads are each coated with a single HLA antigen and yield a list of discrete antibody specificities. Specificities are then compared with HLA frequencies in the donor population to determine the calculated panel-reactive antibody (cPRA), which is presently the best estimate of likelihood of a positive XM/DSA to a randomly selected donor. SAB results further enable virtual crossmatching (VXM) to identify DSA pretransplant, in turn facilitating allocation and risk assessment. VXM has also been used without cell–based XM in some transplant circumstances where legislation permits, or under study conditions, with acceptable (equivalent rejection rates and graft survival at a population level) results.

Additional enhancements of the SAB assay, such as detecting antibodies capable of binding complement component C1q, have been developed to detect potentially more injurious antibodies.

**INTERPRETIVE CONSIDERATIONS OF SOLID-PHASE ASSAYS**

**Defining a Positive Result**

The numeric output of Luminex® SAB is a trimmed MFI (or channel shift in flow cytometry SAB) (Table 1). SAB analysis considers the MFI along with other factors in determining a positive result: laboratories validate their SAB assays with known negative and positive sera, establish a working MFI threshold for antibody detection, and correlate this threshold with positive FCXM results as an important laboratory–specific standardization process within a program. MFI thresholds may be modified on the basis of patient history, control values, different HLA loci, recipient HLA typing, and consideration of epitope/antigen groups. As such, a strict MFI level above which clinically relevant antibodies are consistently identified is challenging to define.

**The Metric Is Not the (Whole) Message**

Ideally, a standard amount of antigen would be present on the bead of interest and would be bound proportionally to antibody amount, yielding a reliable and quantitative MFI result (Figure 1A). Unfortunately, several of the assay properties result in MFI being, at best, a semiquantitative output.

**Antigen Density Is Not Equivalent between Beads**

Antigen density varies between beads both within a single assay (Figures 1B and 2) and between manufacturers. MFI differences between beads in an individual assay may be caused by variable antibody amounts but also, differing amounts of target antigen present.

**Nonspecific Serum Components May Bind to the Bead in Addition to Antibodies of Interest**

Nonspecific binding of non-HLA antibodies to beads (for example, in the presence of drugs, such as intravenous Ig [IVIG], inflammation, or infection) may increase background MFI of both control beads and beads of interest (Figure 1C); isolated MFI of a target bead may be misleadingly high. Control values may provide clues to this issue but are rarely routinely provided in clinical laboratory reports or research studies.

**Interference May Prevent Binding of the Antibody of Interest**

High-titer antibody leading to complement activation and deposition of C1 complex on the bead, IgM antibody, or other serum factors may interfere with binding of the secondary detection reagent, giving false-negative results, and is known as the prozone effect (Figure 1D). Serum dilution or treatment with EDTA/dithiothreitol, osmosis, or heat has been shown to reduce this effect but is often variably.
applied (Figure 3). Interference may also be caused by drugs (e.g., IVIG)50,57,58 or nonspecific serum proteins.59 We further note that dilution does not result in a predictable decline in bead MFI (Figure 4); therefore, the MFI of a single bead in an undiluted serum cannot be assumed to quantitatively or reliably represent antibody amount.

Target Epitopes May Be Shared between Beads
Groups of antigens (and their corresponding SAB) may share common or public epitopes targeted by antibodies (Figure 1E, Table 2). Antibody to a shared epitope may be diluted across these multiple beads, reducing MFI on any single bead of interest26 (Figure 5).

Conformational Changes of Antigens on Beads
Artificial attachment of an antigen to a bead may result in conformational changes of the protein,60,61 potentially leading to exposure of epitopes not normally found in vivo (neoepitopes), yielding false-positive results,62–65 or concealment of immunologically relevant epitopes (cryptic epitopes), giving false-negative results.

Laboratory Assay Modifications
Many laboratories modify the manufacturer methods to enhance assay sensitivity, detect different antibody isotypes, reduce assay time, or reduce reagent costs. These modifications may also affect comparability of MFI between laboratories.

Locus Considerations
It is our laboratory experience that antibodies to Cw and DP antigens must be detected at much higher MFI than DSA at other loci before a positive XM occurs. Other laboratories may use different MFI thresholds for identifying antibodies at different HLA loci on the basis of their own validation studies.

Other Reagent and/or Lot-Specific Variables
Antigens may be represented in the SAB assay by differing numbers of unique beads; there are, for example, in a current reagent lot, three A2 beads and five DQ2 beads but only one C7 bead. Subsaturating antibody may be diluted across multiple beads in a multiplexed assay more so than if only a single bead was present, thereby lowering MFI on any single bead of interest. For higher antibody levels, maximum total bead MFI may be higher for those antigens with multiple beads. We note that there is no validated method of combining MFI across multiple beads, however summing is frequently reported in the literature.

Lot-to-lot variability in bead reactivity, bead specificities, and reagents has additionally been recognized as a confounder to inter- and intralaboratory reproducibility.55,66,67 MFI differences may also originate from differences in instrument type, maintenance, and operators, reagent differences between vendors, and variability in secondary reagent properties.

These considerations do not negate the major role that SAB assays have played in the last decade, permitting significantly more reliable identification of HLA antibody specificities and strengths. Rather we encourage caution when extrapolating research reporting MFI data to other centers, because these data are rarely characterized with sufficient detail of the above factors to appropriately generalize them. Even more importantly, an MFI result as a single metric cannot be analyzed in isolation. The patient’s history of sensitizing events, their clinical status and pathology, their own and their donor’s HLA typing, and other related test results (e.g., FCXM) in the user’s own laboratory must also be considered for comprehensive

Figure 1. MFI of single antigen bead assays has analytic limitations and cannot be used as a quantitative metric of antibody amount. (A) An ideal test should always be able to distinguish antibody binding (green fluorescent signal) from negative control (white) with a clear threshold and no overlap between the MFI distributions. (B) Decreased density of antigen (Ag) on the surface of the bead will result in MFI measurement that underestimates the amount of the antibody present. (C) In contrast, nonspecific binding to the bead can result in artificially high background and signal MFI, with overestimation of antibody. (D) Interfering substances may prevent the detection of the antibody of interest with lower MFI. (E) Epitopes shared between different beads can dilute the amount of antibody bound to any single bead, with an erroneously low MFI on the given bead of interest.
understanding. Additionally, program clinical thresholds for crossing antibodies, regional standards, and regulations for listing of unacceptable antigens as well as differences between organs may affect final recommendations.

**UTILITY OF HLA ANTIBODY TESTING PRETRANSPLANT**

**Waitlist Testing for HLA Antibodies**
Testing for HLA antibodies while waitlisted or during transplant workup serves to identify transplant candidates with potentially reduced access to acceptable donors by virtue of preexisting HLA antibodies acquired through pregnancy, transfusion, or prior transplant. The cPRA serves as an estimate of the percentage of potential

### Table 1. Factors affecting MFI values in SAB HLA antibody assays

<table>
<thead>
<tr>
<th>Factor</th>
<th>Analytic Considerations</th>
<th>MFI Effect and Reporting Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen density can vary up to 3X between beads</td>
<td>Manufacturer information if provided Laboratory validation data</td>
<td>Maximum MFI on a bead of interest may vary on the basis of density of target antigen rather than relative antibody amount MFI may be erroneously elevated</td>
</tr>
<tr>
<td>Other non-HLA antibodies may nonspecifically bind to beads</td>
<td>Negative control beads may have high MFI Beads with patient’s own antigens may have high MFI Positive bead reactions vary from expected epitope patterns</td>
<td>Control values and self-antigen bead values are needed to interpret correctly A validated method to adjust for this increase in background is not known MFI in untreated serum may be erroneously low Serum treatment is neither standardized nor ubiquitously effective Own HLA laboratory serum treatment practices must be known MFI of a single bead of interest may be lower if the epitope targeted by an antibody is present on multiple beads Requires understanding of how laboratory modifies thresholds when epitope patterns are present</td>
</tr>
<tr>
<td>Interference preventing binding of antibody of interest</td>
<td>May have low positive control MFI A positive XM may occur, even if beads with donor antigens are negative Bead MFI may be very different before and after serum treatment</td>
<td>A positive XM may occur, even if beads with donor antigens are negative Bead MFI may be very different before and after serum treatment</td>
</tr>
<tr>
<td>Target epitopes may be shared between beads</td>
<td>Positive or weak positive beads may be present in patterns suggestive of cross-reactive epitope groups</td>
<td>MFI may be erroneously elevated</td>
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<tr>
<td>Differing numbers of beads with the same antigen (different alleles)</td>
<td>Awareness of multiplicity of beads for some antigens but not others</td>
<td>Individual bead MFI may be lower for subsaturating antibody, where more beads are present within a reaction Where multiple target beads are present for some antigens but not others in a single reaction with saturating antibody, there is no validated method for determining the total MFI (summing and other mathematic combinations that are frequently reported in the published literature may erroneously increase total MFI where multiple beads are present)</td>
</tr>
<tr>
<td>Lot-to-lot variability in bead reactions</td>
<td>Lot changes may show changes in background reactivity of some antigens Beads with certain alleles may change between different lots of reagents Different alleles may be present on different vendor products</td>
<td>Laboratories must communicate where MFI affects beads of interest significantly over time A specificity no longer being detected may represent a change in antibody level or simply a change in lot or vendor, such that certain specificities are no longer tested</td>
</tr>
<tr>
<td>Lot-to-lot variability in alleles</td>
<td></td>
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<tr>
<td>Conformational changes of antigens on beads</td>
<td>Antigens are bound to beads potentially in a different conformation in vitro than in vivo Consider if reactivity does not follow known epitope patterns, or strongly positive beads are present with no prior sensitizing events and negative FCXM</td>
<td>Clinically relevant epitopes may be hidden (MFI may be erroneously low) Non-clinically relevant epitopes may become exposed and bind nonspecifically to non-HLA antibodies (MFI may be erroneously high)</td>
</tr>
<tr>
<td>Laboratory-specific modifications</td>
<td>Laboratory method varies from reported literature or manufacturer recommended method</td>
<td>Effect on MFI is variable Communication is essential to understand own laboratory methods</td>
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</table>
donors to whom a recipient will have DSA (with corresponding risk of a positive XM). Beyond application of cPRA in quantifying transplant access for patients and care providers, those patients with the highest cPRA (and lowest access to acceptably mismatched donors) may be prioritized in allocation schema to improve equity of transplant access. Because antibody levels and specificities wax and wane over time, repeated testing (usually 3–12 times per year depending on regional regulations) is performed to obtain the most comprehensive immunologic profile. Even if transient, detected antibodies may represent potential for future immunologic memory responses if targeted to donor antigens and affect subsequent risk assessment.

Testing at the Time of Transplant

Absence of DSA, especially in high titer, has generally been considered a prerequisite for successful transplantation, and at a minimum, a negative pretransplant XM remains desirable. However, below these higher antibody levels1,68 exists a spectrum of antibody specificities and strengths with variable clinical effects, potentially affecting a substantial proportion of waitlisted individuals with broad antibody specificities.50

DSA with a Positive XM: Is Forewarning Always Enough?

A positive XM with solid-phase evidence of HLA DSA is associated with worse short and long-term allograft outcomes8,33,45; conversely, in the absence of HLA DSA, a positive XM may not correlate as strongly with outcomes.69 Identification of pretransplant DSA in living70–77 and deceased donation78 offers an opportunity to lower antibody levels through desensitization and permit transplant; however, long-term outcomes are variable: acute AMR rates of 37%–45% are reported in desensitization series compared with <5% of controls, with transplant glomerulopathy (a form of chronic AMR) occurring in up to one half of patients compared with <10% of controls.79–81

MFI of pretransplant DSA are not, however, consistently predictive of outcomes. Gloor et al.80 showed increased AMR and TG in patients with positive XM at all levels of DSA MFI, and although a clear increase in AMR was seen with total MFI >10,000, the other patients with DSA also had an increase in AMR that was not influenced by MFI. Other studies report graft survival effect at MFI.1500,82 and still others find it at 3000 MFI,83 emphasizing threshold variability. Extreme levels of MFI (>10,000) may correlate with complement-fixing C1q–positive DSA,48,84–86 but these data do not show any increase in adverse outcomes with pretransplant C1q(+)-DSA over standard DSA detection methods.47,86,87

DSA with a Negative XM: Defining Risk Remains Challenging

The increased sensitivity of SAB allows DSA to be detected even with a negative XM.80,88,89 AMR rates of 20%–55% are reported under these circumstances,22,23,83,88,90,91 with AMR negatively affecting the impact of DSA on graft survival in some studies90 but not all.83 In series where DSA are associated with worse allograft survival,82,83,88,92–95 DSA MFI is an imperfect discriminator of outcomes. In contrast, other groups identified no change in rejection or graft survival in patients who were DSA positive but XM negative.96–101 Studies are generally small and retrospective, with difficulty comparing laboratory methods between centers. No DSA features (class, number, and/or MFI) beyond detection alone consistently predict outcomes,83,90 and importantly, many patients with DSA do not have adverse outcome; strategies to better risk stratify pretransplant DSA are needed.

Figure 2. Individual single antigen beads within a single-assay reaction can have significant differences in the density of target antigen affecting maximum MFI. A saturating amount of W6/32 antibody that binds ubiquitously to class I HLA was mixed with single antigen beads. The unadjusted results are shown, with the fluorescence representative of the density of the target antigen on each bead. Wide variation in density is seen.
Despite historical associations of high PRA with immunologically poor outcomes, studies with these improved technologies have shown that patients with high PRA but no DSA have graft- and rejection-free survival comparable with unsensitized recipients. VXM compares recipient antibody specificities with donor antigens, permitting rapid and earlier identification of DSA-negative donors in deceased donor acceptable mismatch strategies and living donor paired exchange programs worldwide.

UTILITY OF HLA ANTIBODY TESTING POST-TRANSPLANT

It has been a decade since the multicenter studies by Terasaki and Ozawa, in which patients with HLA antibodies detected on solid-phase platforms reported higher rates of allograft failure at 1 (6% versus 3%) and 2 (15.1% versus 6.8%) years than those without HLA antibodies. Despite widespread ensuing interest in understanding the role of HLA antibodies and DSA in diagnosing and treating antibody-mediated complications, to date no study has clearly defined optimal timing for antibody testing, characteristics of antibodies likely to be associated with adverse outcomes, or definitive treatment strategies. Studies have been retrospective and nonrandomized, with noncomparable patient phenotypes, differing thresholds for DSA detection, variable testing time points post-transplant, and nonstandard treatment strategies. Despite this, when collectively examined, available studies do provide insights into the relationship of DSA with histopathology and impaired function, the prognostic utility of testing, and potential strategies for treatment and prevention.

Incidence and Prevalence of De Novo DSA and Timing of Outcomes

Examining numerous studies utilizing solid-phase platforms, the median onset to de novo donor-specific HLA antibodies (dnDSAs) varies from 3.8 to 68 months. Cumulative prevalence at 3 years post-transplant is similarly varied from 6% to 38%, with lifetime cumulative prevalence even more disparate. Class II antibodies predominate in most but not all reports. With differences in antibody MFI cutoffs, baseline immunosuppression, and frequency of testing, definitive conclusions remain elusive.

The timing of dnDSA occurrence to onset of graft dysfunction ranges from months to years, suggesting multiple pathways of injury and potentially diverse modifying factors. A comprehensive natural history study coupled to protocol and for-cause biopsies showed the mean time to dnDSA as 4.6 years, with the most important independent predictor being immunosuppressive nonadherence (60% cumulative prevalence versus 20% in adherent patients at 10 years; odds ratio, 8.75). Subclinical antibody-mediated injury (peritubular capillaritis and C4d positivity) occurred in more than one half of patients with stable function, suggesting a period of latency between onset of inflammation and dysfunction that was shortened by nonadherence. dnDSA was associated with significantly reduced graft survival of 50% at 11 years post-transplantation (versus >90% without dnDSA), with similar findings in other series.

**Figure 3.** Removal of interfering factors with serum treatment can significantly increase MFI on beads of interest. (A) Neat serum (no treatment) SAB results of a sensitized renal transplant recipient. MFI appears on the y axis. Each bar represents a single bead. The bar graph illustrates MFI measurement for each HLA allele, with self-antigens (expected to be negative) identified with purple arrows and donor antigens indicated with red arrows. The A2 beads representing donor antigens are clearly negative. An XM would be predicted to be negative, because no antibodies to donor antigens are identified; however, the FCXM was strongly positive. (B) After treatment of the serum with dithiothreitol (DTT), antibody to donor HLA-A2 is detected at MFI > 20,000.
all studies, the relationship between dnDSA and adverse outcome is imperfect; better understanding of which DSAs are more deleterious is urgently needed.

dnDSA and Features of AMR

Late AMR is associated with chronic pathology and worse dysfunction at the time of diagnosis, mixed cellular and humoral features, and subsequently, lower treatment response rates, but it is notable that DSA MFI does not reliably further stratify those individuals who will respond to treatment from those who will do not. Indeed, in one study, those who responded to treatment had higher antibody level as estimated by MFI than nonresponders. Additionally, DSA MFI metrics do not correlate with the chronicity and severity of pathology of AMR. However, both late rejections and concomitant resistance to treatment are strongly associated with class II DSA, especially HLA-DQ, antibody specificities.

C1q Testing

Recent studies have indicated that post-transplant complement-fixing DSA (detected by C1q binding in an SAB assay) may confer greater risk to allografts than noncomplement-binding DSA. In a study of 1016 patients, post-transplant C1q+DSA were associated with worse pathology and 5-year graft survival (54%) than C1q−DSA. Also, those who developed C1q+DSA after transplant but had C1q−DSA before transplant had the worst outcomes. However, another study found C1q+DSA more likely to be of DQ specificity and also, associated with a 30% decrement in 5-year survival. To bind complement, antibodies must be IgG1/3 isotope and be of sufficiently high titer; closer examination of these studies and others indicates that antibody amount (estimated albeit imperfectly by MFI) is the major predictor of C1q positivity and outcomes, and the role of this new assay in addition routine SAB testing remains to be fully determined.

DSA without Dysfunction

With latency between detection of DSA and dysfunction/injury, it is not clearly known how to treat patients with dnDSA in whom no dysfunction or histologic antibody-mediated injury has occurred. One study reports progression of inflammatory pathology, despite optimization of immunosuppression and IVIG. Clinical trials are needed to determine the optimal expectant approaches in these patients and better identify which DSA are likely to have clinical effect.

Non-HLA Antibodies and AMR

Studies continue to explore the role of non-HLA antibodies in AMR outcomes,
although purely non-HLA antibody–mediated rejection remains rare.\textsuperscript{138} Despite early enthusiasm for the role of MICA antibody testing to predict AMR,\textsuperscript{11,139} more recent studies question this association and the benefit of routine MICA testing in addition to routine HLA antibody detection.\textsuperscript{138,140} In recent studies of angiotensin II type 1 receptor antibodies,\textsuperscript{12,141,142} a majority of recipients with an adverse outcome also had HLA antibodies, and again, the incremental value of the test must be weighed against the cost of widespread screening, with many centers using this testing on a case-by-case basis instead.

**Treatment Options: Is There a Role for Monitoring Antibodies?**

Several studies reported on the change in MFI levels of DSA in response to various treatments of AMR.\textsuperscript{134,135} In one, patients who received treatment with IVIG, anti-CD20, and plasmapheresis had greater reductions in MFI compared with patients receiving only IVIG; however, histologic and temporal features suggested that the latter group may have had initially worse rejection features, confounding this association. Reductions in DSA MFI correlated with favorable functional outcome at 3 months post-treatment, a finding replicated in other studies\textsuperscript{133}; however, despite improved creatinine, considerable subclinical inflammation persisted, and longer-term outcomes are not known.

Walsh et al.\textsuperscript{134} studied a combination of rituximab, bortezomib, and plasmapheresis in patients with early and late AMR; treatment was more successful in those with early AMR who concomitantly had

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**Table 2. Examples of common Class I cross–reactive (public) epitope groups**

<table>
<thead>
<tr>
<th>CREG</th>
<th>Antigens Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>A1, 3, 11, 29, 30, 31, 36, 8 0</td>
</tr>
<tr>
<td>A2</td>
<td>A2, A9(23,24), A28(68,69), B17(57,58)</td>
</tr>
<tr>
<td>A10,19</td>
<td>A10(25,26,34,66) A19 (29,30,31,32,33,42,74)</td>
</tr>
<tr>
<td>B12</td>
<td>B12(44,45), B13, B21(49,50), B40(60,61), 41, 84</td>
</tr>
<tr>
<td>B5,18</td>
<td>B5(B51, 52), 18, 35, 53, 78</td>
</tr>
<tr>
<td>B8</td>
<td>B8, B16(38,39), B14(64, 65)</td>
</tr>
<tr>
<td>B15</td>
<td>B62, 63, 71, 72, 75, 76, 77</td>
</tr>
<tr>
<td>B7</td>
<td>B7, 13, B22(54,55,56), 27, 42, 47, 67, 73, 81</td>
</tr>
<tr>
<td>BW6</td>
<td>B7,* 8,* 14, 15,* 16, 18, 22, 35, 39, 40,* 41, 42, 45, 46, 48, 50, 54, 55, 56,* 60, 61, 62, 64, 65, 67, 70, 71, 72, 75, 76, 78, 79, 81, 82</td>
</tr>
<tr>
<td>BW4</td>
<td>A9, 23, 24, 25, 32, B13, B7,* 27,* 27, 37, 38, 44, 47,* 49, 51, 52, 53, 57, 58, 59, 63</td>
</tr>
</tbody>
</table>

Common cross-reactive epitope groups (CREGs) are listed with HLA antigens that belong to each group.\textsuperscript{149,150} Recognition of these patterns of antibodies is important in antibody analysis. Antibodies to epitopes shared among CREG members may be diluted across multiple beads, leading to lower individual bead MFI.

*Exceptions: B*07:27, B*08:02, B*08:03, B*40:13, B*40:19, B*56:07, and select B*15 alleles are in the Bw4 group.

*Exceptions: B*13:09, B27:08, B*27:12, B*27:18, B*44:09, and B*47:02 are in the Bw6 group.

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**Figure 5.** Epitopes targeted by HLA antibodies may be shared across multiple beads in a single assay, lowering the MFI detected on any individual bead. Multiple beads share the Bw6 epitope. In this case, the mismatched donor antigen of interest is B35, with a normalized bead MFI of 1216 (weak). Many centers would predict a negative FCXM with this donor; however, it was strongly positive. Closer examination reveals that the majority of antigens sharing the Bw6 epitope are clustered together in this MFI range. Neither a B7 nor a B5 cross–reactive epitope group explains this reactivity pattern entirely; rather, it is likely that an antibody to Bw6 is diluted across multiple beads sharing this epitope. During in vitro XM, where only the B35 target was present, the antibody can bind without epitope dilution, yielding a positive result. CREG, cross reactive group.
less dysfunction, fewer chronic findings on pathology, less nonadherence, and lower antibody levels with fewer DQ specificities. Everly et al.\textsuperscript{135} report on four different combinations of treatments in AMR, ACR, and mixed rejection with a reduction in DSA MFI associated with improved graft survival at 5 years; however, no clinical feature other than DQ antibody specificity predicted this response, and no treatment regimen was superior. However, other studies confirm that early timing of AMR is the most important factor in predicting response to treatment.\textsuperscript{143} With a multiplicity of confounders, conclusions of treatment efficacy on the basis of antibody characteristics cannot presently be drawn.

A more crucial observation is that antibodies are rarely eradicated (and in many cases, not even significantly reduced), even in patients with clinical improvement\textsuperscript{134,135}; treating AMR to a likely unattainable goal of antibody elimination bears a substantial risk for immunotoxicity.

Equally important is recognizing that the clinical improvement that accompanies reduction in DSA metrics is more easily demonstrable with routine biochemistry testing than an isolated antibody metric, with the limitations outlined earlier in this paper. The role of serial HLA antibody testing to guide treatment after diagnosis remains to be determined.

Finally, the most effective therapeutic strategy for AMR and post-transplant DSA remains unknown, particularly in the absence of dysfunction or inflammation; prudent consideration of the risks and the benefits in individual patients is recommended.

Can DSA Be Prevented?

Given generally poor responses to therapy, strategies to prevent dnDSA development must be considered. Beyond addressing nonadherence\textsuperscript{144–146} or less potent immunosuppression,\textsuperscript{112,118,120,124,125} increasing evidence that class II mismatching, especially at DQ, is a major determinant of DSA development\textsuperscript{114,124,137,145} must prompt considerations of optimizing class II matching. Early cell–mediated inflammatory pathways and infections are also associated with new HLA antibody development\textsuperscript{147,148}; control of these pathways represents additional strategies to be explored (Table 3).

**CONCLUSION**

HLA antibody testing is critical to pre-transplantation and post-transplantation risk assessment. SAB technology has evolved the precision of DSA identification and is the most sensitive method available. It has facilitated greater understanding of humoral mechanisms and refined diagnostics for antibody-mediated injury. Notwithstanding, SAB assays and their MFI metrics must be interpreted with an awareness of their technical intricacies and predictive limitations. In the absence of a perfect SAB test, we must use multiple tests (repeated testing on current and historic sera and complementary tests, including XM, alternative solid-phase platforms, and/or different vendors) in seeking plausible results and to estimate risk in conjunction with and not instead of clinical judgment. Furthermore, in some patients, DSA are not associated with adverse outcomes; caution must be taken before treating an antibody result in isolation. Treatment success cannot be defined by resolution of DSA, which rarely occurs. Most importantly, urgent studies are needed to determine the best screening protocols and treatment strategies for groups of patients defined by the range of DSA clinical phenotypes.

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**Table 3. Clinical considerations in antibody testing and interpretation**

<table>
<thead>
<tr>
<th>Consideration</th>
<th>Effect and Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody testing is a snapshot in time</td>
<td>A single-antibody result either before or after transplant cannot perfectly predict all future events</td>
</tr>
<tr>
<td>Antibody tests are strongly correlated with but do not perfectly predict outcomes</td>
<td>Any antibody test can change within 72 h because of memory B cell responses</td>
</tr>
<tr>
<td>Changes in antibody levels over time may be more important than a single-antibody result</td>
<td>If clinical circumstance changes, new testing may be needed</td>
</tr>
<tr>
<td>The optimal monitoring frequency for post-transplant DSA testing has not been established</td>
<td>Not all patients with a DSA pre- or post-transplant will have an adverse outcome</td>
</tr>
<tr>
<td>DSA and MFI do not reliably predict severity of pathology</td>
<td>Serial testing before and after transplant may be more informative</td>
</tr>
<tr>
<td>DSA and MFI at rejection diagnosis do not reliably predict responsiveness to treatment</td>
<td>The timing of routine testing in patients who are asymptomatic and stable is determined at the program level</td>
</tr>
<tr>
<td>Class II DSAs (especially DQ) correlate with late rejections, worse function at diagnosis, and less responsiveness to treatment</td>
<td>Latency period is modified by many variables, particularly adherence/immunosuppression</td>
</tr>
<tr>
<td>Dynamic changes in MFI with treatment of AMR often reflect change in clinical status</td>
<td>DSA and MFI must be interpreted in the context of clinical function and pathology features</td>
</tr>
<tr>
<td>DSA MFI may decrease with treatment of rejection but rarely disappear altogether</td>
<td>DSA and MFI cannot be used in isolation to diagnose or determine treatment plans</td>
</tr>
</tbody>
</table>

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Utility of HLA Antibody Testing
DISCLOSURES
None.

REFERENCES


BRIEF REVIEW


Utility of HLA Antibody Testing

1501


