Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation

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Background. The introduction of solid-phase immunoassay (SPI) technology for the detection and characterization of human leukocyte antigen (HLA) antibodies in transplantation while providing greater sensitivity than was obtainable by complement-dependent lymphocytotoxicity (CDC) assays has resulted in a new paradigm with respect to the interpretation of donor-specific antibodies (DSA). Although the SPI assay performed on the Luminex instrument (hereafter referred to as the Luminex assay), in particular, has permitted the detection of antibodies not detectable by CDC, the clinical significance of these antibodies is incompletely understood. Nevertheless, the detection of these antibodies has led to changes in the clinical management of sensitized patients. In addition, SPI testing raises technical issues that require resolution and careful consideration when interpreting antibody results.

Methods. With this background, The Transplantation Society convened a group of laboratory and clinical experts in the field of transplantation to prepare a consensus report and make recommendations on the use of this new technology based on both published evidence and expert opinion. Three working groups were formed to address (a) the technical issues with respect to the use of this technology, (b) the interpretation of pretransplantation antibody testing in the context of various clinical settings and organ transplant types (kidney, heart, lung, liver, pancreas, intestinal, and islet cells), and (c) the application of antibody testing in the posttransplantation setting. The three groups were established in November 2011 and convened for a "Consensus Conference on Antibodies in Transplantation" in Rome, Italy, in May 2012. The deliberations of the three groups meeting independently and then together are the bases for this report.

Results. A comprehensive list of recommendations was prepared by each group. A summary of the key recommendations follows. Technical Group: (a) SPI must be used for the detection of pretransplantation HLA antibodies in solid organ transplant recipients and, in particular, the use of the single-antigen bead assay to detect antibodies to HLA loci, such as Cw, DQA, DPA, and DPB, which are not readily detected by other methods. (b) The use of SPI for antibody detection should be supplemented with cell-based assays to examine the correlations between the two types of assays and to establish the likelihood of a positive crossmatch (XM). (c) There must be an awareness of the technical factors that can influence the results and their clinical interpretation when using the Luminex bead technology, such as variation in antigen density and the presence of denatured antigen on the beads. Pretransplantation Group: (a) Risk categories should be established based on the antibody and the XM results obtained. (b) DSA detected by CDC and a positive XM should be avoided due to their strong association with antibody-mediated rejection and graft loss. (c) A renal transplantation can be performed in the absence of a prospective XM if single-antigen bead screening for antibodies to all class I and II HLA loci is negative. This decision, however, needs to be taken in agreement with local clinical programs and the relevant regulatory bodies. (d) The presence of DSA HLA antibodies should be avoided in heart and lung transplantation and considered a risk factor for liver, intestinal, and islet cell transplantation. Posttransplantation Group: (a) High-risk patients (i.e., desensitized or DSA positive/XM negative) should be monitored by measurement of DSA and protocol biopsies in the first 3 months after transplantation. (b) Intermediate-risk patients (history of DSA but currently negative) should be monitored for DSA within the first month. If DSA is present, a biopsy should be performed. (c) Low-risk patients (nonsensitized first transplantation) should be screened for DSA at least once 3 to 12 months after transplantation. If DSA is detected, a biopsy should be performed. In all three categories, the recommendations for subsequent treatment are based on the biopsy results.

Conclusions. A comprehensive list of recommendations is provided covering the technical and pretransplantation and posttransplantation monitoring of HLA antibodies in solid organ transplantation. The recommendations are intended to provide state-of-the-art guidance in the use and clinical application of recently developed methods for HLA antibody detection when used in conjunction with traditional methods.

Keywords: Transplantation, HLA, Antibodies.

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Successful transplantation in the mid-1960s was dependent on developing an understanding of humoral rejection that caused immediate loss of the kidney at the time of transplantation—hyperacute rejection (HAR). The identification of antibodies to human leukocyte antigen (HLA) antigens present on the graft and the subsequent development of a simple and practical test for donor-specific antibodies (DSA)—the complement-dependent lymphocytotoxicity (CDC) cross-matching (XM) test—provided the surgeon and the patient with a reasonable basis for undertaking a transplantation procedure (1).

Developments in technologies and our understanding of antibody reactions since the 1970s have allowed refinement of the methods laboratories can use to aid the prediction of graft rejection. Recognition of autologous and non-HLA antibodies, CDC XM techniques with increased sensitivity, use of flow cytometry, and identification of IgM antibodies with the aid of dithiothreitol (DTT) all provided a sophistication of assessment and development of more accurate prediction of which transplantations might and which might not proceed safely.

Developments of immunosuppression medications in the 1980s and 1990s centered on the control of T-cell alloimmunity, and with increasingly successful and effective protocols, the incidence of acute rejection fell considerably, as did the rate of graft loss. This success has exposed our relative lack of control over antibody-mediated or humoral rejection processes and raised the relative importance of both acute and chronic antibody-mediated rejection (AMR) in graft loss.

Technical developments over the past 10 years have also contributed to an increased understanding of alloimmune biology. The first of these is the relatively reliable identification of complement activation on the graft endothelial cell surfaces using histologic localization of the complement component C4d on transplant biopsies (2, 3). The second has been the use of solid-phase immunoassays (SPI) to identify antibody specificity with precision and sensitivity (4, 5).

These new technologies challenge our established norms but provide opportunities for further improvements through application to clinical practice. The problem we now face is that these technologies are highly sensitive, and we do not fully understand the clinical impact of antibodies detected by these new methods. Current approaches aim at risk stratification based on antibody identification and XM results, taking into account the organ type and clinical considerations, such as urgency, available immunosuppression strategies, and donor factors.

This consensus report is the product of the deliberations of three working groups addressing (a) the technical

issues surrounding the use of SPI for antibody detection and characterization, (b) the application of this as well as conventional technology in the pretransplantation setting, and (c) the role of posttransplantation antibody monitoring. The individuals who comprised each working group are shown in the Appendix. Recommendations are made for the application of current antibody technology in various clinical settings, and suggested future directions in research are outlined. The recommendations are graded according to three levels as follows: Level 1 indicates a procedure that "must" or "should" be performed based on published data and currently proven practice; Level 2 suggests that a certain procedure is of benefit, but when all the evidence is considered, the recommendation is not sufficient to assign Level 1; and Level 3 is a consensus recommendation for which there may not be published data but which the panel of experts deem to be potentially of benefit.

TECHNICAL ASPECTS

The various assays for HLA antibody identification differ greatly in the type of target, format, sensitivity, and specificity. Accurate analysis and clinical interpretation of the results requires both a high degree of expertise and experience and a knowledge of the patient's immunologic history, including sensitizing events and previous transplantation history. Assay targets may be either cells tested in a cytotoxicity or flow cytometry assay or soluble antigens tested in SPI. Because the details of these various assays are widely available, this article will focus mainly on technical highlights of the tests and factors that impact the results.

Comparison of Techniques

Cell-Based Assays

CDC and flow cytometry used for HLA-specific antibody screening and donor XM testing (6, 7) use cellular targets. The CDC assay has lower sensitivity but identifies antibodies that can mediate HAR (1). Technique modifications to increase sensitivity and specificity have been published but are not routinely used in all laboratories (8–10).

The flow cytometry assay detects antibody binding to target lymphocytes through a more sensitive method involving a fluorescent secondary antibody and quantification via a flow cytometer. Flow cytometry XM (FCXM) represents a risk but not necessarily a contraindication to transplantation. Modifications of the flow cytometry assay include the detection of different immunoglobulin classes and subclasses, differentiation of target cells, and Pronase treatment of B-lymphocytes to reduce background nonspecific reactivity.

Solid-Phase Immunoassays

SPI obtained as commercially manufactured kits use solubilized HLA molecules bound to a solid matrix that is either a microtiter plate (enzyme-linked immunosorbent assay [ELISA]) or polystyrene beads (multiplexed multianalyte bead arrays) performed on a conventional flow cytometer or a small footprint fluoroanalyzer (Luminex) (11–13). ELISA results are expressed as optical density ratios compared with a negative control, giving a semiquantitative assessment of antibody binding.

Bead-based array assays use polystyrene beads impregnated with different ratios of two fluorescent dyes

TABLE 1. Methods for antibody screening and cross-matching in solid organ transplantation

Method	Pretransplantation screening	Pretransplantation XM	Comment	Basic information pretransplantation	Posttransplantation	Comment
CDC/CDC modified	+++	+++	Prevention of HAR or early AMR	+	−/+	Donor cells required
FC/FC modified	+++	+++	Prevention of HAR or early AMR	+	+	Donor cells required
ELISA generic	+++	−	Detection of HLA antibodies	+	−/+	Useful only if patient nonsensitized
ELISA specific	+++	−	Specification of HLA antibodies	(Only if patient sensitized)	++	Low level of sensitivity
LUM generic	+++	−	Detection of HLA antibodies	+	−/+	Detection of antibody breadth and level
LUM phenotype	+++	−	Specification of HLA antibodies	+	++	Low level of sensitivity
LUM SAB	+++	−	Comprehensive specification HLA antibodies	+	+++	Comprehensive locus/allele specification

The assays are scored from not valuable (−) to very valuable (+++). The comparative sensitivities are LUM>ELISA/FC>CDC. Note there are no convincing data that demonstrate ELISA is more sensitive than FC.
AMR, antibody-mediated rejection; CDC, complement-dependent lymphocytotoxicity; ELISA, enzyme-linked immunosorbent assay; FC, flow cytometry; HAR, hyperacute rejection; HLA, human leukocyte antigen; LUM, Luminex-based immunoassays (generic, phenotype, and single-antigen beads [SAB]); XM, crossmatch.

(classifier signals) to yield, theoretically, up to a 100 distinguishable bead populations. The antiglobulin reagent in the bead assays is labeled with a third fluorescent dye (the reporter signal) so that, using a dual-laser instrument, the fluorescence signature of each bead can be interrogated to identify the bead population by one laser, whereas the reporter fluorescence simultaneously assesses HLA-specific antibody binding. The bead-based array assay is analyzed on the Luminex platform and is semiquantitative. The level of HLA-specific antibody binding is expressed as the mean fluorescence intensity (MFI) of the reporter signal.

Three types of panels vary in the composition of their target antigens: (a) pooled antigen panels have two or more different bead populations coated with either affinity-purified HLA class I (HLA-A, HLA-B, and HLA-C) or HLA class II (HLA-DR, HLA-DQ, and HLA-DP) protein molecules obtained from multiple individual cell lines and are used as a screening test for the detection of HLA antibody; (b) phenotype panels in which each bead population bears either HLA class I or HLA class II proteins of a cell line derived from a single individual; and (c) single-antigen beads (SAB) in which each bead population is coated with a molecule representing a single cloned allelic HLA class I or II antigen that enables precise antibody specificity analysis. Pooled antigen panels are relatively inexpensive and indicate the presence or absence of antibody to a particular HLA class, but they do not provide specificity nor do they represent all possible antigens. However, these panels may be useful for tracking changes in the level of HLA-specific antibody binding. Phenotype panels are similar to cell-based assays in that more than one HLA specificity is present on each bead population, which requires greater expertise in the interpretation of results than pooled or single-antigen panels. SAB arrays are the most sensitive and specific, providing the highest degree of HLA antibody resolution, and are particularly useful in the accurate identification of antibodies in highly sensitized patients.

Numerous reports show varying degrees of correlation between MFI, antibody level, XM results, and clinical outcomes (14–16) but standardized cutoff values for positivity have not been established. A comparison of the use of cell-based immunoassay versus SPI and their application in different types of organ transplants is found in Tables 1 and 2.

Advantages and Disadvantages of the Techniques

Complement-Dependent Lymphocytotoxicity

The indisputable advantage of the CDC assay for lymphocytotoxic panel reactive antibody (PRA) determination and donor XM testing is the ability to predict (and therefore provide an opportunity to avert) HAR due to HLA DSA (1, 17). Drawbacks are that the assay is not very sensitive, requires a relatively large number of viable lymphocytes, and can yield a positive result due to non-HLA antibodies. The CDC method is difficult to standardize and assessment of antibody breadth in waiting list patients may be confounded by panel composition. Detailed specificity analysis using CDC screening requires access to either large panels of local HLA-typed donors, as practiced in some laboratories (18–20), or the use of frozen commercial cell trays. Importantly, CDC screening cannot distinguish all antibody specificities in highly sensitized patients with complex antibody profiles.

Because the percent PRA is based on how many cells give positive reactions (independent of specificity), the term %PRA used as an indication of the level of a patient's allosensitization can be misleading because centers with different cell panels are likely to achieve different PRA values with the same serum (21). For these reasons and to more accurately assess the probability of a positive XM due to antibodies to either HLA class I or II, %PRA has been replaced by "calculated reaction frequency", calculated PRA (cPRA), or virtual PRA (22, 23).

Flow Cytometry

Although flow cytometry is also subject to reactions caused by non-HLA antibodies, it is appreciably more sensitive than CDC and has been proven useful in identifying patients with weak DSA who are at increased risk of AMR and graft rejection (24). Flow cytometry assays are difficult to standardize due to variability among cytometers, fluorochromes, antiglobulin reagents, and variations in cell-to-serum ratios. The flow cytometry B-cell XM is associated with high background antibody binding, which may be reduced by incubation of target lymphocytes with Pronase (25). However, Pronase treatment may affect HLA expression and lead to false-positive T-cell XM (26). Each center must therefore validate FCXM result thresholds with respect to clinical risk.

TABLE 2. Methods for antibody screening and cross-matching for each type of organ transplant

Organ/single or combined	Pretransplantation screening	Generic methods	Extended methods (SPI-SAB)	XM	Comment
Kidney	+++	SPI±CDC/FC	(All patients)[a]	CDC/FC/vXM[a]	Prevention of HAR or early AMR
Heart	+++	SPI±CDC/FC	All patients	CDC/FC/vXM[a]	Prevention of HAR or early AMR
Lung	+++	SPI±CDC/FC	All patients	CDC/FC/vXM[a]	Prevention of HAR or early AMR
Liver	+	SPI±CDC/FC	.	.	AMR
Pancreas[b]/(islets)	+++	SPI±CDC/FC	All patients	CDC/FC/vXM[a]	Prevention of HAR or early AMR
Intestinal	+++	SPI±CDC/FC	All patients	CDC/FC/vXM[a]	Prevention of HAR or early AMR

[a] Depending on the local/national/organ exchange organization policy.
[b] Pancreatic islet cells can be transplanted with a positive XM.

Test methods to be employed according to the organ transplanted. In case of double transplants, the most stringent method should be used. The assays are scored from not valuable (−) to very valuable (+++). The comparative sensitivities are LUM>ELISA/FC>CDC. Note there are no convincing data that demonstrate ELISA is more sensitive than FC.

AMR, antibody-mediated rejection; CDC, complement-dependent lymphocytotoxicity; ELISA, enzyme-linked immunosorbent assay; FC, flow cytometry; HAR, hyperacute rejection; SPI, solid-phase immunoassays; vXM, virtual crossmatch; XM, crossmatch.

TABLE 3. Features of the Luminex SPI panels provided by the two vendors

Vendor A		Vendor B
Portfolio	Pooled, phenotype, and SAB. HLA class I and II, MICA antigen beads. RUO and in part IVD	Pooled, phenotype, and SAB. HLA class I and II, MICA, and NA antigen beads. IVD
Background assessment	Three negative control beads. NC serum	One negative control bead. NC serum
Antigen sources	Platelets, EBV-transformed lymphocytes. Blood donors, recombinant cell lines	EBV-transformed lymphocytes, recombinant cell lines
Antigen density report	HLA class I and II and MICA	HLA class I
Full automated solution	Available	Available
Special feature	DQ enriched beads in phenotype panels	

EBV, Epstein-Barr virus; HLA, human leukocyte antigen; IVD, in vitro diagnostic; MICA, major histocompatibility complex class I–related chain A; NC, negative control; RUO, research use only; SAB, single-antigen beads; SPI, solid-phase immunoassays.

Solid-Phase Immunoassays

ELISA technology is more sensitive than CDC (11), whereas Luminex bead technologies are more sensitive than both CDC and flow cytometry (12), enabling the detection of low levels of HLA-specific antibody. The comprehensive array of common and many rare HLA alleles for all 11 HLA loci (A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPBA1, and DPB1) present in the Luminex SAB array enables the precise definition of HLA antibodies contained in complex sera (13, 27). The ability to identify epitope-specific antibodies (28–30) and antibodies to HLA-Cw, HLA-DQA, HLA-DPA, and HLA-DPB was not previously possible in most diagnostic routine laboratories and has led to a new realization of the importance of such antibodies in kidney allograft rejection (31, 32).

SPI results are semiquantitative and enable broad categorization of DSA levels into low, intermediate, and high according to the optical density (ELISA), median channel of fluorescence (flow cytometry), or MFI value (Luminex). Luminex phenotype and SAB panels provide large-scale batch testing results within 4 hr, making these tests valuable in supporting a diagnosis of humoral rejection in routine pretransplantation and posttransplantation monitoring and in assessing the efficacy of antibody reduction programs (33, 34). Table 3 displays the features of the Luminex SPI panels provided by two vendors.

SPI, like CDC or flow cytometry, have technical aspects requiring significant expertise in their use and interpretation (Table 4). For SPI, it is relevant to capture both the HLA antibody specificities identified and the level of antibody (MFI). Immunologic risk assessment varies among centers, ranging from listing SAB-defined specificities above a given MFI threshold as unacceptable, listing antibody specificities according to MFI range (low, intermediate, or high), or providing MFI information for each defined antibody specificity (27). It is important to note that although Luminex-based assays can provide a semiquantitative numerical value, they were developed and licensed as qualitative assays.

Interface with Laboratory Databases

The complexity of the data obtained from SAB arrays, particularly in highly sensitized patients, requires each

TABLE 4. Technological advantages and limitations of Luminex HLA SAB

Technological advantages	Technological limitations
Qualitative: enables precise identification of all antibody specificities in complex sera (DSA)	Some positive results can be caused by antibodies to denatured HLA.
Comprehensive: distinguishes antibodies to all common alleles for HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3/4/5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1	Occasional high background binding requiring repeat testing and absorption protocols.
Semiquantitative: enables determination of antibody levels (high, intermediate, and low)	Variable HLA protein density on beads. Blocking factors may cause false-negative or misleading low assessment of antibody levels (prozone?). IgM and C1 can block IgG binding.
Sensitive: enables detection of weak antibody levels	
Rapid: enables real-time antibody monitoring for DSA. HLAi transplantation. Pretransplantation and posttransplantation antibody monitoring (assist diagnosis of AMR). Virtual XM	Lot-to-lot variation requiring validation. Vendor-specific variation.
Enables detection of non-HLA–specific antibodies (e.g., MICA)	
Detection and differentiation between immunoglobulin class and isotype (e.g., complement fixing and noncomplement fixing C4d and C1q)	Reagents not standardized

AMR, antibody-mediated rejection; DSA, donor-specific HLA antibodies; HLAi, HLA incompatible; MICA, major histocompatibility complex class I–related chain A; SAB, single-antigen beads; XM, crossmatch.

laboratory to develop an interface between the Luminex analysis software and the laboratory information system to enable efficient and accurate analysis of antibody data.

Solid-Phase HLA Antibody Detection Assays: Technical Challenges

Effect of Variability in Antigen Quantity and Quality

The relative quantity on beads of a particular antigen differs substantially among pooled antigen, phenotype, and SAB. HLA-Cw, HLA-DQ, and HLA-DP on SAB and DQ on one manufacturer's phenotype panel are characterized by a higher relative antigen density. As a consequence, antibody levels to these antigens run the risk of being overestimated yet may represent only a low immunologic risk for renal transplant rejection (27). Conversely, antibodies against public epitopes such as Bw4 or Bw6 may appear underrepresented because a single antibody may be dispersed across many beads underestimating its actual level. Disparities in antigen quantity exist not only across the different bead formats but also among different HLA molecules on the SAB. Recent improvements in the manufacturing process and quality assurance measures have contributed to a more uniform antigen density across all beads from lot to lot, although this problem has not been completely resolved. In addition, the antibody analysis software available from the manufacturers includes features to normalize the data according to an average antigen density. All these measures contribute to more consistent results.

An issue inherent with the use of soluble HLA molecules is the fact that they are not in their native state and environment. Proper conformation of HLA antigens depends on the bound peptide and associated $\beta 2$ m (class I) and is affected by glycosylation pattern. Purification and coating to the beads can lead to improper conformation of antigens that gives rise to the detection of clinically irrelevant antibodies (35, 36). Thus, deviations in the overall antigen condition (quantity and conformation) due to different methods of preparation may lead to discrepant reactions between phenotype and SAB.

Reports using SAB have suggested the existence of naturally occurring HLA antibodies in males (37, 38), but these antibodies appear to be specific for epitopes on denatured HLA molecules (38, 39). Tests of some of these sera were shown to yield negative results in FCXMs, suggesting the absence of antibodies to HLA antigens in their native conformation. Two recent reports have shown the lack of clinical relevance of antibodies specific for epitopes on denatured antigens (36, 40). Distortion of HLA molecules that results from binding them to a solid matrix represents a dual risk: interpreting positive reactions with cryptic epitopes incorrectly as a contraindication to transplantation and failing to recognize the presence of antidonor HLA antibodies because they do not bind to the distorted molecules. Zachary et al. (41) demonstrated that the identity and level of DSA using phenotype beads was more predictive for CDC and FCXM results than were antibodies based on SAB testing.

Inherent Variability

As with any serologic assay, there is a certain degree of inherent variability in SPI. This variability is seen among different kits, different lots of the same kit, different runs, and different operators. Manufacturers are making efforts to reduce lot-to-lot variability and increase uniformity among the beads within a kit. Users can reduce variability by strict adherence to test conditions and procedures and by the use of robotic liquid-handling instruments. The degree of variability is similar to that of cell-based assays. The identification of strong antibodies is unequivocal and most discrepant results observed with external proficiency schemes occur with weak antibodies.

Interpretation

Reproducibility is a major prerequisite to facilitate the proper interpretation of HLA antibody detection assays. In assigning apparent specificity for DQ or DP, the possibility of specificity for the α chain (DQA or DPA) and for epitopes specific to particular α-β chain combinations must be taken into consideration (42). The interpretation of epitope-specific HLA antibody is very complex and requires personnel with appropriate experience and expertise. Most importantly, the plausibility of antibody assignment must be verified by considering the following: (a) consistency with other antibody tests performed and with test results of other specimens from the same patient, (b) serum donor phenotype to ensure that a self-epitope is not included in the antibody assignment, (c) alloimmunizing events (i.e., transfusion, pregnancies, or previous transplants), and (d) cross-reactivity. The HLA phenotypes of the patient, previous organ donors, and, in the case of known pregnancies, the paternal HLA antigens when obtainable should be considered to verify antibody specificity. High-resolution HLA typing may be required because some patients can form antibodies to epitopes on alleles included in their own antigen group. The HLA type of the serum donor may also be used to define patient- and serum-specific cutoff values. Reliable information on proinflammatory events of the patient is valuable in validating sudden increases in antibody strength or breadth (43).

Assessment of Antibody Level

MFI levels on the beads represent the amount of antibody bound relative to the total antigen present on the beads (degree of saturation), which varies by individual bead. MFI does not represent titer despite the widespread misuse of the term. The MFI has been used successfully in some centers as a means of predicting flow cytometry or CDC XM results (44). This approach is particularly useful in predicting negative XM but may become less reliable with low levels of antibody. The prediction of antibody level is particularly problematic with strongly reacting antibodies. Some diluted sera give MFI equivalent to that found in undiluted sera, indicating saturation of the specific antibody-binding epitope(s) on the HLA molecule bound to the beads (45). In addition, some sera give increased MFI on dilution due to interference by IgM or C1 in undiluted sera (45–47). The relationship between antigen density on the beads and on cells is incompletely understood. These factors compromise the use of MFI as a surrogate marker of antibody level, the estimation of which requires serum dilution or other treatments to remove interfering substances.

Attempts have been undertaken to standardize MFI by conversion to molecules of equivalent soluble fluorochrome

(MESF) using quantification beads known from flow cytometry (48). However, the inherent assay variability from day to day, lot to lot, or among different laboratories could not be eliminated by using MESF.

Accurate quantification of HLA antibody levels is required for therapeutic pretransplantation desensitization and posttransplantation AMR rejection protocols. It has been suggested that the relationship of any given bead to the positive control bead may be useful in determining significant changes or for normalizing MFI values (49, 50). It is suggested that quantification of antibody level is best achieved by titration.

Interference in Solid-Phase Immunoassays

The reactivity due to substances other than the analyte being tested and the reduction in the strength of reactivity with the target analyte are problems inherent to serologic assays. The development of SPI has increased exponentially the sensitivity of tests for HLA antibodies; however, these tests remain susceptible to interference from a variety of substances (51, 52) that may be categorized into two groups: substances present naturally in serum and substances that are administered to patients.

Interference by Substances Inherent in the Serum

Removal or reduction of IgM from sera by hypotonic dialysis increases the reaction strength of the positive control bead, decreases reactivity with the negative control beads, and affects both the strength and the specificity of antibodies detected with the antigen-specific beads (53). Precipitation of IgM in hypotonic dialysis might also trap immune complexes that could bind nonspecifically to beads. Some of the interference is due to reactivity with the polystyrene beads as demonstrated by lack of inhibitory reactivity of some sera when HLA antigens were bound to glass microchips (53). Dilution or treatment of sera with DTT decreases the reactivity of some antibodies and increases the reactivity of others (45); however, it also increases the reactivity with negative control beads and the strength of HLA antibodies from DTT-treated serum does not appear to correlate well with XM results (53). Removal of C1 via dilution, DTT, heat inactivation, or use of a C1 inhibitor can restore masked HLA reactivity on SAB (46), suggesting that the effect of DTT treatment is via cleavage of C1. It is recommended that each laboratory decide under what conditions such treatments may be necessary. This may range from pretreating all samples to having a specific indicator based on testing parameters, such as a low positive control bead value.

Some reactivity with beads coated with HLA class I molecules can be attributed to antibodies to HLA-E that cross-react with the HLA classic class I molecules (54, 55). The reactivity of the HLA-E antibodies is with HLA-B and HLA-Cw, which does not explain the observed reactivity with beads bound with denatured A-locus antigens (see previous section on antigen quantity and quality).

The incidence of interference varies among different groups of patients. For example, non-HLA antibodies that are reactive in SPI assays appear after left ventricular assist device implantation (56).

Interference by Exogenous Substances

Therapeutic reagents used to prevent or treat rejection have been shown to cause interference in SPI for HLA antibodies. Among these agents are intravenous immunoglobulin (IVIg) given at high doses (2 g/kg body weight), antithymocyte globulin, the proteasome inhibitor bortezomib, and eculizumab, a complement C5 inhibitor. It is widely accepted that high-dose IVIg interferes in assays that use an antiglobulin reagent. Reactivity with the negative control beads in SPI was reported to be increased more than fivefold after treatment of patients with IVIg (57). Treatment with antithymocyte globulin results in apparent HLA-specific reactivity (57–59). Within days after treatment with either bortezomib or eculizumab, sera from patients with antibodies yielding strong reactions in SPI showed significant reductions in antibody strength (57). However, after hypotonic dialysis of the sera, there was either a very limited reduction or a slight increase in antibody strength.

SPI are being used widely to guide treatment of transplant patients and to define unacceptable HLA antigens. Thus, it is critical that interference in these assays be recognized and, when possible, reduced or eliminated. Several patterns of reactivity that are indicators of interference are listed below.

Indicators, but not proof of interference in multiplexed microsphere assays include the following:
- High reactivity with the negative control bead,
- Low reactivity with the positive control bead,
- (Each laboratory has to establish what levels of positive and negative controls indicate interference.)
- Sudden change in the pattern of reactivity in sequential sera from a patient in the absence of any specific treatment or event,
- Reactivity that does not reconcile with the results of CDC or FCXM tests, and
- Reactivity with the patient's own HLA antigens.

Modifications to Solid-Phase Immunoassays for Detection and Assessment of Functionality of HLA Antibodies

C4d Assay

The C4d and C1q assays are modifications to SPI designed to distinguish complement fixing from noncomplement fixing antibody. The C4d assay (60–63), which includes spiking with normal human serum as the source of C4d, demonstrated that, for class I and II HLA antibody detection, respectively, this C4d assay has superior specificity compared with CDC (92/100% vs. 79/86%) but inferior sensitivity (61/31% vs. 70/55%) (61). The C4d assay requires complement activation to occur and is influenced by complement regulatory factors. This method has low sensitivity with maximal MFI values in the 3500 range. Clinical data obtained using various modifications of the C4d assay have shown that the presence of C4d$^+$ antibody correlates with graft survival in kidneys (62) and hearts (63). C4d$^+$ antibodies do not appear to be associated with AMR in renal grafts (64), but an association with the presence of C4d$^+$ antibodies and C4d deposition in the peritubular capillaries has been reported (62). More recent reports have demonstrated that de novo DSA is associated with poor patient survival but is independent of either IgG strength (MFI) or the ability to fix C4d (65, 66).

C1q Assay

The C1q assay designed to distinguish complement fixing from noncomplement fixing antibody does not

require complement activation other than the binding of C1q to the antibody (67). It detects antibodies capable of binding complement and initiating the classic pathway irrespective of whether they do so or not. Thus, it is not affected by complement regulatory factors other than, perhaps, C1INH (68). The method uses a standard amount of exogenous purified hC1q added to the patient serum. The C1q assay is highly sensitive, with maximum SAB MFI values of more than 30,000. Although there is a trend for higher MFI values detected by the IgG assay to fix complement, this is not uniformly true and prediction of the complement fixing ability of a given antibody cannot be made from the IgG results (67, 69). The C1q assay detects more IgG antibodies than those detected by CDC but also detects complement fixing IgM. In cardiac transplantations, correlations have been demonstrated between antibodies detected by the C1 q assay and AMR in the first month for both preformed and de novo antibodies (70). Similar correlations with acute rejection and long-term graft outcome have been observed in kidney transplant recipients (71, 72). Analysis of epitopes by both IgG and C1q assays from pretransplantation through AMR resolution showed that C1q$^+$ DSA correlated with the clinical course, whereas the IgG reactivities did not (69). However, Otten et al. (73) found no correlation with clinical course in kidney transplant patients, but the frequency of C1q$^+$ DSA was too low to make meaningful conclusions. Although the C1q assay shows promise, additional studies are required to establish its clinical role as a routine test.

Detection of Antibodies to Non-HLA Antigens

Humoral responses to non-HLA antigens or tissue-specific autoantigens in the setting of solid organ transplantation are primarily to antigens expressed on endothelial cells and epithelial cells. The incidence and clinical consequence of immunization to non-HLA antigens is incompletely understood. A major limitation in our understanding of non-HLA antibodies in transplantation is (a) the lack of knowledge of the identity of the non-HLA targets and (b) a critical need for the development of validated diagnostic screening assays for direct detection of non-HLA antibodies to gain a better understanding of their clinical relevance.

Anti–endothelial cell antibodies (AECA) have been reported to mediate endothelial cell activation, apoptosis, and cell injury (74). AECA represent a heterogeneous group of antibodies comprising both IgM and IgG isotypes and are directed against a variety of antigenic determinants on endothelial cells (75). The endothelial cell is the principal target for the detection of non-HLA antibodies involved in AMR because it expresses antigens that are not present on lymphocytes, which are typically used for the detection of DSA. A major limitation in using endothelial cells as a XM target is the lack of standardized protocols and reagents, including positive and negative controls and anti–endothelial cell reference sera. Historically, different assay systems have been used to identify and characterize AECA, including CDC (76, 77), flow cytometry (78–81), and immunofluoresence (82). These methods differ widely in their sensitivity, specificity, and ability to detect distinct immunoglobulin isotypes. Another limitation is that the endothelial cells used for the detection and characterization of

AECA have been derived from third-party donors, not the transplant donor. These laboratory-developed assays used endothelial cells from different vascular beds, including microvascular (dermal), venous (umbilical cord), and large vessel (aorta), which are known to have differences in protein expression resulting in distinct phenotypes (83–85).

Surrogate endothelial cells can be useful for identifying antibodies to nonpolymorphic antigens or antigens with limited polymorphisms. However, they have limited usefulness in detecting antibodies to highly polymorphic antigens, such as major histocompatibility complex class I–related chain A (MICA), or to antigens that are uncommon or rarely found in human populations. MICA is not constitutively expressed on endothelium; rather, its expression is induced under conditions of cellular stress. Given these caveats, SPI using recombinant MICA protein targets represent a more reliable detection system than primary endothelial cells. Ideally, once AECA are identified, the development of specific SPI should facilitate their detection.

Two recent studies reported the study of AECA using an indirect immunofluoresence method on commercially available slides of human umbilical vein endothelial cells (86, 87). A potential advantage of this method is that it is commercially available and may permit standardization of test results among laboratories using this as a screening test to detect AECA.

A recent study (88) propagated endothelial cells obtained directly from the transplant donor and used them to study the development of posttransplantation endothelial cell DSA after renal transplantation. Interestingly, these DSA could be detected by cytotoxicity, suggesting an Ig isotype that fixes complement (e.g., IgG1 and IgG3).

Antibodies can mediate graft injury via various mechanisms including complement-mediated injury, endothelial cell activation, proliferation and apoptosis, and cellular recruitment (89, 90). Both complement activation and cellular recruitment are regulated by the Fc portion of the immunoglobulin molecule, so it may be important to develop assays that identify the isotype of AECA to understand the pathogenesis of AMR. A recent study showed that donor-reactive IgM AECA did not correlate with rejection, whereas AECA of the IgG2 and IgG4 subclasses that do not activate complement were enriched in recipients with rejection (91).

Lymphocyte XM tests fail to detect AECA. The XM-ONE assay is an Food and Drug Administration (FDA)–approved endothelial FCXM technique that uses endothelial cell precursor cells found in the peripheral blood at a frequency of 1% to 2% (92). A benefit of this test is that it detects DSA and can be used to test for antibodies to T lymphocytes, B lymphocytes, and endothelial cells in the same assay (93). An important question that needs to be addressed is whether AECA detected using XM-ONE also bind to fully differentiated endothelial cells that line the vessels of the allograft.

Discovery and Characterization of Antibodies to Non-HLA Antigens

Proteomic approaches using protein extracts from different sources, including cell lysates and protein microarrays, are being used for antibody screening and identification of specificities. There are several reports of protein arrays for the discovery of non-HLA antigens that can

generate a humoral immune response after renal transplantation and for the discovery of alloantibody and autoantibody targets (*94–97*). Two-dimensional immunoblotting of IgG from patient sera has also been used for the identification of AECA (*98*). Antibodies to non-HLA targets detected after lung transplantation have been described using a novel technique called SEREX (*99*).

There have been a variety of non-HLA targets identified including MICA (*81, 100, 101*), vimentin (*102–106*), angiotensin II type 1 receptor (*107, 108*), tubulin (*109, 110*), myosin (*111, 112*), and collagen (*113, 114*).

Toward Standardization of Methods

Solid-phase HLA antibody assays are complex and variability in manufacturer reagents, assay performance, and data analysis are barriers to harmonization of this assay across laboratories worldwide. It is recommended that laboratories and kit manufacturers standardize critical components that have the potential to influence the results and interpretation of SPI, including the following: HLA source and preparation method, panel composition, and appropriate allele coverage, including DQA1, DPBA, and DPB1. Other considerations for standardization are the antigen density on the bead, antigen integrity (e.g., denatured vs. native antigen), and the anti-human immunoglobulin detection reagents—all critical factors that impact assay performance and interpretation. Technical variance can be reduced by using the same standard operating procedure including the type of plastic trays used (V-bottomed vs. U-bottomed), the serum volume to bead ratio, washing methods (e.g., spin/flick vs. filter tray), vortexing methods, and employing a unified approach to sample preparation to minimize interference in the assay (*53, 115*). If affordable, laboratories should be encouraged to use automated laboratory equipment to achieve uniformity in dispensing reagents and assay washing steps. In addition to optimizing protocols and reagents, fluoroanalyzers and flow cytometers should be calibrated using control particles to achieve similar target values across instruments.

Cell-based antibody detection assays including CDC and flow cytometry also differ widely among laboratories worldwide (*21*). For cooperative studies, or studies attempting to produce comparable data, standardization is necessary. The major areas of standardization include harmonization of standard operating procedures including cell isolation, cell-to-serum ratio, incubation, and wash steps. It is important to define the monoclonal antibodies used for defining T and B lymphocyte populations, secondary antibodies used for the detection of human IgG and negative/positive controls. In addition, instrument setup and data analysis are crucial variables as well. Periodic assessment of the degree to which results are reproducible within a laboratory should be part of the ongoing quality control of every laboratory.

Efforts should be made to standardize the interpretation and reporting of test results using defined reporting algorithms, background normalization, and the potential use of standard fluorescent intensity and MESF to compensate for Luminex and flow cytometer machine differences, respectively. For SPI, it is recommended that test reports include the following critical values: assay type, cPRA, antibody specificity, interpretation of antibody level,

comments on presence or absence of DSA, criteria for positive/negative results, immunoglobulin isotype (e.g., IgG vs. IgM), and any factors that are considered to affect test values and interpretation of results.

A repository of well-characterized HLA polyclonal and monoclonal reference reagents for ongoing technique and reagent validation, monitoring interlaboratory variability, and reproducible quantification of fluorescence values should be developed. This should encompass a diverse set of reference sera to all HLA class I and II antigens representing different titers and isotypes. The reference reagents must be validated in national and international exchange and on cell panels and SPI for all available techniques. Each laboratory must participate in relevant external proficiency testing programs as required by local, regional, and national regulations.

ANTIBODY TESTING PRETRANSPLANTATION

In this section, the pretransplantation challenges encountered in the determination of clinically relevant alloantibodies in sensitized patients, the available options for the timely and successful transplantation of patients with high levels of alloantibodies, and the impact of sensitization against HLA as well as non-HLA antigens on the outcome of kidney and other organ transplants are discussed.

Determination of Unacceptable HLA Antigen Mismatches and Risk Assessment in Kidney Transplantation

A major task of HLA laboratories is the determination of the so-called unacceptable HLA antigen mismatches (UA). Using this information, negative XM prediction or "virtual XM" is possible when a potential donor's complete HLA typing is available. The determination of UA is a critical decision step because the likelihood of an organ offer diminishes with increasing number of UA and all too frequently patients die on the waiting list before they can be transplanted. Conversely, unrecognized UA, due to insensitive or incorrect testing, result in inferior graft survival and futile organ shipments because the XM test in the recipient center is positive.

The introduction of SAB assays has allowed for the precise determination of UA not previously possible, especially in highly sensitized patients (*13*). Assignment of UA should not generally be based on the SAB reactivity alone but whether the antibody reactivity pattern is consistent with a recognized epitope and the patient's history of sensitizing events. Many centers still consider repeat HLA mismatches from previous transplantations as UA even if they give negative results in antibody screenings.

With the advent of more sensitive antibody assays, it is presently unclear which antibody test at what sensitivity level is most appropriate for the determination of UA. Donor-specific IgG HLA antibodies detected by CDC are considered a contraindication for transplantation, whereas DSA detected by other assays represent varying degrees of risk (*116*). Many laboratories no longer use CDC assays to screen for HLA antibodies and therefore depend on SPI alone to determine whether an antigen is unacceptable. Although there are good data for kidney transplants and other organs that preexisting DSA by SPI is associated with an increased risk of rejection,

usually AMR, and inferior outcomes (Tables 5 and 6), it is debatable whether the antibodies that go undetected in CDC and ELISA and are detectable exclusively in SPI bead assays influence outcome (24, 31, 34, 73, 117–134) (Table 5). Currently, individuals with multiple antibody specificities on SAB testing but negative in CDC testing make up a large part of the transplant waiting list. Many recipients with DSA positive only by flow-based or Luminex technology do well posttransplantation and have good long-term graft function (Table 5). Therefore, it is difficult to set a cutoff for acceptable risk. One should also consider that a low MFI might be due to sera being screened at a time remote from the original immunizing event, and in the absence of historical sera, the characteristics of previous more highly reactive sera are unknown. A prospective XM with donor cells and historical serum is frequently not available at the time of organ offer and there is no good test to determine which antigens are likely to trigger an amnestic response. Furthermore, the MFI often does not correlate with strength of XM, indicating that immunologic risk cannot be determined based on this parameter alone.

DSA that persist posttransplantation after desensitization therapy are considered a risk factor for developing transplant glomerulopathy (TG) and subsequent graft loss (119, 135). To truly understand the impact of bead assay-detected pretransplantation DSA, both short-term and longer-term outcomes need to be documented. In addition, these outcomes may be impacted by desensitizing protocols and induction with depleting agents that were not always given to all recipients, so using retrospective data to answer all these questions is often fraught with difficulty.

There are now data that indicate an increased risk of AMR when the transplantation is performed in the presence of DSA (Table 5). Many transplantation programs do not want to transplant across a bead-positive DSA, and as many programs allocate based on a virtual XM, the recipient may be excluded based on the bead result without the opportunity to be cross-matched with donor cells. Most data are from retrospective studies with variable immunosuppressive regimens, so it is difficult to get a true estimate of risk because no control groups exist.

Transplantation of Highly Sensitized Patients

The old dogma that the presence of DSA pretransplantation is a contraindication for transplantation was the reason that highly sensitized patients accumulated on the waiting list because the serologic XM with almost all donors was positive. The introduction of the more sensitive SPI has led to an increase in the number of highly sensitized patients but also to the knowledge that the presence of DSA is not always a contraindication but rather a risk factor. The risk for rejection and graft loss can be decreased in two ways: (a) selection of a donor toward whom the patient has no DSA or (b) removal of the DSA via desensitization protocols.

Strategies to Transplant the Highly Sensitized Patients

Special programs are necessary to increase the chance that a highly sensitized patient can be transplanted with a XM-negative donor without desensitization. For patients waiting for a deceased donor organ, the acceptable mismatch program of Eurotransplant has been shown to be very efficient (18). The basis of the acceptable mismatch program is the precise identification of those HLA antigens toward which the highly sensitized patient did not form antibodies. Similar programs exist internationally where highly sensitized patients are prioritized to receive virtual XM-negative organs. Patients with an incompatible living donor can participate in a paired donor exchange program. In cases where a sensitized patient has a living donor available toward whom the patient has formed DSA, paired donor exchange programs can facilitate transplantation with an alternative XM-negative donor.

If a compatible donor is not identified, desensitization can be performed in combination with other measures. Desensitization protocols aim to lower DSA at the time of transplantation to a threshold considered safe and to maintain the DSA at this level, also with the help of immunosuppression, at least for the first days to weeks after transplantation. Hereby, alloantibodies are removed from the patient's circulation by plasmapheresis or immunoadsorption and their production is suppressed by T-cell immunosuppression, IVIg, rituximab, or the proteasome inhibitor bortezomib based on the rationale that depletion of B lymphocytes or plasma cells may reduce DSA production. An additional approach is the blockage of complement activation by administration of the complement C5 inhibitor eculizumab.

Unfortunately, randomized controlled trials that compare the clinical efficacy of different desensitization strategies are missing. Desensitized patients generally receive more potent immunosuppression than DSA- or XM-negative sensitized patients. It is a matter of debate whether desensitization prevents de novo HLA alloantibody production and results in good long-term outcome. Montgomery et al. (136) reported recently that, compared with waiting for a compatible deceased donor, desensitization is an option for the timely transplantation of sensitized patients with DSA. In this study, living-donor organ transplantation after desensitization provided a significant survival benefit for patients with HLA sensitization (136). Increasing evidence suggests that, in patients carefully selected based on antibody titers, desensitization can be performed safely with good graft and patient survival in living-donor as well as deceased-donor transplantation (137, 138), and a combination of desensitization, acceptable mismatch program, good HLA matching, and other measures in an integrative manner is reported to result in a significant reduction in waiting time and good allograft and patient survival (139).

Impact of Sensitization Against HLA on Outcome of Transplants Other Than the Kidney

As in kidney transplantation, cross-matching is a routine procedure also in pancreas transplantation. Although the determination of UA and virtual cross-matching have become routine procedures at an increasing number of heart and lung transplant centers in recent years, in other organ and islet cell transplantation, more data are required to establish the precise role of pretransplantation DSA on graft outcomes. A selection of published literature on the impact of preexisting HLA antibodies in heart, lung, liver, pancreas, intestine, multivisceral organ, and islet cell transplantation is shown in Table 6 (70, 117, 140–183).

TABLE 5. Literature on the impact of SAB-detected preexisting DSA on kidney transplantation outcome

First author (reference)	Year	DSA+ (n)	n	DSA loci	Method	XM	Donor	Preselection (No.)	AMR	CR	GS
Bryan (117)	2006	10	10	AB	F-PRA/SAB	AHG-CDC, FCT	DD	CDC XM+/AHG XM−	30% but no control	ND	↔
Gibney (118)	2006	20	136	ND	LSC	AHG-CDC	LD/DD	No	↑	ND	→
Patel (119)	2007	21	330	AB DRDQ	LSC/F-PRA	CDC and FC T&B HI	LD	FC−	↑	↔	ND
Burns (120)	2008	70	70	Class I and II, no details	LSC/SAB	FC T&B	LD (2 DD)	Desensitized	↑	ND	ND
Eng (121)	2008	27	83	AB DRDQB	LSC/SAB	CDC	DD	B-cell CDC+	↑	↑	→
Van Den Berg-Loonen (122)	2008	13	37	AB DRDQ	SAB	CDC	DD	AM program	ND	ND	↔
Aubert (123)	2009	11	113	AB DRDQDP	LSC/SAB	CDC	LD/DD	Thymo for high risk	↔	ND	↔
Vlad (124)	2009	27	355	ABC DRDQ	LSC/SAB	CDC	DD	No	↑	ND	↔
Phelan (125)	2009	12	64	ABC DRDQ	LSC/SAB	CDC	LD	Some had prospective FCXM; ELISA −	↔	↑	↔
Gupta (126)	2009	16	121	ND	LSC/SAB	CDC	ND	No	↔	↔	↔
Amico (127)	2009	67	334	AB DRDQDP	SAB	CDC T&B	LD/DD	No	↑	ND	↓ If AMR
Riethmuller (128)	2010	20	155	AB DRDQ	LSC/ELISA/SAB	Luminex	LD	Some had rituximab or ATG for high risk	↑ If class I DSA	ND	ND
Singh (129)	2010	159	237	AB DRDQ	SAB	CDC	DD	No	↑ If class II DSA	ND	↓ If DR DSA
Gloor (130)	2010	117 (12 XM−)	189	AB DRDQ	LSC/SAB	FC T&B	LD/DD	XM+ vs. XM−	↑	↑ If AMR	↓ In XM+
Lefaucheur (131)	2010	83/76 at time of transplantation	402	Class I and II no details	ELISA/SAB	CDC	DD	No	↑	ND	→
Ishida (132)	2011	22	34	AB DR	F-PRA/SAB	CDC	LD	F-PRA+	↑	↑	NA
Higgins (34)	2011	84 (17 CDCXM+, 44 FCXM+, 23 only SAB)	112	ABC DRDQDP	LSC/SAB (most sera)	CDC/FC	LD/DD	Desensitized	↑	NS	→
Susal (133)	2011	53	236	ABC DRDQAB DPAB	SAB	CDC	DD	CDC− and ELISA−; 3-yr GS	ND	ND	NS
Dunn (31)	2011	46	587	ABC DRDQAB DPAB	LSC/SAB	AHG-CDC T&B	LD/DD	Thymo	↑	↑	→
Couzi (24)	2011	30 historic (28 D0)	45	ABC DRDQDP	LSC/ELISA/SAB after 2005	FC T&B	DD	FCXM+	↑	ND	↔
Couzi (24)	2011	11	45	ABC DRDQDP	LSC/ELISA/SAB after 2005	FC T&B	DD	FCXM−	↔	ND	↔
Caro-Oleas (134)	2012	103	892	ABC DRDQDP	SAB	AHG-CDC, FCB	DD	Thymo for high risk	ND	ND	→
Otten (73)	2012	290 (30 C1q+)	837	ABC DRDQ	SAB/C1qSAB	AHG-CDC, FCB	LD/DD	No	ND	ND	↓ If DSA I+ and II+

AHG, anti-human immunoglobulin; AM, acceptable mismatch program; AMR, antibody-mediated rejection; CDC, complement-dependent lymphocytotoxicity; CR, cellular rejection; DSA, donor-specific HLA antibodies; F, flow; FC, flow cytometry; GS, graft survival; HI, highly immunized; LD, living donor; LSC, Luminex screen; NA, not applicable; ND, not determined; PRA, panel reactive antibody; SAB, Luminex single-antigen beads; XM, crossmatch; ↑, increased; ↓, decreased; ↔, no difference.

TABLE 6. Literature on the impact of preexisting HLA antibodies on nonkidney transplants

First author (reference)	Year	n	DSA+ (n)	DSA loci	Method	XM	Preselection	AMR	CR	GS
Preexisting HLA antibodies in heart transplantation										
Bishay (140)	2000	338	Class I=50, class II=144	AB DR	FCXM	FC	No	↑ In class I	↑ In class II	→
Bishay (141)	2000	500	PRA >10% 53	ND	FCXM,	CDC	No	ND	ND	→
Michaels (142)	2003	44	12	ND	FCXM	CDC	Rejection	←	←	ND
Jacobs (143)	2004	60	8	ND	F-PRA	CDC	Ped	↕	ND	→
Di Filippo (144)	2005	45	12	ND	ELISA		Ped	←	↕	ND
Leech (145)	2006	262	35	ND	CDC, F-PRA	CDC, FC	Presensitized	ND	ND	→
Feingold (146)	2007	168	23	ND	CDC, ELISA	CDC	No	ND	←	↕
Opelz (147)	2009	7797	347	ND	NA	CDC	No (1990-99)	ND	ND	↕
Opelz (147)	2009	2326	98	ND	NA	CDC	No (2000-07)	ND	ND	→
Rose (148)	2009	565	19 (11 were C4d+)	ND	LMix, SAB, C4d	CDC, Luminex	No	ND	ND	→
Ho (149)	2009	774	Class I=71, class II=104	AB DRDQ	CDC	CDC	No	←	←	↕
Scott (150)	2011	83	12 (PRA >25%)	ND	F-PRA	CDC	Ped	↕	ND	→
Mahle (151)	2011	1904	397 (PRA >10%), 189 (PRA >50%)	ND	F-PRA, CDC, Luminex	NA	Ped	↕	↕	→
Kobashigawa (152)	2011	523	95	ND	CDC, F-PRA	NA	No	↕	ND	↕
Gandhi (153)	2011	85	34	AB DRDQ	CDC, SAB	CDC-AHG, FC, Virtual	No	←	←	ND
Chin (70)	2011	18	13 (PRA >20%), 3 C1q$^+$	ABC DRDQ	SAB	Virtual	Ped	In C1q$^+$	ND	ND
Preexisting HLA antibodies in lung transplantation										
Lau (154)	2000	200	18	ND	PRA	NA	No	ND	←	→
Appel (155)	2005	380	Class I=9, class II=4	ND	PRA	NA	Desensitized	ND	← After desensitization	ND
Hadjiliadis (156)	2005	656	37 (>10%), 20 (>25%)	ND	CDC	NA	No	ND	ND	→
Appel (157)	2006	341	19	ND	CDC, PRA	NA	Mainly for comparing CDC and PRA	ND	ND	ND
Shah (158)	2008	10,237	240 (>25% PRA)	ND	CDC, PRA	NA	No	ND	ND	←
Stuckey (159)	2011	1	1	ND	PRA	NA	Case report, successful depletion of antibodies with good outcome	ND	ND	ND
Mangi (160)	2011	481	136 (class I PRA >10%), 64 (class II PRA >10%)	ABC DRDQ	CDC, ELISA, PRA	NA	No	←	ND	ND
Preexisting HLA antibodies in liver transplantation										
Fung (161)	1988	12 KL	4	ND	NA	CDC	No	↕	ND	↕
Muro (162)	2005	254	14	AB DR	F-PRA	CDC	No	↕	↕	→
Mosconi (163)	2006	1 KL	1	AB DR	CDC	CDC	No	↕	↕	ND
Castillo-Roma (164)	2008	896	89	AB DR	LMix	CDC	No	ND	←	→
Reichman (165)	2009	1 KL	1	AB DRDQ	SAB	CDC	No	←	ND	→

Study (Ref)	Year	N	n	HLA	Antibody method	Crossmatch	Positive XM			
Opelz (147)	2009	4518	443	ND	NA	CDC T	No (1990-99)	ND	ND	→
Opelz (147)	2009	2645	153	ND	NA	CDC T	No (2000-07)	ND	ND	→
Goh (166)	2010	139	33	AB DR	SAB	CDC	No	↔	↔	→
Girmita (167)	2010	73	21	AB DR	ELISA	CDC	No	ND	←	→
Musat (168)	2010	43	17	AB DR	SAB	NA	No	←	←	ND
Dar (169)	2011	6 KL	6	AB DRDQ	SAB	CDC	No	←	←	ND
O'Leary (170)	2011	39	39	AB DR	SAB	NA	No	ND	←	ND
Askar (171)	2011	2484	PRA 30%	ND	PRA	CDC FC	No	ND	ND	→
Lunz (172)	2012	809	100	AB DRDQ	SAB	CDC	No	←	←	ND
Preexisting HLA antibodies in pancreas transplantation										
Bryan (117)	2006	10 (1 KP)	7	AB	F-PRA	FC-T, AHG-B	4/10 FC-T positive XM	3/10 (1 SPK)	ND	↑↓
Melcher (173)	2006	1	1	ABC DRDQ	SAB and dilutions	AHG-T FC-T	Case report	Yes	ND	ND
Preexisting HLA antibodies in intestine and multivisceral transplantation										
Kato (174)	2006	I 16, M 21	16, M 21	ABDR	F-PRA	CDC	No	ND	←	→
Sindhi (175)	2010	I 103	I 103	ABDR	CDC-PRA	CDC	No	ND	ND	→
Ruiz (176)	2010	I 11	I 1	AB DR	Flow	CDC FC	No	←	ND	ND
Wu (177)	2010	I 134, L+I 76	53	AB DRDQ	ELISA, SAB	CDC	No	←	←	→
Ashokkumar (178)	2010	I 70	ND	ABC DRDQ	ELISA, SAB	CDC	No	ND	←	ND
Tsai (179)	2011	I 4, M 11	9	AB DR	SAB	NA	No	ND	←	ND
Preexisting HLA antibodies in islet cell transplantation										
Mohanakumar (180)	2006	7/12	3/7	AB DR	ELISA, F-PRA	CDC, FC T&B	No	ND	ND	→
Campbell (181)	2007	81/151	11	ABC DRDQ	F-PRA, FSA	AHG-CDC, FC T&B	No	ND	ND	→
Cardani (182)	2007	66 (40 I, 17 IAK, 9 SIK)	7/66 (6 I-BMT), 1-ITA	AB DR	ELISA, LSC	CDC	No	ND	ND	NS
Naziruddin (183)	2011	303	NA	ND	27 centers CDC, ELISA, F-PRA and Luminex	NA	PRA used as a surrogate for sensitization	ND	ND	↔

All studies were retrospective; data on survival and rejection not available.

AHG, anti-human immunoglobulin; AMR, antibody-mediated rejection; BMT, bone marrow transplantation; CDC, complement-dependent lymphocytotoxicity; CR, cellular rejection; DD, deceased donor; DSA, donor-specific HLA antibodies; F, flow; FC, flow cytometry; GS, graft survival; HI, highly immunized; I, intestine alone; IAK, islet and kidney; ITA, islet transplantation alone; KL, combined kidney-liver transplantation; KP, combined kidney-pancreas; L, liver; LD, living donor; LMix, Luminex mix; LSC, Luminex screen; M, multivisceral; NA, not applicable; ND, not determined; Ped, pediatric; PRA, panel reactive antibody; SAB, single-antigen beads (Luminex or flow); SIK, sequential islet and kidney; XM, crossmatch; ↑, increased; ↓, decreased; ↔, no difference.

Although there are no randomized clinical trials giving clear evidence for the impact of preexisting antibodies on heart transplantation outcome, several reports strongly suggest that allosensitization in patients awaiting heart transplantation remains a significant problem largely due to increasing rates of sensitization that result from the administration of blood products after the insertion of assist devices and, in pediatric patients, after the use of allograft tissue in congenital heart surgery (*150, 151*). Sensitization, in particular, the presence of DSA, has been shown to be associated with a higher incidence of rejection and inferior graft outcome (*70, 140–153*) and there is evidence that complement fixing DSA are detrimental for graft outcome (*70, 148*). Recent reports indicate that the successful depletion of alloantibodies can lead to transplantation of highly sensitized heart recipients without HAR (*145, 152*).

There are reports of HAR after lung transplantation across positive XM. Therefore, preexisting antibodies to HLA are generally viewed as detrimental in lung transplantation (*154–157, 160*). Although no evidence is available on the impact of PRA on clinical outcome, a large study reported that PRA of more than 25% has significant negative prediction toward successful functioning of lung allografts (*158*), and in two other studies, pretransplantation desensitization therapy has been associated with improvement in clinical parameters, including acute rejection and bronchiolitis obliterans (*155, 159*).

In liver transplantation, the impact of allosensitization on outcome is still controversial, and pretransplantation HLA testing and cross-matching are currently not routine procedures. More recent studies, however, indicate that pretransplantation cross-matching may be relevant in the setting of liver transplantation and that especially a positive T-cell XM and the presence of pretransplantation DSA are associated with poor graft survival (*147, 162–170, 172*). Preformed HLA antibodies were also associated with poorer survival of retransplants (*166*). Lunz et al. (*172*) found in a recent study that acute cellular rejection was more common in patients who were DSA and XM positive and demonstrated C4d positivity in the biopsies of these patients as early as 3 weeks after transplantation.

In combined liver-kidney transplantation, it has been proposed that the liver will offer immunologic protection against rejection of the kidney allograft (*161*). However, recent studies including a large analysis of over 2484 combined liver-kidney transplant recipients indicate that presensitization has a negative impact on both overall patient survival and kidney graft survival (*163, 165, 169, 171*). Although in sensitized recipients of combined liver kidney transplants the liver was thought to be protective and prevent AMR in the presence of preformed HLA antibodies (*161*), in more recent studies, preformed DSA were reported to promote AMR in the kidney (*165*) and it was shown that, in sensitized patients with preformed class II DSA, the liver may not be fully protective (*169*).

There are limited data on the impact of pretransplantation antibodies on pancreas graft function. Most pancreas transplants are performed as simultaneous pancreas-kidney transplants and the data in this group have mostly focused on the kidney because the pathologic criteria for AMR in the kidney were more clearly defined (*117*). Early graft loss from thrombosis is not uncommon in pancreas transplantation, but hyperacute or accelerated acute rejection may not be considered a factor in early graft loss, and as a result, the impact of preexisting antibody against donor HLA may have been underestimated (*173*). The criteria for diagnosis of AMR in the pancreas have only recently been established. This will allow for better diagnosis of AMR and will lead to clarification of the role of antibody in the risk for AMR and impact on graft outcomes (*184*).

Data on intestinal transplantation are limited. In isolated intestinal allografts, preformed HLA antibodies have been shown to have significant adverse effects on allograft survival (*174, 175*). Furthermore, a case of immediate AMR was also documented in an intestinal transplant with DSA and positive XM (*176*), and pretransplantation and posttransplantation de novo DSA were significantly associated with the frequency and severity of acute cellular rejection (*177–179*).

Whereas two studies reported that pretransplantation DSA detected by flow beads and or CDC were associated with inferior transplant survival of islet cell transplants (*180, 181*), in another study, the same association could not be found conceivably because the patients were often transplanted in conjunction with a kidney or received donor bone marrow with the islet infusion (*182*). In a multicenter registry report, pretransplantation PRA (DSA was not determined) was shown not to be predictive of islet cell transplant failure, whereas posttransplantation PRA was associated with poor outcome (*183*). Although there are few data available, the studies that measured DSA in islet cells alone recipients showed a negative impact of preexisting HLA antibodies.

Impact of Pretransplant Non-HLA Antibodies on Organ Transplantation Outcomes

Although, as shown in Table 7, several groups reported on a possible impact on transplantation outcomes of pretransplantation antibodies against non-HLA targets, such as MICA, endothelial cell antigens, islet cells antigens, collagen, K-α1 tubulin, cardiac myosin, and vimentin, at present, there are no studies giving clear evidence for a strong role of such non-HLA antibodies in solid organ or islet cell transplantation (*91, 92, 108, 111, 112, 185–212*). An important group of non-HLA antibodies are the isoagglutinins to ABO blood group antigens. Traditionally, ABO-incompatible transplants have been avoided but currently numerous living-donor transplants are performed across blood group mismatches, and many studies indicate that conventional immunosuppression can achieve comparable outcomes to ABO-compatible transplants provided that isoagglutinin titers are reduced pretransplantation (*192, 193, 196, 197, 206*).

In kidney transplantation, results obtained in a large cohort of patients from the Collaborative Transplant Study Group (*187*) indicated that, even if the donor was an HLA-identical sibling, a significant number of patients with high panel reactivity pretransplantation had lower graft survival, suggesting a role for immune responses to non-HLA antigens in allograft rejection. The presence of preexisting antibodies to MICA has been shown to correlate with kidney graft outcome in some reports (*186, 188, 194, 198, 199*) but not in other

TABLE 7. Impact of preexisting non-HLA antibodies on organ transplantation outcomes

First author (reference)	Year	n	Antibody+ (n)	Antibody detected	Method	Preselection	AMR	CR	GS	EL
Preexisting non-HLA antibodies in kidney transplantation										
Shin (185)	2001	58	NA	AECA	ELISA	No	↑	ND	ND	II-2
Sumitran-Holgersson (186)	2002	139	20	MICA	FC	No	↑	ND	→	II-2
Opelz (187)	2005	4048	803 (PRA 1–50%), 244 (PRA >50%)	Non-HLA	PRA	HLA-id	ND	ND	↓ At 10 yr	II-2
Zou (188)	2007	1910	217	MICA	Luminex	No	↑	ND	→	II-2
Grandtnerová (189)	2008	2	2	AECA	FC	Case Report	↑	ND	ND	II-2
Han (190)	2009	392	62	AECA	Cell-based ELISA	No	↑	ND	ND	II-2
Breimer (92)	2009	147	35	AECA	FCXM-ONE	No	↑	ND	ND	II-2
Ismail (191)	2009	60	40	AECA	Cell-based ELISA	No	↑	↑	→	II-2
Montgomery (192)	2009	60	60	ABO	Tube	ABO-i	↑	ND	↕	II-2
Toki (193)	2009	57	57	ABO	Latex Agglutination	ABO-i	↑ Only if titer >1:32	ND	↕	II-2
Reinsmoen (108)	2010	97	32	AT1R	ELISA	No	↑	ND	ND	II-3
Narayan (194)	2011	1	1	MICA	Luminex	Case report	↑	ND	ND	II-3
Kerman (195)	2011	52	NA	AT1R	ELISA	No	↑	ND	→	II-2
Jackson (91)	2011	60	14	AECA	FC	No	↑	ND	ND	II-2
Flint (196)	2011	37	37	ABO	Tube	ABO-i	↕	ND	↕	II-2
Chung (197)	2011	14	14	ABO	Tube	ABO-i	↕	↑	→	II-2
Cox (198)	2011	442	17	MICA	Luminex	No	ND	ND	ND	II-2
Solgi (199)	2012	40	8	MICA	FC	No	↕	ND	↕	II-2
Preexisting non-HLA antibodies in heart transplantation										
Latif (200)	1995	129	29	Myosin	SDS, Western blot	No	↑	ND	ND	II-2
Warraich (112)	2000	117	32	Myosin	ELISA	Yes, patients with DCM	↑	ND	ND	II-2
Morgun (111)	2004	41	NA	Myosin	ELISA	No	ND	ND	→	II-2
Suarez-Alvarez (201)	2006	31	2	MICA	CDC, Luminex	No	↕	ND	ND	II-2
Suarez-Alvarez (202)	2007	44	3	MICA	CDC, Luminex	No	↕	ND	↕	II-2
Roche (203)	2008	21	21	ABO	NA	ABO-I Ped	↕	ND	→	II-2
Smith (204)	2009	616	69	Non-HLA	CDC, Luminex	No	ND	ND	↑	II-2
Smith (205)	2009	491	70	MICA	Luminex	No	↕	ND	↕	II-2
Dipchand (206)	2010	80	35	ABO	Agglutination	Ped	↕	ND	↕	II-2
Preexisting non-HLA antibodies in lung transplantation										
Smith (207)	1995	85	27	AECA	CDC	No	↕	ND	→	II-2
Iwata (208)	2008	10	NA	ColV	ELISA	Nested case control assessed PGD	ND	ND	ND	II-3
Bharat (209)	2010	142	41 total, 33 (ColI), 30 (ColV)	ColI, ColV, Kα1T	ELISA	No	ND	↑	ND	II-2
Preexisting non-HLA antibodies in islet cell transplantation										
Jaeger (210)	1999	10 IAK, 6 IK, 5 I	6 GAD 2 ICA	GAD, ICA	RIA	No	ND	ND	→	II-2
Huurman (211)	2000	21	10 (4 GAD+IA-2, 3 GAD alone, 3 IA-2 alone)	GAD, IA-2	RIA	No	ND	ND	↕	II-2
Bosi (212)	2001	33 IAK, 3 I	1	GAD, IA-2	RIA	No	ND	ND	↕	II-2

ABO; ABO-incompatible; AECA, anti-endothelial/epithelial cell antibody; AMR, antibody-mediated rejection; AT1R, angiotensin II type 1 receptor; DCM, dilated cardiomyopathy; EL, evidence level; CDC, complement-dependent lymphocytotoxicity; ColI, anti-collagen 1; ColV, anti-collagen V; CR, cellular rejection; FC, flow cytometry; GAD, antibodies against glutamic acid decarboxylase; GS, graft survival; HLA-id, HLA-identical; I, islet cell transplantation; IA-2, islet antigen 2 antibodies; IHD, ischemic heart disease; IAK, islet cells after kidney transplantation; ICA, islet cell antibodies; IHD, ischemic heart disease; IK, simultaneous islet and kidney transplantation; Kα1T, anti-K-α1 tubulin; MICA, major histocompatibility class I-related chain A; NA, not applicable; ND, not determined; Ped, pediatric; PGD, primary graft dysfunction; RIA, radioimmunoassay; SDS, sodium dodecyl sulfate polyacrylamide gel; XM, crossmatch.

studies (*213*). There are also reports demonstrating that antibodies against angiotensin II type 1 receptor (*108, 195*) and endothelial cell antigens are associated with kidney transplant rejection (*91*).

In heart transplantation, antibodies to cardiac myosin have been shown to be strongly associated with poor graft outcome in several studies (*111, 112, 200*), and in a large study, the presence of lymphocytotoxic IgM autoantibodies in the patient's sera was identified as an independent risk factor for graft rejection (*204*). No association was found between pretransplantation DSA to MICA and function of heart transplants (*205*).

In liver transplantation, data on a possible impact of pretransplantation non-HLA alloantibodies other than ABO isoagglutinins are not available.

In lung transplantation, antibodies to extracellular matrix proteins expressed on lung tissue, such as collagen I and V, and a gap junction–associated protein K-α1 tubulin were significantly associated with higher rates of primary graft dysfunction, development of HLA antibodies, and chronic rejection (*208, 209*). There is also evidence suggesting that antibodies against undefined epithelial cell antigens have a deleterious effect on outcome of lung transplantations (*207*).

In islet cell transplantation, antibodies to glutamic acid decarboxylase, islet cells antibodies, and insulin autoantibodies are commonly seen, and there is evidence that they may correlate with graft outcome either in association with recurrence of original disease or as part of the rejection process (*210–212*).

ANTIBODY TESTING POSTTRANSPLANTATION

It is well accepted HLA DSA are associated with allograft rejection and graft failure of transplanted organs. Considerable experimental and clinical evidence points to a causal effect of DSA. Whether to anticipate and monitor donor-directed antibodies depends on the characteristics, demographics, and risk factors of the patient being transplanted (e.g., primary recipient vs. regraft recipient, male vs. female, nulliparous vs. multiparous, unsensitized vs. sensitized, and transfused vs. nontransfused). In this section, the evidence supporting (or refuting) the clinical rationale for posttransplantation monitoring of solid organ transplant recipients has been tabulated and annotated. Although there has been consensus of this committee on how or whether to proceed in each situation, it was not unanimous. Clearly, there are inherent reasons to support or refute aggressive posttransplantation approaches should patients begin to display DSA that were not originally present or show evidence of graft dysfunction. Part of the controversy on monitoring derives from the lack of validated therapy once DSA are detected in a stable patient. Although it is easy to get caught up in the emotion of posttransplantation monitoring, more evidence-based studies will be essential and critical to reaching uniform consensus.

Introduction

Standard-of-care monitoring of an allograft recipient consists of repeated physiologic assessment of allograft function (e.g., serum creatinine, FEV-1, ECHO, C-peptide, liver enzymes, and bilirubin levels). It is also standard of care to perform a diagnostic biopsy of the allograft when dysfunction arises that cannot be explained by other causes (e.g., obstruction via renal ultrasound or infection via urine culture). This is true for all transplant patients regardless of their a priori risk for rejection based on their pretransplantation assessment.

Currently, two clinical tests are required to make a diagnosis of AMR in kidney recipients: a serum test to detect DSA and a biopsy to detect evidence of antibody-mediated injury in the allograft. For kidney transplantation, the criteria for acute AMR were summarized in the Banff '03 report (*214*), which required both a circulating DSA (to donor HLA or other endothelial antigens) and histology with C4d$^+$ and morphologic evidence of acute tissue injury (i.e., acute tubular necrosis–like minimal inflammation, glomerulitis and peritubular capillaritis, or arterial-transmural inflammation/fibrinoid change). For chronic AMR, the histologic features are TG, multilamination of the basement membranes of the peritubular capillaries, and transplant arteriopathy. The C4d and DSA criteria are the same as for acute AMR (*215*). In contrast to acute AMR, chronic AMR has a long subclinical phase measured in months to years. In 2011, the Banff working group recognized (*216*) that there is growing evidence for C4d-negative AMR in kidney allografts, particularly in the late or chronic phase (*135, 217*). Detection of C4d in the graft microvasculature has the advantage of high specificity for DSA and demonstrates complement fixation at the level of the endothelium, independent of the antigenic target. It has the major disadvantage of requiring local complement fixation by the antibody. Two markers have been shown to detect antibody interaction with the microvasculature, increased endothelial gene expression (*218*), and peritubular capillaritis (*135*). Although these are less specific than C4d for circulating DSA, they may afford increased sensitivity and a Banff working group is looking at further refinement of the pathologic definition of AMR.

The pathologic, clinical, and serologic definition and prerequisites for AMR in the heart are quite different and recently published as an International Society of Heart and Lung Transplantation consensus (*219*). An important paradigm shift from this consensus was that a clinical definition for AMR (cardiac dysfunction and circulating DSA) was no longer believed to be required due to recent publications demonstrating that asymptomatic (no cardiac dysfunction) biopsy-proven AMR is associated with subsequent greater mortality and greater development of cardiac allograft vasculopathy. Additionally, DSA was not always detected during AMR episodes because the antibody may be adsorbed to the donor heart or may be to non-HLA antigens (these limitations also apply to the kidney and other organs). Although AMR occurs in transplanted lungs, livers, pancreas, islets, and multivisceral organs (*164, 168, 170, 220–226*), the criteria to diagnose such rejection are less well established.

If donor-directed antibodies are identified posttransplantation, the question of what to do with this information becomes paramount. Is there suspicion of graft dysfunction (e.g., rising creatinine in a renal transplant recipient, dyspnea or arrhythmias in a cardiac allograft recipient, right upper quadrant and flank tenderness, or elevated liver function tests in a liver transplant patient)? Because there now appears to be reasonable evidence of C4d-negative AMR, what should be done if a C4d test is negative? What if no

donor-directed HLA antibody is detected but a "for cause" biopsy shows histologic evidence of AMR (presumably non-HLA antibodies or adsorption by the graft)? Should treatment to eliminate antibodies that cannot be identified still be initiated? If one of the goals for transplant patients is to minimize immunosuppression, should this be a complete contraindication if DSA are present even if there is no evidence of dysfunction or pathology? Similarly, if DSA are present without any accompanying symptoms of graft rejection, should treatment to eliminate the antibodies be initiated? These and other questions have no simple answers because the data needed for these queries typically do not exist, except for case reports and anecdotes. As such, there are no generally accepted guidelines of posttransplantation monitoring for AMR. In the following sections, evidence-based recommendations to monitor for AMR in solid organ allograft recipients are provided. Recommendations vary depending on whether a patient is considered at low, intermediate, or high risk to present with AMR. For the purposes of this discussion, early posttransplantation was defined as within the first 6 months, whereas late posttransplantation refers to events after 6 months.

Serum HLA Antibody Testing in the Face of Allograft Dysfunction

AMR occurs in 10% to 20% of cardiac allograft recipients and correlates with an increased incidence of hemodynamic compromise, rejection, greater incidence of cardiac allograft vasculopathy, and death (142, 227–229). However, not all solid organ allograft recipients are at equal risk for the development of AMR, at least in the early posttransplantation phase. For example, in renal allograft patients who are transplanted in the face of immunologic memory (e.g., in the presence of DSA pretransplantation), there is an increased incidence of AMR (clinical and subclinical) early posttransplantation in the range of 21% to 55% (127, 130, 230–232). By comparison, in the absence of a DSA pretransplantation, the incidence of early AMR is much lower, which is reported to be 1% to 6% in the first year (127, 130, 230–232). Moreover, in patients with cPRA of 80% or more, the evidence that they are at an increased risk for AMR and graft loss is lacking in the absence of a DSA (16). Therefore, when considering the utility of serum HLA antibody testing early posttransplantation, one must take into consideration the risk profile of the patient in question.

Causes of late allograft loss in kidney transplantation are largely identifiable and primarily immune mediated related to (a) recurrent or de novo autoimmune disease, (b) AMR with chronic injury (TG and peritubular capillary basement membrane multilayering), or (c) interstitial fibrosis and tubular atrophy (IFTA) with or without cell-mediated rejection or polyomavirus nephropathy (217, 233–236). As such, the utility of serum HLA antibody testing may have a greater likelihood of detecting a DSA with the onset of late graft dysfunction (i.e., increase in serum creatinine or new onset proteinuria after 6 months after transplantation). This is especially true when a patient has been documented to be nonadherent to immunosuppression (236, 237).

Protocol Biopsy Screening for Antibody-Mediated Rejection Posttransplantation

For the sake of this discussion, a protocol biopsy is defined as one performed in a stable graft without evidence of physiologic dysfunction (e.g., proteinuria) or in follow-up to a posttreatment intervention. The use of protocol biopsies in cardiac allograft recipients is well accepted (219), but their role in renal and liver transplant recipients is still debated.

Renal and cardiac patients (and likely liver and lung patients) transplanted with a DSA, whether immunomodulated or not pretransplantation, have a higher incidence of AMR early posttransplantation (see above) compared with patients without DSA. Moreover, studies performing protocol biopsies in renal and cardiac patients have found an increased incidence of subclinical AMR. Furthermore, in renal recipients, subclinical TG can be identified, which is associated with chronic injury and late graft dysfunction (120, 127, 238, 239).

In addition to performing protocol biopsies to detect subclinical AMR, protocol biopsies can be conducted after an AMR event to determine the effectiveness of therapy (i.e., persistent C4d$^+$, glomeruli, peritubular capillaries, or vessels) and to identify prognostic indicators of outcome (i.e., TG and peritubular capillary basement membrane multilayering) (230, 236, 240, 241). Conversely, in the absence of a pretransplantation DSA, the likelihood of detecting subclinical AMR early posttransplantation by protocol biopsy in renal transplant recipients is low (127, 236, 242) but may be more readily apparent in hearts (219).

Recently, Wiebe et al. (236) performed "protocol biopsies" in patients in whom the only abnormality was a de novo DSA in the serum; whether these should be considered "protocol" or "for cause" is a matter for debate. Nonetheless, this group demonstrated that 10 of 14 biopsies in stable kidney transplant patients with a de novo DSA had evidence of microvascular inflammation (glomeruli, peritubular capillaries, and vessels with or without C4d$^+$) consistent with subclinical AMR.

The significance of de novo HLA antibody in the absence of de novo DSA HLA antibody is debatable. Groups have reported excellent outcomes in patients with pretransplantation or de novo HLA antibody (16, 236, 243, 244), whereas others have suggested an increased risk for graft loss, citing the DSA as potentially being sequestered in the allograft preventing its detection in the circulation (244–251). What is often debated is the significance of de novo HLA antibodies that are not donor directed. Lacking is a study whereby a protocol biopsy was initiated based on a de novo HLA antibody without a DSA being present to document whether any evidence of antibody-mediated injury is present. It is notable in the serial study by Wiebe et al. (236) that only 9% who developed de novo DSA had an earlier serum where only de novo HLA antibody was detectable, suggesting that de novo HLA antibody is generally not a marker of de novo DSA in the kidney allograft.

Experience with the use of protocol biopsies to study patients undergoing immunosuppression withdrawal is limited. However, a recent study demonstrated that patients who were switched from cyclosporine to everolimus at 3 or 4.5 months after transplantation had a higher rate of de novo DSA and AMR after cyclosporine withdrawal compared with patients maintained on cyclosporine (252). Similarly, nonadherence, a form of immunosuppressive withdrawal, is associated with a higher rate of de novo DSA and accelerated graft loss associated with AMR (236, 237). Taken together, it would suggest that the reduction of immunosuppression could carry an increased risk for AMR.

Serial Serum HLA Antibody Screening Posttransplantation

Kidney patients transplanted in the face of a DSA pretransplantation have an increased incidence of AMR early posttransplantation as well as subclinical AMR and TG (see above). Several groups have demonstrated in such patients that the persistence of, or an increase in, DSA in the serum correlates with a poor graft outcome regardless of whether the early graft function is stable or not (120, 216, 230, 253, 254). Similarly, failure of the DSA to decrease posttreatment for AMR has been associated with a poor graft outcome (253, 255, 256).

The development of de novo DSA after renal transplantation has been associated with increased risk for graft loss in many large series (35, 236, 243–247, 257–259). Of note, in many of these reports, although de novo class I DSA occurs, most patients develop de novo class II DSA directed at donor DR, DQ, and, occasionally, DP antigens.

A recent study followed the evolution of de novo DSA and demonstrated that the mean time to appearance is 4.6 years after transplantation with a tendency to appear sooner in the face of nonadherence (236); the prevalence of de novo DSA at 10 years was 20% in adherent patients and 60% in nonadherent patients. A similar high prevalence of noncompliance in patients with chronic AMR was recently reported (237). In addition to drug minimization or nonadherence, other risk factors for the development of de novo DSA are HLA-DR mismatching, early cell-mediated rejection (both clinical and subclinical), and younger recipient ages (236, 245, 252, 260).

With regard to cardiac transplantation, the deleterious effect of alloantibodies on graft survival, particularly in patients who developed de novo alloantibodies posttransplantation, was recently reported. In a group of 950 patients studied over a 15-year period, graft survival was highest in patients who never developed alloantibodies (70%) or who displayed them only pretransplantation (71%); graft survival was lower in recipients who showed antibodies both pretransplantation and posttransplantation (56%) or only posttransplantation (47%). De novo antibodies appearing more than 1 year after transplantation had the poorest survival. The development of AMR had a significant negative impact on graft survival (16% in AMR vs. 63% in no AMR patients) (261).

Among lung transplant recipients, the presence of HLA antibodies has been associated with persistent recurrent acute rejection (220). Importantly, rejection is the most significant risk factor for the development of bronchiolitis obliterans, which has an incidence as high as 50% posttransplantation (223–225) and is associated with the development of DSA (221). Whether treatment to eliminate/reduce DSA mitigates the risk of lung rejection is still unclear (221). In liver allograft recipients, although HLA antibodies have recently emerged as contributing factors to episodes of acute and chronic rejection (164, 168, 170), whether antibody depletion has any beneficial effect has not yet been reported. This is also true for recipients of other organ transplants.

Other Considerations

Should Non-HLA Antibodies Be Considered for Posttransplantation Assessment?

The question of whether other non-HLA DSA should be considered posttransplantation is one of growing interest.

AMR has been reported, rarely, in HLA-identical sibling renal allografts, presumably due to non-HLA antigenic targets (262, 263). There have been a number of reports of MICA antibodies associated with poor graft survival (188, 264, 265). The prevalence of MICA antibodies after kidney transplantation was 8.9% versus 26.8% for HLA antibodies in one series (264) and MICA antibodies were present pretransplantation in 11.4% of patients in another series (188). The issue with these articles is that donor specificity is not proven and correlation with a pathologic outcome was absent. Therefore, although the data support the association with allograft failure, the hypothesis that MICA antibodies are causal has not been proven.

Dragun et al. (107) reported angiotensin II type 1 receptor activating IgG antibodies in renal allograft rejection. However, it should be noted that these patients tended to have a unique presentation with malignant hypertension, graft dysfunction, and histology showing endarteritis and fibrinoid necrosis that occurred at a median of 4 days after transplantation (range, 2–1217).

AECA of undefined specificity must also be considered as potential mediators of graft rejection. Recent studies have suggested that preexisting and de novo AECA are associated with a risk of early graft rejection (74, 87, 90, 91). Similar to the MICA antibodies described above, the donor specificity of these antibodies has not been conclusively proven.

Recently, groups using protein microarray technology have been reporting the association of autoantibodies (e.g., anti-peroxisomal-trans-2-enoyl-coA-reductase) with the development of TG or IFTA in kidney allografts (95, 97). Whether any of the above antibodies are the cause or consequence of the pathogenesis of TG and IFTA has yet to be determined.

Consensus Recommendations

Technical Group

1) Antibody identification
   a. At least one SPI should be used to detect and characterize HLA class I and II–specific antibodies. A SAB immunoassay should be performed at least once pretransplantation in HLA-immunized patients. This is particularly important for the characterization of antibodies directed at Cw, DQA, DPA, and DPB, which are not adequately defined by other techniques. [1]
   b. Use both SPI and cell-based assays to assess antibody status to the intended donor. [1]
   c. Laboratories must correlate the level of antibody detected by SPI with cell-based assays to establish the likelihood of a positive XM. [1]

2) Standards for cell-based assays (CDC)
   a. CDC assays for antibody identification and cross-matching should be performed using target cells that permit identification of antibodies to both HLA class I and II antigens. [1]
   b. Nonspecific reactivity must be recognized. [1]
   c. Consider modifications to increase sensitivity and specificity including wash steps, changes in incubation times, addition of antiglobulin, and serum modification steps to remove or inactivate IgM and C1. [2]

3) Standards for flow cytometry cell-based assays (flow cytometry)
   a. Differentiation of T and B cells should be performed by a three-color fluorescence technique. [1]
   b. Consider modifications such as Pronase use to increase sensitivity and specificity. [3]
4) Standards of practice
   a. The laboratory performing tests on transplant patients must have documented expertise in antibody assessment and interpretation. [1]
   b. Each laboratory must establish its own threshold for antibody specificity assignment and clinical interpretation. [1]
   c. Each center should define changes in MFI values between sera from the same patient that are clinically meaningful. [2]
   d. The patient history must be considered for the interpretation of antibody screening and interpretation of test results. Factors include the history of parity in female patients and previous graft HLA mismatches. Such information indicating a possible state of presensitization despite low levels of antibodies can put greater clinical emphasis on low-level antibodies than would normally occur. Consideration of prior immunologic history can also assist in the recognition of naturally occurring antibodies to denatured HLA antigens in patients who have no obvious cause of sensitization. [1]
   e. In determining antibody specificity, the laboratory should consider the possibility of antibodies to epitopes on any polymorphic chains (including DQA and DPA) as well as epitopes resulting from combinations of different α and β chains. [2]
   f. HLA typing of donor and recipient must be performed at a level required for accurate antibody interpretation. [1]
   g. Store donor material in the form of frozen cells and DNA for posttransplantation DSA investigations. [1]
5) Interfering factors in interpretation of SPI
   a. Consideration must be given to the following variables when performing and assessing HLA antibody results: antigen density on beads and condition (i.e., denatured Ag); reactivity of control sera and control beads; reduction of test interference (i.e., EDTA, DTT, and hypotonic dialysis); and when saturation of target antigens may have occurred, sera should be tested under conditions where meaningful changes in antibody levels can be detected (e.g., serum dilutions). [1]
6) Assay standardization
   a. Laboratories should follow standardized operating procedures and policies that minimize test variability including, wherever possible, robotic processing, temperature control, consistency in washing procedures, and instrument calibration. [1]
   b. Quality-control procedures must be introduced to monitor interassay and intraassay variability. [1]
   c. Each laboratory must participate in relevant external proficiency testing programs as required by local, regional, and national regulations. [1]
7) Reporting of results
   a. The following points should be included in the reporting format:
   1. Sample and assay dates
   2. Assay name

3. Calculated reaction frequency/cPRA/virtual PRA indicates the frequency of donors with unacceptable HLA antigen mismatches.
4. Specificity assignment and assessment of antibody level. Note that SPI have not been approved for reporting of quantitative measurements. As such, MFI values do not necessarily reflect antibody titer.
5. Comments on presence/absence of DSA if a specific donor is being assessed
6. Immunoglobulin class and isotype if available
7. Assay or serum modification employed. [3]

Pretransplantation Group

8) Transplantation risk stratification categories should be developed based on antibody identification and XM results. [3]
9) Information regarding prior sensitizing events should be considered in interpreting antibody testing results. [2]
10) DSA detected by CDC antibody screening and crossmatching in the most recent serum collected must be avoided because they are associated with a high risk for AMR and graft loss. [1]
11) To minimize risk of sensitization and antibody-mediated allograft damage, administration of blood products pretransplantation should be avoided if possible. [1]
12) When a patient is sensitized, precise characterization of HLA antibodies and complete HLA typing of the donor pretransplantation must be performed. [1]
13) HLA antibody screening should be performed at a frequency that accommodates the likelihood of an imminent transplantation based on local waiting times and the immunologic risk of adverse outcome such as in highly sensitized patients. [3]
14) A minimum of two sera obtained at different time points should be tested to confirm presence or absence of HLA antibodies. [3]
15) Sera should be tested after known sensitizing events, proinflammatory events, and at regular intervals once listed for transplantation. [1]
16) Kidney
   a. Unacceptable HLA antigens should be a part of kidney allocation algorithms. [2]
   b. Accurate XM prediction depends on complete HLA typing. To minimize the incidence of unexpected positive XM in paired exchange registries, the donor should be typed at HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA, HLA-DQB, HLA-DPA, and HLA-DPB loci. [2]
   c. A renal transplantation can be performed without a prospective pretransplantation CDC or flow XM if SAB testing indicates the consistent absence of DSA against HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA, HLA-DQB, HLA-DPA, and HLA-DPB locus antigens. Each center needs to develop its policy in agreement with regulatory bodies and clinical programs. [3]
   d. Risk assessment should include HLA antibody specificities identified in historic sera. [3]
   e. In renal transplantation, if DSA is present but the CDC XM against donor T and B cells is negative, this should

be regarded as an increased immunologic risk but not necessarily a contraindication to transplantation, especially after elimination of DSA by desensitization. [2]

f. To optimize access to transplantation of highly sensitized recipients, inclusion of patients in special programs, such as kidney paired donation, AM, or desensitization, should be considered. [1]

g. HLA matching should be part of the allocation procedures to reduce the probability of developing HLA antibody, rejection, and graft loss. [2]

h. ABO incompatibility is no longer an absolute contraindication in kidney transplantation and ABO-incompatible transplants can be successfully performed in recipients in whom isoagglutinin titers have been lowered to acceptable levels. [1]

i. Based on current evidence, no recommendation can be made for routine pretransplantation testing for non-HLA antibodies other than ABO. [2]

17) Heart

a. In both pediatric and adult heart transplantation, determination of pretransplantation DSA must be performed because it is critical to improve short-term outcomes and preventing early acute rejection. [1]

b. Desensitization therapy should be considered in sensitized heart transplant recipients. [2]

18) Lung

a. Pretransplantation DSA in recent serum should be avoided in lung transplantation whenever possible. [1]

19) Liver

a. The liver allograft may be partially resistant to antibody-mediated damage; however, high-level DSA antibody may be associated with inferior outcomes and should be considered as a risk factor for graft dysfunction. [2]

b. Pretransplantation screening for HLA antibodies is recommended in liver transplant recipients for risk stratification. [3]

c. Donor tissue should be collected and stored in liver transplantation. [3]

d. An XM should be performed in sensitized liver transplant recipients. [2]

e. In sensitized recipients of combined liver-kidney transplantation, the liver may not confer full protection for preventing AMR in the kidney and should be included in risk assessment. [2]

20) Pancreas

a. Recommendations for kidney transplantation should apply to the pancreas for simultaneous pancreas-kidney transplantation. [1]

b. Pancreas is at risk for AMR and pretransplantation DSA should be avoided whenever possible. [1]

c. In pancreas transplantation, AMR should be considered in the differential diagnosis of early graft thrombosis and graft dysfunction. [2]

21) Intestine

a. In intestinal transplantation, pretransplantation HLA antibodies should be determined. The risk assessment should be based on the level of DSA. [2]

22) Islets

a. Based on the available literature, pretransplantation DSA are associated with impaired islet cells function posttransplantation and should be avoided. [2]

Posttransplantation Group

23) Pretransplantation

a. DNA must be available on all donors for identification of donor antigens. This is essential for accurate DSA assessment. [1]

b. Store frozen pretransplantation serum from recipients (acceptable is −20°C; recommended is −80°C). The most current serum is acceptable; day of transplant serum recommended. [1]

24) Posttransplantation (months 0–12)

a. Very high risk patients (desensitized): These patients are recognized to be at high risk for early clinical or subclinical AMR and as such are treated with a desensitization protocol. Such protocols are not standardized and are center specific. Monitor DSA and conduct protocol biopsies in the first 3 months after transplantation. [1]

1. If there is evidence of clinical or subclinical AMR, the patient should be treated. Efficacy of treatment is reflected as normal graft function and is associated with a reduction of DSA levels (*253*, *266*). [2]

2. If there is a rapidly increasing level of DSA accompanied by a biopsy showing no rejection, initiation of therapy to reduce the DSA levels should be considered. [3]

b. High-risk patients (DSA positive/XM negative): These patients are recognized to be at risk for early clinical or subclinical AMR. Monitor DSA and conduct a protocol biopsy in the first 3 months after transplantation. [1]

1. If biopsy is positive for AMR, the objective is to treat. Efficacy of treatment is reflected as normal graft function and is associated with a reduction of DSA levels (*253*, *266*). [2]

2. If there is a rapidly increasing level of DSA accompanied by a biopsy showing no rejection, initiation of therapy to reduce the DSA levels should be considered. [3]

3. DSA persists in the absence of biopsy proven rejection, immunosuppression should not be reduced and additional monitoring should be considered. [3]

4. If the DSA and biopsy are negative, follow as if low risk (see d.1.) unless there is an inflammatory event, in which case additional monitoring for DSA is recommended. [2]

c. Intermediate-risk patients: Includes history of sensitization to donor antigen(s) by CDC and SPI but currently negative and history of sensitization with at least one positive test for HLA antibodies. Monitor for DSA within the first month. [2]

1. If a DSA present, then perform a biopsy. A biopsy is recommended because of published data that document an association between DSA and clinical or subclinical rejection. [2]

2. If biopsy is positive for rejection, the objective is to treat. Efficacy of treatment is reflected as normal graft function and is associated with a reduction of DSA levels (*253*, *266*). [2]

3. In the absence of biopsy-proven rejection, additional DSA monitoring should be considered within the first year. [3]

4. Patients with a DSA in the absence of biopsy-proven rejection should not be considered for reduction in immunosuppression. [3]

5. In the absence of a DSA follow-up as if low risk (see d.1.). [2]

d. Low-risk patients (nonsensitized, first transplantation)

1. Screen for DSA under the following circumstances:

a) at least once 3 to 12 months after transplantation. [2]

b) whenever significant change in maintenance immunosuppression is considered (e.g., minimization/withdrawal/conversion). [2]

c) suspected nonadherence. [2]

d) graft dysfunction. [2]

e) before transfer of care to a remote center outside the transplant center. [3]

2. If DSA present, then perform a biopsy. A biopsy is recommended because of published data that document an association between DSA and clinical or subclinical rejection. [2]

3. If the biopsy is positive for rejection the objective is to treat. Efficacy of treatment is reflected as normal graft function and is associated with a reduction of DSA levels (253, 266). [2]

4. In the absence of biopsy-proven rejection additional DSA, monitoring should be considered within the first year. [3]

5. Patients with a DSA in the absence of biopsy-proven rejection should not be considered for reduction in immunosuppression. [3]

6. If no DSA present, then no additional testing in the first year is recommended in the absence of circumstances listed under point 1 above. [2]

25) Posttransplantation (month 12 onward)—applies to all risk categories

a. Store at least one serum sample per year (i.e., on the transplantation anniversary). [3]

b. Evaluate DSA in a current serum if any of the following conditions occur:

1. Significant change in maintenance immunosuppression is considered (e.g., minimization/withdrawal/conversion). [2]

2. Suspected nonadherence. [2]

3. Graft dysfunction. [2]

4. Before transfer of care to a remote center outside the transplant center. [3]

c. If de novo DSA present or if there is an increase in previous DSA levels, perform a biopsy. A biopsy is recommended because of published data that document an association between DSA and clinical or subclinical rejection. [2]

1. If biopsy is positive for AMR, the objective is to treat. Efficacy of treatment is reflected as normal graft function and is associated with a reduction of DSA levels (253, 266). [2]

2. If biopsy is negative (no sign of rejection) monitor the DSA and monitor for a change in graft function. [3]

3. Patients with a DSA even without biopsy proven rejection should not be considered for reduction in immunosuppression. [3]

Note regarding low-risk patients: The recommendations of posttransplantation monitoring beyond the first year represent the majority opinion of the group. There was, however, a minority dissenting voice that supported at least annual testing on the collected sample(s). The rationale of the minority was that early detection of DSA would allow the clinician to optimize patient care (e.g., perform a biopsy and avoid immunosuppressive minimization). The majority voice focused on the high cost of additional testing on all patients when the anticipated incidence of de novo DSA is less than 5% per year coupled with the lack of proven effectiveness of early intervention for late AMR (267).

Additionally, the aforementioned recommendations refer to noncardiac transplantation monitoring. In the case of low-risk heart transplant patients, the reader is referred to the recently published consensus article in the *Journal of Heart and Lung Transplantation* (219).

Recommendation grades are shown in brackets at the end of each statement. (See Introduction for explanation of grades.) The grades are based on those outlined by Uhlig K, McLeod A, Craig J et al. Grading evidence and recommendations for clinical practice guidelines in nephrology: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2006;70:2058.

Future Directions and Research

Technical Group

1. A coordinated search for a selected bank of HLA reference antibodies to be used for assessing the interlaboratory variability in SPI antibody testing should be undertaken. Antibodies to MICA and relevant non-HLA antigens should be included.

2. The C1q modified single-antigen antibody identification requires more research and validation to understand its application to risk assessment and monitoring efficacy of treatment.

3. If production of the SPI donor-specific XM kit is continued, further research will be required to establish the clinical role of such a test. Specifically, the sensitivity of the XM in relation to SAB testing requires further investigation.

4. The clinical endothelial cell XM for the detection of anti-endothelial antibodies needs further investigation.

5. SPI for the detection of non-HLA anti-endothelial antibodies require development.

6. Multicenter studies are required to establish the clinical utility of testing for antibodies to tissue-specific non-HLA antigens such as the angiotensin II type 1 receptor. Further development of SPI for the detection of these antibodies is warranted based on initial findings.

7. The role of antibodies to epitopes found on HLA-E merits further investigation.

8. The short-term and long-term clinical effect of low levels of HLA antibodies detected by SPI requires further investigation.

Pretransplantation Group

9. More systematic studies for immunologic risk assessment that include long-term outcome data are required.

10. Randomized controlled studies to analyze the efficacy and safety of different desensitization protocols are required.

11. Additional studies are needed to determine the role of preexisting complement fixing versus noncomplement fixing antibodies to HLA and their role in organ transplantation.
12. Efficacy of desensitization in achieving good long-term graft survival needs to be established in heart transplantation.
13. Efficacy of desensitization in achieving good long-term graft survival needs to be established in lung transplantation.
14. More systematic studies are required on the impact of DSA on pancreas transplant alone or in combination with a kidney.
15. Detailed analysis is required to establish the impact of pretransplantation DSA on outcomes in intestine and multivisceral transplantation.
16. Further studies are needed to determine the risk of preexisting DSA in islet cell transplantation.
17. Standardization of the methodology for determining ABO isoagglutinin titers is required.
18. Future studies are required to define the role of preexisting antibodies to non-HLA and self-antigen on the outcome of solid organ and cellular transplantations.

Posttransplantation Group

A need is recognized for the following:

19. Serial screening of serum to determine timing of onset of de novo DSA before onset of graft dysfunction.
20. Protocol biopsies at first appearance of de novo DSA to document pathologic correlation.
21. Assessment of DSA for complement fixing activity and correlation with clinical events (e.g., DSA C1q binding and IgG subclass specificity of DSA).
22. Clinical trials that include serial DSA monitoring posttreatment and posttreatment biopsies to correlate DSA levels with histologic response to therapy and long-term outcome.
23. Clinical trials to prevent production of DSA.

Future Directions

Although DSA are routinely found in patients who experience immunologic graft loss, whether the antibodies are causal or a consequence of other process(es) was, at least until recently, an unanswered question. As such, it has been a challenge to know what, if anything to do when antibodies are identified posttransplantation. Clearly, reemergence of DSA that were present immediately pretransplantation, which required the recipient to be desensitized in order for the patient to be transplanted, requires an immediate response to try and prevent a catastrophic event.

The response of any given patient to therapy can span a huge spectrum, ranging from complete elimination of DSA and the accompanying symptoms of graft rejection to a failure to eliminate or even reduce the DSA while the patient steadily proceeds to graft rejection. Studies over the past several years have indicated that certain patients (e.g., those with pretransplantation CDC titers of DSA >1:16) (268) do extremely poorly after transplantation even when the antibodies appear to be completely eliminated pretransplantation after desensitization and therefore should not be entered into a desensitization program. Thirty percent of patients who undergo desensitization still experience AMR and are at risk to develop TG and ultimately graft loss (269). To date, there is no predictive test to identify into which category (the 70% of patients who do well or the 30% who experience AMR) the patient will fall (269). Newly developed tests aimed at detecting and quantifying memory B cells (270) and plasma cells (271) that produce DSA may eventually be used to help categorize a patient's posttransplantation risk to have transplant threatening DSA reappear. But until those tests become reliable, the risk of any given desensitized patient cannot be accurately assessed. So, too, is the risk of any posttransplantation patient to develop DSA. Collective experience indicates that 15% to 20% of patients will develop de novo DSA posttransplantation (236, 272). How frequently posttransplantation candidates without pretransplantation DSA should be monitored and what to do if antibodies are identified are "hot button" questions.

Experimental studies undeniably document the pathologic role of DSA in allograft rejection in animal models. One of the most convincing studies was described by Russell et al. (273) who used a severe combined immunodeficient mouse model of heart transplantation where cellular rejection was obviated. They observed that the passive transfer of alloantisera containing antibodies to donor histocompatibility antigens led to the development of obstructive coronary lesions, the equivalent of transplant coronary artery disease in humans. The result of this study cannot be extrapolated to humans and although the data supporting the humoral theory of rejection are indirect and subtler, the collective data have become quite compelling (reviewed in (272) particularly when using SPI approaches to identify donor-directed HLA antibodies). Specifically, DSA are found in most of the kidney (249, 258, 274), heart (65, 275), lung (275), islet (226), and liver (164, 168) transplant patients who have rejected their grafts, and most studies demonstrate that the appearance of DSA precedes graft loss, lending credence to the tenet that these antibodies, although not necessarily causal of rejection, are not a consequence of rejection. Although recent studies have examined the effect of antibody depletion (with bortezomib and combination therapy with plasmapheresis/IVIg and rituximab) and have shown to curtail the symptoms of AMR (266), the long-term benefit of such treatment has not been documented.

A recent FDA workshop that focused on the treatment and prevention of AMR raised a number of concerns about how data are generated, interpreted, and used (267). The need to standardize and refine antibody detection assays was recognized to be paramount. Although such tests are currently being used to define DSA (and, by extension, diagnose AMR), they are far from perfect. Although there is a desire to use these tests to quantify antibodies and to monitor the effectiveness of therapy, it was noted that these assays have not been validated or approved for quantitative assessment (267). Finally, emerging (although controversial) data suggest that the analysis of antibody function (e.g., complement fixation) may be a useful indicator of AMR risk (71, 276).

Determining clear surrogate endpoints for effective AMR treatment has also been called for by the FDA; whether antibody levels can serve this purpose is as yet unclear. Compounding the problems is the lack of an effective treatment option for late-developing AMR (241). Can early

intervention prevent graft loss due to AMR? Answering that question will ultimately require multicenter randomized control trials, which is exactly what the participants in the FDA workshop on AMR concluded. It is only with such foresight that effective strategies can be developed to treat, reverse, and prevent AMR.

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Appendix
The composition of the three working groups who contributed to this consensus report is as follows:

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3. Posttransplantation Group
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