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ISHLT CONSENSUS STATEMENTS

The management of antibodies in heart transplantation: An ISHLT consensus document

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Despite the successes from refined peri-operative management techniques and immunosuppressive therapies, antibodies remain a serious cause of morbidity and mortality for patients both before and after heart transplantation. Patients awaiting transplant who possess antibodies against human leukocyte antigen are disadvantaged by having to wait longer to receive an organ from a suitably matched donor. The number of pre-sensitized patients has been increasing, a trend that is likely due to the increased use of mechanical circulatory support devices. Even patients who are not pre-sensitized can go on to produce donor-specific antibodies after transplant, which are associated with worse outcomes. The difficulty in managing antibodies is uncertainty over which antibodies are of clinical relevance, which patients to treat, and which treatments are most effective and safe. There is a distinct lack of data from prospective trials. An international consensus conference was organized and attended by 103 participants from 75 centers to debate contentious issues, determine the best practices, and formulate ideas for future research on antibodies. Prominent experts presented state-of-the-art talks on antibodies, which were followed by group discussions, and then, finally, a reconvened session to establish consensus where possible. Herein we address the discussion, consensus points, and research ideas.
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The presence of circulating antibodies in heart transplantation (called sensitization) impacts clinical outcomes. Due to different clinical implications, sensitization can be

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Table 1 Results of Pre-conference Online Survey[a]

On pre-transplant antibody detection:

- Asked which antibody test(s) were done pre-transplantation for patients with detected antibodies but who were not highly sensitized: 86% did a virtual crossmatch; 30% did a prospective flow cytometry; 32% did a prospective CDC crossmatch; 11% responded other; and 4% did none. (56/56 centers responded)
- The most frequently cited MFI cut-off for determining which corresponding antigens to avoid was 5,000, with a range from >0 to 10,000. (56/56)
- The frequency with which respondents monitored antibodies pre-transplant was: every 3 months for 34% of respondents; every 6 months for 19%; monthly for 15%; never if PRA was 0% at listing for 5%; with the remaining 27% of respondents varying the frequency based on waitlist status, PRA level, or for MCS patients. (56/56)
- 70% of respondents did not have a different approach for avoiding Class I vs Class II anti-HLA antibodies pre-transplant. (56/56)
- 56% of respondents routinely submitted unacceptable antibodies to their regulatory agency to use during the virtual crossmatch, and 44% did not. (55/56)
- Asked when the respondent would order a prospective crossmatch: 22% would do so for PRA >25%; 19% would never do so if the virtual crossmatch was negative; 8% would for PRA >80%; 5% would for PRA >70%; 5% would for PRA >50%; and 41% either always did one, did one for any sensitized patient, or used other factors such as the distance from the donor. (56/56)
- 48% of respondents would decline a donor heart if the flow cytometry crossmatch was positive, and 75% would decline if the CDC crossmatch was positive. (52/56)

On sensitized patients:

- 75% of respondents desensitized patients before transplantation, and 25% did not. (56/56)
- Asked which antibody detection method respondents used for deciding to initiate desensitization therapy: 62% used cPRA; 13% used general PRA; and 25% relied on other factors, such as an MFI cut-off, antibody specificity, CDC positivity after EDTA, complement levels, IgG/IgM levels, or the probability of transplantation. (52/56)
- When asked which PRA or cPRA values trigger desensitization therapy: 21% used >80%; 21% used >50%; and the remainder used values that ranged from >10% to >90%. Some centers combined MFI values, the frequency of positive crossmatches, blood group status, and the likelihood of being transplanted. (56/56)
- 48% of respondents believed that sensitized VAD patients posed the same risk as sensitized non-VAD patients; 29% thought sensitized VAD patients were less at risk; and 23% did not know. (56/56)
- The most commonly used treatments for desensitization therapy were IVIg, plasmapheresis, rituximab, and bortezomib. (54/46)
- 78% of respondents did not change their desensitization treatments for Class I vs Class II antibodies pre-transplant, and 22% did. (55/56)
- 73% of respondents used special treatments for sensitized patients peri-operatively and 27% did not. (56/56)
- Of the respondents who did use special treatments, the most commonly used therapies were ATG, plasmapheresis, and IVIg. (47/47)

On antibody treatment:

- 78% of respondents did not change their approach for treating Class I vs Class II DSA, and 22% did. (55/56)
- When asked, which therapy was first-line in the treatment of antibodies: 79% used IVIg; 62% used plasmapheresis; 52% used rituximab; 19% used ATG; 8% used bortezomib; and 17% used other therapies, including carfilzomib, MMF, cyclophosphamide, and immunoadsorption. (51/56)
- On the treatment of DSA, 69% of respondents would treat if there was cardiac dysfunction; 58% would treat with biopsy-proven AMR Grade 1 or 2; 15% would treat only with high-level DSA; 11% would treat for all cases; 11% would treat only if the DSA were $C1q^{+}$, and 2% did not treat DSA. (54/56)
- The most commonly used therapies for treating DSA were plasmapheresis, rituximab, IVIg, augmenting corticosteroids, ATG, and bortezomib. (54/56)
- The most commonly used first-line therapies for treating DSA were: plasmapheresis, IVIg, and rituximab. (54/56)
- Respondents were asked whether their approach to treating DSA differed <1 year post-transplantation vs >1 year transplantation: 65% did not change their approach, and 35% did. (55/56)
- 78% of respondents did not treat Class I vs Class II DSA post-transplant differently, and 22% did. (55/56)

On post-transplant antibody surveillance:

- 73% of respondents routinely monitored antibodies post-transplantation; 18% would only monitor antibodies if they were detected pre-transplant; and 9% would do so in patients with biopsy-proven AMR or PGD. (56/56)
- 80% of respondents monitored antibodies at 1, 3, 6, and 12 months post-transplantation; 28% also did so at 9 months; and a small minority did so every month in the first year. (50/56)
- After the first year post-transplantation, 41% of respondents monitored antibodies annually; 29% did not monitor at all; 20% did so on a for-cause basis; 14% did so every 6 months; and 6% did so every 3 months. (54/56)
- For DSA-positive patients, 35% of respondents checked if the DSA could fix complement, and 65% did not. (55/56)
- For patients with DSA, 52% of respondents would perform a biopsy if there was also cardiac dysfunction; 29% did so in all cases; 15% did so if the DSA level was high; 12% did not do a biopsy; and 9% did so only if the C1q was positive. (54/56)
- 67% of respondents did not view non-HLA antibodies as concerning, and 33% did. (55/56)
- Respondents who monitored for non-HLA antibodies looked at anti-MICA, anti-MICB, anti-AT1 receptor, anti-vimentin, and anti-endothelial antibodies. (21/21)

AMR, antibody-mediated rejection; AT1, angiotensin 1; ATG, anti-thymocyte globulin; CDC, complement-dependent cytotoxicity; cPRA, calculated panel-reactive antibody; DSA, donor-specific antibody/antibodies; EDTA, ethylene-diamine tetraacetic acid; HLA, human leukocyte antigen; IVIg, intravenous immunoglobulin; MCS, mechanical circulatory support; MFI, mean fluorescence intensity; MICA/B, MHC Class I polypeptide-related sequence A/B; MMF, mycophenolate mofetil; PGD, primary graft dysfunction; PRA, panel-reactive antibody; VAD, ventricular assist device.

[a]There were 56 participating centers.

divided into the pre- and post-transplant periods. Pre-transplant antibodies can reduce the likelihood of obtaining a compatible donor heart and may increase the risk of rejection after heart transplantation. In the post–heart transplant setting, there is now considerable evidence demonstrating the detrimental effects of anti–human leukocyte antigen (anti-HLA) donor-specific antibodies (DSA) on outcomes such as rejection, cardiac allograft vasculopathy (CAV), and survival.[1–5]

An international consensus conference endorsed by the International Society for Heart and Lung Transplantation (ISHLT) took place on April 25, 2016, in Washington, DC, to ascertain the current practices of heart transplant centers in identifying and managing antibodies before and after cardiac transplantation. The conference was attended by 103 participants from 75 centers around the world with extensive clinical and research experience in a broad range of specialties, including cardiology, cardiac surgery, pediatric cardiology, nephrology, immunology, and pathology (see Appendix).

Before the conference, participants were invited to take part in a survey. Respondents from 51 of the 75 centers submitted data on their pre- and post-transplant antibody management practices. Aggregate survey data from 1,243 heart transplant patients revealed that 186 (15%) of these patients developed DSA in the first year post-transplant. There were other important survey results as well. For sensitized waitlist patients, the most frequently cited mean flourescence intensity (MFI) cut-off for determining which corresponding antigens to avoid (for the virtual crossmatch) was 5,000 MFI; when asked which pre-transplant PRA or cPRA values trigger desensitization therapy, 21% used >80% and 21% used >50%; respondents' first-line treatment of pre-transplant antibodies included intravenous immunoglobulin (IVIg) (79%), plasmapheresis (62%), rituximab (52%), anti-thymocyte globulin (ATG) (19%), bortezomib (8%), and other therapies (17%); on the treatment of post-transplant DSA, the percentage of respondents who would treat if there was cardiac dysfunction was 69%, biopsy-proven antibody-mediated rejection (AMR) Grade 1 or 2 was 58%, only with high-level DSA was 15%, for all cases was 11%, only if the DSA were C1q positive was 11%, and would not treat DSA was 2%. The remainder of the survey results are summarized in Table 1.

There is no standard of care for treating circulating antibodies. Questions that need to be answered for formulating management guidelines revolve around detecting and quantifying circulating antibodies, and validating the efficacy of therapeutic agents. Such unanswered questions include:

- Which antibodies are clinically significant?
- What are the best methods for detecting antibodies?
- Are non-HLA alloantibodies clinically significant?
- Which treatments against antibodies are effective?
- What is the impact of treating antibodies?

In this report we summarize the pre-conference survey data, current state-of-the-art practices, discussions that took place during the conference, and, finally, the consensus statements and conclusions of the participants.

Understanding antibodies and mechanisms of injury

AMR is a major limitation to long-term cardiac transplant survival and is mainly driven by antibodies directed against the mismatched HLA Class I and Class II antigens expressed on the allograft.[6] However, more recently, antibodies to non-HLA antigens, such as major histocompatibility complex (MHC) Class I polypeptide-related sequence A (MICA) or antibodies to self-antigens, have also been shown to mediate AMR.[7] HLA antibodies act through multiple interconnected mechanisms that synergize in a "perfect storm" to promote acute and chronic AMR.[8] The 2 major pathways of antibody-mediated injury are governed by Fc-receptor–dependent and Fc-receptor–independent effector functions. Several factors influence the capacity of the antibody to elicit effector functions, including the antibody subclass, titer and affinity, and level of HLA antigen expression on the graft. Understanding these characteristic features of HLA antibodies and their effector functions should allow for the identification of antibodies that are more pathogenic and likely to promote AMR and to guide treatment.

Fc-dependent effector functions of HLA antibodies

HLA antibodies can mediate injury to the allograft by activating the classical complement pathway or by binding to the Fc-receptor of inflammatory cells. The subclass and glycosylation of an immunoglobulin (Ig) molecule determines whether or not the antibody can activate complement or recruit inflammatory cells via Fc-gamma receptors (FcγRs).[9] The antibody titer and affinity, immunoglobulin G (IgG) subclass, and level of HLA antigen expressed on the allograft will also impact whether an antibody can activate complement. Complement-activating antibodies trigger the classical pathway through binding of C1q, resulting in production of anaphylatoxins C3a and C5a, which can augment leukocyte recruitment and T-cell responses.[10] Monocytes, neutrophils, and natural killer (NK) cells express FcγRs, which can interact with the heavy chain of HLA antibodies bound to donor endothelial cells. FcγR functions mediate phagocytosis and antibody-dependent cytotoxicity. Recipient polymorphisms in FcγR may influence the degree to which NK and myeloid cells bind to the allograft.[9,11]

Fc-independent effector functions of HLA antibodies

Antibodies activate endothelial cells by binding to, and crosslinking, HLA Class I and Class II molecules via the F $(ab')_2$ region of the Ig molecule.[12,13] Upon crosslinking, HLA molecules associate with integrin β4,[14] facilitating the phosphorylation of kinases in the mammalian target-of-rapamycin (mTOR) pathway and leading to cytoskeletal remodeling, proliferation, cell migration, and recruitment of leukocytes.[15] Antibodies can also exacerbate inflammation during AMR by rapidly mobilizing Weibel–Palade bodies, resulting in a rapid increase in cell-surface P-selectin and

subsequent adhesion of neutrophils and monocytes to endothelium.[16,17] Finally, HLA crosslinking activates transcription factors, such as c-AMP response element binding protein (CREB) and non-canonical nuclear factor-kappaB (NF-κB), resulting in increased protein expression of late-phase adhesion molecules, including cytokines, chemokines, interleukin-6 (IL-6), IL-8, C-X-C motif chemokine 10 (CXCL10), C-C motif chemokine 2 (CCL2), and CCL5.[18]

Taken together, the pleiotropic functions of HLA antibodies on the endothelial cells of the allograft act in concert to mediate microvascular inflammation and graft injury characteristics of AMR. Identifying the features that are critical for effector functions may help identify antibodies more likely to cause rejection.

Current methods for HLA antibody detection

There are several HLA antibody screening methods, each with varying sensitivities, specificities, and clinical usefulness. They can be used pre-transplant to assess for sensitization and risk of AMR, post-transplant for monitoring DSA, and for monitoring the efficacy of desensitization therapy.

The complement-dependent cytotoxicity (CDC) assay, based on work by Patel and Teresaki,[19] is the oldest, most variable, least sensitive, least specific, but most clinically relevant, method for preventing accelerated acute and hyperacute rejection.[20] Recipient serum is mixed with T- and B-lymphocytes from the donor and a source of complement (usually from rabbits) is added. A cytotoxic reaction suggests the presence of complement-fixing DSA against donor HLA. The sensitivity of the CDC assay was enhanced by the addition of anti-human globulin (AHG), which allowed adsorption-positive, cytotoxic-negative anti-HLA antibodies to be detected. However, reactive antibodies cannot be defined by their HLA alleles.

The introduction of microparticles (beads) coated with HLA antigens has revolutionized the process of identifying anti-HLA antibodies. Individual beads can be distinguished by slight variations in internal fluorescence, which identifies the antigen(s) present on the bead. This means that many beads can be pooled together in a single reaction with the serum, run on a flow cytometer or Luminex (LMX) instrument, and stained for the presence of bound antibody. Results are expressed as MFI.[21] Of note, MFI does not represent antibody titer but instead is a measure of antibody-antigen binding strength or bead saturation, which may not correlate with clinical significance. One reason for this is that the density of HLA on the bead may not reflect the true expression of HLA on in-vivo donor cells. Occasionally, diluted sera yield the same MFI result, indicating that the antibody titer may be higher than suggested by undiluted sera due to bead saturation. Diluting sera is also useful for eliminating the effects of IgM or complement 1 (C1), which can interfere with antibody binding.[21]

Flow PRA uses panels of beads coated with the equivalent of a whole cell's Class I (A, B, C) or Class II (DR, DQ, DP) HLA. Often used for initial screening, it gives a qualitative (positive or negative) result on an incomplete panel. It is more sensitive than CDC but lacks allele specificity other than class, and gives a crude measure (usually an underestimation) of PRA. This screening method requires expert interpretation and it is possible to miss antibodies.[21]

LMX PRA uses panels of beads coated with a full cell's equivalent of Class I or II HLA. It is more sensitive than CDC but less sensitive than single antigen beads (SAB). It is non-specific, underestimates PRA, and requires expert analysis as with CDC. Broadly reactive sera can be difficult if not impossible to analyze. It has the advantage of leaving antigens in their theoretically native configuration.[21]

LMX SAB IgG uses beads coated with a unique HLA antigen/allele on each bead. Bound antibody is detected by an anti-IgG antibody (Figure 1). SAB are the most sensitive, specific, and unambiguous of the bead assays, but are often considered overreactive, with equivocal clinical

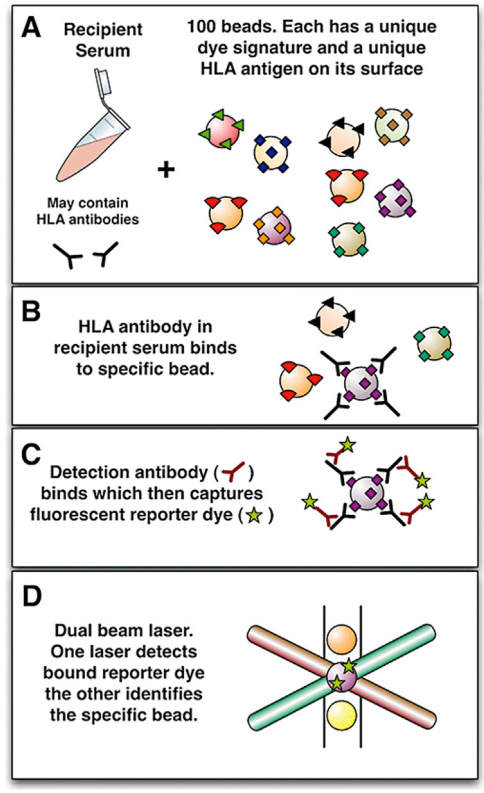

Figure 1 Single-antigen bead assay. Recipient serum potentially containing anti-HLA antibodies is added to a mixture of synthetic beads. Each bead is coated with a set of antigens (screening beads) or for more precise detail, with a single antigen (single-antigen beads). A unique dye signature (up to 100) specifies the identity of each bead (A). If anti-HLA antibodies are present these will bind to the appropriate bead (B) and a detection antibody can subsequently bind and capture a reporter dye (C). Each unique bead can then be interrogated for the presence of the reporter dye on its surface using a dual beam laser (D). A profile of antibodies can thus be identified in the recipient and compared with the known HLA identity of any potential donor, allowing a prediction of the crossmatch result. Reprinted with permission from: Mulley WR, Kanellis J. Understanding crossmatch testing in organ transplantation: a case-based guide for the general nephrologist. *Nephrology* 2011;16(2):125-33.

significance. When HLA molecules are prepared and coated on beads, they can become denatured, exposing antigens that are not present on native HLA. Antibodies against these antigens, known as cryptic antigens, are controversial. A recent study in heart transplantation demonstrated that antibodies against cryptic antigens do not usually fix complement and do not correlate with a positive flow cytometry crossmatch. Antibodies to cryptic antigens were found in 21% of patients on the transplantation waiting list. Therefore, removal of these antigens from the list of unacceptable antigens would meaningfully increase the size of the donor pool for a significant proportion of wait-listed patients.[22] Conversely, a retrospective analysis of 975 renal transplant recipients showed that graft survival was significantly lower for patients with antibodies against cryptic antigens if they fixed complement.[23] When antibodies to denatured HLA are suspected, laboratories may use surrogate donors expressing the HLA specificity of the "antibody in question" for flow cytometry crossmatch to demonstrate that the antibodies are not clinically relevant and should not be included in the list of unacceptable antigens. SAB assays yield a calculated PRA (cPRA)—a percentage of actual incompatible donors based on specificity and ethnicity, not on the number of reactions. A cPRA of 75% means that 75% of donors in the population will express HLA antigen to which the recipient from whom the blood is drawn already has relevant quantities of pre-formed antibodies.

Although solid-phase assays are more sensitive than cell-based assays, their adoption in clinical trials is impeded by intra- and interlaboratory variability. Differences can result from the test conditions, laboratory technician performance, and kit manufacturing processes. Reed et al found that standardization of operating procedures can significantly reduce these differences, as demonstrated by reduced MFI variation.[24]

LMX C1q defines antibodies capable of binding the first component of the complement cascade, C1q. Antibodies capable of binding C1q can potentiate targeted cell killing. A major observation is the lack of a correlation between antibody-binding strength, as represented by MFI, and an antibody's ability to bind complement.[25] C1q SAB is the most sensitive, specific, and clinically relevant bead assay for AMR in the first month after heart transplantation.[26–29] It can be used to monitor desensitization using IVIg therapies pre- or post-transplantation, because C1q, not IgG, is detected. It does not require specialized serum as a complement source. Other methods to detect antibodies that can fix complement have been developed and include modified SAB assays for detecting C3d- and C4d-binding anti-HLA antibodies. The C4d assay has clinical relevance for long-term outcomes in heart transplantation but requires complement activation with specialized serum sources.[30] In a recent study, the presence of C3d-binding antibodies at the time of AMR diagnosis correlated with an increased risk of graft loss in kidney transplant recipients.[31]

Both the IgG and C1q assays are subject to a prozone effect (a false negative from a high antibody titer that is shielded from detection by complement complex formation). The use of ethylene-diamine tetraacetic acid (EDTA) in the blood sample tubes reduces the prozone effect as it will bind excess complement. Also, dilution such as 1:8 will dilute excess complement and expose the antibodies, resulting in detection.

Significance of pre-transplant antibodies

Pre-transplant sensitization reduces access to ABO-compatible donors, thus increasing wait times to transplant. It also negatively impacts post-transplant survival, and increases the risk of rejection and cardiac allograft vasculopathy in adults[1,32,33] and children.[34] The usual cut-off to be considered sensitized is PRA $>10\%$. Sensitization rates have doubled over the past 2 decades.[35] Risk factors for the development of antibodies include prior blood transfusions, pregnancies, prior organ transplantation, and use of human homograft tissue for vascular reconstruction in children and adults with congenital heart disease.[34] Implantable mechanical circulatory support (MCS) devices have evolved into an increasingly common risk factor. There are several possible reasons for increased sensitization in those receiving MCS, including increased use of peri-operative blood products and a possible immunologic response to the textured surface of some devices. With increasing numbers of patients being bridged to transplant with MCS,[36] the high prevalence of sensitization among MCS patients is of significant clinical concern. Surprisingly, recent studies have suggested that sensitization among MCS recipients does not seem to have the same post-transplant impact as the sensitization seen in non-MCS patients.[37–39]

A retrospective review of the United Network for Organ Sharing (UNOS) database, including 871 patients bridged to transplant, showed longer wait times to transplant in sensitized MCS patients, without impact on post-transplant rejection or survival at 1-year follow-up.[40] However, higher Class II PRA was associated with increased primary graft dysfunction.[40] A more recent study of the UNOS database (2006 to 2012), including 11,840 heart transplant recipients, of whom 4,167 were bridged to transplant with MCS, showed that MCS was associated with increased allosensitization. As shown in previous studies, MCS recipient allosensitization was not associated with increased mortality post-transplant (hazard ratio [HR] 1.07, 95% confidence interval [CI] 0.89 to 1.28, p = not statistically significant [NS]). In contrast, sensitization was associated with increased mortality post-transplant in non-MCS patients.[41] The differences in post-transplant risk with allosensitization seen in patients with and without MCS are unclear. One possible mechanism is that removal of the MCS device at transplant may decrease the inflammatory milieu, thus limiting an immune response to the graft.[41] Overall, the mechanism is poorly understood and further study is required.

There are no agreed-upon protocols for desensitization therapy and several trials are underway assessing the safety and efficacy of agents. Many centers treat pre-transplant PRA $>50\%$ (average threshold 35%, range 10% to 100%). Approximately 45% of treated sensitized patients achieve

significant (≥50%) reduction in circulating antibodies, and 73% undergo successful heart transplantation.[42] However, in sensitized heart transplant candidates, no single strategy has been identified as optimal.

Most programs will not transplant across a positive prospective crossmatch and use criteria to ensure negative crossmatches and no detectable DSA. Other programs utilize clinical experience and novel immunosuppression protocols that enable transplantation across weakly positive donor-specific crossmatches. In these cases, vigilant post-transplant monitoring to detect rising DSA is necessary to allow for clinical intervention aimed at minimizing graft injury. Acceptable outcome results demonstrate the utility and feasibility of these approaches.

Significance of post-transplant antibodies

In a well-described series, Tambur et al[4] evaluated HLA antibodies in 71 heart transplant recipients. The presence of HLA antibodies was assessed using flow PRA. Twenty-five recipients were found to have HLA antibodies after transplant, of whom 7 had DSA. There was a strong association between the presence of de-novo HLA antibodies, particularly Class I antibodies, and cellular rejection, although no association was found with AMR. Class II antibodies were highly prevalent among patients with CAV and were associated with CAV at 3 years; 55% of patients with CAV had Class II antibodies compared with 14% in those who did not have CAV. There was also a strong correlation between transplant-related death and the presence of Class II antibodies ($p < 0.008$, $r = 0.98$).

Ho et al[43] studied the effects of de-novo antibodies in a large cohort of 950 heart transplant recipients. Survival in patients who developed de-novo antibodies after the first year was significantly worse (40%) ($p < 0.001$) compared with no de-novo antibodies (70%) and de-novo antibodies during the first year (52%) ($p < 0.05$) at 15 years post-transplantation. In another study of 122 pediatric heart transplant recipients, de-novo HLA antibodies were found to be more common with increasing age.[44] Class II antibodies were significantly less frequent in infants after ABO-incompatible transplant compared with ABO-compatible transplant ($p < 0.05$), suggesting that tolerance of the non–self-blood group antigens may extend to reducing the immune response against allograft HLA.

Smith and colleagues similarly evaluated the impact of post-transplant DSA.[2] In their single-center study, 57 of 243 recipients transplanted between 1995 and 2004 developed de-novo DSA. The majority of the antibodies were Class II (65% Class II and 21% mixed). On univariate analysis, post-transplant DSA and persistent DSA, present in 2 consecutive serum samples, were both strong predictors of mortality (HR 3.198, $p = 0.0018$, and HR 4.351, $p = 0.002$). On multivariate analysis, de-novo persistent DSA was the strongest independent predictor of mortality (HR 4.331, $p < 0.0004$). Transient DSA and non-specific antibodies had no impact on survival. In contrast to other studies, Smith and colleagues did not find any association between post-transplant DSA, either de novo or persistent, with angiographic CAV.[2]

A significant point of contention is whether the presence of DSA should be a *sine qua non* of AMR. The problem with doing so is that not all clinically relevant antibodies have been identified and antibodies against the allograft may be undetectable with current methods. A recent study looking at the long-term outcomes of patients with biopsy-proven AMR showed that 29% of patients diagnosed with AMR had no DSA. Although the presence of DSA increased the odds of graft dysfunction (odds ratio [OR] = 5.37, 95% CI 1.34 to 21.47, $p = 0.018$), the sensitivity of DSA to detect AMR was only 54.3%.[45]

The timing of AMR post-transplant may provide prognostic information—with late AMR associated with worse outcomes. In a retrospective analysis, Hodges and colleagues evaluated outcomes for 15 patients with de-novo DSA who developed AMR a median of 4.5 years after transplant.[46] Patients were diagnosed between November 2005 and August 2011. The median survival was only 0.8 year after diagnosis of AMR. Similarly, Coutance et al[47] retrospectively studied outcomes in patients who developed AMR at least 1 year after transplant at a single center between November 2006 and February 2013. Survival was only 50% at 1 year. At 3 months post-diagnosis, 33% of patients had left ventricular dysfunction and 17% were found to have CAV.

Although it is known that DSA portend graft dysfunction and poor survival, there is a lack of agreement regarding asymptomatic AMR and whether these patients should be treated. The deposition of C4d, as determined by immunofluorescence or immunohistochemistry, may assist in determining which asymptomatic patients with DSA should be treated. Frank et al[48] examined 109 EMB samples for C4d deposition and correlated findings with DSA, histology, and the clinical picture. Patients with DSA against Class I or both Class I and Class II HLA tended to have a positive C4d stain on immunofluorescence and were more likely to develop graft failure compared with patients who had DSA against Class II alone or no DSA.

Complement-fixing properties of antibodies may lend additional prognostic information. In a small series comprised of pediatric heart transplant recipients,[26] patients with $C1q^+$ DSA within the first month after transplant all developed AMR. In a separate series, the presence of circulating $C1q^+$ DSA post-transplant significantly predicted AMR, but not acute cellular rejection (ACR), with a positive predictive value of 87.5% and negative predictive value of 100%. Persistent $C1q^+$ DSA was associated with persistent clinical AMR, including C4d deposition.[49] Similarly, Farrero-Torres and colleagues demonstrated that, although $IgG^+/C1q^-$ DSA post-transplant were significantly associated with ACR (HR 8.8, $p < 0.001$), $IgG^+/C1q^+$ antibodies were significantly associated with AMR (HR 11.6, $p < 0.001$).[50]

The need to treat post-transplant DSA is determined by several factors, including the clinical presentation and endomyocardial biopsy findings and the timing of first detection of antibodies (early vs late after transplantation).

DSA developing long after transplant may arise from recurrent sub-clinical or unrecognized rejection processes and reflect sub-optimal immunosuppression or non-adherence.[51] Assessment of appropriate intake of the maintenance immunosuppression and possible struggles in adherence should be evaluated in patients with late-occurring de-novo DSA. A survey of heart transplant specialists conducted by Chih et al[52] showed that most practitioners treated newly diagnosed DSA when there was hemodynamic compromise or biopsy-proven AMR of ISHLT Grade 2 and higher. Further, the majority of practitioners would not treat de-novo DSA without histologic or clinical evidence of graft impairment. Transplant specialists disagree over how to manage patients who have low-grade AMR, or who have equivocal findings, such as AMR with no detectable antibodies.

Non-HLA antibodies, specifically antibodies against MICA, endothelial cells, and angiotensin receptor antibodies, have also been associated with alloreactivity, CAV, and AMR.[2,53–56] Anti-MICA antibodies have been found in severe rejection after heart transplantation and, more recently, donor-specific anti-MICA antibodies were suggested to be strongly associated with AMR and CAV.[57,58] Although the assays used in those studies are not readily available at all centers, consideration should be given to evaluation for these antibodies in the setting of graft dysfunction, particularly when there is no evidence of HLA antibodies. In the case of angiotensin 1 (AT1) receptor antibodies, targeted therapy may be useful; losartan blocked the effects mediated by AT1 receptor antibodies in a small series of kidney transplant recipients, however this has not yet been demonstrated in heart transplant recipients and removal or blocking of AT1 receptor antibodies has not yet been demonstrated to have a clinical impact.[59]

Desensitization strategies

Similar desensitization protocols are used both pre- and post-transplantation. Desensitization aims to eliminate circulating antibodies, increase the size of the donor pool, and improve post-transplant outcomes. Most therapeutic agents target antibodies, B-cells, plasma cells, and the complement pathway to temper the humoral response. A full list of treatment modalities is presented in Table 2.

Common desensitization treatments include IVIg,[60] plasmapheresis (PP),[61] immunoadsorption,[62–64] and rituximab.[65] Overall, IVIg (2 g/kg) appeared more effective than PP with a better safety profile (PP required longer treatment and was associated with more infections).[66] IVIg at a high dose (3 g/kg) in patients resistant to 2 g/kg is effective in reducing sensitization, but is associated with a high incidence of reversible renal insufficiency.[66]

Cyclophosphamide has traditionally been used in desensitization regimens in combination with IVIg and PP, although its use has declined in recent years.[67] Rituximab (375 mg/m^2 per dose) has been used successfully as combination therapy with IVIg (with or without PP) in cardiac desensitization protocols.[65] Bortezomib 1.3 mg/m^2 is a selective proteosome inhibitor that has been shown to induce apoptosis of plasma cells. In all desensitization therapies, infectious complications and adverse side effects may limit their utility.[68] Based on the current data, none of which are derived from prospective, randomized studies, it is difficult to ascertain whether the advantages of desensitization outweigh the side effects.

Table 2 Treatment Options for Sensitized Patients Awaiting Heart Transplantation

Removal of antibodies:
- Plasmapheresis or immunoadsorption[73]

Intravenous immunoglobulin:
- Is thought to work in multiple ways, including Fc-receptor blockade, complement inhibition, downregulation of β receptors, neutralizing circulating antibody and cytokines[74]

Immunosuppressive agents[75]:
- Corticosteroids
- Rituximab (anti-CD20), depletes B-cells[76]
- Bortezomib (proteasome inhibitor), depletes plasma cells[77]
- Alemtuzumab (anti-CD52), partly depletes T- and B-cells
- Eculizumab (C5 inhibitor) blocks antibody-mediated complement activation
- Anti-thymocyte globulin (depletes thymic cells: T-cells and T-precursor cells, and partly B-cells)
- Cyclophosphamide (cytostatic, rarely used)

Other modalities:
- Photopheresis[78]
- Total lymphoid irradiation (rarely used)[79]

In highly sensitized patients awaiting heart transplantation, a combined approach may be necessary: desensitization therapy and virtual crossmatch.[69] Methods to determine only those antibodies that are pathogenic may lessen the restricted donor pool for sensitized patients.[70] The conventional goal of desensitization therapy is to achieve a negative crossmatch before proceeding with a transplant. There is, however, an alternative strategy, whereby transplantation proceeds despite a positive crossmatch and therapies are initiated to mitigate the impact of DSA. This strategy is particularly useful in the pediatric setting where the negative impact of post-transplant survival outweighs the high waiting list mortality suffered by children awaiting an appropriate match.[71] Cornell et al[72] reported good outcomes for 30 renal transplant recipients transplanted with a positive crossmatch and treated with eculizumab—a monoclonal antibody that inhibits C5 and thus blocks formation of the complement membrane attack complex. In that study there was a significant reduction in early AMR compared with an historic control group.

Summary of the discussions at the consensus conference

The discussions throughout the day highlighted how equivocal the evidence is, pertaining to not only the treatment of antibodies but even the identification of clinically relevant

antibodies. Participants were in agreement over the detrimental effects of antibodies both pre- and post-transplant. Attendees were, for the most part, comfortable with using the virtual crossmatch to transplant most sensitized patients, but were in less agreement over highly sensitized patients. Centers ranged in their practices from always performing a prospective crossmatch to proceeding with transplantation if the virtual crossmatch was negative.

Desensitization was another point of contention. Although there is evidence that desensitization likely confers a benefit to the patients, participants had varying views regarding when to desensitize. Conference participants thought it would be useful if centers could agree on similar protocols so outcomes could be compared on a like-for-like basis. There was debate over whether it is better to strive for a negative prospective crossmatch or proceed with the first available organ and mitigate the possible negative impact of DSA post-operatively.

Most attendees followed ISHLT guidelines when monitoring for DSA in the first year post-transplantation, checking at 1, 3, 6, and 12 months post-operatively, with the justification being that antibodies may or may not be transient and the only way to identify transience is to monitor frequently. Another reason for this monitoring protocol is the belief that early antibodies may be easier to treat, and, by treating antibodies before graft dysfunction becomes clinically evident, patients avoid irreversible injury. Although some participants did not believe it necessary to monitor DSA in low-risk patients after 1 year, the conference attendees agreed that it would be beneficial to continue gathering data through routine monitoring—perhaps annually. It was also agreed that there exists sufficient evidence to show that late both DSA and persistent DSA are associated with adverse outcomes. In the future, patients could be on different antibody monitoring schedules according to their risk profile.

The conference participants agreed that there is no single assay capable of definitively quantifying the risk of any specific antibody. With existing technologies, several assays in conjunction with the clinical presentation and biopsy findings remain the preferred course for determining clinically relevant antibodies. Participants agreed that single-antigen bead and C1q assays are useful for identifying clinically relevant antibodies. Most thought that MFI was only semi-quantitative rather than an exact measure of antibody strength. Using diluted patient serum with the SAB test improves the assessment of antibody removal therapy.

Participants indicated that not all DSA need to be treated. Most participants would not treat DSA unless there was also evidence of graft dysfunction. Asymptomatic DSA would, however, lead to more frequent monitoring of both antibodies and graft function. There was consensus that $C1q^+$ antibodies, graft dysfunction, and restrictive physiology suggest a need for antibody treatment. Chronic Class II HLA antibodies were seen as the most difficult to eradicate. There have been few changes to antibody treatments in recent years. Newer therapies are being investigated, particularly in the field of kidney transplantation. In heart transplantation, bortezomib is the latest addition to the mainstream armamentarium and has led to modest improvements in reducing the PRA of the most highly sensitized patients. The conference participants were uncertain about non-HLA antibodies. Participants only felt comfortable treating in the context of graft dysfunction. It was agreed that too few data exist and further research is needed. Although yet to be proven, there was consensus that antibodies probably cause, or at least worsen, CAV.

Consensus statements for the management of antibodies in transplantation

- Solid-phase assays, such as the Luminex SAB assay, are recommended to detect circulating antibodies.
- Standardization of operating procedures and manufacturing processes for solid-phase assays is needed to decrease the inter- and intralaboratory variability of assay results, so that they may be used in multicenter clinical trials.
- Patients at risk for sub-optimal outcome post-transplant are defined as having a PRA $>10\%$ or donor-directed antibodies at the time of transplantation.
- Post-transplantation monitoring for DSA should be performed at 1, 3, 6, and 12 months post-operatively in accordance with ISHLT guidelines. Patients who are low risk should be monitored annually for DSA after the first year. Sensitized patients should be monitored more frequently.
- DSA testing should be performed for any patient presenting with symptoms or signs of graft dysfunction.
- DSA with graft dysfunction and restrictive physiology should be considered for treatment.
- DSA that remain at higher dilutions, $C1q^+$ DSA antibodies, DSA that persist, and DSA arising late after transplantation have been associated with adverse outcomes. Further research is required to determine whether treating antibodies in these situations would improve outcomes.
- The clinical significance of antibodies against non-HLA antigens such as MICA are equivocal and require further validation.
- First-line therapies for desensitizing patients include IVIg, plasmapheresis, immunoadsorption, and rituximab.
- Randomized, controlled trials are needed to assess the benefit of treatment in both pre- and post-transplant sensitized patients. Ideally, centers would agree on and use the same desensitization protocols so that data derived from treated patients would be comparable.
- Other future studies will include: identifying which antibodies require treatment; whether sensitization in MCS device patients requires different treatment approaches; assessing the role of non-HLA antibodies; and validating the suspected causal link between antibodies and CAV.
- An antibody registry is suggested to assist in the facilitation of research.

The antibody consensus conference framed specific questions in the approach to the management of circulating

antibodies in both pre- and post-transplant patients. Although some questions were addressed, there remain many others that will require further study, and perhaps randomized clinical trials, for answers. Truly, this conference is only a prelude to the future understanding of circulating antibodies in heart transplantation. As such, the consensus statements in this article will be revisited and updated as our understanding of antibodies evolves.

Disclosure statement

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Appendix. : Participants of the consensus conference

Consensus conference participants included: Keith Aaronson, University of Michigan Medical Center; Arezu Aliabadi, Medical University of Vienna (Austria); Arne Andreassen, Oslo University Hospital (Norway); Annalisa Angelini, Universita di Padova (Italy); Francisco Arabia, Cedars-Sinai Medical Center; David Baran, Newark Beth Israel Medical Center; Marcus Barten, Univeristy Heart Center Hamburg (Germany); Tuvia Ben Gal, Rabin Medical Center (Israel); Gerald Berry, Stanford University; Alejandro Bertolotti, Hospital Universitario Fundación Favalor (Argentina); Geetha Bhat, Advocate Christ Medical Center; Patrick Bruneval, Hopital European Georges Pompidou (France); Bernard Cantin, Hôpital de Laval (France); Javier Carbone, Gregorio Maranon Hospital (Spain); Michel Carrier, Montreal Heart Institute (Canada); Marilia Cascalho, University of Michigan; Patricia Chang, University of North Carolina at Chapel Hill; Sandra Chapparo, University of Miami/Jackson Memorial Hospital; Robert Cole, Emory University; Monica Colvin, University of Michigan; Maria Crespo-Leiro, Unidad de Insuficiencia Cardiaca (Spain); Lawrence Czer, Cedars-Sinai Medical Center; Juan Delgado Jimenez, Hospital Doce de 12 Octubre (Spain); Anthony Demetris, University of Pittsburgh; David DeNofrio, Tufts Medical Center; Shashank Desai, Inova Fairfax Hospital; Duska Dragun, Charité–Universitätsmedizin Berlin (Germany); Sitaramesh Emani, Ohio State Medical Center; Stephan Ensminger, HDZ-NRW (Germany); Eric Epailly, Les Hôpitaux Universitaires de Strasbourg (France); Fardad Esmailian, Cedars-Sinai Medical Center; Maryjane Farr, Columbia University; Marny Fedrigo, Universita di Padova (Italy); David Feldman, University of Cinncinati; Dan Fishbein, University of Washington; Michael Fishbein, David Geffen School of Medicine at UCLA; Alan Gass, Westchester Medical Center; Lee Goldberg, University of Pennsylvania; Einar Gude, Hospital Rikshospitalet (Norway); Shelley Hall, Baylor University Medical Center; Elizabeth Hammond, LDS Hopsital; Manfred Hummel, Deutsches Herzzentrum (Germany); Ulrich Jorde, Montefiore Medical Center; Andrew Kao, St. Luke's Hospital; Michael Kiernan, Tufts Medical Center; Daniel Kim, University of Alberta (Canada); Jon Kobashigawa, Cedars-Sinai Medical Center; Ornella Leone, Policlinico Sant'Orsola-Malpighi (Italy); Ugolino Livi, Ospedale Santa Maria della Misericordia (Italy); Daniel Luthringer, Cedars-Sinai Medical Center; Peter MacDonald, St. Vincent's Hospital; Joren Madsen, Massachusetts General Hospital; Pradeep Mammen, University of Texas Southwestern; Donna Mancini, New York Presbyterian Hospital; Fabiana Marcondes, Instituto do Coração (Brazil); David Markham, Emory University; Mandeep Mehra, Brigham & Women's Hospital; Dylan Miller, Intermountain Central Laboratory; Paul Mohacsi, Inselspital–Universitätsspital Bern (Switzerland); James Mudd, Oregon Health Sciences; Takeshi Nakatani, National Cardiovascular Center (Japan); Sriram Nathan, University of Texas, Houston; David Nelson, Integris Baptist Medical Center; Anju Nohria, Brigham & Women's Hospital; Jayan Parameshwar, Papworth Hospital (UK); Jignesh K. Patel, Cedars-Sinai Medical Center; Daniel Pauly, Truman Medical Center; Margarita Peradejordi, Hospital Universitario Fundación Favaloro (Argentina); Michael Pham, Stanford University; Richard Pierson, University of Maryland - Baltimore; Sean Pinney, Mt. Sinai Hospital; Barbara Pisani, Wake Forest University; Luciano Potena, University of Bologna (Italy); Vivek Rao, Toronto General Hospital (Canada); Elaine Reed, David Geffen School of Medicine at UCLA; Nancy Reinsmoen, Cedars-Sinai Medical Center; Monica Revelo, University of Utah; E. Rene Rodriguez, Cleveland Clinic; Eulalia Roig, Hospital de Sant Pau (Spain); Heather J. Ross, Toronto General Hospital (Canada); Uwe Schulz, Heart Center North-Rhine Westphalia (Germany); Marc Semigran, Massachusetts General Hospital; Scott Silvestry, Washington University; Mark Slaughter, University of Louisville; Randall C. Starling, Cleveland Clinic; Jose Tallaj, University of Alabama at Birmingham; Anat Tambur, Northwestern University; David Taylor, Cleveland Clinic Foundation; Jeffrey Teuteberg, University of Pittsburgh; Kathryn Tinckam, University Health Network (Canada); Dolly Tyan, Stanford University; Patricia Uber, Virginia Commonwealth University; Walter Uber, Medical University of South Carolina; Nir Uriel, University of Chicago; Simon Urschel, University of Alberta (Canada); Adrian Van Bakel, Medical University of South Carolina; Johan Vanhaecke, Universitaire Ziekenhuizen Leuven (Belgium); Shoei-Shen Wang, National Taiwan University (Republic of China); Xiaohai Zhang, Cedars-Sinai Medical Center; Emmanuel Zorn, Columbia University; Mark Zucker, Newark Beth Israel Medical Center; Andreas Zuckermann, Medical University of Vienna (Austria).

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