REVIEW

OPEN

Clinically relevant interpretation of solid phase assays for HLA antibody

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Purpose of review
Accurate and timely detection and characterization of human leukocyte antigen (HLA) antibodies are critical for pre-transplant and post-transplant immunological risk assessment. Solid phase immunoassays have provided increased sensitivity and specificity, but test interpretation is not always straightforward. This review will discuss the result interpretation considering technical limitations; assessment of relative antibody strength; and the integration of data for risk stratification from complementary testing and the patient's immunological history.

Recent findings
Laboratory and clinical studies have provided insight into causes of test failures – false positive reactions because of antibodies to denatured HLA antigens and false negative reactions resulting from test interference and/or loss of native epitopes. Test modifications permit detection of complement-binding antibodies and determination of the IgG subclasses. The high degree of specificity of single antigen solid phase immunoassays has revealed the complexity and clinical relevance of antibodies to HLA-C, HLA-DQ, and HLA-DP antigens. Determination of antibody specificity for HLA epitopes enables identification of incompatible antigens not included in test kits.

Summary
Detection and characterization of HLA antibodies with solid phase immunoassays has led to increased understanding of the role of those antibodies in graft rejection, improved treatment of antibody-mediated rejection, and increased opportunities for transplantation. However, realization of these benefits requires careful and accurate interpretation of test results.

Keywords
antibody-mediated rejection, HLA epitopes, HLA-specific antibodies, solid phase immunoassays

INTRODUCTION

Over the last two decades, there has been a steadily increasing understanding of the role of HLA antibodies in allogeneic transplantation. For more than 35 years, cell-based assays were the universal method for determining antibody specificity and strength. However, these assays were not specific for HLA antibodies, were time consuming, and required a sufficient supply of viable lymphocytes. These problems led to unexpected positive crossmatches, impeded the development of desensitization and organ sharing programs, and delayed effective treatment of unrecognized antibody-mediated rejection. The development of solid phase immunoassays (SPI) in the mid-1990s, starting with enzyme-linked immunosorbent assay using soluble HLA targets, represented a quantum level improvement in antibody testing. However, these assays were quickly replaced when multiplexed bead testing on the Luminex platform became available. These tests offered even greater sensitivity, specificity, speed, and sample efficiency. Luminex-based assays, using microbeads coated with soluble HLA protein as targets, are currently the most widely used. SPI use has supported great advances in transplantation such as virtual crossmatches [1] and desensitization protocols [2]. However, technical limitations both common to all serologic assays

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KEY POINTS

- The use SPI has advanced our understanding of the role of HLA antibodies in allograft rejection and led to improved treatment of antibody-mediated rejection and increased opportunities for transplantation.
- The interpretation of test results is challenging because of technical limitations and the complexity of the HLA antibody mediated immune response.
- Integration of data from SPI, other complementary testing, and the patient's immunological history are fundamental for accurate pre-transplant and post-transplant immunological risk assessment.

and unique to the bead based assays have manifested [3,4▪]. These limitations when considered together with the complexity of data analysis indicate the need of considerable expertise for accurate result interpretation [5▪]. Furthermore, the clinical impact of antibodies detected exclusively by SPI, though being actively investigated, is not yet fully understood.

HLA ANTIBODY TESTING: CELL-BASED VERSUS SOLID PHASE ASSAYS

Cell-based assays are currently used mainly for donor-specific crossmatches, in which recipient's serum is incubated with donor's cells followed by cell-bound antibody detection. The basic complement-dependent-cytotoxicity crossmatch (CDCXM), used without the addition of an antihuman globulin (AHG), detects complement-activating antibodies. In the more sensitive flow cytometric crossmatch (FCXM), fluorochrome-conjugated AHG is used to detect all cell-bound antibodies. Main disadvantages of cell-based assays are the need of viable lymphocytes, low sensitivity, low specificity because of detection of non-HLA antibodies, and test result variability even within centers.

Antibody screening and characterization is currently done mainly by Luminex-based assays. Main advantages are high specificity and sensitivity, semi-quantitation, multiplexing capability, and the opportunity for automation. The targets are suspension arrays of differentially fluorescently labeled microbead populations coated with soluble HLA antigens. After incubation with patient's serum and addition of AHG conjugated with a reporter fluorochrome, HLA bound antibody is detected by analysing the beads with a flow-cytometry based Luminex analyser. The mean fluorescence intensity (MFI) of the reporter signal is used to make a semi-quantitative estimation of antibody level. Kits come in three formats according to their targets: antigens pooled from multiple cell lines (screening assays), panels of individual class I or class II phenotypes (phenotype beads), and panels of HLA class I or class II antigens, in which each bead population is coated with multiple copies of a single recombinant antigen (single antigen beads or SABs). Screening assays are used for detecting HLA class I or II antibody presence or absence and for monitoring changes in antibody level. Phenotype and SAB beads are used for assessing antibody specificity and level [3].

SOLID PHASE ASSAYS: STRENGTHS AND WEAKNESSES

SAB arrays include alleles of the HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1 loci. They are instrumental for definition of antibody specificities in sera containing a complex mixture of HLA antibodies, identification of epitope specificity, and detection of HLA-Cw, HLA-DQA, HLA-DQB, HLA-DPA, and HLA-DPB antibodies as well as antibodies specific for epitopes resulting from specific DQ or DP α and β chain combinations. Denaturation or misfolding of soluble HLA molecules can lead to loss of native epitopes, yielding false negative reactions and to the creation or exposure of new or 'cryptic' epitopes that may bind non-HLA antibodies yielding false positive reactions [6]. False negatives can result in inappropriate shipment of donor organs, unexpected positive crossmatches, or transplantation with an organ from a donor not recognized as incompatible. False positives may lead to inaccurate assignment of unacceptable antigens for sensitized patients and cause unjustified exclusion of potential donors [7–9]. Furthermore, antibody strength is difficult to assess when several donor-specific antibody (DSA) present as MFI values are not algebraically additive.

Phenotype panels, with targets that are closer to a physiological condition, are less prone to artifacts because of denaturation, contain more than one representation of several alleles, and allow a better estimation of antibody strength. However, identification of all antibody specificities present may be difficult or impossible for serum from broadly sensitized patients, and HLA-Cw and DP antibodies are not readily detected.

The use of the combination of both assays facilitates a more accurate antibody assessment, with SABs providing increased specificity and sensitivity whereas phenotype beads provide a better assessment of strength and are less affected by interference and by protein denaturation.

ANTIBODY SPECIFICITY ASSESSMENT

Interpretation of SPI results requires understanding of HLA molecular structure and polymorphism and of serologic reactivity with epitopes shared among HLA molecules that result in cross reactive groups (CREGS). HLA antibodies recognize structural motifs (epitopes) that contain a set of amino acids present in the donor and not in the recipient. Each HLA molecule contains multiple epitopes and many epitopes are shared among different HLA antigens. Identifying patterns of reactivity against shared epitopes allows accurate specificity assignment and assessment of antibody complexity. For antibodies against DQ or DP molecules, in which genes coding for both alpha (HLA-DQA1, DPA1) and beta (DQB1, DQB1) chains are polymorphic, specificity for the alpha chain and for epitopes specific to particular alpha-beta chain combinations may be determined [10▪▪]. Pattern recognition is also essential for distinguishing true positive and true negative reactions. A single positive bead may be identified as false positive, if an expected epitope-sharing reactivity is not observed or, in the case of phenotype panels, if other beads bearing the same allele are negative. Epitope reactivity patterns may continue below MFI thresholds commonly considered as positive, and false negatives can be assigned if MFI values are used as the only criterion for positivity.

ANTIBODY LEVEL ASSESSMENT

SPI are semi-quantitative assays and therefore MFI values do not translate directly into antibody level. Variability in the amount of protein coating different bead populations confounds the comparison of HLA antibody levels across specificities in the same array and of the same specificity across lots. The protein concentrations for HLA-C, HLA-DQ, and DP are higher than for other HLA antigens, and therefore their MFI values are inflated. MFI values may lead to underestimating antibody levels when the epitope is distributed across multiple beads, as in the case of the public epitopes Bw4 and Bw6. Further, binding of non-HLA antibodies will confound interpretation, just as it does with cell-based assays. Therefore, MFI values taken out of context are not meaningful. Despite these shortcomings, MFI values are being used successfully to predict crossmatch results and assess immunological risk. Correlation of MFIs with CDCXM and FCXM are used to assess clinical relevance of DSA and thresholds are tailored according to the immunological risk acceptable to each individual transplantation program [1,5▪,11▪].

Antibody quantitation is also complicated by bead saturation and serum interferences. Strong antibodies may saturate beads and the actual antibody level may be revealed only with serum dilutions. In addition, some sera render increased MFI upon dilution because of the presence of interfering substances, such as IgM and complement that may dilute to undetectable levels sooner than the HLA antibody. Several serum treatments, such as hypotonic dialysis, 1,4-dithiothreitol (DTT), and EDTA may be used to reduce interference [12,13]. Some centers favor the use of titration as the best method for antibody level assessment as it resolves both bead saturation and interference [14▪]. Two recent studies provide evidence that the complement component C3 may play an important role in the phenomenon of interference [15,16].

RESULT INTERPRETATION

Ultimately, SPI results must be combined with additional data from cell-based assays and from the patient's immunologic history in order to provide accurate assignment of antibody specificity and level. Results must be consistent with other antibody tests performed and with test results of other specimens from the same patient. HLA antibodies are dynamic and sensitization assessment should include the tracking of antibody specificity and level over time. Changes in antibody patterns should be investigated for which close communication with the clinical teams is fundamental. For example, an apparent sudden increase in antibody strength and breadth may be validated by the occurrence of proinflammatory events such as surgery or infection or of sensitizing events such as blood transfusions [17,18]. The patient's HLA type must be considered to avoid self-epitope assignment and may be useful in defining cut-off values in the presence of high background. The donor's HLA type is used for assigning donor-specific antibodies. High-resolution phenotyping of recipient and donor may be required to define some antibodies.

The patient's immunological history should also be taken into account. Previous immunizing events (pregnancies, transfusions, transplants) may help to not only define antibody specificity, but also recognize increased risk even in absence of antibody. Accordingly, HLA typing of previous organ and blood donors and, in the case of known pregnancies, the HLA typing of offspring, when available, should be taken into account [5▪]. Detection of memory B cells, although ideal for assessment of humoral immunological memory, is still being used mainly in the research setting because of cost and of the technical expertise required to implement methods for their detection [19–22].

ANTIBODY FUNCTIONALITY

Recognition of the great variability in the pathogenic potential of HLA antibodies has led to efforts to develop assays that may discriminate clinically relevant antibodies. Given that the complement cascade contributes to allograft damage, there are SPI assays that have been modified to detect complement-binding antibodies. Currently, the assays most commonly used detect: C1q (a fragment generated at the beginning of the classical pathway), C3d (a split product of C3 that is amplified midway in the complement cascade), and IgG subclasses, IgG1 and 3 being the strongest complement binders. A study of a large cohort of kidney patients found an association of C1q-binding antibodies with diminished allograft survival [23]. By contrast, a study in a smaller patient cohort found that C1q was a marker of high-titer antibodies and not an independent risk factor [24]. The significance and utility of the C1q assay are still under investigation [25–27]. Sicard *et al.* [28] found in a large cohort of kidney patients that C3d binding DSA was associated with higher risk of graft loss. O'Leary *et al.* [29] reported that IgG3 de-novo DSA is associated with worse outcome in a large cohort of liver patients. Everly *et al.* [30] identified worse allograft survival in a subgroup of kidney patients with IgG3 and IgM persistent DSA. In conclusion, although these new assays are valuable in the research setting, it is not yet clear whether they will be of utility in the clinical setting. Furthermore, it is important to note that noncomplement-binding antibodies should not be assumed to be harmless. There is increasing evidence that complement-independent mechanisms play a fundamental role in the pathology of antibody mediated rejection (AMR) [31▪▪].

EPITOPE MAPPING, EPITOPE MATCHING, ANTIGEN MATCHING

The use of SABs, allele level HLA typing, monoclonal antibodies, and molecular modeling has greatly enhanced our ability to map epitopes. The knowledge of serological cross-reactivity or CREGS, accumulated through 45 years of histocompatibility testing, combined with epitope mapping is revealing a great complexity in HLA antibodies. Three main approaches are currently being used to identify epitopes. Duquesnoy [32] developed the Match Maker application that uses amino acid sequence comparison and molecular modeling to infer putative epitopes called eplets. El-Awar *et al.* [33] have used patterns of SAB reactivity of monoclonal antibodies or alloantibodies adsorbed, then eluted from recombinant single antigen cell lines to define shared epitopes. Mallon *et al.* [34] have used computational methods to assess surface electrostatic potential of HLA molecules in order to explain serological patterns of alloantibody binding and HLA Bw4 and Bw6 antigenicity.

How best to apply this knowledge in the clinical practice to improve transplant outcomes is under intense debate. Currently, most renal transplants involve mismatched antigens and the development of DSA may lead to poor long-term survival and need for a new transplant. A higher risk of allosensitization has been shown with increased number of mismatches, both at the epitope [35] and antigen [36,37▪▪] levels. However, not all mismatches are immunogenic. Understanding permissible mismatches at the antigen and epitope level may lead the path to better matching of patients and improve long-term outcome [37▪▪,38].

In the USA, the renal allocation program is based on unacceptable antigens, which are used to calculate the calculated panel reactive antibodies (CPRA) or percentage of donors who express the HLA antigens reactive with the sensitized patient's antibodies. Patients with higher CPRAs receive extra points in the allocation system. It has been proposed that defining HLA mismatch acceptability at the allele level would benefit sensitized patients waiting for a cadaveric donor by allowing epitope matching and hence improving probability of transplantation and better outcomes [39]. The counter-argument has been that determining epitope specificity in complex sera is not straightforward and that epitope matching will not improve sensitized patients' chances of having access to a compatible organ. Furthermore, the extra burden of performing time consuming and expensive high-resolution typing on the donor would not be justified [40].

HLA ANTIBODIES AND REJECTION

Despite great advances in preventing acute rejection in kidney transplantation, 10-year allograft survival has not improved. Advances in pathology and HLA antibody monitoring have shown that chronic allograft rejection has a strong antibody component and chronic AMR is the cause of loss of allografts [41,42]. Several studies show that renal recipients with de-novo DSA experience worse graft survival and over-representation of HLA-DQ antibodies [43–45]. Loupy *et al.* [46] report that protocol biopsies and DSA monitoring may predict rejection before clinical manifestations and irreversible kidney allograft damage. New cohort and case studies show a deleterious impact of DSA directed against HLA-Cw and DP antigens [47–50]. Involvement of DSA in cardiac transplant rejection is supported by several studies [51–53] and is included as a risk

factor in the American Heart Association guidelines [54]. Reinsmoen *et al.* [55] report correlation between both HLA-DSA and AT1R antibodies with AMR and cell-mediated rejection (CMR), Three centers report association of de-novo DSA with bronchiolitis obliterans syndrome in lung transplants [56–58] and recent reports also show an association of DSA with poor outcomes in intestine/multivisceral transplants [59,60]. Several new studies continue to support the role of HLA antibodies in liver allograft pathology [61–65,66▪▪]. Finally, the use of microarrays and microfluidic platforms that allow analysis of large number of mRNA and micro RNA are providing new insights into the pathophysiology of AMR but have not been yet incorporated into routine clinical use [67–70].

CONCLUSION

HLA antibody assessment requires careful evaluation of SPI results in the context of complementary testing and the patient's sensitization history. Given that the level of acceptable immunological risk varies according to the type of allograft and the specific transplantation protocol, both the testing algorithm and interpretative report must be tailored not only to each patient/donor pair but also to each transplantation program served by the histocompatibility laboratory. New tests, including modified SPI, memory B cell characterization, and microarray detection of biomarkers of rejection at the RNA level are advancing understanding of allograft rejection.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

1. Zachary AA, Sholander JT, Houp JA, Leffell MS. Using real data for a virtual crossmatch. Hum Immunol 2009; 70:574–579.
2. Zachary AA, Leffell MS. Desensitization for solid organ and hematopoietic stem cell transplantation. Immunol Rev 2014; 258:183–207.
3. Zachary AA, Vega RM, Lucas DP, Leffell MS. HLA antibody detection and characterization by solid phase immunoassays: methods and pitfalls. Methods Mol Biol 2012; 882:289–308.
4. ▪ Gebel HM, Bray RA. HLA antibody detection with solid phase assays: great expectations or expectations too great? Am J Transplant 2014; 14:1964–1975.

This review covers the technical strengths and limitations of SPI and provides practical solutions for their use in clinical transplantation.

5. ▪ Tait BD, Susal C, Gebel HM, *et al.* Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. Transplantation 2013; 95:19–47.

This article provides excellent guidelines from an international consensus conference on the use of SPI in conjunction with cell-based assays for meaningful assessment of clinically relevant HLA-specific antibodies.

6. Visentin J, Guidicelli G, Moreau JF, *et al.* Deciphering allogeneic antibody response against native and denatured HLA epitopes in organ transplantation. Eur J Immunol 2015; 45:2111–2121.
7. Gombos P, Opelz G, Scherer S, *et al.* Influence of test technique on sensitization status of patients on the kidney transplant waiting list. Am J Transplant 2013; 13:2075–2082.
8. Middleton D, Jones J, Lowe D. Defining HLA-specific antibodies. Am J Transplant 2014; 14:236.
9. Visentin J, Guidicelli G, Bachelet T, *et al.* Denatured class I human leukocyte antigen antibodies in sensitized kidney recipients: prevalence, relevance, and impact on organ allocation. Transplantation 2014; 98:738–744.
10. ▪▪ Tambur AR, Rosati J, Roitberg S, *et al.* Epitope analysis of HLA-DQ antigens: what does the antibody see? Transplantation 2014; 98:157–166.

These authors have shown the complexity of the humoral response against the combinatorial epitopes of DQ alpha and beta chains using epitope analysis of de-novo HLA-DQ antibodies from kidney patients who lost their allograft.

11. ▪ Baxter-Lowe LA, Cecka M, Kamoun M, *et al.* Center-defined unacceptable HLA antigens facilitate transplants for sensitized patients in a multicenter kidney exchange program. Am J Transplant 2014; 14:1592–1598.

This article demonstrates how definition of unacceptable antigens based on different center-specific criteria can facilitate kidney exchanges.

12. Zachary AA, Lucas DP, Detrick B, Leffell MS. Naturally occurring interference in Luminex assays for HLA-specific antibodies: characteristics and resolution. Hum Immunol 2009; 70:496–501.
13. Schnaidt M, Weinstock C, Jurisic M, *et al.* HLA antibody specification using single-antigen beads – a technical solution for the prozone effect. Transplantation 2011; 92:510–515.
14. ▪ Tambur AR, Herrera ND, Haarberg KM, *et al.* Assessing antibody strength: comparison of MFI, C1q, and titer information. Am J Transplant 2015; 15:2421–2430.

This study shows that titration is a viable solution for the challenge of HLA antibody quantitation.

15. Schwaiger E, Wahrmann M, Bond G, *et al.* Complement component C3 activation: the leading cause of the prozone phenomenon affecting HLA antibody detection on single-antigen beads. Transplantation 2014; 97:1279–1285.
16. Visentin J, Vigata M, Daburon S, *et al.* Deciphering complement interference in antihuman leukocyte antigen antibody detection with flow beads assays. Transplantation 2014; 98:625–631.
17. Locke JE, Zachary AA, Warren DS, *et al.* Proinflammatory events are associated with significant increases in breadth and strength of HLA-specific antibody. Am J Transplant 2009; 9:2136–2139.
18. Leffell MS, Kim D, Vega RM, *et al.* Red blood cell transfusions and the risk of allosensitization in patients awaiting primary kidney transplantation. Transplantation 2014; 97:525–533.
19. Zachary AA, Lucas DP, Montgomery RA, Leffell MS. Rituximab prevents an anamnestic response in patients with cryptic sensitization to HLA. Transplantation 2013; 95:701–704.
20. Crespo M, Heidt S, Redondo D, Pascual J. Monitoring B cell subsets and alloreactivity in kidney transplantation. Transplant Rev (Orlando) 2015; 29:45–52.
21. Chong AS, Sciammas R. Memory B cells in transplantation. Transplantation 2015; 99:21–28.
22. Lucas DP, Leffell MS, Zachary AA. Tetramer staining for the detection of HLA-specific B cells. Methods Mol Biol 2013; 1034:313–318.
23. Loupy A, Lefaucheur C, Vernerey D, *et al.* Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013; 369:1215–1226.
24. Yell M, Muth BL, Kaufman DB, *et al.* C1q binding activity of de novo donor-specific HLA antibodies in renal transplant recipients with and without antibody-mediated rejection. Transplantation 2015; 99:1151–1155.
25. Loupy A, Legendre C. From mean fluorescence intensity to C1q-binding: the saga of anti-HLA donor-specific antibodies. Transplantation 2015; 99:1107–1108.
26. Schaub S, Honger G, Koller MT, *et al.* Determinants of C1q binding in the single antigen bead assay. Transplantation 2014; 98:387–393.
27. Peacock S, Kosmoliaptsis V, Bradley AJ, Taylor CJ. Questioning the added value of Luminex single antigen beads to detect C1q binding donor HLA-specific antibodies. Transplantation 2014; 98:384–386.
28. Sicard A, Ducreux S, Rabeyrin M, *et al.* Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss. J Am Soc Nephrol 2015; 26:457–467.
29. O'Leary JG, Kaneku H, Banuelos N, *et al.* Impact of IgG3 subclass and C1q-fixing donor-specific HLA alloantibodies on rejection and survival in liver transplantation. Am J Transplant 2015; 15:1003–1013.
30. Everly MJ, Rebellato LM, Haisch CE, *et al.* Impact of IgM and IgG3 anti-HLA alloantibodies in primary renal allograft recipients. Transplantation 2014; 97:494–501.

31. Valenzuela NM, McNamara JT, Reed EF. Antibody-mediated graft injury: ■■ complement-dependent and complement-independent mechanisms. Curr Opin Organ Transplant 2014; 19:33–40.

An excellent review of current understanding of the molecular mechanisms underlying allograft injury.

32. Duquesnoy RJ. HLA epitope based matching for transplantation. Transpl Immunol 2014; 31:1–6.
33. El-Awar N, Terasaki PI, Cai J, *et al.* Epitopes of HLA-A, B, C, DR, DQ, DP and MICA antigens. Clin Transpl 2009; 295–321.
34. Mallon DH, Bradley JA, Winn PJ, *et al.* Three-dimensional structural modelling and calculation of electrostatic potentials of HLA Bw4 and Bw6 epitopes to explain the molecular basis for alloantibody binding: toward predicting HLA antigenicity and immunogenicity. Transplantation 2015; 99:385–390.
35. Wiebe C, Nevins TE, Robiner WN, *et al.* The synergistic effect of class II HLA epitope-mismatch and nonadherence on acute rejection and graft survival. Am J Transplant 2015; 15:2197–2202.
36. Kosmoliaptsis V, Gjorgjimajkoska O, Sharples LD, *et al.* Impact of donor mismatches at individual HLA-A, -B, -C, -DR, and -DQ loci on the development of HLA-specific antibodies in patients listed for repeat renal transplantation. Kidney Int 2014; 86:1039–1048.
37. Lucas DP, Leffell MS, Zachary AA. Differences in immunogenicity of HLA ■■ antigens and the impact of cross-reactivity on the humoral response. Transplantation 2015; 99:77–85.

Experimental evidence of different immunogenicity of HLA antigens, of the influence of donor-recipient sharing of cross-reactive antigens in their phenotype, and of the directionality of this effect.

38. Glotz D, Tambur A. Stratifying patients based on epitope mismatching: ready for primetime? Am J Transplant 2015; 15:2021–2022.
39. Duquesnoy RJ, Kamoun M, Baxter-Lowe LA, *et al.* Should HLA mismatch acceptability for sensitized transplant candidates be determined at the high-resolution rather than the antigen level? Am J Transplant 2015; 15:923–930.
40. Cecka JM, Reed EF, Zachary AA. HLA high-resolution typing for sensitized patients: a solution in search of a problem? Am J Transplant 2015; 15:855–856.
41. Djamali A, Kaufman DB, Ellis TM, *et al.* Diagnosis and management of antibody-mediated rejection: current status and novel approaches. Am J Transplant 2014; 14:255–271.
42. Haas M, Sis B, Racusen LC, *et al.* Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014; 14:272–283.
43. Devos JM, Gaber AO, Teeter LD, *et al.* Intermediate-term graft loss after renal transplantation is associated with both donor-specific antibody and acute rejection. Transplantation 2014; 97:534–540.
44. Kim JJ, Balasubramanian R, Michaelides G, *et al.* The clinical spectrum of de novo donor-specific antibodies in pediatric renal transplant recipients. Am J Transplant 2014; 14:2350–2358.
45. Tagliamacco A, Cioni M, Comoli P, *et al.* DQ molecules are the principal stimulators of de novo donor-specific antibodies in nonsensitized pediatric recipients receiving a first kidney transplant. Transpl Int 2014; 27:667–673.
46. Loupy A, Vernerey D, Tinel C, *et al.* Subclinical rejection phenotypes at 1 year post-transplant and outcome of kidney allografts. J Am Soc Nephrol 2015; 26:1721–1731.
47. Aubert O, Bories MC, Suberbielle C, *et al.* Risk of antibody-mediated rejection in kidney transplant recipients with anti-HLA-C donor-specific antibodies. Am J Transplant 2014; 14:1439–1445.
48. Bachelet T, Martinez C, Del Bello A, *et al.* Deleterious impact of donor-specific anti-HLA antibodies toward HLA-Cw and HLA-DP in kidney transplantation. Transplantation 2016; 100:159–166.
49. Cippa PE, Gaspert A, Etter C, *et al.* Late antibody-mediated rejection by de novo donor HLA-DP-specific antibody after renal transplantation: a case report. Hum Immunol 2014; 75:462–465.
50. Mierzejewska B, Schroder PM, Baum CE, *et al.* Early acute antibody-mediated rejection of a negative flow crossmatch 3rd kidney transplant with exclusive disparity at HLA-DP. Hum Immunol 2014; 75:703–708.
51. Starling RC, Stehlik J, Baran DA, *et al.* Multicenter analysis of immune biomarkers and heart transplant outcomes: results of the clinical trials in organ transplantation-05 study. Am J Transplant 2016; 16:121–136.
52. Frank R, Molina MR, Goldberg LR, *et al.* Circulating donor-specific anti-human leukocyte antigen antibodies and complement C4d deposition are associated with the development of cardiac allograft vasculopathy. Am J Clin Pathol 2014; 142:809–815.
53. Tran A, Fixler D, Huang R, *et al.* Donor-specific HLA alloantibodies: impact on cardiac allograft vasculopathy, rejection, and survival after pediatric heart transplantation. J Heart Lung Transplant 2016; 35:87–91.
54. Colvin MM, Cook JL, Chang P, *et al.* Antibody-mediated rejection in cardiac transplantation: emerging knowledge in diagnosis and management: a scientific statement from the American Heart Association. Circulation 2015; 131:1608–1639.
55. Reinsmoen NL, Lai CH, Mirocha J, *et al.* Increased negative impact of donor HLA-specific together with non-HLA-specific antibodies on graft outcome. Transplantation 2014; 97:595–601.
56. Kauke T, Kneidinger N, Martin B, *et al.* Bronchiolitis obliterans syndrome due to donor-specific HLA-antibodies. Tissue Antigens 2015; 86:178–185.
57. Morrell MR, Pilewski JM, Gries CJ, *et al.* De novo donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation. J Heart Lung Transplant 2014; 33:1288–1294.
58. Safavi S, Robinson DR, Soresi S, *et al.* De novo donor HLA-specific antibodies predict development of bronchiolitis obliterans syndrome after lung transplantation. J Heart Lung Transplant 2014; 33:1273–1281.
59. Gerlach UA, Lachmann N, Sawitzki B, *et al.* Clinical relevance of the de novo production of anti-HLA antibodies following intestinal and multivisceral transplantation. Transpl Int 2014; 27:280–289.
60. Kubal C, Mangus R, Saxena R, *et al.* Prospective monitoring of donor-specific anti-HLA antibodies after intestine/multivisceral transplantation: significance of de novo antibodies. Transplantation 2015; 99:e49–e56.
61. Del Bello A, Congy-Jolivet N, Danjoux M, *et al.* De novo donor-specific anti-HLA antibodies mediated rejection in liver-transplant patients. Transpl Int 2015; 28:1371–1382.
62. Del Bello A, Congy-Jolivet N, Muscari F, *et al.* Prevalence, incidence and risk factors for donor-specific anti-HLA antibodies in maintenance liver transplant patients. Am J Transplant 2014; 14:867–875.
63. Del Bello A, Congy-Jolivet N, Rostaing L, Kamar N. Incidence of anti-HLA donor specific antibodies in liver-transplant patients given mTOR inhibitors without calcineurin inhibitors. J Hepatol 2014; 61:963–965.
64. Grabhorn E, Binder TM, Obrecht D, *et al.* Long-term clinical relevance of de novo donor-specific antibodies after pediatric liver transplantation. Transplantation 2015; 99:1876–1881.
65. Iacob S, Cicinnati VR, Lindemann M, *et al.* Donor-specific anti-HLA antibodies and endothelial C4d deposition-association with chronic liver allograft failure. Transplantation 2015; 99:1869–1875.
66. O'Leary JG, Kaneku H, Demetris AJ, *et al.* Antibody-mediated rejection as a ■■ contributor to previously unexplained early liver allograft loss. Liver Transpl 2014; 20:218–227.

This study shows association of high-level pretransplant HLA donor-specific antibodies with liver AMR.

67. Hayde N, Broin PO, Bao Y, *et al.* Increased intragraft rejection-associated gene transcripts in patients with donor-specific antibodies and normal biopsies. Kidney Int 2014; 86:600–609.
68. Suviolahti E, Ge S, Nast CC, *et al.* Genes associated with antibody-dependent cell activation are overexpressed in renal biopsies from patients with antibody-mediated rejection. Transpl Immunol 2015; 32:9–17.
69. Vitalone MJ, Sigdel TK, Salomonis N, *et al.* Transcriptional perturbations in graft rejection. Transplantation 2015; 99:1882–1893.
70. Xu Z, Nayak D, Yang W, *et al.* Dysregulated microRNA expression and chronic lung allograft rejection in recipients with antibodies to donor HLA. Am J Transplant 2015; 15:1933–1947.